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Original Review

The Neuropeptide Substance P is Elevated in Sickle Cell Disease and is a Marker of Severity of Vaso-occlusive Crisis

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Abstract

Background: Acute episode of pain is the most frequent symptom for which patients with sickle cell disease (SCD) seek medical attention. The neuropeptide Substance P (SP) has been suggested as a possible aetiologic factor. This study compared the serum levels of SP in SCD subjects in painful vaso-occlusive crisis with those in steady state and normal HbAA subjects.

Methodology: This case-controlled study investigated eighteen SCD patients in vaso-occlusive crisis (VOC) and eighteen in steady state, while fourteen HbAA subjects were recruited as controls. Blood was collected in plain bottles and subsequently, the serum was separated for SP assay using the ELISA technique. Each sample was run, and results were confirmed in duplicate. Optical density was read at an absorbance of 450nm.

Results: The study showed that SP was significantly higher in SCD patients in steady state (184.79±18.67ng/L versus 104.17±19.24ng/L) compared to the controls (t=2.97, p=0.006); while the values obtained in those in VOC (375.78±76.21ng/L) were also significantly higher (t=2.433, p=0.02) than those in steady state (184.79±18.67ng/L). The SP value in the SCD patients in VOC was almost twice as much as those in steady state and about three times as much as the value in the controls and the differences were statistically significant (t=7.72, p=0.001).

Conclusion: The study showed significantly higher SP levels in VOC compared to steady state or HbAA subjects suggesting that SP may be a marker for pain sensitisation.

Keywords: Neuropeptide Substance P; Sickle Cell Disease; Severity; Vaso-occlusive Crisis.

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Quick Response Code:

Introduction

Sickle cell disease (SCD) is the most prevalent genetic disease in the world.[1] It is estimated that more than 300,000 babies are born with severe forms of haemoglobinopathies worldwide each year. While 75 percent of all patients with SCD live in Sub-Saharan Africa, Nigeria alone accounts for more than 100,000 new births every year,[2, 3] With a prevalence rate of 1-3%, the countryhas one of the highest concentrations of patients with this disorder in the world.

Pain is a frequent complaint of people living with SCD.[4,5,6,7]Over half of SCD patients have one to two episodes annually, and 1% of patients have more than 10 episodes each year.[8]Pain crises are one of the common causes of distress among patients with SCD, and account for 90% of SCD-related hospital admissions.[9,10]SP is an attractive candidate to explain both the clinical findings and the cytokine elevations present during vaso-occlusive pain crises. SP is a potent chemotactic factor[11], thatmediates neurogenic pain and inflammation.[12, 13] and induces therelease of histamine from mast cells[14] which may mediate pain. It also promotes thesecretion of cytokines such as IL-1, IL-6, IL-8, and TNF-α.[15-18]Therefore, Substance P may be a measurable laboratory marker to assess the severity of thevaso-occlusive crisis and, therefore, anti-SP receptor antagonist may have a therapeutic potential in the treatment of vaso-occlusive events in SCD patient.

Materials and Methods

Study design and setting

This study which was a prospective case-controlled survey, was carried out at hospitals in Osogbo, the capital city of Osun State in Southwestern Nigeria. Study sites included Ladoke Akintola University (LAUTECH) Teaching Hospital and State Hospital, Asubiaro, both in Osogbo, Osun State, Nigeria.

The sickle cell disease patients were recruited from haematology clinics, haematology day wards, and the accident and emergency units at the study sites, while controls who were otherwise well, were recruited from the general population around the study sites.

Sample size and method

The sample size was determined using the Kish Leslie formula.[19] A confidence level of 95%, and an expected prevalence of 3.0% was obtained from a similar study[2]. The minimum calculated sample size was 45, which was increased to 50 to account fora10% attrition rate.

Participants recruitment

The study population consisted of consenting previously diagnosed sickle cell adult patients (HbS and HbSC) in steady state and vaso-occlusive crises as cases. Participants included in this study included SCD patients in steady state, those in painful vaso-occlusive crisis, and control subjects with HbA phenotype who were haematologically normal. Those excluded were those that have received a blood transfusion within the previous 3 months, and SCD patients on Hydroxyurea or medications other than vitamin BCo, Folate, or Paludrine. Patients or controls with other painful chronic inflammatory disorders such as rheumatoid arthritis, gout, and ankylosing spondylitis were also excluded from the study.

Sample collection

Three millilitres of venous blood was collected by standard phlebotomy technique through venipuncture via the antecubital vein using a plastic syringe into plain bottles. The samples were allowed to clot and centrifuged at 3000 rpm for 5 minutes at room temperature to separate serum from cells. The serum was separated into a separate plain tube with a cap and stored frozen at -40^{0}Cbeforeanalysis.

Methodology

The SP was assayed using an antigen competition, enzyme-linked immunosorbent assay (Bioassay kit). Each sample was run, and results were confirmed in duplicate. Optical density was read at an absorbance of 450 nm. Data entry and processing were done using the statistical package for the social sciences (SPSS), version 20.0 (SPSS Chicago Inc., IL, and USA). The data were presented using descriptive statistics of frequency distribution, means, standard deviations, and percentages. The relationship was tested using the Pearson correlation coefficient. The level of significance was taken as p values less than 0.05.

Results

A total of 50 participants made up of 18 SCD subjects in vaso-occlusive crises, 18 SCD subjects in steady state, and 16 healthy age and sex-matched controls were investigated. Three subjects were evaluated both in the VOC and steady state. The subjects were 26% females in both the VOC and steady-state groups while the males were 10% in both groups. In the control group, females were 16% while males were 12%.

The mean age for the VOC group was 21.60 ± 4.81 years, 22.94± 3.17 years for the steady state, and 21.79± 2.91 years for the control groups. There was no significant age difference in the subjects with VOC, those in steady state, and the control group (p = 0.606).

The study showed that SP was significantly higher in SCD patients in steady state (184.79 ± 18.67ng/L versus 104.17 ± 18.67ng/L) compared to the controls (t = 2.97, p = 0.006); while the values obtained in those in VOC (375.78±76.21ng/L) were also significantly higher (t = 2.433, p = 0.02) than those in steady state (184.79± 18.67ng/L). The SP value in the SCD patients in VOC was almost twice as much as those in steady-state and about three times as much as the value in the controls and the differences were statistically significant (t = 7.72, p = 0.001). Three subjects were sampled both in the steady state and VOC, which confirmed the findings that SP increased significantly during VOC compared to their baseline levels.

Table 1: Comparison of SP between VOC, Steady State, and Controls

Group Compared	Mean ± SD (ng/L)	T value	P value
VOC vs.	375.78 ± 76.21	2.433	0.020
Steady State	184.79 ± 18.67		
VOC vs.	375.78 ± 76.21	3.074	0.004
Controls	104.17 ± 19.24		
Steady State vs.	184.79 ± 18.67	2.970	0.006
Controls	104.17 ± 19.24		

Discussion

Sickle cell vaso-occlusion, which may involve both the micro- and microvasculature, is the most important pathophysiologic event in SCD and explains most of its clinical manifestation. Patients with SCD in baseline health have significantly higher SP levels compared to healthy controls. SP levels

Significantly increase during acute pain in patients with SCD compared to baseline levels.[12,23]This was similar to the findings of Michaels et al[12], Douglas[22], and Brandow et al.[23] In humans with pain disorders other than SCD, higher plasma SP levels are associated with pain and disease severity.[23] Thus, the significant elevation of SP in patients with SCD during baseline health and the further increase during acute pain supports existing data that the peripheral and/or central nervous system is sensitized inSCD.[8,23-24]This is similar to the findings of this study which showed that SP was significantly higher in SCD patients in steady state compared to the controls. The SP value in the SCD patients in VOC was almost twice as much as those in steady state and about three times as much as the value in the controls.

the significant elevation of SP in patients with SCD during baseline health and the further increase during acute pain supports existing data that the peripheral and/or central nervous systems are sensitized in SCD.[8,24-25] Furthermore, the knowledge that SP is elevated in baseline health suggests that the levels are chronically increased and thus could contribute to the development of peripheral and/or central nervous system sensitization.[23]

SP has both neuromodulating and immunomodulating effects that could contribute to the development of severe unrelenting pain in SCD. Interestingly, many inflammatory cytokines that are involved in neurogenic inflammation are significantly increased in patients with SCD compared to controls and increase further during acute vaso-occlusive crisis.[4,26-27]All these findings are suggestive that SP is a potential biomarker for SCD pain and vaso-occlusive crises.

Significantly elevated SP levels in patients with SCD during baseline health (steady state) compared to controls suggest that SP may be a mediator of, or marker for, pain sensitization. SP levels further increase during acute pain suggesting SP also plays a role in mediating or marking acute pain events. Thus, estimating the serum levels of SP may be useful in confirming or establishing the resolution of VOC in patients with SCD with prior knowledge of the baseline level, especially those already addicted to analgesic use.

Conflict of Interest: None declared.

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References

1. Aliyu ZY, Kato GJ, Taylor J, Babadoko A, Mamman AI, Gordeuk VR, et al. Sickle cell disease and pulmonary hypertension in Africa: a global perspective and review of epidemiology, pathophysiology, and management. Am J Hematol. 2008;83(1):63-70.
2. World Health Organization Fifty-Ninth World Health Assembly A59/9, Provisional Agenda Item 11.4 24 April 2006.
3. Piel FB, Hay SI, Gupta S, Weatherall DJ, Williams TN. Global burden of sickle cell anaemia in children under five, 2010–2050: modelling based on demographics, excess mortality, and interventions. PLoS Med. 2013;10(7):e1001484.
4. Pathare A, Al Kindi S, Alnaqdy AA, Daar S, Knox-Macaulay H, Dennison D. Cytokine profile of sickle cell disease in Oman. Am J Hematol. 2004;77(4):323-328.
5. Makis A, Hatzimichael E, Bourantas K. The role of cytokines in sickle cell disease. Ann Hematol. 2000;79(8):407-413.
6. Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on the production of inflammatory cytokines by human monocytes. Science 1988;241:1218-1221.

7. Rees DC, Olujohungbe AD, Parker NE, Stephens AD, Telfer P, Wright J. Guidelines for the management of the acute painful crisis in sickle cell disease. Br J Haematol 2003;120(5):744-752.
8. Kohli DR, Li Y, Khasabov SG, Gupta P, Kehl LJ, Ericson ME, et al. Pain-related behaviours and neurochemical alterations in mice expressing sickle haemoglobin: modulation by cannabinoids. Blood 2010;116(3):456-465.
9. Ohaeri JU, Shokunbi WA. The psychosocial burden of sickle cell disease on caregivers in a Nigerian setting. J Natl Med Assoc 2002;94(12):1058-1070.
10. Smith WR, Penberthy LT, Bovbjerg VE, McClish DK, Roberts JD, Dahman B, et al. Daily assessment of pain in adults with sickle cell disease. Ann Intern Med 2008;148(2):94-101.
11. Haines K, Kolasinski S, Cronstein B, Reibman J, Gold L, Weissmann G. Chemoattraction of neutrophils by substance P and transforming growth factor-beta 1 is inadequately explained by current models of lipid remodelling. J Immunol 1993;151(3):1491-1499.
12. Michaels LA, Ohene-Frempong K, Zhao H, Douglas SD. Serum levels of substance P are elevated in patients with sickle cell disease and increase further during vaso-occlusive crisis. Blood 1998;92(9):3148-3151.
13. Moussaoui S, Carruette A, Garret C. Further evidence is that substance P is a mediator of both neurogenic inflammation and pain: Two phenomena inhibited either by postsynaptic blockade of SP receptors or by presynaptic action of opiate agonists. Regul Neuropept 1992;37:424-425.
14. Okayama Y, Ei-Lati SG, Leiferman KM, Church MK. Eosinophil granule proteins inhibit substance P-induced histamine release from human skin mast cells. J Aller Clin Immunol 1994;93(5):900-909.
15. Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on the production of inflammatory cytokines by human monocytes. Science 1988;241:1218-1221.
16. Rameshwar P, Ganea D, Gascon P. In vitro stimulatory effect of substance P on hematopoiesis. Blood 1993;81(2):391-398.
17. Serra M, Calzetti F, Ceska M, Cassatella M. Effect of substance P on superoxide anion and IL-8 production by human PMNL. Immunology. 1994;82(1):63-69.
18. Lee H, Ho W, Douglas S. Substance P augments tumor necrosis factor release in human monocyte-derived macrophages. Clin Diagn Lab Immunol 1994;1(4):419-423.
19. Kish L. Survey Sampling. John Wiley and Sons. 1965:83.
20. Mabayoje V, Adeyemo M, Akinola N. Case Review: Drug addiction in sickle cell disease, a possible ongoing challenge in the management of pain? J Global Biosci. 2015;4(4): 2021-2025.
21. Duits A, Schnog J, Lard L, Saleh A, Rojer R. Elevated IL-8 levels during sickle cell crisis. Eur J Haematol. 1998;61(5): 302-305.
22. Douglas SD. Substance P and sickle cell disease—a marker for pain and novel therapeutic approaches. Br J Haematol 2016;175(2):187-188.
23. Brandow AM, Wandersee NJ, Dasgupta M, Hoffmann RG, Hillery CA, Stucky CL, et al. Substance P is increased in patients with sickle cell disease and is associated with haemolysis and hydroxycarbamide use. Br J Haematol 2016;175(2):237-245.
24. Brandow AM, Stucky CL, Hillery CA, Hoffmann RG, Panepinto JA. Patients with sickle cell disease have increased sensitivity to cold and heat. Am J Hematol 2013;88(1):37-43.
25. Vincent L, Vang D, Nguyen J, Gupta M, Luk K, Ericson ME, et al. Mast cell activation contributes to sickle cell pathobiology and pain in mice. Blood. 2013;122(11):1853-1862.
26. Keikhaei B, Mohseni AR, Norouzirad R, Alinejadi M, Ghanbari S, Shiravi F, et al. Altered levels of pro-inflammatory cytokines in sickle cell disease patients during vaso-occlusive crises and the steady state condition. Eur Cytokine Netw 2013;24(1):45-52.
27. Sarray S, Saleh LR, Saldanha FL, Al-Habboubi HH, Mahdi N, Almawi WYJC. Serum IL-6, IL-10, and TNFα levels in pediatric sickle cell disease patients during vasoocclusive crisis and steady-state condition. Cytokine 2015;72(1):43-47.