Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Homozygous Deletion Alpha-Thalassemia and Hereditary Persistence of Fetal Hemoglobin, Two Genetic Factors Predictive the Reduction of Morbidity and Mortality During Pregnancy in Sickle Cell Patients. A Report from the Democratic Republic of Congo

Tite Minga Mikobi[1,2], Prosper Tshilobo Lukusa[1,3], Jean-Marie Mbuyi Muamba[4] and Tozin Rhama[5].

[1] Center for Human Genetics, Faculty of Medicine, University of Kinshasa, Democratic Republic of Congo, DRC.
[2] Division of Gynecology Obstetrics, Center for Sickle Cell Anemia, Kinshasa, DRC.
[3] Division of Pediatrics, Hospital University, Faculty of Medicine, University of Kinshasa, DRC.
[4] Division of Internal Medicine, Service of Immuno hemato Rheumatology, Hospital University, Faculty of Medicine, University of Kinshasa, DRC.
[5] Division of Gynecology Obstetrics, Hospital University, Faculty of Medicine, University of Kinshasa, DRC.

Competing interests: The authors have declared that no competing interests exist.

Abstract. ***Objective*: to determine the beneficial role of Fetal Hemoglobin (FHb) and alpha-thal on fetal and maternal morbidity during pregnancy in sickle cell patients.**
***Study site:* the study was conducted at the sickle cell center of Kinshasa between 2008 and 2018**
***Setting and study population*: this is a documentary and analytical study that included 980 deliveries of homozygous sickle cell patients.**
***Methods*: the diagnosis of SCD and the quantification of FHb were performed with the capillary electrophoresis technique. The molecular test confirmed the diagnosis of SCD. The diagnosis of alpha-thal was made with the multiplex ligation-dependent probe amplification (MLPA) technique. Sickle cell pregnancies were followed according to the protocol of care in force in the University of Kinshasa Hospital service. The variables of interest were: hematological variables, sickle cell crises during pregnancy, maternal and fetal complications.**
***Statistics*: statistical analyses were performed with SPSS 20.0 software. Means and standard deviations were compared with the Student's t and ANOVA tests. The value of p <0.05 was considered the significance level.**
***Results*: the Hb-SS / alpha-thal and HbSS / HPFH genotypes were observed in 101 and 121 women, respectively. Otherwise, 758 women had HbSS genotype. The morbidity related to sickle cell complications in the mother and fetus were less frequent in the Hb-SS / alpha-thal and HbSS / HPFH groups than in HB-SS group. The differences were statistically significant.**
***Conclusion*: this study showed a significant protective effect of alpha-thal and HPFH during pregnancy in sickle-cell pregnant women.**

Keywords: Alpha-thal, HPFH, Morbidity, Pregnancy, Sickle cell disease.

Citation: Mikobi T.M., Lukusa P.T., Muamba J.M.M., Rhama T. Homozygous deletion alpha-thalassemia and hereditary persistence of fetal hemoglobin, two genetic factors predictive the reduction of morbidity and mortality during pregnancy in sickle cell patients. a report from the democratic republic of congo. Mediterr J Hematol Infect Dis 2019, 11(1): e2019039, DOI: http://dx.doi.org/10.4084/MJHID.2019.039

Published: July 1, 2019 **Received: December 18, 2018** **Accepted: May 22, 2019**

Correspondence to: Tite Minga Mikobi. Center for Human Genetics, Faculty of Medicine, University of Kinshasa, Democratic Republic of Congo, DRC. E-mail: tite.mikobi@unikin.ac.cd

Introduction. Sickle cell disease (SCD) is a constitutional hemoglobinopathy with autosomal recessive inheritance. The characteristic of this mutation is a transversion of a purine base [A] by a pyrimidine base in the beta-globin gene. The consequence of this mutation is the substitution of glutamic acid by valine at position 6 in the beta globin chain.[1,2] The substitution of a hydrophilic amino acid (glutamic acid) by a hydrophobic amino acid (valine) results in the production of abnormal hemoglobin called HbS. Indeed, in concentrated solution and under the influence of a decrease in oxygen partial pressure, HbS undergoes a supramolecular polymerization process.[3] Hemoglobin S is today the most widespread structural abnormality of hemoglobins in the world.[4,5] Equatorial Africa is the area of maximum incidence. Clinically SCD is characterized by recurrent vaso-occlusive ischemic events, chronic hemolysis, and high susceptibility to infections. The best management begins with early detection, preventive care against encapsulated bacterial infections and especially the administration of hydroxyurea. The association of SCD and pregnancy is characterized by high maternal and fetal morbidity.[6,7] Indeed, it is well established that there is a reciprocal influence between pregnancy and SCD. During pregnancy, there is a worsening of maternal anemia, an increase in the frequency of VOC, and a high risk of infection.[8] The fetus is at high risk of spontaneous abortions, prematurity, intrauterine growth retardation, hypotrophy, and in utero death.[9-11] Genetically, two factors showed their modulatory effect on the sickle cell phenotype. These are fetal hemoglobin (FHb: $\alpha_2\gamma_2$) and alpha-thalassemia. A high level of FHb slows down the polymerization, which results in a reduction of the number of VOC and hemolysis. The mechanism of this protection is explained by the formation of hybrid polymers ($\alpha_2\beta^s\gamma$) that stop the growth of the phenomenon of polymerization. This property has since been used in the treatment of SCD following empirical evidence that hydroxyurea stimulated the production of FHb.[12,13] The protective effect of alpha-thalassemia is related to the reduction of Hb concentration in the erythrocytes, which results in the microcytic anemia.[14] Indeed, Mikobi et al. showed that homozygous alpha-thalassemia and an FHb level > 15% had a protective effect on SCD in Congolese patients.[15] The objective of the present study was to determine the beneficial role of genetic modulating factors of sickle cell disease in fetal and maternal morbidity and mortality during pregnancy in sickle cell patients.

Patients and Methods.

Subjects. In this is a transversal study, 980 records of SCD pregnant women, who gave birth between 2008 and 2018, were analyzed. The review was conducted at the Sickel Cell Center in Kinshasa, Democratic Republic of Congo (DRC). The patients were divided into three genotypic subgroups. The first subgroup consisted of SCD pregnant with associated homozygous alpha-thalassemia (Hb-SS/alpha-thal), the second group consisted of SCD with hereditary persistence of fetal hemoglobin (HbSS / HPFH), and the third group consisted of gestants without association with one of the two pathologies mentioned above (HbSS).

Operational definitions. In this study, the group of gestational Hb-SS /alpha-thal included only patients who had the deletion alpha-thalassemia homozygous (-α, -α or --, αα) and the group HbSS / HPFH the patients with an FHb level > 15%. However, all sickle cell patients with heterozygous alpha-thalassemia (-α, αα or αα, -α) or with FHb < 15% were excluded from this study.

Laboratory tests. The diagnosis of SCD was made by the technique of capillary electrophoresis; the device used was the Mini cap flex piercing (Sebia, France). This technique also made it possible to quantify the fraction of FHb and to make the diagnosis of HPFH. The diagnosis of HPFH was retained for an FHb > 15% after three dosages within three months. The diagnosis of SCD was confirmed by a molecular test based on the restriction fragment-length polymerization technique (RFLP). The diagnosis of alpha-thalassemia has been made by the Multiplex Ligation-dependent Probe Amplification (MLPA) technique. The procedures, as well as the reagents used for both techniques (RFLP and MLPA), have been carefully described by Mikobi et al..[15,16]

Protocol for the management of sickle cell disease. Transfusion and pain management. Our protocol advocates only therapeutic transfusions during pregnancy. Prophylactic transfusions which systematically transfuse sickle cell disease from the 24th weeks of pregnancy are not practiced in our department for two main reasons: economic and lack of consensus in the real benefit of systemic prophylactic transfusion. However, we will resort to occasional transfusion exchanges during the pregnancy when there is an indication. Vaso-occlusive pain at levels 1 and 2 was managed with analgesics according to the recommendations of the WHO. Complicated VOC with Stage 3 pain was managed with a multidisciplinary team consisting of anesthesia intensive care and hematologist.

Maternal pregnancy supplements and fetal monitoring. All pregnant women were given a systematic supplementation of iron and folic acid, and anti-oxidants (omega 3 fatty acids and magnesium pidolate) from the 12th week of amenorrhea. Prophylactic anti-

malarial treatment was given between the 24th and 32nd weeks, and anti-helminthic deworming was given between 28 and 32 weeks. No patients received hydroxyurea during pregnancy. Fetal surveillance was primarily based on ultrasound scans.

Childbirth. Delivery was systematically scheduled for the 37th week of amenorrhea after the completion of the biophysical manning score.

Variables of interest for the study. In this study, the following general maternal variables were assessed: gestational age, menarche age, parity, weight gain. The pregnancy weight gain (ΔP) was calculated from the following formula: ΔP = Pf - Pi (Pf weight of the pregnant woman at the time of delivery, Pi weight before pregnancy). The evolution of the Hb rate allowed to appreciate the validity of a punctual transfusion. Maternal morbidity was assessed by the analysis of sickle cell complications: VOC and hemolysis as a function of gestational age, including pregnancy complications: preeclampsia, acute chest syndrome (ACS), parasitic infections (malaria), bacterial infections (urinary tract infections, pneumonia, sepsis) and postpartum, endometritis. Fetal morbidity was assessed by analysis of abortion rates, prematurity, low birth weight, and in utero death.

Statistical analyses. Statistical analyses were performed using SPSS version 20.0.T. (2016). We had determined the distribution of the study population, which was normal. We have determined also means and standard deviations. Mean of two groups were compared by the Student's t-test and those of three groups by the ANOVA test. The value of $p < 0.05$ was considered the only one of significance.

Results. Our study showed that 101 (10.30%) of our sickle cell deliveries had a homozygous alpha-thal deletion, while 121 (12.34%) of the women had an HPFH. Besides, 758 or 77.34% of the deliveries had none of the two associated genetic factors.

Table 1 gives the general characteristics of sickle-cell pregnancies according to their genotype. The analysis in the table shows that the Hb-SS / alpha-thal and HbSS / HPFH women had their menarche before those of the HbSS genotype. In addition, these gestants (HbSS/alpha-thal and HbSS/HPFH) had a better weight gain. Statistical differences between the first two groups and the third group were highly significant ($p < 0.001$).

Table 2 shows the biological variables during pregnancy. From the analysis in this table, the gestants of the HbSS genotype had higher levels of WBC, platelets, and reticulocytes than those with an alpha-thal or HPFH combination. The differences were highly significant ($p<0.001$). In contrast, serum iron and Hb were similar in all three groups.

Figure 1 shows the distribution of sickle cell crises during pregnancy. The chart analysis shows that VOC

Table 1. General Characteristics of Sickle Cell Pregnancy by Genotype.

Variables	HbSS/alpha-thal (n = 101)		HbSS/HPFH (n = 121)		HbSS (n = 758		*P (anova)*
	Mean	SD	Mean	SD	Mean	SD	
Maternal age (year)	23.2	3.4	24.6	2.5	20.9	1.2	0.06
Menarche age (year)	12	1.2	13	1.4	16	1.9	<0.001
Parity (n)	2	1.5	3	1.5	2	1.5	0.09
Gestational age (weeks)	37	1.9	36	1.5	35	2.2	0 .06
Gravitational weight gain (Kg)	6.2	1.1	5.4	1.9	3.1	1.4	<0.001

Table 2. The biological variables of the pregnant.

Variables	HbSS/alpha-thal (n = 101)		HbSS/HPFH (n = 121)		HbSS (n = 758)		*P (anova)*
	Mean	SD	Mean	SD	Mean	SD	
Hemoglobin (g/dl)	6.5	1.1	9.5	1.9	7.1	2.2	0.05
WBCs ($x10^3/\mu l$)	8.1	0.2	6.5	0.9	15.7	1.3	<0.001
Platelets ($x10^3/\mu l$)	250.35	111.1	272.44	102.4	360.41	134.6	<0.001
Reticulocytes (%)	7.56	2.34	9.74	4.11	15.47	7.27	<0.001
Serum iron (μmol/l)	15.75	2.65	18.5	4.2	17.7	3.4	0.08

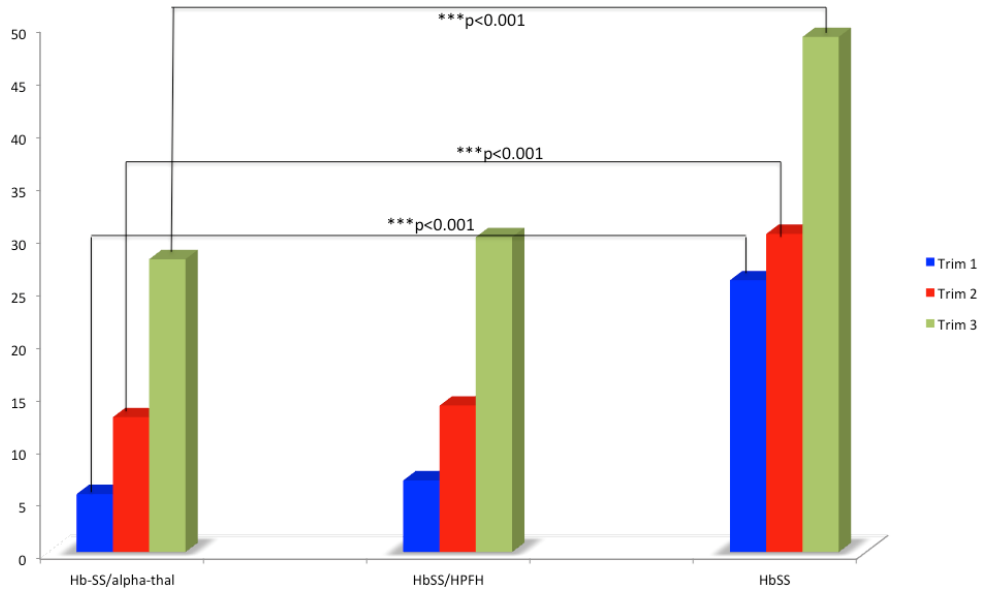

Figure 1. Evolution of VOC and worsening of anemia during pregnancy.

Table 3. Frequencies of maternal and fetal complications.

Complications	HbSS/alpha-thal (n = 101)		HbSS/HPFH (n = 121)		HbSS (n = 758)		P (anova)
	Mean	SD	Mean	SD	Mean	SD	
Pulmonary complication ACS (%)	8.4	1.2	9.2	1.4	12.1	1.2	0.12
Preeclampsia (%)	9.2	0.9	4.2	1.5	10.5	3.6	<0.001
Abortion (%)	5.2	0.9	4.2	1.5	10.5	3.6	<0.001
Prematurity (%)	12.7	2.1	14.1	1.9	22.2	2.9	<0.001
Fetal weight (g)	2850.9	250.2	2950.5	655.2	2350.8	222.4	<0.001
Death in utero (n)	2	0.2	4	0.4	25	1.2	<0.001
Maternal Death (n)	1	0.2	1	0.1	15	0.5	<0.001

and hemolysis increase steadily with gestational age. They reach their maximum in the third trimester. However, the HbSS genotype is more affected than the other two groups. Statistical differences with the Hb-SS/alpha-thal group are highly significant (p<0.001).

Table 3 presents the frequencies of maternal and fetal complications. The analysis in this table shows that the frequencies of ACS and pre-eclampsia were similar in all three groups. In contrast, spontaneous abortions, prematurity, low birth weight, in utero death and maternal death have been more observed in the HbSS genotype. Statistical differences with the other two genotypes were highly significant (p <0.001).

Figure 2 shows the distribution of parasitic and bacterial infections during pregnancy. It appears that the frequencies of malaria were similar in the three groups. In contrast, bacterial infections (urinary tract infections, pneumonia, sepsis, and endometritis) were more common in the HbSS genotype. The statistical differences were highly significant (p <0.001).

Delivery route. In our series, 49% of Caesarean sections were performed in patients with the HbSS genotype. Whereas the rates of the cesarean section of pregnant women with the HbSS / alpha and HbSS / HbF genotypes were 24% and 27% respectively.

Discussion. FHb and alpha-thal are recognized as modulatory factors for the clinical expression of SCD.[17] However, their beneficial effect during pregnancy is not well known in subtropical Africa.

In this study, sickle cell patients with HbSS genotypes had their menarche late at 16 years of age. Puberty delay is usually observed in SCD and is proportional to the severity of the disease.[18] The delivery was programmed at the 37th week; this attitude is the one reported by many authors.[19,20] Our study showed a significant difference in weight gain in favor of the HbSS / PHHF and HbSS / alpha-thal genotypes. The observed difference could be associated

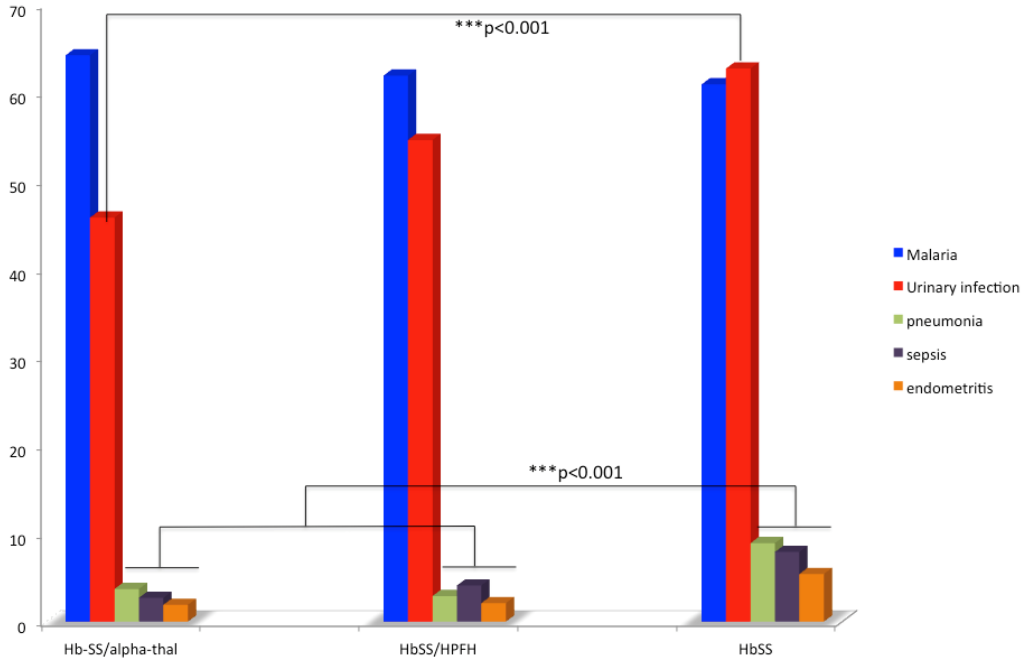

Figure 2. Distribution of parasitic and bacterial infections during pregnancy.

with the protective effects of HbF[21] and alpha-thal.[22] From the hematological variables, the HbSS genotype has a high number of WBCs, reticulocytes, and platelets. These high biological variables can explain the high morbidity of these patients during pregnancy.

VOC episodes were the leading cause of morbidity during pregnancy in SCD, as also reported by other authors.[23] These VOC increase with gestational age and are more frequent and severe in the perinatal period. In our series, VOC seizures were more common in the HbSS group. During pregnancy, several authors report a high frequency of complications such as acute thoracic syndrome and pre-eclampsia.[23,24] Their frequencies (9 to 16%) vary from one series to another.[20,23,24] In our series, the frequencies of these two complications are similar to those reported by other authors on the one hand and similar between the three genotypes on the other hand. During pregnancy, spontaneous abortions, prematurity, hypotrophy, and in utero fetal death are the main complications found in SCD.[20,25] The frequencies are different from one series to another; they are also proportional to the severity of the disease during pregnancy. In our series, these complications are more common in the HbSS group.

Infection is ranked as the second leading cause of morbidity during pregnancy in SCD. In our series, malaria was the first parasitic infection encountered because of the geographical situation of DRC. Indeed, DRC is located in the area with the highest prevalence of malaria. This infection alone constitutes the first cause of maternal and fetal morbidity.[26] In tropical Africa, malaria is ranked as the leading cause of fetal hypotrophy. The high morbidity of malaria during pregnancy is associated with maternal anemia that Plasmodium falciparum can cause.[27] In our series, the frequency of malaria was similar in all three genotypes. Maternal morbidity has also been influenced by bacterial infections such as urinary tract infection, pneumonia, sepsis, and endometritis. Their frequencies are similar to those reported by other authors.[20] However, in our series, these infections were more common in the HbSS group. The high frequency of cesarean section in SCD is related to peripartum complications. In our series, 49% of pregnant women had delivered by cesarean section. This frequency is similar to those reported by other authors.[20] However, the group with the HbSS genotype was more exposed to cesarean section than the other two. The protective effect of HbF and alpha-thal observed in our series, is similar to that reported by other authors in the associations S/β(0) thalassemia, S/β(+) thalassemia,[21] or other major sickle cell syndromes like SC, SD.

Conclusions. Homozygous alpha-thal and PHHF have shown their protective effect on sickle cell disease in general. This study shows that these genetic factors modulating sickle cell phenotype can significantly reduce maternal and fetal morbidity during pregnancy.

References:

1. Ingram VM. A specific chemical difference between the globins of normal human and sickle cell anemia hemoglobin. Nature, 1956; 178: 792-4. https://doi.org/10.1038/178792a0 PMid:13369537
2. Ingram VM. Abnormal hemoglobin. The chemical difference between normal and sickle cell hemoglobins. Biochim - Biophys Acta, 1959; 36: 402-411. https://doi.org/10.1016/0006-3002(59)90183-0

3. Perutz MF, Mitchison JN. State of hemoglobin in sickle cell anemia. Nature, 1950; 166: 677-679. https://doi.org/10.1038/166677a0
4. Serjeant GR - Sickle cell disease. Lancet, 1997; 350: 725-730. https://doi.org/10.1016/S0140-6736(97)07330-3
5. Serjeant GR. The Natural History of Sickle Cell Disease. Cold Spring Harb Perspect Med, 2013; 3:a011783 https://doi.org/10.1101/cshperspect.a011783 PMid:23813607 PMCid:PMC3784812
6. Hendrickse JPdeV, Harrison KA, Watson-Williams EJ, Luzzatto L, Ajabor LN. Pregnancy in homozygous sickle-cell anemia. J Obstet Gynecol Br Com- monw 1972;79:396-409. https://doi.org/10.1111/j.1471-0528.1972.tb14177.x
7. Baum KF, Dunn DT, Maude GH, Serjeant GR. The painful crisis of homozygous sickle cell disease. A study of risk factors. Arch Intern Med 1987;147:1231-4. https://doi.org/10.1001/archinte.1987.00370070045007 PMid:3606281
8. Koshy M, Burd L. Management of pregnancy in sickle cell syndrome. Hematol Oncol North Am 1991;5(3):585-96. https://doi.org/10.1016/S0889-8588(18)30433-7
9. Powars DR, Sandhu M, Niland-Weiss J et al. Pregnancy in SSD. Obstet Gynecol 1986; 67:217-28. https://doi.org/10.1097/00006250-198602000-00012 PMid:3945432
10. Sun PM, Wilburn W, Raynor D et al. SSD in pregnancy: twenty years of experience at Grady Memorial Hospital, Atlanta, Georgia. Am J Obstet Gynecol 2001;184:112-30. https://doi.org/10.1067/mob.2001.115477 PMid:11349177
11. Serjeant GR, Loy LL, Crowther M et al. Outcome of pregnancy in homozygous SSD. Obstet Gynecol 2004;103(6):1278-85. https://doi.org/10.1097/01.AOG.0000127433.23611.54 PMid:15172865
12. Nagel RL, Bookchim RM, Johnson J et al. Structural bases of the inhibitory effects of hemoglobin F and hemoglobin A2 on the polymerization of hemoglobin S. Proc Natl Acad Sci USA, 1982;76: 670 - 2. https://doi.org/10.1073/pnas.76.2.670 PMid:284392 PMCid:PMC383012
13. Cannas G, Poutrel S, Thomas X. Hydroxycarbamine: from an Old Drug Used in Malignant Hemopathies to a Current Standard in Sickle Cell Disease. Mediterr J Hematol Infect Dis. 2017 Feb 15;9(1):e2017015. doi: 10.4084/MJHID.2017.015. eCollection 2017. Review. https://doi.org/10.4084/mjhid.2017.015 PMid:28293403 PMCid:PMC5333733
14. Higgs DR, Aldridge BE, Lamb J et al. The interaction of alpha-thalassemia and homozygous sickle cell disease. N Engl J Med., 1982; 306: 1441 - 6. https://doi.org/10.1056/NEJM198206173062402 PMid:6176865
15. Mikobi TM, Lukusa PT, Aloni MN, et al. Association between sickle cell anemia and alpha thalassemia reveals a high prevalence of the α3.7 triplication in congolese patients than in worldwide series. J Clin Lab Anal. 2017;00:e22186. https://doi.org/10.1002/jcla.22186 PMid:28276593
16. Mikobi TM, Lukusa Tshilobo P, Aloni MN, Akilimali PZ, Mvumbi-Lelo G, and Mbuyi-Muamba JM. Clinical phenotypes and the biological parameters of Congolese patients suffering from sickle cell anemia: A first report from Central Africa. J Clin Lab Anal. 2017;00:e22140. https://doi.org/10.1002/jcla.22140 PMid:28116772
17. Jit BP, Mohanty PK, Purohit P, Patel S, Meher S, Mohanty JR, Sinha S, Behera RK, Das P. Association of fetal hemoglobin level with frequency of acute pain episodes in sickle cell disease (HbS-only phenotype) patients. Blood cells Mol Dis. 2019 Mar;75 :30-34. Epub 2018 Dec 20. https://doi.org/10.1016/j.bcmd.2018.12.003 PMid:30597429
18. Yacobovich J, Tamary H : Thalassemia major and sickle cell disease in adolescents and young adults. Acta Haematol. 2014;132(3-4) :340-7. Epub 2014 Sep 10. https://doi.org/10.1159/000360235 PMid:25228560
19. Chang JN, Magann EF, Novotny SA, Cooley CE, Gauss CH, Parrish MR, Morrison JC. Maternal/Perinatal Outcome in Women with Sickle Cell Disease: A Comparison of Two Time Periods. South Med J. 2018 Dec;111(12) :742-745. https://doi.org/10.14423/SMJ.0000000000000900 PMid:30512127
20. Silva-Pinto AC, de Oliveira Domingues Ladeira S, Brunetta DM, De Santis GC, de Lucena Angulo I, Covas DT. Sickle cell disease and pregnancy: analysis of 34 patients followed at the Regional Blood Center of Ribeirão Preto, Brazil. Rev Bras Hematol Hemoter. 2014 Sep-Oct;36(5):329-33. Epub 2014 Jul 16. https://doi.org/10.1016/j.bjhh.2014.07.002 PMid:25305164 PMCid:PMC4318372
21. Sokolova A, Mararenko A, Rozin A, Podrumar A, Gotlieb V. Hereditary persistence of hemoglobin F is protective against red cell sickling. A case report and brief review. Hematol Oncol Stem Cell Ther. 2017 Oct 16. pii: S1658-3876(17)30115-2. https://doi.org/10.1016/j.hemonc.2017.09.003 PMid:29079125
22. Resende Cardoso PS, Lopes Pessoa de Aguiar RA, Viana MB. Clinical complications in pregnant women with sickle cell disease: prospective study of factors predicting maternal death or near miss. Rev Bras Hematol Hemoter. 2014 Jul-Aug; 36(4):256-63. Epub 2014 May 29. https://doi.org/10.1016/j.bjhh.2014.05.007 PMid:25031164
23. Cardosa D, Ridout A, Nanda S, Howard J, Robinson SE, Oteng-Ntim E. Maternal sickle cell disease and twin pregnancy: a case series and review of the literature. Hematology. 2019 Dec;24(1):148-158. Epub 2018 Oct 21. https://doi.org/10.1080/10245332.2018.1535534 PMid:30345909
24. Chambers J, Smith N, Sehring M, Chittivelu S. Acute Chest Syndrome Progressing to ARDS in a Patient of 25-Week Gestation. Case Rep Crit Care. 2018 Jan 30;2018:4243569. eCollection 2018. https://doi.org/10.1155/2018/4243569 PMid:29666710 PMCid:PMC5831955
25. Burgos Luna JM, Páez Rúa DM, Ruiz Ordoñez I, Fernández PA, Escobar Vidarte MF. Description of criteria for near miss in high-complexity obstetric population with sickle cell anemia: an observational study. J Matern Fetal Neonatal Med. 2018 Sep 19:1-6. https://doi.org/10.1080/14767058.2018.1510912 PMid:30231783
26. McGann PT, Williams AM, Ellis G, McElhinney KE, Romano L, Woodall J, Howard TA, Tegha G, Krysiak R, Lark RM, Ander EL, Mapango C, Ataga KI, Gopal S, Key NS, Ware RE, Suchdev PS. Prevalence of inherited blood disorders and associations with malaria and anemia in Malawian children. Blood Adv. 2018 Nov 13;2(21):3035-3044. https://doi.org/10.1182/bloodadvances.2018023069 PMid:30425067 PMCid:PMC6234379
27. Maier AG, Matuschewski K, Zhang M, Rug M. Plasmodium falciparum. Trends Parasitol. 2018 Dec 27. pii: S1471-4922(18)30248-4.