Original Article

THE ROLE OF LUTHERAN BLOOD GROUP ANTIGEN /BASAL CELL ADHESION MOLECULE POSSESSION IN VASO-OCCLUSION AMONG SICKLE CELL ANAEMIA SUBJECTS IN LAGOS, NIGERIA

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Abstract

Background: Vaso-occlusive painful crises are the hallmark of sickle cell anaemia responsible for the significant morbidity and mortality associated with the disease. This study is aimed to determine if there is an association between levels of Lutheran /Basal Cell Adhesion Molecule possession and vaso-occlusive painful crisis in sickle cell anaemia subjects. **Materials and Methods**: It was an analytical, prospective study in which Enzyme-Linked Immunosorbent Assay was used to identify serum Lutheran /Basal Cell Adhesion Molecule glycoprotein. Lutheran /Basal Cell Adhesion Molecule was evaluated in sickle cell anaemia subjects during a crisis, the same set of subjects was invited three months after during steady-state and HbAA blood donors were also used as controls. Disease severity and analogue pain scores were assessed in the sickle cell anaemia subjects. Data were analyzed using the statistical package for social science version 23. **Results**: A total of forty-nine (49) sickle cell anaemia subjects and forty-seven (47) HbAA controls were recruited, the mean ages were 26.55±7.31 years and 31.13±8.51years respectively. The concentrations of Lutheran /Basal Cell Adhesion amongst the sickle cell anaemia subjects in crisis, steady-state and HbAA controls were 1.61± 0.23ng/ml, 1.46±0.21ng/ml, and 1.35±0.17 ng/ml respectively. The concentrations in crisis / steady and crisis /controls were statistically significant at p=0.01 each. **Conclusion**: The mean concentration of Lutheran /Basal Cell Adhesion is most elevated in SCA subjects in a crisis state followed by steady-state and HbAA controls.

Keywords: Sickle cell anaemia, Lutheran /Basal Cell Adhesion, Vaso-occlusive crisis, Analog pain scale score

INTRODUCTION

Sickle red cell has an abnormal haemoglobin structure which causes red blood cells to be characteristically sickled under hypoxic conditions because of a point mutation in which adenine is replaced by thymine in the DNA structure resulting in the replacement of the normal glutamic acid with a neutral amino acid, valine.[1]

Vaso-Occlusive Crises (VOC)

VOC is the painful obstruction of microcirculation which is a telltale sign of SCA. Pain ranges from mild self-limiting to severe pain lasting for days and associated with known complications of SCA like bone pain crisis, avascular necrosis of head of femur, acquired functional asplenia, acute chest syndrome, priapism etc. Detailed knowledge of VOC pathophysiology is desirous in order to increase therapeutic options available for treatment. A targeted therapeutic approach is likely to produce a better yield than symptomatic treatment presently in use which

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include intravenous fluids, analgesics and oxygen supplementation.

Steady-State

Steady-state is a period during which the patient is free of any acute painful crisis or any changes due to therapy as defined by Ballas [2]

Lutheran /Basal Cell Adhesion Molecule (Lu/BCAM)

Two membrane glycoproteins 85 kDa and 78 kDa are encoded by the Lutheran gene. [3] The gene is located on chromosome 19q13.2.[3,4] (Figure 1). The Lu gp of 85 kDa and B-CAM gp of 78 kDa represent isoforms of the same protein encoded by two mRNA spliceoforms of the LU gene.[4] Comparing the nucleotide sequence of Lu gp 85 kDa with 78 kDa, the former was identical to Basal Cell Adhesion Molecule (BCAM) 78 kDa.[5] The BCAM antigen 78 kDa has the same NH_2-terminal amino acid sequence as Lu gp of 85 kDa, but differs in the last 40 COOH-terminal amino acids which is absent in the 78kDa isoform but present in 85 kDa. The short tail, 78 kDa (BCAM) and the long tail, 85 kDa (Lu) are obtained by alternative splicing of introns 13 resulting in two transcripts spliceoforms that encodes both the long and short tails.[4] It was also demonstrated that the Chinese hamster ovary cells expressing both the long and short tails of the glycoproteins reacted equally with both anti-Lu as well as anti-B-CAM.[6] The implication of this is that Lu and B-CAM antigens are held by the same glycoproteins under two isoforms of 85 and 78kDa. Apart from red blood cells, the Lu and B-CAM antigens are expressed in many other tissues.[7] Lu/BCAM are red blood cell (RBC) glycoproteins whose receptors are laminins α5, Lu/BCAM binds laminins α5.[8] through its aspartic acid 343. Figure 2

Lu/BCAM glycoproteins are integral to the RBC membrane cell skeleton. In addition to its laminin-binding, it also through its cytoplasmic domain, interacts with the spectrin-actin-protein 4.1 complex by binding directly to spectrin α4 repeat via the lu/BCAM RK 573-574 motif. [0, 10]

Figure 2. In 2010, it was reported that spectrin deficiency in hereditary spherocytosis is associated with high Lu/BCAM-mediated RBC adhesion to laminin. [11]

Laminin interacts with Lu/BCAM by binding to its D343 motif while spectrin-actin-protein 4.1 interacts with the RK 573-574 motif of Lu/BCAM.

If the association between Lu/BCAM protein and vaso-occlusive painful crises can be established, it will be possible in future to genetically edit or silence the gene of Lu/BCAM to reduce its expression in sickle cell anaemia patients or to manufacture an inhibitor e.g., monoclonal antibody that blocks the binding of Lu/BCAM glycoprotein to laminin thus achieving a targeted therapy.

This research is aimed at evaluating the levels of Lu/BCAM glycoprotein levels using enzyme-linked immunoassay tests (ELISA) in SCA patients in vaso-occlusive painful crisis and steady-state and comparing it with HbAA controls.

MATERIALS AND METHODS

Study Location

The adult Haematology clinic of Lagos State University Teaching Hospital (LASUTH) was the study location.

Study Design

This was a descriptive, comparative and cross-sectional study

Study Population

SCA subjects were enrolled during VOC painful crises, and steady-state along with blood donors with HbAA Phenotype who served as controls.

Study Duration

This study was carried out for two years between March 2019 and February 2021.

Laboratories Used

A blood sample was drawn at the laboratory attached to the Haematology clinic, while high-performance liquid chromatography (HPLC) for quantification of HbA_0, HbSS, HbF and HbA_2 for all the SCA participants and haemoglobin electrophoresis of all control participants were done at the Haematology main laboratory at LASUTH. Enzyme-Linked Immunosorbent Assay (ELISA) of Lu/BCAM Protein was done at LASUTH's transfusion transmissible infection screening room, Ikeja.

Sample Size Determination

Sample size was determined using the statistical formula that applies to comparative studies. [13]

$N= (Z_{\alpha/2} + Z_{\beta})^2 * (p_1(1-p_1) + p2\,(1-p2))/(p_1-p_2)^2$

Where n= Sample size

$Z_{\alpha/2}$ =critical value of Normal distribution at $\alpha/2$ for a confidence level of 95%, α =0.05 and the critical value is 1.96

Z_{β} = critical value of the normal distribution at β for a power of 80%, β is 0.2 and the critical value is 0.84

p1 and p2 are the expected proportions of the two groups

Because the proportions are not known, values close to 50% are used as p1=70%, p2=40%, where p1 is the SCA in crisis and p2 is SCA in steady state.

$N= (1.96+0.84)^2* (70(1-70) + 50(1-50))/70-50)^2$

Sample size for each group is 40.

With an estimated non-response rate of 10%, attrition factor = 100/100 – x. [14]

$$\text{Attrition factor} = \frac{100}{100-10} = \frac{100}{90} = 1.11$$

Sample size for each of the two groups is 41

Sampling Technique

Participants were recruited using non-purposive, consecutive recruitment. Haemoglobin phenotype of all controls was performed using the alkaline haemoglobin electrophoresis method while all the SCA patients had haemoglobin quantification done before the Lu/BCAM ELISA

Patient Selection

Subjects/Participants

- Adults with HbSS phenotype.
- Blood donors with HbAA phenotype.

Exclusion Criteria for HbSS in Steady State

1. HbSS Phenotype Patients not in steady state
2. Other Hb phenotypes (e.g. HbSC, SD, Etc).
3. SCA with elevated levels of HbF and HbA_2

Exclusion Criteria for HbSS in bone Pain crisis

1. SCA Phenotype Patients not in painful crisis
2. Other Hb phenotypes (e.g. HbSC, SD, etc).
3. SCA with elevated levels of HbF and HbA_2

Exclusion Criteria for blood donors

1. Other Hb phenotypes (e.g. AS, AC etc).
2. Non-fasting participants.
3. HbAA controls on lipid-lowering medications.
4. HbAA controls those who are hypertensive or diabetics.

Clinical severity Assessment

The clinical severity of every SCA subject was assessed with the disease severity score using a modified scoring system by Hedo *et al*. [15]

The total severity score was calculated as Mild SCA [$\leq$ 3], Moderate SCA [≥ 3 but ≤ 7] and Severe SCA [≥ 7]. (Table 1)

Numerical Rating Scale (NRS)

NRS was used for all SCA subjects in vaso-occlusive painful crises. Participants were asked to rate their pain by circling the number commensurate to their pain. (Table 2)

Specimen Collection

During a vaso-occlusive painful crisis, from intravenous access, under aseptic conditions using a vacutainer needle, 5mls of blood was collected into a plain vacutainer bottle to obtain serum for Lu/BCAM ELISA of all participants, and sera were frozen after separation. Another 4.5mls was also obtained in an Ethylene Diamine Tetra-acetic Acid (EDTA) bottle for full blood count which was done on the same day of collection. Three months after the VOC, patients were invited back to the clinic, 3 mls of blood sample was collected into a vacutainer EDTA bottle from all of them and properly mixed with the anticoagulant. The latter sample was used for high-performance liquid chromatography (HPLC) for quantification of HbSS, HbA_0, HbF and HbA_2 for all the SCA participants and 5mls of blood was collected again during steady-state into a plain vacutainer bottle to obtain serum for Lu/BCAM ELISA of all of them. Alkaline Hb electrophoretic analysis of controls ensured the control participants were HbAA and 5mls of blood was collected from them into a plain vacutainer bottle to obtain serum for Lu/BCAM ELISA assays.

Sample Analysis

Haemoglobin Quantification

A Bio-Rad haemoglobin quantification machine (manufactured in the USA) was used for the quantification of the haemoglobin. It is a fully automated machine that combines quantification of HbS, C, A_2, F and A1c testing on a single high-performance liquid chromatography platform.

Lu/BCAM ELISA Procedure

Sera of all the plain bottle –samples collected frozen at -40^{o}C were used for BCAM Elisa assay (manufactured by Melsin Medical Co. Limited, Jilin Province, China) LOT number: P20201204 and CAT Number: EKHU-3048. Prior to the ELISA procedure, all samples that were not HbSS or HbAA following HPLC of SCA samples and haemoglobin electrophoresis of HbAA control samples were discarded from the stock and excluded from the study.

Full Blood Count

A total of 4.5mls was collected from SCA into an EDTA anticoagulant bottle for full blood count analysis done on the same day of sample collection. A Sysmex KN-21 N (manufactured by Sysmex Corporation, Kobe Japan with a serial number B6991 was used for the assay. It is a three-part auto-analyzer able to run 19 parameters per sample which include white blood cell count, haematocrit, red blood cell count, mean cell volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration among others.

Questionnaire Administration and History Taking

With the use of an interviewer-administered questionnaire, each participant was interviewed to obtain relevant socio-demographic and clinical data like age at diagnosis, time of last crisis, most frequent type of crisis, history of blood transfusion and drug history particularly use of hydroxycarbamide.

Ethical Considerations

Ethics committee approval for the study was obtained at the Health Research Ethics Committee of Lagos State University Teaching Hospital, Ikeja, Lagos, Reference

number; LREC/06/10/1141. The initial of all participants was used to guarantee confidentiality. The participants were informed about the study, as well as their rights and benefits. Written informed consent was obtained by means of a voluntarily signed consent form. No participant was coerced in any way to participate in the study, which was at no cost to them.

Statistical Analysis

Data were analyzed using SPSS version 23.0 (Statistical Package for Social Sciences, Inc., Chicago, Ill). The continuous variables were given as means ± standard deviation (SD). A paired-t test was used for data obtained from subjects during the crisis and a steady-state, independent t test was used on data obtained in SCA in crisis and HbAA controls, while a one-way analysis of variance (ANOVA) was used to test the hypothesis of VOC and Lu/BCAM concentrations. P-value was considered to be statistically significant when ≤ 0.05.

RESULTS

A total of 51 SCA participants were recruited for the study but two were excluded based on the haemoglobin quantification reports showing they were AS and SC. Only 49 SCA (cases) were finally enlisted. Similarly, one hundred and three (103) volunteer blood donors were initially recruited but following alkaline haemoglobin electrophoresis of their samples, all volunteers who had other haemoglobin phenotypes apart from HbAA and failed other eligibility criteria were excluded leaving only 47 for the study.

Table 1: Modified Disease Severity Scoring System for Sickle Cell Anaemia

CLINICAL AND LABORATORY FEATURES				SCORE
Crisis number(s) per year?	0-1 [0]	2- 3 [1]	[2]	
Acute chest syndrome		Yes [1]	No [0]	
Osteomyelitis		Yes [1]	No [0]	
Renal Failure		Yes [1]	No [0]	
Heart Failure		Yes [1]	No [0]	
Avascular necrosis of femoral head		Yes [1]	No [0]	
Pneumonia		Yes [1]	No [0]	
Pigment gall stone &Jaundice		Yes [1]	No [0]	
Dehydrated		Yes [1]	No [0]	
Anaemia Hb	[0]	1 0 g/dl	g/dl [2]	
		g/dl [3]	Hb < 4 g/dl [4]	
			Total Severity Score	

The mean ages were 26.55±7.31 years (cases) and 31.13±8.51years (controls). In the cases, 24 (49%) were males and 25 (51%) were females while males were 44 (93.6%) and females were 3 (6.4%) in the controls.
Majority of the cases 41(83.7%) were single while only 8 (16.3%) were married. Only 1 (2%) of the cases had primary education, 14 (28.6%) had secondary school education and 34 (69.4%) had tertiary school education

Table 2: Numerical Rating Scale

0	1	2	3	4	5	6	7	8	9	10

No pain Worst possible pain
Numerical Rating Scale: Please circle the number that best describe your pain

Table 3: The Haematological Parameters of All SCA Subjects

	Minimum	Maximum	Mean	Range
Full Blood Count				
Packed Cell Volume%	9	38	23.04±5.19	29.00
White Blood Cell*10^9/L	5.30	103.60	17.81±15.30	98.30
Neutrophil%	23.0	90.30	63.35±15.66	67.30
Lymphocyte%	2.7	75.30	29.04±15.20	72.60
Platelet*10^9/L	108.0	987.0	365.42 ±190.78	879.00
MCV (FL)	54.4	100.8	82.51±8.70	46.40
MCH (pg)	16.3	35.2	27.71±3.59	18.90
MCHC (g/dL)	17.7	39.5	33.13±3.12	21.80
Haemoglobin Quantification				
HbA_0	1.3	8.60	3.61±1.35	7.3
HbA_2	0.9	4.70	3.25±0.87	3.8
HbF	1.0	11.90	3.82±2.53	10.90
HbS	73.40	94.10	87.11±4.69	20.70

Key: MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin; MCHC: Mean Cell Haemoglobin Concentration; Hb: Haemoglobin

Table 4: Concentrations of Lu/BCAM in SCA and Controls

Concentrations (ng/ml)	Males	Females	All Participants
SCA Crisis	1.59±0.09	1.62±0.31	1.61± 0.23
SCA Steady state	1.50±0.16	1.42±0.25	1.46±0.21
HbAA Controls	1.35±0.17	1.46±0.11	1.35±0.17

Table 5: Paired T-Test Between Lu/BCAM Concentration of SCA Subjects in Crisis and Steady- State

SCA in Crisis	SCA in Steady state	P value	95% CI
1.61±0.23	**1.46±0.21**	**0.01**	

Table 6: Independent T-Test between Lu/BCAM concentration in SCA Crisis and Controls, Lu/BCAM concentration in Crisis and HbF in SCA and Lu/BCAM concentration in SCA subjects in Crisis and mean severity score

SCA in Crisis	Control	P value	95% CI
1.61 ± 0.23	1.35 ± 0.17	0.01	
SCA in Crisis	HbF in SCA	P value	95% CI
1.61 ± 0.23	3.82 ± 2.53	0.01	
SCA in Crisis	Mean total severity score in SCA	P value	95% CI
1.61 ± 0.23	3.57 ± 1.55	**0.01**	

Table 7: SCA Severity Score in Crisis

SCA Severity score in in group	Frequency	Percent
Mild < 3	25	51
Moderate 3-7	23	46.9
Severe > 7	1	2.0

Overall, 27 (55.1%) of the cases had a crisis in the last 3 months before the recruitment while 22 (44.9%) had a crisis greater than 3 months.

In more than half of the cases, 34 (69.4%) had a vaso-occlusive painful crisis at least 2-3 times per year,7 (14.3%) had a crisis more than 4 times a year and 8 (16.3%) had a vaso-occlusive painful crisis at least once per year.

A predominance of 36 (73.5%) have had a blood transfusion while 13 (26.5%) never had a blood transfusion before recruitment. In the majority of the cases, 12 (24.5%) had numerical pain scores of 6, 7, and 8 each, while 4 (8.2%) had scores of 5, 9, and 10 each, and only 1 (2%) had a score of 1. The mean numerical pain score was 7.18±1.43.

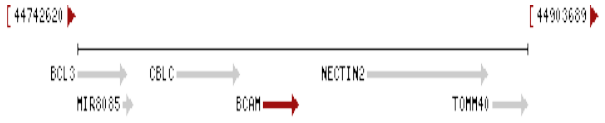

Figure 1: Chromosome 19
Chromosome 19 - NC_000019.10

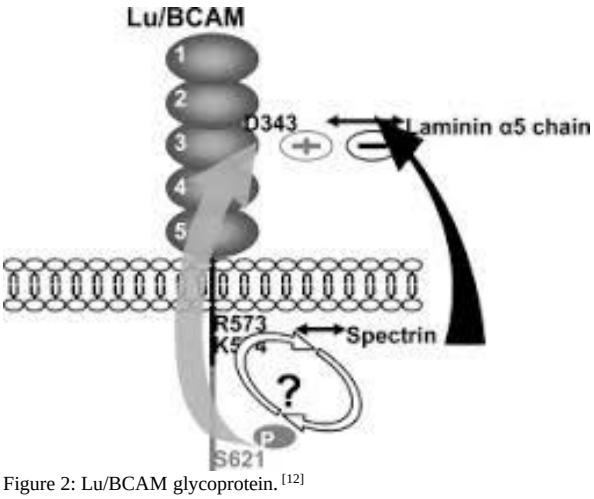

Figure 2: Lu/BCAM glycoprotein. [12]

A total of 28(59.6%) of controls were single, 18 (38.3%) married and 1 (2.1%) was divorced. The majority of the controls,30(63.8%) had tertiary school education followed by 15 (31.9%) with at least a secondary school education and only 2 (4.3%) had a primary school education.

The haematological parameters of the SCA subjects are shown in Table 3

Concentrations of Lu/BCAM in SCA subjects and Controls is presented in Table 4, and Paired T Test between Lu/BCAM concentration of SCA in Crisis and Steady-state in Table 5

Table 6 presents all the independent T-tests

SCA Severity Score in crisis is presented in Table 7

One-way analysis of variance (ANOVA) between the mean concentrations of SCA in crisis/ steady-state and numerical pain scale score was not statistically significant with a p-value of 0.91

ANOVA between frequency of crisis and Lu/BCAM concentration of SCA in crisis did not reach a significant value. p =0.15

DISCUSSION

This study evaluated the association of vaso-occlusive painful crisis with Lu/BCAM which is known to be overexpressed in SCA. [16] The mean ages of the cases and controls were closely related and were statistically significant, age could therefore not have impacted on results obtained in this study.

Our study established the mean concentration of Lu/BCAM was highest in SCA subjects in vaso-occlusive painful crisis, followed by SCA in steady-state and least in HbAA controls confirming that the concentration is overexpressed in SCA and the concentration is also more elevated when they are in painful crisis compared with steady-state.

The Lu/BCAM mean concentration was found to be statistically significant between the values in painful crisis and steady-state using a paired t-test. This confirms that the mean concentration is elevated in painful crises compared with the same set of patients in steady states and may contribute to the pathogenesis of pain in vaso-occlusive painful episodes. Independent t-test between the mean total severity score and mean concentration of Lu/BCAM in SCA crisis was also statistically significant corroborating the association of Lu/BCAM concentration with clinical severity. However, a one-way analysis of variance between Lu/BCAM mean concentrations in crisis/steady-state and numeric pain score did not reach a significant p-value. The lack of association between numeric pain scores and mean concentrations of Lu/BCAM in SCA may be due to reliance on information regarding pain scale scores provided by the subjects who may not have appropriately scored the pain episodes. A statistically significant level was also obtained when the Lu/BCAM concentrations were compared between values in SCA and HbAA controls.

It is, however, noteworthy that a statistically significant level was obtained using an independent t-test on Lu/BCAM mean concentration in crisis and mean HbF concentration of participants. Several studies. [17-21] have reported that the higher the HbF concentration of the HbSS red blood cells, the less severe the crisis because HbF is known to retard sickling of red blood cells and ameliorate vaso-occlusive painful episodes. A high HbF level may thus reduce the adhesiveness of Lu/BCAM glycoprotein to the vascular endothelium thereby reducing impairment of blood flow and microvascular obstruction. Apart from the many mechanisms of action hydroxycarbamide (HC) uses to ameliorate vaso-occlusive painful episodes. [22] Chaar *et al.* [23] reported a novel mechanism of action of HC involving Lu/BCAM in 2014, HC down-regulates phosphodiesterase 4A, thus decreasing cAMP levels leading to less Lu/BCAM phosphorylation and reduced SCA RBC adhesion to vascular endothelium.

It was observed that SCA participants had higher values of Lu/BCAM concentrations than HbAA controls confirming the theory of overexpression in SCA. Similarly, male SCA subjects had a lower concentration of Lu/BCAM than females in crisis and a lower mean value of HbF concentrations which is contrary to what is expected. There are possibly other factorsassociated with the vaso-occlusive painful crisis in males apart from the level of HbF. In other similar studies. [24,25] evaluating gender-specific vaso-occlusive painful crisis in SCA, males were more affected than females and were admitted more often after initial stabilization for pain controls, justifying other factors apart from high concentrations of Lu/BCAM in males contributing to a more severe form of the SCA.

A study limitation is the reliability of the information on the frequency of crisis provided by the SCA subjects, that they had no crisis between the last vaso-occlusive painful crises and when they came three months after during steady-state and reliance on numerical pain scale score provided by the SCA subjects which may not be completely objective.

CONCLUSIONS

The mean concentration of Lu/BCAM is most elevated in the SCA crisis state followed by steady-state and HbAA controls. Though levels of Lu/BCAM in SCA during a crisis may not be related to the severity of pain using the numerical pain score, it is directly related to the clinical severity score.

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Conflict of Interest:

The authors declare no conflict of interest.

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