International Journal of Scientific Reports
Uche E et al. Int J Sci Rep. 2023 Jan;9(1):5-10
http://www.sci-rep.com

pISSN 2454-2156 | eISSN 2454-2164

DOI: https://dx.doi.org/10.18203/issn.2454-2156.IntJSciRep20223370

Original Research Article

Protein S activity in sickle cell anaemia patients in steady state and crisis

Ebele Uche[1]*, Olufunto Kalejaiye[2], Festus Olowoselu[3], Timothy Ekwere[4], Rafatu Bamiro[5], Abdulateef Kareem[6], Benjamin Augustine[7], Hassan Odebiyi[8], Abdulhafeez Balogun[9], Akinsegun Akinbami[1]

[1]Department of Haematology and Blood Transfusion, Lagos State University, College of Medicine, Lagos, Nigeria
[2]Department of Medicine, [3]Department of Haematology and Blood transfusion, College of Medicine, College of Medicine, University of Lagos, Lagos, Nigeria
[4]Department of Haematology, College of Health Sciences, University of Uyo, Akwa Ibom State. Nigeria
[5]Department of Haematology and Blood Transfusion. General Hospital Marina, Lagos, Nigeria
[6]Department of Family Medicine, Lagos State University Teaching Hospital, Lagos, Nigeria
[7]Department of Haematology and Blood Transfusion, Ahmadu Bello University Zaria, Kaduna State, Nigeria
[8]Department of Haematology and Blood Transfusion, Federal Medical Center, Birnin Kudu, Jigawa State, Nigeria
[9]Newday Medical Centre, Akure, Ondo State, Nigeria

Received: 02 November 2022
Revised: 10 December 2022
Accepted: 13 December 2022

***Correspondence:**
Dr. Ebele Uche,
E-mail: Eifeyinwa2000@yahoo.com

ABSTRACT

Background: In sickle cell anaemia, there is an ever-present risk for haemostatic abnormalities which may result in reduced levels of naturally occurring coagulation inhibitors. Protein S is a vitamin K dependent, γ-carboxy glutamic acid-containing glycoprotein that potentiates the action of protein C when activated resulting in anticoagulation. This study measured serum Protein S activity in SCA patients during vaso-occlusive crisis and in steady-state, and this was compared with the activity in HbAA controls with a view to establishing if protein S plays a role in VOC in SCA patients.
Methods: This was an analytical prospective study comprised of 30 HbSS patients and 30 HbAA controls. Using ELISA method, Protein S levels were measured in the HbSS study group during crisis and in the HbAA control group; When the HbSS patients were in steady state, Protein S levels were also measured.
Results: Protein S levels were highest in the HbAA control group (5.27±0.47 ng/ml) followed by the HbSS patients in steady state (5.08±0.56 ng/ml) and lowest in the HbSS patients in crisis (4.96±0.97 ng/ml). However, this difference was not statistically significant.
Conclusions: Protein S levels are reduced in HbSS patients when compared with HbAA controls, and the levels are lower during a VOC compared with steady state.

Keywords: Protein S, Sickle cell anaemia, Crisis, Steady state, Control

INTRODUCTION

The burden of sickle cell disease (SCD) is high in Nigeria, with a prevalence of between 2-3% of the population being homozygous HbSS and with the trait being seen in up to 20% of the population. One of the tell-tale signs of SCD is pain during a vaso-occlusive crisis (VOC) and many researches are ongoing on the pathogenesis of pain and the complications of VOC. In sickle cell anaemia, there is an ever-present risk for

haemostatic abnormalities which may result in reduced levels of naturally occurring coagulation inhibitors. Protein S is a Vitamin-K dependent, γ-carboxy glutamic acid-containing glycoprotein that potentiates the action of protein C when activated resulting in anticoagulation. It is a naturally occurring coagulation -inhibitor produced by the liver and exists as both free forms (40% of total) or bounded to C4b-binding protein (60% of total).[1] In theory, it has been postulated that in sickle cell disease, levels of naturally occurring coagulation inhibitors such as protein C and protein S are markedly reduced when compared to the healthy population.[2,3] This decrease, though not clearly understood, is said to be related to the cycle of overuse and/or reduced production (consequent of hepatic dysfunction) that occurs in sickle cell disease.[4] Activated protein C with cofactor protein S inactivates activated factors V and VIII, which blocks thrombin generation.[5] The deficiency is implicated in the pathogenesis of clinical complications of sickle cell anaemia including chronic leg ulcers, pulmonary infarcts and sickle cell-associated cerebral complications and contributing to increased morbidity and mortality within this population.[3] This study measured serum protein S activity in SCA patients during a crisis and in steady-state, and this was compared with the activity in HbAA controls with a view to establishing if protein S plays a role in VOC in SCA patients.

METHODS

Study population

This comprised of SCA patients (study group) attending the adult sickle cell clinic of The Lagos state university teaching hospital (LASUTH) and HbAA volunteer participants attending general outpatients and blood donor clinics (control group). Steady-state is defined as the period free of crisis extending from at least three weeks since the last clinical event and three months or more since the last blood transfusion, to at least one week before the start of a new clinical event.[6]

Study design and duration

This was an analytical prospective study in which enzyme-linked immunosorbent assay (ELISA) was used to assay Protein S levels in SCA patients during a crisis, and the same set of patients were invited three months after during steady-state and Protein S levels was assayed again. HbAA individuals were used as controls. This study was done over a period of six months between June 2019 and January 2021.

Sample size determination

Sample size was determined using the statistical formula that applies to comparative studies.[7]

$$N = (Z\alpha/2 + Z\beta)2 * (p1(1-p1) + p2(1-p2))/(p1-p2)2$$

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 $_\beta$ for a power of 80%, β is 0.2 and the critical value is 0.84, p1 and p2 are the expected sample means of the two groups. Because the means 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 a steady state. Sample size for each group was thus calculated to be 40. However, due to financial constraints as this study was fully self-funded by the authors, 30 participants were used for each arm of the study making a total of 90 participants.

Sampling technique

Steady-state SCA patients attending the out-patient clinic and patients admitted for painful crises in the clinic day-care three months earlier as well as consenting blood donors of LASUTH were recruited consecutively into the study. Only consenting blood donors who had HbAA phenotype and who met other inclusion criteria were eventually used as the control population. Haemoglobin phenotypes of all controls were done using the alkaline haemoglobin electrophoresis method while all the SCA patients had haemoglobin quantification done before the Protein S ELISA was done.

Inclusion criteria

Adults who are HbSS phenotype using haemoglobin quantification at LASUTH haematology clinic and HbAA blood donors served as controls were included in the study.

Exclusion criteria

Exclusion criteria for HbSS in steady state; adult HbSS phenotype patients not in steady state, non-consenting HbSS patients, other Hb phenotypes (e.g. HbSC, SD, Etc) and adult SCA patients with elevated levels of HbF and HbA_2. Exclusion criteria for HbSS in bone pain crisis; adult SCA Phenotype Patients not in painful crisis, non-consenting SCA patients, other Hb phenotypes (e.g. HbSC, SD, etc), adult SCA patients with elevated levels of HbF and HbA_2. Exclusion criteria for blood donors; non-consenting participants, other Hb phenotypes (e.g., AS, AC etc), non-fasting participants, HbAA controls on lipid-lowering medications and HbAA controls who are hypertensive or diabetics.

Sample collection

A total of 5 mls of blood was collected from each subject from the antecubital vein under aseptic techniques. The blood was dispensed into plain sterile tubes and allowed to clot and retract. This was centrifuged at 3500 rpm for five minutes and the serum samples transferred into 2mls cryovial and stored at -80°C till analysis. The samples were run using enzyme-linked immunosorbent assay

(ELISA) kits for measurement of protein S activity with a kit manufactured by Melsin Medical Co., Limited, Jiin Province China.

Participant's informed consent

The participants were informed about the study, as well as their rights and benefits. Written informed consent was obtained using a voluntarily signed consent form. No participant was coerced in any way to participate in this study, which was at no cost to them.

Confidentiality

The names and initials of all participants were not used to guarantee confidentiality. Participants were assigned unique identification numbers. Paper records were stored in a cabinet in a secured room. Electronic data were password protected.

Questionnaire administration and history taking

With the use of an interviewer-administered questionnaire, each participant was interviewed to obtain relevant demographic and clinical data. Some of the questions asked in the questionnaire include the age of diagnosis of sickle cell anaemia, history of blood transfusion, frequency of crisis per year, time of last acute painful crisis, history of last hospital admission, drug history and most frequent type of crisis to determine steady-state status of the HbSS participants and history of other concomitant illnesses.

Statistical analysis

Data were analysed by IBM SPSS (Statistical Package for Social Sciences, Inc.) statistics for windows version 20.0 Armonk, New York, USA. The continuous variables are presented as means±standard deviation (SD). The Pearson chi-squared tested for association between discrete variables. Independent t-test and analysis of variance (ANOVA) were used between the two groups p value was considered to be statistically significant when ≤ 0.05.

RESULTS

This study comprised of 30 HbSS patients and 30 HbAA controls. The overall mean age for the total group was 29.12+8.07 years whilst the mean ages were 27.23±8.02 years and 31.00±7.81 years for HbSS patients and HbAA controls respectively.

A total of 42 (70%) males (13 HbSS and 29 HbAA) and 18 (30%) females (17 HbSS and 1 HbAA) were recruited into the study. In both groups, most of the participants had tertiary education 21 (70%) and 18 (60%) for the test and control groups respectively. The others had only primary or secondary education. Among the study group participants, majority (26; 86.7%) were single whilst 4 (13.3%) of them were married. In the control group, 17 (56.7%) of them were single, 12 (40.0%) were married and 1 (3.3%) of them was divorced/separated. The haematological parameters of the study group participants are shown in (Table 2). The concentrations of Protein C amongst the SCA patients in crisis/steady-state and HbAA controls (Table 3). This is also shown in (Figure 1-3). The paired t-test between the Protein C concentration in SCA patients during crisis and in steady state is depicted in (Table 4). The Independent t-test between HbSS in crisis and HbAA control is shown in (Table 5). The Independent t-test between mean Protein C concentration of SCA in crisis and mean concentration of HbF in SCA is shown in (Table 6).

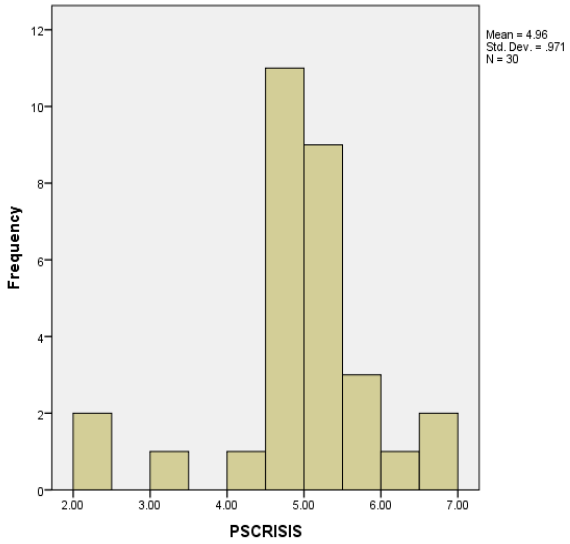

Figure 1: Protein S concentration of HbSS patients in crisis.

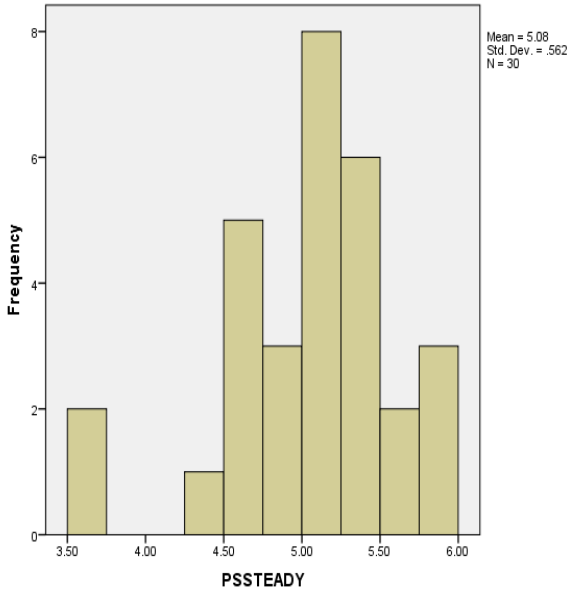

Figure 2: Protein S concentration of HbSS patients in steady state.

Table 1: Sociodemographic characteristics of study participants.

Variable	Study group	Control group	P value
Mean age (years)	26.6±7.95	31.0±7.80	0.88
Males N (%)	14 (46.7)	29 (96.7)	
Females N (%)	16 (53.3)	1 (3.3)	
Primary education N (%)	1 (3.3)	1 (3.3)	
Secondary education N (%)	8 (26.7)	11 (36.7)	
Tertiary education N (%)	21 (70)	18 (60)	

Table 2: Haematological parameters of the sickle cell anaemia patients.

Parameters	Minimum	Maximum	Mean
Full blood count			
Haematocrit (%)	12.70	38.80	23.00±4.77
MCV (fl)	62.90	100.80	82.68±8.38
MCH (pg)	19.50	241.00	34.65±39.11
MCHC (g/dl)	29.30	36.30	33.02±1.96
White blood cells (10^9/l)	5.3	49.80	15.76±8.74
Neutrophils (%)	23	88	64.84±14.49
Lymphocytes (%)	5.0	75.3	28.62±14.99
Mixed (%)	1.7	11.1	6.48±2.45
Platelets (10^9/l)	108.00	987.0	333.50±190.74
Haemoglobin quantification (%)			
HbA	2.10	6.90	3.34±0.79
HbA2	0.90	4.70	3.26±0.88
HbF	1.00	11.90	4.00±2.83
HbS	80.60	91.70	88.16±3.18
Others	0.20	9.60	1.54±1.79

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

Table 3: Concentrations of Protein S in SCA patients and HbAA controls.

Parameters	Concentration (ng/ml)
SCA crisis	4.96±0.97
SCA steady state	5.08±0.56
HbAA controls	5.27±0.47

Table 4: Paired t-test between protein s concentration in crisis and steady-state.

Parameters		
SCA in crises	**SCA in steady state**	**P value at 95%CI**
4.96±0.97	5.08±0.56	0.07

Table 5: Independent t test between HbSS in crisis and HbAA control.

Parameters		
SCA in crises	**Control**	**P value at 95%CI**
4.96±0.97	5.27±0.47	0.13

Table 6: Independent t-test between protein C levels in crisis and HbF in SCA.

Parameters		
SCA in crises	**HbF in SCA**	**P value at 95%CI**
4.96±0.97	4.00±2.83	0.08

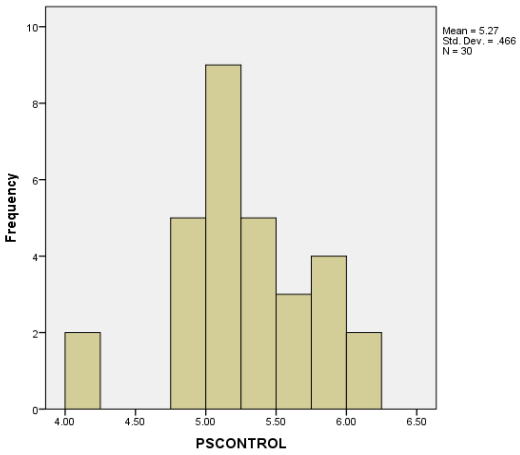

Figure 3: Protein S concentration in HbAA control subjects.

DISCUSSION

This study aimed to determine Protein S activity in SCA and its association (if any) with a vaso-occlusive painful crisis. The SCA patients were recruited during a vaso-occlusive painful crisis and three months after (when they were in steady-state), they were re-invited for repeat Protein S measurement; this eliminated any inter-patient differences that may have been a study limitation. HbAA individuals were also recruited to serve as controls. Vaso-occlusive crises (VOC) is a painful condition that occurs in sickle cell disease as a consequence of an obstruction of microvasculature which results in the clogging of these vessels by red blood cells which are adhesive and sickled and results in ischaemic injury in the organs resulting in pain.[8] The clinical manifestations and complications of sickle cell anaemia are varied and extensive, affecting virtually organ or system in the body often as a consequence of the vaso-occlusive seen in the microvasculature.[9] The results are multi-systemic manifestations often presenting in forms of conditions like sickle cell nephropathy, sickle cell hepatopathy, acute chest syndrome and even coagulopathy.[10,11] Patients with SCA are usually in a hypercoagulable state characterized by an imbalance in the haemostatic system with resultant inappropriate or excessive fibrin and/or platelet deposition with thrombotic clinical consequences.[2] Many laboratory studies have consistently reported increased thrombin generation and increased fibrinolytic activity as well as reduced levels of Naturally Occurring Anticoagulants (NOAC) such as Protein S and Protein C.[12-14] It has been postulated that in sickle cell disease, levels of naturally occurring coagulation inhibitors such as protein C and protein S are markedly reduced when compared to the healthy population.

Various etiological factors have been suggested to explain the hypercoagulability observed in SCD; this varies from phosphatidylserine exposure as a result of continuous red blood cell sickling with membrane vesiculation and endothelial tissue factor expression to cytokine-induced clotting activation.[15,16] Thrombosis in large blood vessels causes and/or contributes to SCD-related cerebral and pulmonary complications, and some studies have suggested that reduced levels of protein S and C are associated with increased stroke risk in patients with SCD. [3,17-19]. In our study, the levels of Protein S were lowest in SCA patients during crisis and highest in HbAA controls and this pattern has been documented by other researchers. In a cross-sectional review of 17 children with sickle cell anaemia in steady-state who were matched with 16 children who were apparently healthy to assess for their coagulation and fibrinolytic properties. [3] It was reported that the protein S levels in children with sickle cell anaemia in steady-state were lower than healthy controls. Similar results were seen in various other studies.[2,12] In contrast, in some other studies, Protein S levels (along with other NOAC) were not significantly different in HbSS patients during steady-state and in crisis. In their study, Schnog and colleagues investigated the relationship between Protein S and C levels with clinical SCD- related events. They found no difference in the levels of Protein S and C in patients in steady-state and during the crisis.[4] NOAC levels were however seen to be lower in HbSS patients compared with HbSC patients, lending credence to the fact that patients with more severe phenotype have lower NOAC levels. One explanation for this may be the fact that endothelial perturbation, perhaps as a result of (and contributing to) vaso-occlusion, occurs continuously even in clinically asymptomatic, as depicted by elevated levels of endothelial activation markers in the ‘‘steady-state’’.[4] Even though Protein S levels were higher during steady-state compared with during crisis, using the paired t-test, this difference failed to reach statistical significance. Similarly, an independent t-test between mean Protein S levels during a crisis and among the HbAA control group also failed to reach any statistical significance. This may be attributed to the small sample size used in our study which may have led to a Type 1 statistical error. Perhaps if the study is repeated with larger sample size, a significant difference will be obtained. There is usually a great variability in SCA severity among patients and even in the same patient over time. Over the years, several disease modifiers have been identified about SCA and one of such modifiers is fetal haemoglobin (HbF). A study by Xu et al reported a reduction in the symptoms associated with SCA in patients with accentuated expression of y globin genes, resulting in high levels of HbF.[20] Several studies have confirmed that HbF levels are inversely related to the degree of severity of clinical symptoms in SCA patients. High HbF has been shown to retard polymerization of sickled red cells in the deoxygenated state. It does this by reducing HbS concentration thus inducing a lower rate of vaso-occlusive crises, leg ulcers, avascular necrosis of the neck of femur, acute chest syndrome, and ultimately a reduced disease severity.[21] Using the independent t-test on the mean Protein S levels in SCA patients during a crisis and the mean HbF concentration, there was no statistical significance.

Limitations

Limitation of current study was the small sample size used due to financial constraints as this study was self - funded by the researchers.

CONCLUSION

The mean Protein S level is highest in SCA crisis state followed by steady-state and HbAA controls though these differences failed to reach statistical significance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. El-Hazmi MAF, Warsy AS, Bahakim H. Blood Proteins C and S in Sickle Cell Disease. Acta Haematol. 1993;90(3):114-9.
2. Francis RB. Protein S deficiency in sickle cell anemia. J Lab Clin Med. 1988;111(5):566-70.
3. Bayazit AK, Kilinc Y. Natural coagulation inhibitors (protein C, protein S, antithrombin) in patients with sickle cell anemia in a steady state. Pediatr Int. 2001;43(6):592-6.
4. Schnog JB, Mac Gillavry MR, van Zanten AP, Meijers JCM, Rojer RA, Duits AJ, et al. Protein C and S and inflammation in sickle cell disease. Am J Hematol. 2004;76(1):26-32.
5. Bezeaud A, Venisse L, Helley D, Trichet C, Girot R, Guillin MC. Red blood cells from patients with homozygous sickle cell disease provide a catalytic surface for factor Va inactivation by activated protein C. Br J Haematol 2002;117(2):409-13.
6. Akinola NO, Stevens SM, Franklin IM, Nash GB, Stuart J. Sublinical Ischaemi episodes during the steady state of sickle cell anaemia. J Clin Pathol 1992;45:902-6.
7. Wang, H and Chow, SC. Sample size calculation for comparing proportions. Wiley Encyclopedia of Clinical Trials. USA: Wiley interscience; 2007:3-4.
8. Manwani D, Frenette PS. Vaso-occlusion in sickle cell disease: pathophysiology and novel targeted therapies. Hematology Am Soc Hematol Educ. 2013;122(24):362-9.
9. Ballas SK, Lieff S, Benjamin LJ, Dampier CD, Heeney MM, Hoppe C, et al. Definitions of the phenotypic manifestations of sickle cell disease. Am J Hematol. 2010;85(1):6-13.
10. Ebert E, Nagar M, and KH-C gastroenterology, 2010 undefined. Gastrointestinal and hepatic complications of sickle cell disease. Clin Gastroenterol Hepatol. 2010;8(6):483-9.
11. Ilesanmi OO. Pathological basis of symptoms and crises in sickle cell disorder: implications for counseling and psychotherapy. Hematol Rep. 2010; 2(1):e2.
12. Westerman MP, Green D, Gilman-Sachs A, Beaman K, Freels S, Boggio L, et al. Antiphospholipid antibodies, proteins C and S, and coagulation changes in sickle cell disease. J Lab Clin Med. 1999;134(4): 352-62.
13. Tomer A, Harker LA, Kasey S, Eckman JR. Thrombogenesis in sickle cell disease. J Lab Clin Med. 2001;137:398-407.
14. Westerman MP, Green D, Gilman-Sachs A, et al. Antiphospholipid antibodies, proteins C and S, and coagulation changes in sickle cell disease. J Lab Clin Med. 1999;134:352-62.
15. Helley D, Eldor A, Girot R, Ducrocq R, Guillin MC, Bezeaud A. Increased procoagulant activity of red blood cells from patients with homozygous sickle cell disease and b-thalassemia. Thromb Haemost. 1996; 76:322-7.
16. Lane PA, O'Connell JL, Marlar RA. Erythrocyte membrane vesicles and irreversibly sickled cells bind protein S. Am J Hematol. 1994;47:295-300.
17. Francis RB. Large-vessel occlusion in sickle cell disease: pathogenesis, clinical consequences, and therapeutic implications. Med Hypotheses. 1991; 35:88-95.
18. Khanduri U, Gravell D, Christie BS, Al Lamki Z, Zachariah M, Cherian E. Reduced protein C levels a contributory factor for stroke in sickle cell disease. Thromb Haemost. 1998;79:879-80.
19. Tam DA. Protein C and protein S activity in sickle cell disease and stroke. J Child Neurol. 1997;12:19-21.
20. Schnog JB, Gillavry MRM, van Zanten AP, Meijers JCM, Rojer RA, Duits AJ, et al. Protein C and S and Inflammation in Sickle Cell Disease. Am J Heme. 2004;76(1):26-32.
21. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. Disorders of haemoglobin: genetics, pathophysiology, clinical management. 2nd ed. Cambridge, United Kingdom: Cambridge University Press; 2009.

Cite this article as: Uche E, Kalejaiye O, Olowoselu F, Ekwere T, Bamiro R, Kareem A, et al. Protein S activity in sickle cell anaemia patients in steady state and crisis. Int J Sci Rep 2023;9(1):5-10.