Original Article

Urea A Significant Clinical Marker of Vaso-Occlusive Crises in Sickle Cell Disease

Clinical Marker of Vaso-Occlusive Crises in Sickle Cell Disease

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ABSTRACT

Objective: To measure serum urea level of Sickle Cell Disease (SCD) patients in pain free steady state and compare it with the levels taken at start, during and on recovery of Vaso-occlusive crises (VOC).

Study Design: Prospective observational study

Place and Duration of Study: This study was conducted at the Lifecare Molecular & PCR Lab Services from May 2018 till May 2020 for a period of more than two years.

Materials and Methods: 25 diagnosed cases of SCD aged 12 to 22 years were included. It was a multidisciplinary approach study including the anesthetist for pain management when required. UCE (Urea, creatinine and electrolytes) was taken in the steady pain free state, at the start, during and on resolution of VOC. The steady state urea level was compared with the other samples taken during VOC using a student's unpaired two-tailed 't' test, a p value <0.005 was taken as significant.

Results: 36 episodes of VOC were analyzed. Mean serum urea level in steady state was 2.8 +- 0.8 mmol/L which dropped to 1.9 +- 1.1 at the time of presentation, reached to 1.5 +- 0.9 ($p< 0.0005$) during VOC with gradual return to steady state level as the crises settled.

Conclusion: A significant drop in urea levels during VOC with a return to steady state level was seen defining VOC and its resolution.

Key Words: Sickle cell disease, Urea, VOC, Painful crises, Nitric Oxide, Arginine

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INTRODUCTION

SCD is amongst the most common genetic disease in United States, effecting 1 in 500 African Americans. About 1 out of every 12 African Americans carry autosomal recessive mutation, with approximately 300,000 effected children born annually [(1)]. We could not come across any study on the epidemiology of SCD in Pakistan. SCD is one of the common hemoglobinopathy seen especially in Africa, black Americans and people living in middle-east Asia.

It has numerous presentations but one of the most common is Painful crises or Vaso-occlusive crises (VOC) of the bones, which may be mild to severe and usually lasts for few days to one week. SCD is a group of hereditary genetic disorder in which there is mutation of one or both beta globin chains resulting in the formation of abnormal hemoglobin (Hb) [(2)]. Either both the chains have sickle cell mutation or one chain has sickle cell abnormality while other chain has an abnormality for some other hemoglobinopathy. SCD has two groups, one homozygous group where both chains have sickle cell genes, then it is called sickle cell anemia or homozygous sickle cell disease, while the other is heterozygous group where if sickle cell gene combines with some other hemoglobinopathy like Hb-C, D, E or quantitative globin chain disorders like beta thalassemia trait, then it becomes heterozygous SCD or named according to the other hemoglobin disorder [(3)]. In both cases one abnormal gene has to be sickle cell gene. The abnormality of sickle gene occurs due to the replacement of glutamic acid with valine in position six on the β -chains [(4)]. During deoxygenation phase of Hb in peripheral tissues and organs, red cells containing sickle Hb precipitate, becomes rigid and cell assumes a sickle appearance [(5)]. This rigid and abnormal shaped red cell can obstruct blood flow through small vessels especially in bones, thereby causing painful Vaso-occlusive crises (VOC), which is the most common clinical presentation of the disease leading to loss or working days in addition to bone, tissue, and organ damage. This hemoglobinopathy causes a significant

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decrease in life span of the sickle cell resulting in chronic hemolytic anemia [6]. Although this appears simple but there is great variability in the clinical expression of the disease even in same family with same phenotype [7]. This variability can be due to epigenetic, genetic, environmental and social factors either alone or in various combinations. Most frequent presenting symptoms in SCD is pain during the VOC process for which NSAIDS, Opioids both oral and parenteral and sometimes hospital admission is required. Due to better understanding and treatment of the disease, patients now live longer but the disease has become more complex and sometimes it is difficult to differentiate real VOC from pain due to other reasons like pain due to avascular necrosis of bones and joints. Some of these patients also become dependent on pain medications especially morphine or may use this symptom for some gain. It is thought sickling of red cells initiate the beginning of VOC [8]. These sickle cells are adhesive and binds to several endothelial receptors and proteins like endothelial cell P -selectin, E -selectin, intercellular adhesion molecule -1, vascular cell adhesion molecule -1, CD36 leading to activation and recruitment of monocytes, polymorphonuclear neutrophils and platelets[9]. Thus, occlusion of microcirculation on a background of chronic hemolysis is important factors that stimulate the cytokine storm leading to the clinical and subclinical VOC episode [10]. NO is a soluble gas with a half-life of seconds and is synthesized continuously in endothelial cells from the amino acid L-arginine by isoforms of the NO synthase enzyme producing relaxation of smooth muscles causing vasodilation, and increased regional blood flow [11]. NO also induces cellular events in a coordinated program which promotes blood flow, primarily by decreasing aggregation of platelet, expression of cell adhesion molecules on endothelial cells, and secretion of procoagulant proteins [12]. This reduction in NO also contributes to VOC. Biochemically Arginine is catabolized by enzyme arginase to urea which is then cleared by the kidneys [13]. Arginine is required to produce NO by enzyme NO synthetase. Arginine can also be acted upon by enzyme Arginase to produce ornithine and urea [14]. This arginase enzyme is released by the hemolysis of red cells particularly the young cells which are predominant in SCD. As a result, the concentration of arginine decreases in the blood leading to a probable decrease in serum Urea [15]. The ongoing chronic hemolysis in these patients may also be a contributing factor for a decrease in serum urea particularly during and recovery from VOC. Early detection and treatment of VOC is important in the management of VOC as it may lead to further complications of the disease. Our aim was to compare the variation of urea concentration in steady state, at the start of VOC, during and on recovery. In addition, it may also help us to clinically differentiate true painful crises of VOC from non-VOC pains like joint pains and or malingering by the patient.

MATERIALS AND METHODS

25 SCD patients, 18 males and 7 females attending Lifecare Molecular & PCR Lab Services, Karachi for their blood investigations were included in the study between May 2018 & April 2020 after Informed consent. The normal range of urea nitrogen in blood or serum is 5 to 20 mg/dl, or 1.8 to 7.1 mmol urea per liter. The wide range in this level is due to protein intake, endogenous protein catabolism, state of hydration, hepatic urea synthesis, and renal urea excretion. Their steady state urea, electrolytes, creatinine & urea (UCE) was collected in the steady state (the pain free period between the VOCs) as a baseline value for comparison when the same parameters were taken at the start, during and at recovery from VOC. Blood was collected in plain tube by venipuncture and UCE was done on chemical analyzer of total lab automation. Statistical significance was calculated using the student's unpaired two -tailed 't' test and the data presented as mean + standard deviation. A p value of <0.05 was considered as significant.

RESULTS

Table 1 shows a total of 25 patients with SCD completed enrollment, and because the enrollment was consecutive, no attempt was made to choose the patients by genotype. They comprised of 18 male (28 episodes) and 7 females (8 episodes). Eight males had one episode and ten had two episodes while from seven females six had one and one had two episodes. Patient's age ranged from 12-22 years (mean 16.25 years). Table 2 shows patient's serum urea dropped significantly to mean level of 1.5 +- 0.9 mmol/l from steady state level of 2.8 +- 0.8 mmol/l ($p<0.0005$) and then remained at this value for nearly two or three days to regain its near steady state level on fourth to sixth day for recovery.

Table No.1: Demographic & VOC numbers of SCD patients [n=25]

SCD n = 25	**Age in years**			**Total VOCs n =36**		
	Range	Mean	Median	Pts with only one episode of VOC	Pts with multiple episodes of VOC	Total VOCs
Males n = 18	12-22	16.7	16	8	10 (10 X 2 VOC =20)	28
Females n =7	12-18	14.37	15	6	01 (1 X 2 VOC =02)	08

Table No.2: Details of serum urea level as per stage of VOC

Serum Urea (NV 3.2 – 7.3 mmol/L)			**Phases of VOC**			**P value. Comparison b/w**	
		Steady State	Start of VOC	During VOC	Recovery from VOC	Steady state & during VOC	Steady state & recovery state
Total Patients n=25	Mean +- SD Range	2.8 +- 0.8 1.1-5.3	1.9 +- 1.1 0.3 - 5.3	1.5 +- 0.9 0.5 – 5.0	2.5 +- 1.0 0.7 – 5.4	p <0.0005	p .091
Male Patients n=18	Mean +- SD Range	3.5-0.7 2-53	2.4 +- 1 0.4 – 4.8	2.4 + - 1.1 0.6 - 5.0	3.0 +- 1.2 1.1 – 5.4	p <0.0005	p 0.066
Female patients n=7	Mean +- SD Range	2.4 + 0.6 1.1 – 3.6	1.6 +- 0.9 0.3 – 4.3	1.8 +- 0.7 0.4 – 3.8	2.1 +- 0.9 0.6 – 4.2	p <0.0005	P 0.143
Pts with single episodes n=19	Mean +- SD Range	2.8 + 0.9 1.1 – 5.3	2.0 +- 1.0 0.3 – 5.3	2.1 +- 0.7 0.4 – 5.2	2.6 +- 1.2 0.6 – 5.4	p <0.0005	p 0.059
Pts with multiple episodes n=6	Mean +- SD Range	2.8 + 0.7 1.6 – 4.3	1.4 +- 0.7 0.4 – 3.4	1.6 + - 0.7 0.5 – 3.5	2.3 +- 1.0 0.6– 4.3	p <0.0005	P =0.047

DISCUSSION

To the best of our knowledge, we have not come across any study which deals with urea and sickle cell disease during VOC. The major findings in our study were the low serum urea levels in the steady state and a drop during VOC and return to baseline steady level on resolution of VOC. We have observed this phenomenon in sickle cell patients presenting with VOC. Many factors contribute to low serum urea levels in the steady pain free state in these patients. A study by Olaniran [(16)] shows that a major factor of low urea levels is the kidney damage done by the SCD commonly known as sickle cell nephropathy (SCN), but under-recognized complication. In an original article by Geraldo [(17)], the main abnormalities identified, were urinary concentrating and incomplete distal acidification defect. There was also an increase in the potassium transport and decrease in water reabsorption, evidencing the occurrence of distal tubular dysfunction leading to a lower serum urea concentration, particularly in acute hemolysis on background of chronic hemolysis which is seen in SCD patients for most of the time during crises [(17)]. Our study shows that it is worth monitoring serum urea concentrations during steady phase and VOCs when it is related to the improvement in the painful crises due to VOC. One study was done in Basra Iraq where AL-Nama et al mentioned that serum urea in pediatric sickle cell patients is lower than the normal children, but they did not mention the cause of this decrease [(18)]. Another study was done by Michael et al which shows that SCD significantly changes Kidney structure and function causing multiple renal syndromes and diseases [(19)]. Such variety of renal issues shows the significant and unique but complex vascular abnormalities of SCD and the tendency sickled red cells in the renal medulla due to its hypoxic, acidotic, and hyperosmolar conditions. These all abnormalities cause a low serum urea concentration even in steady state.

The second factor which causes low serum urea and the variation particularly during a VOC episode is the decreased availability of arginine amino acid during VOC. Our observation has been substantiated by a study done by A. Kyle Macka [(20)]. A study by Nadr et al has shown that arginine amino acid is metabolized into ornithine through one path and via a second pathway into urea. When sickled red cell is hemolyzed particularly in VOC, an enzyme arginase is released in the blood which degrades arginine causing a decreased production of urea during the episode when hemolysis increases. Hemolysis also releases free Hb which is a great scavenger of Nitric Oxide. This NO is a vasodilator and its deficiency causes more damage during the VOC [(21)]. Our opinion is that at the start of VOC these changes start and causes urea level to decrease gradually and remains low as long as the process is continuing Once the process starts improving and majority of sickled cells are consumed in the episode, new RBC enter the circulation and sickling and hemolysis decreases thereby the concentration of arginase and free Hb also decreases. This may result in the upsurge of serum urea level near or at the steady state level. This bouncing back of serum urea level indicates that the process of VOC is improving. It has

also been seen in a study in which they measured GFR of two groups and found that absolute values for GFR corrected for BSA were significantly higher in SCD group compared to normal group and SCD children had more tendency to hyperfiltrate than normal children [22]. Our study is further supported by a study from Nath KA and et al [23] on renal functions in sickle cell disease patients and shows development of renal interstitial disease which seems to cause problems in urea and other constituents when the renal concentrating capability is compromised. This study supports our view that on a background of sickle cell induced nephritis, an acute VOC further aggravates it and causes a decrease in serum urea concentration.

CONCLUSION

The phenomenon of significant drop in serum urea levels occurs during a VOC and its serial monitoring may be a useful marker to judge the commencement, progress & resolution of the VOC. Improvements in serum urea towards steady state levels proves to be a good indicator for the recovery from VOC. It is also recommended that levels of arginine in the blood in SCD patients in the steady and VOC stages should also be conducted to further strengthen study.

Author's Contribution:

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