ORIGINAL RESEARCH PAPER

Biochemistry

DETERIORATING ORGAN FUNCTION IN SICKLE CELL DISEASE PATIENTS EVEN IN A STEADY STATE

KEY WORDS: Sickle Cell Disease, Steady state, Blood Urea, Serum Creatinine, Uric acid

DR. Apoorva V. Shivankar Assistant professor, Department of Biochemistry, Govt. Medical college, Nagpur

Dr. Mahavir R. Mundra* Assistant professor, Department of Biochemistry, Govt. Medical college, Nagpur*Corresponding Author

Dr. Archana V. Dhotre Associate professor, Department of Biochemistry, Govt. Medical college, Nagpur

ABSTRACT

This study proposes that a sickle cell disease patient even though in good health, maintained in a steady state on drugs is not truly stable internally. A continuous deterioration in the functioning at an individual organ level is always there, necessitating a routine monitoring to avoid future complications. This is a comparative study between the sickle cell disease patients in a steady state and their normal counterparts with respect to the kidney and liver functioning. Blood urea, Serum Creatinine and Uric acid levels were compared between 100 diseased and 100 healthy controls in Government Medical College and Hospital (GMCH), Nagpur. Results showed significantly lower values of Blood Urea, Serum Creatinine whereas increased levels of Serum Uric acid in the diseased compared to controls indicating decreased organ function in SCD patients even in a steady state.

1.Introduction:

Sickle cell disease (SCD) is an inherited autosomal recessive hemoglobinopathy characterized by the presence of sickle shaped red blood cells (RBCs) and presents with hemolytic anemia, increased susceptibility to infections and vaso-occlusive episodes leading to a reduced quality and expectancy of life.[1]

Approximately 5% of the world's population carries trait genes for hemoglobin disorders, mainly, sickle-cell disease and thalassemia as stated by World Health Organization (WHO, 2006). According to WHO, over 3,00,000 babies are born per year worldwide with these hemoglobinopathies. The governing bodies of WHO have adopted special resolutions on hemoglobin disorders. The resolution on sickle-cell disease, from the 59th World Health Assembly in May, 2006 meeting of the WHO Executive Board, called upon affected countries and the Secretariat of WHO to strengthen their response to this condition. In addition, a resolution on the prevention and management of birth defects, including sickle-cell disease and thalassemia, was adopted by the 63rd World Health Assembly in May, 2010.[2]

In sickle cell disease, hemoglobin, the red blood pigment contained in the erythrocytes, is the one to be affected. It is a protein whose major function is to transport oxygen throughout the body. A molecule of this hemoglobin is made up of two identical α chains and two identical β chains ($\alpha_2\beta_2$ tetramer). It is a genetic disease caused by a single base pair substitution wherein the Glutamic acid is replaced by Valine at β6 position. Erythrocytes containing such mutated hemoglobin assume an irregular crescent like shape under stressful conditions like low oxygen concentration usually present in blood capillaries. It is these sickled red blood cells (RBCs) which are responsible for entire pathophysiology of the disease. Due to their sickled shape, smaller blood vessels are blocked and thus, the corresponding organs are devoid of oxygen supply leading to their impaired functioning. Also this sickling can lead to production of reactive oxygen species (ROS), namely superoxide, hydroxyl, and peroxide radicals within and outside RBCs.[3] All these changes along with chronic activation and damage of endothelial cells by sickled RBCs, heme, polymorphonuclear neutrophils (PMNs) and inflammatory mediators contribute to progressive microvascular damage in all organs, including the brain, liver and kidneys.[1,3-6] All these organs are the highly perfused organs of the body and cannot sustain an oxygen deprived state. The acidic, hypertonic and hypoxic environment of renal medulla favours polymerization of hemoglobin S and thus occlusive renal damage.[7,8]

The debilitating effects of this disease are such that, before the latter half of the twentieth century, individuals with sickle-cell anemia rarely survived to maturity. As urea and creatinine need both liver and kidneys for their metabolism that is liver for synthesis and kidneys for excretion, monitoring of urea and creatinine levels in serum can depict the extent of liver and renal involvement in the disease and can help in planning the management strategy so as to prevent complications. This hyper metabolic state, hemolysis induced oxidative stress and renal involvement in sickle cell disease can also cause alterations in the uric acid levels of the body. The limited solubility of uric acid particularly in the acidic environment of distal nephron may thus be of great concern in humans in case of its accumulation.[9-11]

Additionally, only a few studies were conducted in an endemic area like Central India and most of these studies were confined to the pediatric age group or the patients already in crisis phase. In an endemic area like Vidarbha, a larger sickle cell disease population belonging to an adult age group maintained in a steady state is also found. If this population is targeted for proper management, a better result in the form of decreased sufferings, reduced complications and longer lifespan can be achieved. So, to confirm the involvement of organs like liver and kidney, this study was conducted involving estimation of blood urea, serum creatinine and uric acid levels in steady state homozygous sickle cell disease patients in an age group of 14 to 26 years in Central India.

2. Material and Methods:

The present study was conducted in the Department of Biochemistry at a tertiary health care centre (GMCH, Nagpur) with the help of Medicine Department over a period of one and a half year.

2.1 Study design:

Hospital based cross sectional study with comparison groups.

2.2 Study population:

100 diagnosed and registered cases of homozygous sickle

cell disease in a steady state belonging to an age group of 14 to 26 years were taken as cases and another 100 normal age matched healthy volunteers as controls. All subjects of case population were attending the sickle cell outpatient department of the tertiary health care centre.

The study subjects were diagnosed as sickle cell disease (HbSS) cases on the basis of solubility test and hemoglobin electrophoresis.

2.3 Inclusion Criteria :

Registered cases of sickle cell disease (HbSS) in steady state, 14 to 26 years of age as study cases and normal age matched healthy individuals as controls.

2.4 Exclusion Criteria :

Age < 14 years and > 26 years, heterozygous sickle cell anemia (HbAS), mixed types of sickle cell disease, other hemoglobinopathies, sickle cell patients in crisis phase, documented chronic infection, recent hospitalization, blood transfusion in a period of four months prior to blood sampling, other hemolytic disorders, hepatic or renal disorders, mineral or vitamin supplementation, antibiotics or corticosteroids intake prior to blood sampling.

2.5 Specimen collection and preservation:

About 5 ml of blood was collected in a clean plain bulb by venepuncture. The serum was separated by centrifugation. All the tests were performed on this serum. Blood urea, Serum creatinine and Serum uric acid levels were estimated in the study by kit method on semiautoanalyser. All the parameters were estimated on the same day of collection of the sample

2.6. ICONWP Semi-Automatic Analyzer

Parameters were estimated with methods as follows:

Sr. No.	PARAMETERS	METHODS
1.	Blood Urea	Berthelot's method (Urease enzymatic method), [12]
2.	Serum Creatinine	Jaffe's method, [13]
3.	Serum Uric Acid	Uricase Enzymatic method, [14]

2.7. Statistical Analysis :

- Demographic parameters (age, height, weight), clinical parameters and biochemical parameters were presented as mean ± SD.
- Statistical data was recorded on Microsoft Excel programme 2007.
- Unpaired t-test was performed to compare demographic, clinical and biochemical parameters between homozygous sickle cell disease patients and control groups.
- Pearson's correlation coefficient (r) was calculated to assess the correlation between biochemical parameters.
- Tests were two sided. P value <0.05 was considered as statistically significant. The p value < 0.001 was considered as highly significant and the p value > 0.05 was taken as non-significant (NS).
- Graph Pad Prism version 6.0 was used for statistical analysis.

3. Results :

3.1 Demographic and anthropometric data:

Cases and controls were matched for age. They showed statistically significant difference in their height, weight and BMI.

Parameters	Cases (100) [Mean ± SD]	Control (100) [Mean ± SD]	P value	Significance
Mean Age (years)	19.59 ± 3.18	19.35 ± 3.57	0.6164	not significant
Height (m)	1.59 ± 0.09	1.63 ± 0.08	0.0012**	significant
Weight (kg)	42.51 ± 7.83	47.6 ± 9.08	<0.0001***	significant
BMI(kg/m^2)	16.67 + 1.90	17.71 ± 2.41	0.0009***	significant

3.2 Vital parameter Haemoglobin:

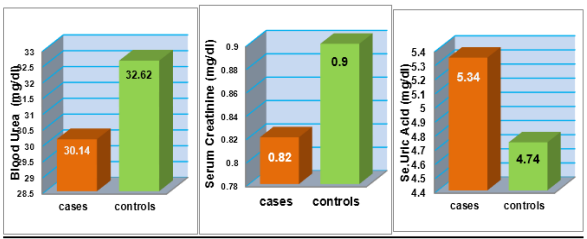
Haemoglobin levels were significantly lower in sickle cell disease cases

Vital parameter	Cases [mean ± SD] gm/dl	Controls [mean ± SD] gm/dl	p-value	Significance
Hemoglobin	8.12 ± 0.87	12.23 ± 2.04	<0.0001***	significant

3.3 Biological Parameters

Blood urea and serum creatinine were found to be significantly reduced whereas serum uric acid levels were increased in sickle cell patients as compared to normal controls.

Parameter	Cases (n=100) [mean ± SD]	Controls (n=100) [mean ± SD]	p value	Significance
Blood Urea (mg/dl)	30.14 ± 7.66	32.62 ± 5.63	0.0098**	significant
Serum Creatinine (mg/dl)	0.82 ± 0.14	0.90 ± 0.17	0.0015**	significant
Serum Uric Acid (mg/dl)	5.34 ± 0.89	4.74 ± 0.96	<0.0001***	significant



4. Discussion:

The present study was undertaken to compare the changes which occurred in the levels of blood urea, serum creatinine and uric acid in sickle cell disease adult patients in steady state and matched controls to assess the ongoing changes in the diseased even in a steady state. Here, the cases and controls were comparable with each other with respect to age. But a significant difference was observed with respect to anthropometric parameters like weight, height and body mass index.

4.1 Blood Urea

Urea, an end product of protein metabolism is produced in liver and excreted by the kidneys. Sickle cell disease is characterized by a progressive liver injury and decreased liver function owing to repeated vaso-occlusive damage by the time adulthood is reached. The smaller number of sickle shaped cells found in the hepatic vein after passage through the liver suggests that the cells most susceptible to sickling are already trapped by their rigidity and engulfed by phagocytes during their passage through the hepatic sinusoids, where the oxygen content of the blood is extremely low. This leads to liver dysfunction in about one-third of patients with sickle cell disease.[15] Other factors that may compound the pathophysiology of the liver involvement in this disease are iron overload and cholelithiasis.[9,16-19] As liver is thus damaged, urea cycle is restricted to some extent, thereby reducing urea formation.

Zinc is a cofactor for ornithine transcarbamylase,[20-22] which is

an important enzyme of urea cycle. Thus, the zinc deficiency which is present in sickle cell disease can be a reason for decrease in ornithine transcarbamylase activity thereby inhibiting urea cycle.[9,20,21,23,24]

In sickle cell disease due to volume overload glomerular filtration rate increases which further increases the urine flow rate. As the urine flows at a higher rate in the renal tubule, very less time is available for passive diffusion of urea and hence a larger amount of it is washed away with urine thereby decreasing blood urea levels.

Also some studies have shown that the nephron damage and impaired tubular reabsorption seen in sickle cell disease as a result of vaso-occlusion and oxidative damage may be responsible for decreased blood urea levels.[25]

4.2 Creatinine

Creatinine is largely formed and stored in muscles by irreversible and non-enzymatic removal of water from creatine phosphate.[26] Thus, a lesser muscle mass indicates low serum creatinine levels explaining the reason behind the lowered serum creatinine in the lean sickle cell patient. Mohsen et al. (1991)[27] stated that along with reduced muscle mass, increase in plasma volume reported in sickle cell anemia patients by increasing GFR can also result in an overall decrease in serum creatinine concentration.[27-29] Endogenous creatinine is excreted by filtration through the glomerulus and small but significant tubular secretion. Renal involvement in sickle cell disease presents with supranormal proximal tubular function which leads to an increased secretion of creatinine in the proximal convoluted tubule. Thus, excretion of creatinine is increased resulting in its lower serum levels.[7,9,28-30]

4.3 Uric Acid

Sickled RBCs' have a reduced life span as they are readily destroyed in the body. Thus, in an attempt to maintain the blood supply to various organs, bone marrow attains a hypermetabolic state. This leads to markedly increased red cell turnover giving rise to increased nucleic acid [purine] formation followed by their breakdown during hemolysis which in turn results in increased production of uric acid.[9,28,31-33] Decreased breakdown of uric acid in the gut may also contribute to a smaller extent to the increased uric acid levels in sickle cell patients[32,34]

Also due to recurrent red cell hemolysis, free hemoglobin is released which catalyzes the Fenton reaction generating free radicals which precipitate oxidative stress.[35-38] The recurrent ischemia-reperfusion injury, higher auto-oxidation of hemoglobin S, the chronic proinflammatory state of the disease, production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and an enhanced lipid per-oxidation are the various factors adding to the oxidative damage in these patients.[35-38] The elevation of serum uric acid in sickle cell disease may be a protective response against this oxidative stress as uric acid itself is capable of opposing harmful effects of free radicals and oxidative stress by scavenging the free radicals in human serum.[39]

The involvement of proximal convoluted tubules can lead to improper reabsorption, secretion and post secretory reabsorption of uric acid and thus may reduce excretion of uric acid.[40]

5.Conclusion:

In the present study the following findings were concluded

- Reduced blood urea and serum creatinine levels in sickle cell patients indicated the ongoing organ damage even in steady state necessitating routine investigations and timely actions to prolong the development of crisis phase and prevent permanent damage thereby giving the patient a better health profile.
- Increased uric acid levels in sickle cell patients indicated increased metabolic turnover and built up of oxidative stress in them which can then be reduced by timely supplementing appropriate antioxidants.

Presently the healthcare cost in the management of patients with sickle cell disease is disproportionately higher adding an additional economic burden to the sufferings of the patients. Both these can be taken care of to some extent with the timely monitoring and supplementations and thus their quality of life can be improved.

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