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Nitric Oxide Dysregulation and Vaso-Occlusive Crisis in Sickle Cell Anemia: A Review

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Abstract

Sickle cell anemia (SCA) is a genetic disorder characterized by the production of abnormal hemoglobin S (HbS), leading to chronic hemolysis and recurrent vaso-occlusive crises (VOCs). VOCs are acute, painful episodes caused by the obstruction of blood flow due to sickled red blood cells (RBCs), resulting in tissue ischemia and organ damage. Nitric oxide (NO) plays a crucial role in vascular homeostasis, and its dysregulation is a significant factor in the pathophysiology of SCA, particularly in VOCs. In SCA, chronic hemolysis releases free hemoglobin into the plasma, which scavenges NO and reduces its bioavailability. Additionally, increased arginase activity depletes L-arginine, the substrate for NO synthesis, further diminishing NO production. Oxidative stress exacerbates NO degradation and endothelial dysfunction, amplifying the risk of VOCs. The interplay between NO deficiency, oxidative stress, and endothelial dysfunction creates a vicious cycle that perpetuates vascular damage and increases the frequency and severity of VOCs. This review explores the mechanisms underlying NO dysregulation in SCA and its impact on vascular function. It also discusses potential therapeutic interventions aimed at modulating NO pathways to prevent or reduce VOCs. These interventions include NO donors, L-arginine supplementation, phosphodiesterase inhibitors, antioxidant therapy, and arginase inhibitors.

Keywords: Sickle cell anemia, vaso-occlusive crisis, nitric oxide dysregulation, hemolysis, endothelial dysfunction, oxidative stress, therapeutic interventions.

Introduction

Sickle cell anemia (SCA) is a severe hereditary hemoglobinopathy resulting from a single nucleotide mutation in the β-globin gene, which substitutes valine for glutamic acid at the sixth position of the β-globin chain.[1-2] This alteration leads to the production of hemoglobin S (HbS) instead of the normal hemoglobin A (HbA). Under deoxygenated conditions, HbS polymerizes, causing red blood cells (RBCs) to adopt a characteristic sickle shape. These deformed RBCs exhibit increased rigidity, reduced deformability, and a propensity to adhere to the endothelium, contributing to various clinical complications, including hemolysis, chronic anemia, and vaso-occlusive crises (VOCs).[3-5] VOCs are the hallmark of SCA and are responsible for the acute, severe pain episodes experienced by patients. These crises occur when sickled RBCs obstruct blood flow in the microvasculature, leading to ischemia and reperfusion injury in tissues and organs. The frequency and severity of VOCs vary among patients and significantly impact the quality of life and overall prognosis. While the polymerization of HbS and the resulting RBC sickling are central to the pathophysiology of VOCs, recent research has highlighted the crucial role of nitric oxide (NO) dysregulation in exacerbating these crises.[6-10] Nitric oxide is a critical signaling molecule involved in various physiological processes, including vasodilation, inhibition of platelet aggregation, and modulation of inflammation.[11] In the vascular system, NO is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS) and plays a vital role in maintaining vascular homeostasis.[12] NO diffuses from endothelial cells to the underlying smooth muscle cells, where it activates soluble guanylate cyclase, increasing cyclic guanosine monophosphate (cGMP) levels and causing vasodilation. This mechanism is essential for regulating vascular tone and blood flow.

In SCA, several factors contribute to NO dysregulation, leading to reduced NO bioavailability and impaired vascular function. Chronic hemolysis is a major contributor, as the breakdown of sickled RBCs releases free hemoglobin into the plasma. Free hemoglobin rapidly scavenges NO, forming inactive nitrate and methemoglobin, thus depleting NO levels in the bloodstream. This process, known as hemolysis-associated NO scavenging, significantly impairs endothelial-dependent vasodilation and promotes vascular occlusion. Another critical factor in NO dysregulation in SCA is the increased activity of arginase, an enzyme that hydrolyzes L-arginine to ornithine and urea. Elevated arginase activity in SCA patients reduces the availability of L-arginine for eNOS, limiting NO synthesis. This competition for L-arginine between arginase and eNOS further diminishes NO production and contributes to endothelial dysfunction. Additionally, elevated levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of eNOS, have been observed in SCA, further reducing NO synthesis.[13-17] Oxidative stress also plays a significant role in NO dysregulation in SCA. Increased production of reactive oxygen species (ROS) results from chronic inflammation, recurrent hemolysis, and ischemia-reperfusion injury associated with VOCs. ROS can react with NO to form peroxynitrite, a highly reactive and damaging molecule, effectively reducing NO bioavailability. Moreover, oxidative stress can uncouple eNOS, causing it to produce superoxide instead of NO, further exacerbating oxidative damage and endothelial dysfunction. Endothelial dysfunction in SCA is characterized by increased expression of adhesion molecules, such as P-selectin, E-selectin, and vascular cell adhesion molecule-1 (VCAM-1), which promote the adhesion of sickled RBCs to the endothelium. NO deficiency exacerbates endothelial activation and adhesion molecule expression, facilitating the recruitment of leukocytes and sickled RBCs to the vascular wall. This process not only contributes to the initiation of VOCs but also perpetuates inflammation and vascular injury.[18-24]

Mechanisms of Nitric Oxide Dysregulation in Sickle Cell Anemia

Chronic hemolysis is a hallmark of sickle cell anemia (SCA), resulting from the frequent rupture of sickled red blood cells (RBCs). This process releases free hemoglobin into the plasma, which rapidly binds to and scavenges nitric oxide (NO). Hemoglobin has a high affinity for NO, converting it into nitrate and methemoglobin, thereby reducing NO bioavailability. The loss of NO impairs its vasodilatory function, leading to increased vascular tone and reduced blood flow. This mechanism, known as hemolysis-associated NO scavenging, is a primary contributor to endothelial dysfunction and vaso-occlusive crises (VOCs) in SCA.[25-26]

Arginase Activity

Arginase, an enzyme that hydrolyzes L-arginine into ornithine and urea, plays a significant role in NO dysregulation in SCA.[27] Patients with SCA exhibit increased arginase activity, which competes with endothelial nitric oxide synthase (eNOS) for L-arginine, the substrate necessary for NO synthesis. Elevated arginase activity depletes L-arginine levels, limiting NO production. This competition exacerbates endothelial dysfunction by reducing NO availability, thereby impairing vasodilation and promoting vascular occlusion.

Oxidative Stress

Oxidative stress is markedly elevated in SCA due to chronic inflammation, recurrent hemolysis, and ischemia-reperfusion injury associated with VOCs. Reactive oxygen species (ROS) generated during these processes degrade NO and uncouple eNOS, further diminishing NO synthesis. ROS can react with NO to form peroxynitrite, a highly reactive and damaging molecule, effectively reducing NO bioavailability. This oxidative environment not only depletes NO but also damages endothelial cells, amplifying vascular dysfunction and increasing the propensity for VOCs.[28-30]

Endothelial Dysfunction

Endothelial cells in SCA patients are frequently in an activated state, characterized by increased expression of adhesion molecules such as P-selectin, E-selectin, and vascular cell adhesion molecule-1 (VCAM-1). These adhesion molecules facilitate the adherence of sickled RBCs and leukocytes to the endothelium, promoting vascular occlusion. NO deficiency exacerbates this endothelial activation, leading to heightened inflammation and vascular injury. Reduced NO levels also impair endothelial repair mechanisms, perpetuating endothelial dysfunction and contributing to the chronic vascular complications seen in SCA.[31-35]

Role of Asymmetric Dimethylarginine (ADMA)

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of eNOS that competes with L-arginine, reducing NO production.[36] Elevated levels of ADMA have been observed in SCA patients, further impairing NO synthesis. ADMA inhibits eNOS activity by mimicking L-arginine and binding to the enzyme, effectively decreasing NO availability. The combination of increased ADMA and reduced L-arginine exacerbates NO deficiency, contributing to the pathophysiology of VOCs.

Hemolysis-Driven Inflammation

Hemolysis not only scavenges NO but also triggers an inflammatory response that further disrupts NO homeostasis.[37] Free heme and hemoglobin released during hemolysis activate toll-like receptors on endothelial cells and leukocytes, promoting the release of pro-inflammatory cytokines. This inflammatory milieu enhances oxidative stress, further depleting NO and promoting endothelial dysfunction. The interplay between hemolysis, inflammation, and NO dysregulation creates a vicious cycle that perpetuates vascular damage and increases the frequency and severity of VOCs.

Uncoupling of eNOS

Under normal conditions, eNOS synthesizes NO from L-arginine.[38] However, in the presence of oxidative stress and low L-arginine levels, eNOS can become uncoupled, producing superoxide instead of NO. This phenomenon not only reduces NO production but also generates additional ROS, exacerbating oxidative stress and vascular damage. Uncoupled eNOS contributes significantly to the endothelial dysfunction observed in SCA, further promoting VOCs.

Platelet Activation and Aggregation

NO plays a crucial role in inhibiting platelet activation and aggregation, processes that are exacerbated in SCA. NO deficiency leads to increased platelet activation, contributing to thrombus formation and vascular occlusion. Activated platelets release additional pro-inflammatory mediators, further enhancing endothelial dysfunction and NO dysregulation. This pro-thrombotic state is a significant factor in the pathogenesis of VOCs.[37]

Vascular Smooth Muscle Cell (VSMC) Proliferation

NO inhibits the proliferation of vascular smooth muscle cells (VSMCs), a process that is dysregulated in SCA. Reduced NO levels due to hemolysis and oxidative stress lead to increased VSMC proliferation, contributing to vascular remodeling and stiffness. This vascular remodeling further impairs blood flow and exacerbates VOCs. The loss of NO's regulatory effect on VSMCs highlights its critical role in maintaining vascular homeostasis.[39-42]

Therapeutic Interventions Targeting Nitric Oxide Pathways

NO Donors

Nitric oxide (NO) donors, such as nitroglycerin and isosorbide dinitrate, provide exogenous sources of NO, directly enhancing vasodilation and improving blood flow.[43] These compounds release NO or related molecules that convert to NO in the body, thereby increasing NO bioavailability. Clinical studies have shown that NO donors can reduce the frequency and severity of vaso-occlusive crises (VOCs) by improving vascular tone and reducing endothelial adhesion of sickled red blood cells (RBCs). However, the clinical use of NO donors is limited by the development of tolerance, where the body becomes less responsive to the effects over time, and potential side effects such as headaches, hypotension, and methemoglobinemia.

L-Arginine Supplementation

L-arginine is the substrate for endothelial nitric oxide synthase (eNOS) in the production of NO. Supplementing L-arginine aims to increase NO synthesis by providing more substrate for eNOS. Studies have shown that L-arginine supplementation can improve endothelial function, enhance NO production, and reduce oxidative stress. However, clinical trials have produced mixed results, with some showing benefits in reducing VOCs and improving vascular health, while others have shown no significant effect. The variability in outcomes may be due to differences in dosages, patient populations, and study designs, indicating the need for further research to optimize treatment protocols.[44-47]

Phosphodiesterase Inhibitors

Phosphodiesterase (PDE) inhibitors, such as sildenafil, tadalafil, and vardenafil, work by preventing the degradation of cyclic guanosine monophosphate (cGMP), a downstream mediator of NO signaling.[48] By inhibiting PDE, these drugs maintain higher levels of cGMP, thereby enhancing NO-induced vasodilation and improving blood flow. PDE inhibitors have been shown to reduce pulmonary hypertension, a common complication in sickle cell anemia (SCA), and may have potential in reducing VOCs. However, their long-term efficacy and safety in SCA patients require further investigation through well-designed clinical trials.

Antioxidant Therapy

Antioxidants aim to reduce oxidative stress, a significant contributor to NO dysregulation in SCA. Compounds such as N-acetylcysteine, vitamin E, and omega-3 fatty acids can neutralize reactive oxygen species (ROS), thereby preserving NO bioavailability and protecting endothelial function. Antioxidant therapy has shown promise in preclinical studies and early-phase clinical trials, demonstrating reduced oxidative stress and improved vascular function. The effectiveness of antioxidants in reducing VOC frequency and severity needs to be validated in larger, randomized controlled trials.[49-53]

Arginase Inhibitors

Arginase inhibitors, such as N-hydroxy-nor-L-arginine (nor-NOHA) and CB-1158, block the activity of arginase, an enzyme that competes with eNOS for L-arginine.[54] By inhibiting arginase, these compounds increase the availability of L-arginine for NO synthesis, enhancing NO production and improving endothelial function. Preclinical studies have shown that arginase inhibitors can improve vascular reactivity and reduce endothelial adhesion in SCA models. Ongoing clinical trials are investigating the potential benefits of arginase inhibitors in reducing VOCs and improving overall vascular health in SCA patients.

Hydroxyurea

Hydroxyurea is a well-established treatment for SCA that increases fetal hemoglobin (HbF) levels, reduces hemolysis, and decreases the frequency of VOCs. While its primary mechanism is through the induction of HbF, hydroxyurea also has effects on NO metabolism. It reduces the release of free hemoglobin, thereby decreasing NO scavenging, and has been shown to increase NO bioavailability indirectly. The combined effects of hydroxyurea on hemoglobin synthesis and NO regulation make it a cornerstone therapy in SCA management.[55-58]

Statins

Statins, commonly used for their cholesterol-lowering effects, also possess anti-inflammatory and endothelial-protective properties. They can enhance eNOS expression and activity, increasing NO production and improving endothelial function. Statins have shown promise in preclinical studies and small clinical trials in reducing inflammation, improving vascular reactivity, and potentially reducing VOCs.[59] Further research is needed to establish their role and efficacy in the routine management of SCA.

Hemoglobin-Based Oxygen Carriers (HBOCs)

Hemoglobin-based oxygen carriers (HBOCs) are designed to serve as blood substitutes, providing oxygen delivery without the risk of transfusion-related complications. Some HBOCs have been engineered to reduce NO scavenging by modifying the hemoglobin molecule. These modified HBOCs can potentially serve as both oxygen carriers and NO donors, improving oxygen delivery and vascular function in SCA patients.[60] Clinical development and trials are ongoing to evaluate their safety and efficacy in reducing VOCs and improving overall outcomes in SCA.

Endothelin Receptor Antagonists

Endothelin-1 (ET-1) is a potent vasoconstrictor that contributes to endothelial dysfunction and NO dysregulation in SCA.[61] Endothelin receptor antagonists, such as bosentan and ambrisentan, block the effects of ET-1, reducing vasoconstriction and improving endothelial function. These drugs have been effective in treating pulmonary hypertension and may have potential in reducing VOCs by mitigating endothelial dysfunction and enhancing NO bioavailability. Clinical trials are needed to assess their specific benefits in SCA.

Conclusion

Nitric oxide (NO) dysregulation plays a critical role in the pathogenesis of vaso-occlusive crises (VOCs) in sickle cell anemia (SCA), significantly impacting the clinical outcomes and quality of life for patients. The complex interplay between hemolysis, increased arginase activity, oxidative stress, and endothelial dysfunction culminates in reduced NO bioavailability and impaired vascular function. Current therapeutic approaches focus on enhancing NO bioavailability, reducing oxidative stress, and improving endothelial function. NO donors, L-arginine supplementation, phosphodiesterase inhibitors, antioxidant therapy, and arginase inhibitors represent promising strategies, each addressing different aspects of NO dysregulation. Additionally, established treatments like hydroxyurea and emerging interventions such as statins, hemoglobin-based oxygen carriers (HBOCs), and endothelin receptor antagonists offer potential benefits by indirectly or directly modulating NO pathways.

References

1. Alenzi FQ, AlShaya DS. Biochemical and molecular analysis of the beta-globin gene on Saudi sickle cell anemia. Saudi Journal of Biological Sciences. 2019;26(7):1377-1384. https://doi.org/10.1016/j.sjbs.2019.03.003 PMid:31762599 PMCid:PMC6864391

2. Williams TN, Thein SL. Sickle cell anemia and its phenotypes. Annual review of genomics and human genetics. 2018;19(1):113-147. https://doi.org/10.1146/annurev-genom-083117-021320 PMid:29641911 PMCid:PMC7613509

3. Obeagu EI, Ochei KC, Nwachukwu BN, Nchuma BO. Sickle cell anaemia: a review. Scholars Journal of Applied Medical Sciences. 2015;3(6B):224422-52.

4. Obeagu EI. Erythropoeitin in Sickle Cell Anaemia: A Review. International Journal of Research Studies in Medical and Health Sciences. 2020;5(2):22-28. https://doi.org/10.22259/ijrsmhs.0502004

5. Obeagu EI. Sickle Cell Anaemia: Haemolysis and Anemia. Int. J. Curr. Res. Chem. Pharm. Sci. 2018;5(10):20-21.

6. Obeagu EI, Muhimbura E, Kagenderezo BP, Uwakwe OS, Nakyeyune S, Obeagu GU. An Update on Interferon Gamma and C Reactive Proteins in Sickle Cell Anaemia Crisis. J Biomed Sci. 2022;11(10):84.

7. Obeagu EI, Ogunnaya FU, Obeagu GU, Ndidi AC. Sickle cell anaemia: a gestational enigma. European Journal of Biomedical and Pharmaceutical Sciences. 2023;10((9): 72-75

8. Obeagu EI. An update on micro RNA in sickle cell disease. Int J Adv Res Biol Sci. 2018; 5:157-158.

9. Obeagu EI, Babar Q. Covid-19 and Sickle Cell Anemia: Susceptibility and Severity. J. Clinical and Laboratory Research. 2021;3(5):2768-2487.

10. Obeagu EI. Depression in Sickle Cell Anemia: An Overlooked Battle. Int. J. Curr. Res. Chem. Pharm. Sci. 2023;10(10):41-.

11. Gkaliagkousi E, Ritter J, Ferro A. Platelet-derived nitric oxide signaling and regulation. Circulation research. 2007 Sep 28;101(7):654-662. https://doi.org/10.1161/CIRCRESAHA.107.158410 PMid:17901370

12. Tran N, Garcia T, Aniqa M, Ali S, Ally A, Nauli SM. Endothelial nitric oxide synthase (eNOS) and the cardiovascular system: in physiology and in disease states. American journal of biomedical science & research. 2022;15(2):153. https://doi.org/10.34297/AJBSR.2022.15.002087

13. Obeagu EI, Obeagu GU. Evaluation of Hematological Parameters of Sickle Cell Anemia Patients with Osteomyelitis in A Tertiary Hospital in Enugu, Nigeria. Journal of Clinical and Laboratory Research.2023;6(1):2768-0487.

14. Obeagu EI, Dahir FS, Francisca U, Vandu C, Obeagu GU. Hyperthyroidism in sickle cell anaemia. Int. J. Adv. Res. Biol. Sci. 2023;10(3):81-89.

15. Njar VE, Ogunnaya FU, Obeagu EI. Knowledge And Prevalence of The Sickle Cell Trait Among Undergraduate Students Of The University Of Calabar. Prevalence.;5(100):0-5.

16. Swem CA, Ukaejiofo EO, Obeagu EI, Eluke B. Expression of micro RNA 144 in sickle cell disease. Int. J. Curr. Res. Med. Sci. 2018;4(3):26-32.

17. Obeagu EI. Sickle cell anaemia: Historical perspective, Pathophysiology and Clinical manifestations. Int. J. Curr. Res. Chem. Pharm. Sci. 2018;5(11):13-15.

18. Obeagu EI, Obeagu GU. Sickle Cell Anaemia in Pregnancy: A Review. International Research in Medical and Health Sciences. 2023 Jun 10;6(2):10-13.

19. Obeagu EI, Mohamod AH. An update on Iron deficiency anaemia among children with congenital heart disease. Int. J. Curr. Res. Chem. Pharm. Sci. 2023;10(4):45-48.

20. Edward U, Osuorji VC, Nnodim J, Obeagu EI. Evaluationof Trace Elements in Sickle Cell Anaemia Patients Attending Imo State Specialist Hospital, Owerri. Madonna University journal of Medicine and Health Sciences ISSN: 2814-3035. 2022 Mar 4;2(1):218-234. https://doi.org/10.59298/NIJRMS/2023/10.2.1400

21. Umar MI, Aliyu F, Abdullahi MI, Aliyu MN, Isyaku I, Aisha BB, Sadiq RU, Shariff MI, Obeagu EI. Assessment Of Factors Precipitating Sickle Cell Crises Among Under 5-Years Children Attending Sickle Cell Clinic Of Murtala Muhammad Specialist Hospital, Kano. blood.;11:16.

22. Obeagu EI. Vaso-occlusion and adhesion molecules in sickle cells disease. Int J Curr Res Med Sci. 2018;4(11):33-35.

23. Ifeanyi OE, Stella EI, Favour AA. Antioxidants In The Management of Sickle Cell Anaemia. Int J Hematol Blood Disord (Internet) 2018 (cited 2021 Mar 4); 3. Available from: https://symbiosisonlinepublishing. com/hematology/hema tology25. php. 2018 Sep.

24. Buhari HA, Ahmad AS, Obeagu EI. Current Advances in the Diagnosis and Treatment of Sickle Cell Anaemia. APPLIED SCIENCES (NIJBAS). 2023;4(1). https://doi.org/10.59298/NIJBAS/2023/1.1.11111

25. Obeagu EI, Obeagu GU. Hemolysis Challenges for Pregnant Women with Sickle Cell Anemia: A Review. Elite Journal of Haematology. 2024;2(3):67-80.

26. Obeagu EI, Obeagu GU, Hauwa BA. Optimizing Maternal Health: Addressing Hemolysis in Pregnant Women with Sickle Cell Anemia. Journal home page: http://www. journalijiar. com.;12(01).

27. Vilas-Boas W, Cerqueira BA, Zanette AM, Reis MG, Barral-Netto M, Goncalves MS. Arginase levels and their association with Th17-related cytokines, soluble adhesion molecules (sICAM-1 and sVCAM-1) and hemolysis markers among steady-state sickle cell anemia patients. Annals of hematology. 2010; 89:877-882. https://doi.org/10.1007/s00277-010-0954-9 PMid:20405289 PMCid:PMC2908460

28. Nnodim J, Uche U, Ifeoma U, Chidozie N, Ifeanyi O, Oluchi AA. Hepcidin and erythropoietin level in sickle cell disease. British Journal of Medicine and Medical Research. 2015;8(3):261-265. https://doi.org/10.9734/BJMMR/2015/17480

29. Obeagu EI. BURDEN OF CHRONIC OSTEOMYLITIS: REVIEW OF ASSOCIATIED FACTORS. Madonna University journal of Medicine and Health Sciences. 2023;3(1):1-6.

30. Aloh GS, Obeagu EI, Okoroiwu IL, Odo CE, Chibunna OM, Kanu SN, Elemchukwu Q, Okpara KE, Ugwu GU. Antioxidant-Mediated Heinz Bodies Levels of Sickle Erythrocytes under Drug-Induced Oxidative Stress. European Journal of Biomedical and Pharmaceutical sciences. 2015;2(1):502-507.

31. Obeagu EI, Obeagu GU. Sickle Cell Anaemia in Pregnancy: A Review. International Research in Medical and Health Sciences. 2023;6 (2):10-13.

32. Obeagu EI, Ogbuabor BN, Ikechukwu OA, Chude CN. Haematological parameters among sickle cell anemia patients' state and haemoglobin genotype AA individuals at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. International Journal of Current Microbiology and Applied Sciences. 2014;3(3):1000-1005.

33. Ifeanyi OE, Nwakaego OB, Angela IO, Nwakaego CC. Haematological parameters among sickle cell anaemia... Emmanuel Ifeanyi1, et al. pdf• Obeagu. Int. J. Curr. Microbiol. App. Sci. 2014;3(3):1000-1005.

34. Obeagu EI, Opoku D, Obeagu GU. Burden of nutritional anaemia in Africa: A Review. Int. J. Adv. Res. Biol. Sci. 2023;10(2):160-163.

35. Ifeanyi E. Erythropoietin (Epo) Level in Sickle Cell Anaemia (HbSS) With Falciparum Malaria Infection in University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. PARIPEX - INDIAN JOURNAL OF RESEARCH, 2015; 4(6): 258-259

36. Tsikas D. Does the inhibitory action of asymmetric dimethylarginine (ADMA) on the endothelial nitric oxide synthase activity explain its importance in the cardiovascular system? The ADMA paradox. Journal of Controversies in Biomedical Research. 2017;3(1):16-22. https://doi.org/10.15586/jcbmr.2017.22

37. Martins R, Knapp S. Heme and hemolysis in innate immunity: adding insult to injury. Current opinion in immunology. 2018; 50:14-20. https://doi.org/10.1016/j.coi.2017.10.005 PMid:29107115

38. Wu G, Meininger CJ, McNeal CJ, Bazer FW, Rhoads JM. Role of L-arginine in nitric oxide synthesis and health in humans. Amino acids in nutrition and health: Amino acids in gene expression, metabolic regulation, and exercising performance. 2021:167-87. https://doi.org/10.1007/978-3-030-74180-8_10 PMid:34251644

39. Ifeanyi OE, Nwakaego OB, Angela IO, Nwakaego CC. Haematological parameters among sickle cell anaemia patients in steady state and haemoglobin genotype AA individuals at Michael Okpara, University of Agriculture, Umudike, Abia State, Nigeria. Int. J. Curr. Microbiol. App. Sci. 2014;3(3):1000-1005.

40. Ifeanyi OE, Stanley MC, Nwakaego OB. Comparative analysis of some haematological parameters in sickle cell patients in steady and crisis state at michael okpara University of agriculture, Umudike, Abia state, Nigeria. Int. J. Curr. Microbiol. App. Sci. 2014;3(3):1046-1050.

41. Ifeanyi EO, Uzoma GO. Malaria and The Sickle Cell Trait: Conferring Selective Protective Advantage to Malaria. J Clin Med Res. 2020; 2:1-4. https://doi.org/10.37191/Mapsci-2582-4333-2(2)-026

42. Obeagu EI, Obeagu GU. Oxidative Damage and Vascular Complications in Sickle Cell Anemia: A Review. Elite Journal of Haematology, 2024; 2 (3).:58-66.

43. Roberts BW, Mitchell J, Kilgannon JH, Chansky ME, Trzeciak S. Nitric oxide donor agents for the treatment of ischemia/reperfusion injury in human subjects: a systematic review. Shock. 2013;39(3):229-339.

https://doi.org/10.1097/SHK.0b013e31827f565b PMid:23358103

44. Obeagu EI, Obeagu GU. Addressing Myths and Stigmas: Breaking Barriers in Adolescent Sickle Cell Disease Education. Elite Journal of Health Science. 2024;2(2):7-15. https://doi.org/10.2147/JBM.S462758 PMid:39081620 PMCid:PMC11288316
45. Obeagu EI, Obeagu GU. Implications of climatic change on sickle cell anemia: A review. Medicine. 2024 Feb 9;103(6):e37127. https://doi.org/10.1097/MD.0000000000037127 PMid:38335412 PMCid:PMC10860944
46. Obeagu EI. Chromium VI: A Silent Aggressor in Sickle Cell Anemia Pathophysiology. Elite Journal of Haematology, 2024; 2 (3).:81-95.
47. Obeagu EI. Maximizing longevity: erythropoietin's impact on sickle cell anemia survival rates. Annals of Medicine and Surgery. 2024:10-97. https://doi.org/10.1097/MS9.0000000000001763 PMid:38463100 PMCid:PMC10923353
48. Samidurai A, Xi L, Das A, Kukreja RC. Beyond erectile dysfunction: cGMP-specific phosphodiesterase 5 inhibitors for other clinical disorders. Annual review of pharmacology and toxicology. 2023;63(1):585-615. https://doi.org/10.1146/annurev-pharmtox-040122-034745 PMid:36206989
49. Obeagu EI, Ubosi NI, Obeagu GU, Egba SI, Bluth MH. Understanding apoptosis in sickle cell anemia patients: Mechanisms and implications. Medicine. 2024;103(2):e36898. https://doi.org/10.1097/MD.0000000000036898 PMid:38215146 PMCid:PMC10783340
50. Obeagu EI, Ayogu EE, Anyanwu CN, Obeagu GU. Drug-Drug Interactions in the Management of Coexisting Sickle Cell Anemia and Diabetes. Elite Journal of Health Science. 2024;2(2):1-9.
51. Obeagu EI, Obeagu GU. Dual Management: Diabetes and Sickle Cell Anemia in Patient Care. Elite Journal of Medicine. 2024;2(1):47-56.
52. Obeagu EI, Obeagu GU, Hauwa BA. Optimizing Maternal Health: Addressing Hemolysis in Pregnant Women with Sickle Cell Anemia. Journal home page: http://www. journalijiar. com.;12(01).
53. Obeagu EI, Obeagu GU. Synergistic Care Approaches: Integrating Diabetes and Sickle Cell Anemia Management. Elite Journal of Scientific Research and Review. 2024;2(1):51-64.
54. Grzywa TM, Sosnowska A, Matryba P, Rydzynska Z, Jasinski M, Nowis D, Golab J. Myeloid cell-derived arginase in cancer immune response. Frontiers in immunology. 2020; 11:938. https://doi.org/10.3389/fimmu.2020.00938 PMid:32499785 PMCid:PMC7242730
55. Obeagu EI, Obeagu GU. Improving Outcomes: Integrated Strategies for Diabetes and Sickle Cell Anemia. Int. J. Curr. Res. Chem. Pharm. Sci. 2024;11(2):20-9.
56. Obeagu EI, Obeagu GU. The Role of Parents: Strengthening Adolescent Education for Sickle Cell Disease Prevention. Elite Journal of Public Health. 2024;2(1):15-21. https://doi.org/10.2147/JBM.S462758 PMid:39081620 PMCid:PMC11288316
57. Obeagu EI, Obeagu GU. Hemolysis Challenges for Pregnant Women with Sickle Cell Anemia: A Review. Elite Journal of Haematology, 2024; 2 (3).:67-80.
58. Obeagu EI, Obeagu GU. Overcoming Hurdles: Anemia Management in Malaria-Affected Childhood. Elite Journal of Laboratory Medicine. 2024;2(1):59-69.
59. Bontempo P, Capasso L, De Masi L, Nebbioso A, Rigano D. Therapeutic Potential of Natural Compounds Acting through Epigenetic Mechanisms in Cardiovascular Diseases: Current Findings and Future Directions. Nutrients. 2024;16(15):2399. https://doi.org/10.3390/nu16152399
60. Cao M, Zhao Y, He H, Yue R, Pan L, Hu H, Ren Y, Qin Q, Yi X, Yin T, Ma L. New applications of HBOC-201: a 25-year review of the literature. Frontiers in Medicine. 2021; 8:794561. https://doi.org/10.3389/fmed.2021.794561 PMid:34957164 PMCid:PMC8692657
61. Brun M, Bourdoulous S, Couraud PO, Elion J, Krishnamoorthy R, Lapoumeroulie C. Hydroxyurea downregulates endothelin-1 gene expression and upregulates ICAM-1 gene expression in cultured human endothelial cells. The Pharmacogenomics Journal. 2003;3(4):215-226. https://doi.org/10.1038/sj.tpj.6500176 PMid:12931135