SMU
Sikkim Manipal University
SMU Medical Journal
ISSN : 2349 – 1604 (Volume – 3, No. 1, January 2016) Research article
Indexed in SIS (USA), ASI (Germany), I2OR & i-Scholar (India) and SJIF (Morocco) databases
Impact Factor: 3.835 (SJIF)

Alteration of Leptin and C-peptide Status in Homozygous Sickle Cell Anaemia

***[1]Nnodim Johnkennedy.,[2]Meludu Samuel C., [3]Dioka CE., [4]Martin Ifeanyichukwu., [1]Okorie Hope and [5]Uloneme GC**

[1]Department of medical laboratory science, faculty of health science, Imo State University Owerri, Imo State, Nigeria, [2]Department of Human Biochemistry, College of Health Sciences, NnamdiAzikiwe University, Nnewi Campus, PMB 5001 Nnewi, Anambra State, Nigeria.[3]Department of Chemicalpathology, College of Medicine and Health Science, NnamdiAzikiwe University Nnewi Campus, Anambra State Nigeria Nigeria. [4]Department of Medical Laboratory Science NnamdiAzikiwe University Nnewi Campus.[5]Department of Human Anatomy Imo State University Owerri, Imo State, Nigeria..

[*]Corresponding auther: Dr. JohnkennedyNnodim
Phone:+2348034237000

Manuscript received : 11.09.2015
Manuscript accepted: 18.10.2015

Abstract

The study was carried out to determine the level of leptin and C-peptide in sickle cell anemia.Thirty confirmed sickle cell patients in steady state(HbSS-SS) and thirty normal haemoglobin (HbAA) as well as sixteen sickle cell disease in crises(HbSS-cr) between the ages of 15 to 30 years were selected in this study. Leptin and C-peptide were measured by commercially available ELISA kits. The results obtained showed that the levels of leptin and

C-peptide in Sickle cell anaemia patients were significantly elevated when compared with HbAA subjects (P<0.05). However, the level of leptin and C-peptide in sickle cell steady state were highly significant when compared with those in crisis(P<0.05). This may probably implies that leptin and C-peptide decrease could enhance sickle cell crisis. Also, it could provide the understanding of the possible role of leptin and C-peptide in the pathophysiology of sickle cell anaemia.

Keywords :Leptin, C-peptide ; sickle cell anaemia; pathophysiology

Introduction

Sickle cell anaemia is a genetic disorder caused by the substitution of valine for glutamic acid at the sixth position of the amino acid β-chain of the haem molecule. It is characterized by the possession of sickle cell haemglobin. The abnormal haemoglobin is less soluble and tends to crystallise out resulting in the deformation of the cells which instead of being round become sickle shaped [1]. It is the main cause of morbidity and mortality among Sickle cell anaemia. Sickle cell anaemia associated with hormonal regulation. One important energy hormone that regulate energy homeostasis in sickle cell anaemia is leptin. It is a protein that is made in the fat cells, circulates in the blood stream and goes to the brain[2]. Leptin is a hormone that regulates the amount of fats stored in the body. This is done through adjusting energy expenditures and sensation to hunger[3]. Leptin is mainly derived from the adipose tissue and is a prominent biological factor of energy homeostasis whose circulating levels are a reflection of adiposity. It binds to the hypothalamus through the leptin receptor and acts via the JAK/STAT pathway to prevent the expression norexigenic factors and as well activate the expression of anorexigenic factors to suppress appetite, food intake and weight gain[5]. The regulation of fat stores is considered to be the main function of leptin. It is equally important in physiological processes. Leptin is located on chromosome 7 in humans. It is a 16kDa protein of 167 amino acids[6]. Leptin plays a critical role in adaptive response to starvation.It acts on leptin receptors in the hypothalamus where it inhibits hunger via counteracting the effects of neuropeptide Y which is a potent hunger promoter secreted by cells in the gut and hypothalamus[7]. Hunger could be inhibited by leptin through

counteracting the effect of anandamide which is a potent hunger promoter. In the same vein, hunger could be inhibited though the promotion of the synthesis of α-MSH(melanocyte stimulating hormone) which is a hunger suppressant. It is worthy to note that the absence of leptin or its receptors leads to uncontrolled hunger and hence resulting to obesity[8]. Leptin receptors are expressed not only in the hypothalamus but also in other parts of brain regions, especially in the hippocampus. Hence, someleptin receptors in the brain are grouped into central (hypothalamic) and some as peripheral (non-hypothalamic)[9]. The deficiency of circulating plasma leptin has been linked with cognitive changes associated with anorexia as well sickle cell anaemia[10].

On the other hand, C-peptide is a short 31-amino-acid protein that connects insulin's A-chain to its B-chain in the proinsulin molecule. A C-peptide test measures the level of this peptide in the blood. It is mainly found in amounts equal to insulin because insulin and C-peptide are linked when first synthesized by the pancreas. Insulin aids the body use and control the amount of glucose in the blood. Insulin permits glucose to enter body cells where it is used for energy[11]. The level of C-peptide in the blood can indicate how much insulin is being synthesized by the pancreas. A C-peptide test is mainly done when diabetes has just been found and it is not clear whether type 1 diabetes or type 2 diabetes is present. A person whose pancreas does not make any insulin, that is type 1 diabetes has a low level of insulin and C-peptide. A person with type 2 diabetes can have a normal or high level of C-peptide[12].

A C-peptide test can also unravel the cause of low blood sugar or hypoglycemia, such as excessive use of medicine to treat diabetes or a noncancerous growth in the pancreas (insulinoma). Because man-made (synthetic) insulin does not have C-peptide, a person with a low blood sugar level from taking too much insulin will have a low C-peptide level but a high level of insulin. An insulinoma causes the pancreas to release too much insulin, which causes blood sugar levels to drop (hypoglycemia). A person with an insulinoma will have a high level of C-peptide in the blood when they have a high level of insulin[13].

The aim of this study was to evaluate the levels of leptin and C-peptide in sickle cell anaemia.

Materials and methods

Thirty HbSS diagnosed by haemoglobinelectrophoresis(15 males and 15 females) aged 10 to 30years were selected for the study while 16(8males and 8 females) were in crisis. These patients were attending General Hospital Owerri. Thirty HbAA normal subjects(15 males and 15 females) were used as control.

Blood sample

In all subjects , 5ml of veinous blood was collected into a non anticoagulated tubes. The samples were spun in a Wisterfugecentrifuge(model 684) at 1000g for 10 minutes and the serum collected into bijou bottle. Informed consent of the participants was obtained and was conducted in line with the ethical approval of the hospital.

Biochemical assay

Theleptin and C-peptide were measured by enzyme linked immunoabsorbent assay (ELIZA) using standard commercial kits(Biosource International Inc. Camarillo CA)

Statistical analysis

The results were expressed as mean±standard deviation. Statistical significance was calculated using Student t-test. The level of significance was calculated at $P<0.05$.

Results and Discussion

Sickle cell anaemia results from single amino acid substitution (Valine for Glutamine) at the sixth position in each of the two beta globin chain of haemoglobin and has remained a genetic disease[14]. It produces a myriad of metabolic, haematological, nutritional and clinical effects. This is responsible for erythrocyte dysfunction in these patients[15]. In this study, it was observed that leptin in sickle cell anaemia were significantly decreased when compared with HbAA. This is in line with the work of Iwalokun et al.[16]. The low level of

Table 1.leptin and C-peptide levels in HbAA,homozygous sickle cell anaemia in steady state and crisis

PARAMETERS	HbAA	HbSS	HbSS-crisis
Leptin(ng/mL)	10.41± 1.01	6.3±3.5*	4.1 ±2.01**
C-peptide (ng/mL),	3.00 ±2.1	1.80±0.9*	1.10±0.99*

*Significantly different from HbAA at P<0.05.
**Significantly different from HbSS-crisis at P<0.05

could be associated with inflammation, infection and body fat levels. It is well-established that leptin is associated with the regulation of the inflammatory response in sickle cell anaemia. It has been noted that the main role of leptin is to act as a starvation signal when levels are low, to help maintain fat stores for survival during times of starvation, rather than a satiety signal to prevent overeating[17,18]. However, Leptin over-expression in adipose tissue has been observed in a number of individuals with morbid obesity. There is evidence that leptin contributes to erythropoiesis and erythropoietin production by the kidney as well as potentiates platelet activation. Since sickle cell anaemia patients are deficient in cellular immunity, prone to anorexia and growth deficit, and inflammatory episodes, which supports the deficiency of leptin[19,20]. The decreased plasma leptin level that was exacerbated in sickle cell crisis represents a balance of these antagonistic events. In fact, considering the role of leptin in haematopoiesis and effects of adipocyte metabolism on leptinsythesis, the value of haematological parameters and other adipogenic factors cannot be over-emphasized[21,22].

In the same vein, the level of C-peptide was significantly decreased in sickle cell anaemia when compared with the apparently healthy HbAA. This is in line with the work of

Shiraj et al,[23]. C- peptide has been shown to bind to specific cell surface receptors in cultured cells derived from human renal tubules, mesangium, skin fibroblasts, and saphenous vein endothelium[24]. Data suggest that it acts through G-protein-coupled receptors to activate calcium-dependent signaling pathways through the C-terminal pentapeptide portion of the molecule. Also, C-peptide activation of calcium signaling is thought to elevate the activity of Na+-K+-ATPase, which has been found to be reduced in patients with sickle cell anaemia. Likewise, the middle segment of the peptide has been known to activate Na+-K+-ATPase, but not as strongly as the full peptide or the pentapeptidesequence[25].

The low level of leptin and C-peptide could enhance sickle cell crisis. Also, it could provide the understanding of the possible role of leptin and C-peptide in the pathophysiology of sickle cell anaemia.

References

[1] Nnodim JK, Meludu SC, Dioka CE, Onah C, Ihim A and Atuegbu C(2014). Trace elements deficiency in patients with homozygous sickle cell disease. British Journal of Medicine & Medical Research. 4(21), 3879-3888

[2] Pratley R, Nicolson M, Bogardus C and Ravussin E (1997). Plasma leptin responses to fasting in Pima Indians. Am. J. Physiol. 273 (3 Pt 1), E644–9.

[3] Mars M, De GraafC, De Groot C, van Rossum C and Kok F (2006). "Fasting leptin and appetite responses induced by a 4-day 65%-energy-restricted diet". International journal of obesity (Lond). 30 (1), 122–128

[4] Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R and Ranganathan S (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1 (11), 1155–61

[5] Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL and Caro JF (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334 (5), 292–5.

[6] Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI and Friedman JM (1996). Abnormal splicing of the leptin receptor in diabetic mice. Nature. 379 (6566), 632–5

[7] Chan JL, Heist K, DePaoli AM, Veldhuis JD and Mantzoros CS (2003). The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J. Clin. Invest. 111 (9), 1409–1421

[8] Malendowicz W, Rucinski M, Macchi C, Spinazzi R, Ziolkowska A, Nussdorfer GG and Kwias Z (2006). Leptin and leptin receptors in the prostate and seminal vesicles of the adult rat. Int. J. Mol. Med. 18 (4), 615–8

[9] Aka N, Atalay S, Sayharman S, Kiliç D, Köse G and Küçüközkan T (2006). Leptin and leptin receptor levels in pregnant women with hyperemesis gravidarum. The Australian & New Zealand journal of obstetrics &gynaecology. 46 (4), 274–277.

[10 Myers MG, Cowley MA and Münzberg H (2008). Mechanisms of leptin action and leptin resistance.Annu. Rev. Physiol. 70 (1), 537–556.

[11] Wahren J and Ekberg K, Johansson J, (2000). Role of C-peptide in human physiology. Am J PhysiolEndocrinolMetab. 278(5), E759-68.

[12] Johansson BL, Borg K, Fernqvist-Forbes E, Kernell A, Odergren T and Wahren J. (2000) Beneficial effects of C-peptide on incipient nephropathy and neuropathy in patients with Type 1 diabetes mellitus. Diabet Med. 17(3), 181-9.

[13] Wiedmeyer HM, Polonsky KS and Myers GL, (2007). International comparison of C-peptide measurements.ClinChem. 53(4), 784-7.

[14] Sagir G, Ahmed SG, Bukar AA and JolayemiB(2010): Hematological Indices of Sickle Cell Anaemia Patients with Pulmonary Tuberculosis in Northern Nigeria. Medit J Hemat Infect Dis. 2,201

[15] Prasad R, Hasan S, Castro O, Perlin E and Kim K(2003): Long-term outcomes in patients with sickle cell disease and frequent vasoocclusive crises. Am J Med Sci, 325,107-109.

[16] Iwalokun BA, Senapon O I, Semande O H, Ayoola O A and Phillip U A(2011)Serum levels of leptin in Nigerian patients with sickle cell anaemia . BMC Blood Disorders. 11,2

[18] Oswal A and Yeo G (2010). Leptin and the control of body weight: a review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. Obesity (Silver Spring). 18 (2), 221–9.

[19] Friedman JM and Halaas JL(1998): Leptin and the regulation of body weight in mammals. Nature. 395,763-770.

[20] Caner I, Selimoglu MA, Yazgi H and Ertekin V(2006): Serum Leptin Levels in Children with Acute Viral Hepatitis A. West Indian Med J. 55,409-413.

[21] Koc E, Bideci A, Cinaz P, Ergenekon E and Atalay Y(2001): Relationships between levels of leptin and hematological parameters in healthy term infants. J PediatrEndocrinolMetab. 14, 1129-32

[22] Ye XL and Lu CF (2013). Association of polymorphisms in the leptin and leptin receptor genes with inflammatory mediators in patients with osteoporosis.Endocrine. 44 (2), 481–8

[23] Shiraj E, Reddy S, Scherbaum WA, Abdulkadir J, Hammel JP and Faiman C(2002). Basal andpostglucagon C-peptide levels in Ethiopians with diabetes. Diabetes Care. 25, 453-57.

[24] Hills CE and Brunskill NJ (2008).Intracellular signalling by C-peptide. Exp Diabetes Res 635158.

[25] Sima AA, Zhang W and Sugimoto K, (2001). C-peptide prevents and improves chronic Type I diabetic polyneuropathy in the BB/Worrat. Diabetologia. 44 (7), 889–897

Authors Column

Dr. JohnkennedyNnodim is a Lecturer in the Department of Medical Laboratory Science, Faculty of Health Science, Imo State University, Owerri, Nigeria. He holds a Ph.D in chemicalpathology. He has several research papers already published in local and international scientific journals.

SMU Medical Journal, Volume – 3, No. – 1, January, 2016, PP. 556-564