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MICROSCOPIC INVESTIGATIONS OF URINE OF SICKLE CELL ANEMIC PATIENTS

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ABSTRACT

The present study was carried out to investigate the normal and abnormal microscopic constituents of urine of sickle cell anemic patients of district Amravati, MS India in order to study the physiological status of the kidney in such patients. In total 67 samples were investigated. The normal and abnormal cellular components like presence of RBCs, WBCs, Epithelial cells, Renal tubules cells, Bacteria, Yeast and Protozoan were studied. Noncellular components/crystals like Tyrosine, Cholesterol, A/T Phosphate, Leucine, B-Granule, Sulfa-amide, Mucus, Fibers Hyline, Uric Acid, Oxalate and Cystine were studied. Out of the total samples, RBC_s were found in 69.7% patients, WBC_s in 66.7% samples , Epithelial cells in 96.9% patients, Renal tubules cells in 39.4% patients, Bacteria in 10.6% patients and Yeast in 9.09% patients. different normal and abnormal crystal were observed in the urine sample of sickle cell anemia. Tyrosine was observed in 15 %, cholesterol in 16 %, phosphate in 40%, sulphamide in 11%, mucus in 2%, while hyline crystals were found in 53% patients ,uric acid in 34% , oxalate in 53% and cystine in 5% Sickle Cell Anemia urine samples. Thus present study shows that SCA patients of district Amravati, MS India are under the threat of Glomerulonephritis, urinary tract infection, urinary tract inflammation, Heavy proteinuria, tubular necrosis etc. We propose the proper management of the disease to protect this very important and vital organ.

KEY WORDS: Sickle Cell Anemia, Microscopic Study, Urine, Crystal,Cellular Components

INTRODUCTION

In India, sickle cell gene is mostly constrained to tribal and scheduled caste population , carrier frequencies of which range between 5-40%. (Bhatia and Rao 1987). Rao (1988) estimated the expected figure of sickle homozygotes as 1,31,375 while expected number of sickle cell heterozygotes as 24,34,170. Sickle cell anemia is one of the genetic disorderd caused by defective gene for hemoglobine (HbS). Abnormal hemoglobin polymerizes forming sickled RBCs after deoxygenation. This damages the membrane of red blood cells and RBCs stick in blood vessels. Thus downstream tissues deprive of the oxygen and causes ischemia and infarction. The well known sickle cell crisis are acute chest syndrome .

Sickle cell anemia was among the first diseases to be understood at a molecular level, yet more than 50 years after the genetic basis of the disorder was first described. Many details of its primary pathophysiology are still not understood. Sickle cell Disease (SCD) is an inherited blood disorder for which the optimal treatment remains uncertain. The genetic abnormality is due to a substitution of the amino acid Valine for glutamic acid at the sixth position on the beta globin chain and was first described over one hundred years ago (Herrick, 1910;Pauling et al., 1949). Based on the World Health Organization published global prevalence map of SCD and other data, it was estimate that about 20-25 million individuals worldwide have homozygous SCD; 12-15 million in sub-Saharan Africa, 5-10 million in India and about 3 million distributed in different parts of the world (Serjeant, 2006).

It is observed that over 300,000 babies with severe form of sickle cell disease are born every year worldwide. The majority of which is the middle and low income countries. Approximately 5% of the world's populations are the carriers of gene for sickle cell disease; the percentage may be as high as 25% in some area. A high concentration of the disease is in Asia, the Mediterranean basis, the Middle East and the Africa (WHO, 2006).

Evidence exists that for those with sickle cell syndromes "kidney damage starts very early and progresses throughout life" (Rossi-Espagnet et al., 1968; Eckman and Platt, 1991; Saborio and Scheinman, 1999).Various renal abnormalities have occurred in association with sickle cell (SS) disease (McCoy 1969; Schlitt. and Keitel, 1960 and Sweeney et.al. 1962). The renal features of sickle cell disease (SCD) include hematuria, proteinuria, tubular disturbances and chronic kidney disease (Pham et.al., 2000). In this era, the most common causes of death in adults from sickle cell disease reported are pulmonary hypertension, sudden death of unknown etiology, renal failure and infection (Darbari et.al., 2006).

Microscopic Examination of urine is the cheapest and the easiest technique to determine the physiology of the kidney. The purpose of the present study was to investigate the normal and abnormal microscopic components of the urine of sickle cell disease patients and correlate its association with the physiologic status of the kidney.

MATERIAL AND METHODS

Study Population

The study population consisted of 67 randomized subjects with Sickle cell anemia from the district Amravati, Maharashtra, India. This study was approved by Institutional Human Ethics Committee. Written consent from each participant was taken. This study was carried out in the year 2011-12.

Collection of Sample

A clean catch urine sample was obtained. Urine sample was collected from the participants by the standard spontaneous voiding procedure as stated by Corwin (1996). Urine was collected at about noon to avoid contamination of urine by the contents of urethra or vagina and therefore its constituents are more likely to reflect kidney origin. First void sample was also collected, because this is usually, a concentrated specimen and most informative. Urine was collected in a sterile, labeled bottle containing 4% formaldehyde. Urine was transported to the laboratory immediately in an ice bag. All the analysis was performed within a week (European Confederation of Laboratory Medicine, 2000; Skobe. 2004).

Preparation of the Urine Sample

One ml of urine was centrifuged at 3,000 rpm for 5 minutes. Following aspiration of the supernatant to a marked level, the pellet was agitated into a homogeneous mixture followed by a sampling pipette provided by the manufacturer (Tarson). This was followed by macroscopic analysis of urine using Magnus inclined trinocular microscope (Make-Olympus).

Microscopic Analysis of Urine

A drop of prepared urine sample was pipetted onto a slide and a coverslip was placed. The slide was then examined with or without staining. The urine sediment is routinely examined by bright field microscopy under both low and high power without staining. For the present study, prepared slide was observed under the Magnus inclined trinocular microscope (Make- Olympus). After that, RBCs, WBCs and ECs were examined using a high-power field (HPF).The normal and abnormal cellular components like presence of RBCs, WBCs, Epithelial cells, Renal tubules cells, Bacteria,

Yeast and Protozoan were studied. Non-cellular components like Tyrosine, Cholesterol, A/T Phosphate, Leucine, B-Granule, Sulfa -amide, Mucus, Fibers Hyline, Uric Acid, Oxalate and Cystine were observed.

RESULTS

In total, 67 samples were collected at the different timings as mentioned above in the methodology.

Cellular Components of Urine of SCA

The normal and abnormal cellular components like RBCs, WBCs, Epithelial cells, Renal tubules cells, Bacteria, Yeast and Protozoan were studied. Out of the total samples, RBCs were found in 69.7% patients, WBCs were found in 66.7% samples , Epithelial cells were found in 96.9% patients, Renal tubules cells were observed in 39.4 % patients, Bacteria were found in 10.6 % patients, Yeast in 9.09% patients and we could not observed any Protozoan in urine of SCA. Percentage distribution of normal and abnormal Cellular components of urine in SCA is shown in fig.1.

Non-Cellular Components of Urine in SCA

Non-cellular components like Tyrosine, Cholesterol, A/T Phosphate, Leucine, B-Granule, Sulfa -amide, Mucus, Fibers, Hyline, Uric Acid, Oxalate, Cystine were studied in the urine sample of sickle cell anemic patients. Percentage distribution of normal and abnormal non-Cellular components of urine in SCA are shown in fig. 2. We observed different normal and abnormal crystal in the urine sample of SCA. We observed Tyrosine in 15 %, cholesterol in 16 %, phosphate in 40%, sulphamide in 11%, mucus in 2%, while hyline was found in 53% patients, uric acid in 34% , oxalate in 53% and cystine in 5% SCA patients. It was observed that all most all patients suffer from glomerulonepritis, interstitial nephritis and tubular nepritis . This is the most significant observations of this study.

DISCUSSIONS

In the present study various abnormal cellular and non-cellular components in the urine of SCD patients were observed. In combination with dipstick analysis, microscopic analysis can help distinguish patients with UTI or medical renal disease. In the present microscopic investigation on the urine of SCD, we observed RBCs in 70% samples which may be because of the Glomerulonephritis, vasculitis and inflammation. Sometimes hematouria can become life threatening (Hori and Thiruchelvam, 2012).White blood cells are observed in 66% patients which may appear in the urine in Interstial nephritis and pylonephritis. Epithelial cells are observed in 96% patients. Thus, the most of the patients of SCA suffers from the Acute tubular necrosis, interstitial nephritis and glomerulonephritis. Renal tubular cells were observed in 40% patients while 11% patients were infected by bacteria. Yeasts were found in 9% urine samples.

Non-cellular/ crystalline components in urine of SCD also express various abnormal conditions. Presence of Uric acid, tyrosine, oxalate, A/T Phosphate, Hyline etc. is the indication of acute nephropathy. Appearance of cholesterol in urine indicates heavy protein urea may be due to improper filtration at Glomerulus. Presence of Cystine in the urine of SCD indicates the abnormal metabolic process in the body. On the basis of microscopic analysis of urine of SCA it could be concluded that the Kidney of such patients are under risk and suffering from various complications like nephrites, glomerular damage, hematourea etc. Various possible complications in SCA of district Amravati MS India are shown in fig.3. On the basis of cellular and non-cellular components of urine of SCA patients of district Amravati, the probable percent frequency of physiological complications going on inside the important organs like kidney, liver , urinary bladder ect was evaluated. It was found that SCA patients are suffered by various complications. Above 69.7% patients of SCA suffers from Cystisis and Urinary Tract Inflammation, 96.9% patients suffer from Interstitial nephritis, Acute Tubular

Necrosis and Glomerulonephritis. It was observed that 66.7% SCA patients suffer from Pylonephritis which means inflammation of renal pelvis. 16% SCA patients suffer from Chyluria. In 15% SCA patients Atrophy and Cirrosis of Liver could be seen while 53% patients suffers from liver dysfunction.

Many researchers stated the abnormal functioning of kidney in SCA patients. Microalbuminuria and albuminuria are common in the sickle cell disease and can occur in up to 80% of patients resulting in a glomerulopathy (Alvarez et. al., 2008 and Bray et.al., 2006). Approximately 15% of patients will advance to end stage renal disease by their third decade of life. Guasch et.al., 2006 stated that 25% of patients with hemoglobin SS disease have renal insufficiency defined as a reduced creatinine clearance of < 90 ml/min. The kidneys may display normal echogenicity (89% of patients); may be diffusely, mildly echogenic (5%); or may exhibit increased medullary echogenicity with normal cortical echogenicity (3%). Overtime, the kidneys may shrink if renal failure ensues (Harrow et.al.,1963). Sickle cell nephropathy is indicated by sickled erythrocytes, with the consequent effects of decreased medullary blood flow, ischemia, microinfarct and papillary necrosis (Pham et.al., 2000). Evaluation by a nephrologist rather than a urologist include proteinuria, red cell casts and dysmorphic red blood cells, especially if the serum creatinine level is more.(Grossfeld., et. al. 2001). Many medical renal conditions (eg, glomerulonephritis) and hemoglobinopathies (eg, sickle cell trait) cause blood in the urine. The kidney of the homozygous sickle cell anemia (Hb SS) patient is affected by the hemodynamic changes of chronic anemia and by the consequences of vaso-occlusion, especially in the renal medulla (Serjeant 1992 ; Mapp et.al 1987).The prevalence rate of proteinuria in patients with Hb SS has been reported to vary from 17 to 33% in studies in which proteinuria was determined by the dipstick method (Aoki and Saad, 1995). Allen stated that haematuria occurs in 3-4% of patients with sickle cell disease (Allen, 1964). The present study support above observations.

CONCULSIONS

Urine of SCA of district Amravati, MS, India exhibits many abnormal cellular and non-cellular components. Present study shows that the SCA patients are under the threat of Glomerulonephritis, urinary tract infection, urinary tract inflammation, Heavy proteinuria , tubular necrosis, Interstitial nephritis, Pylonephritis etc. Thus it can be concluded that the kidney of the SCA is in risk might be due to hypoxic condition in SCA, hence proper and timely precautions should be taken to lessen the damage to this important organ. SCA should be managed properly as soon as it is detected so that the vital organs could be protected and saved from the deterioration.

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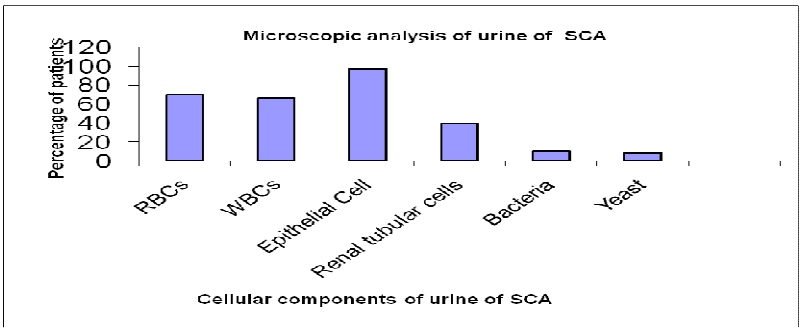
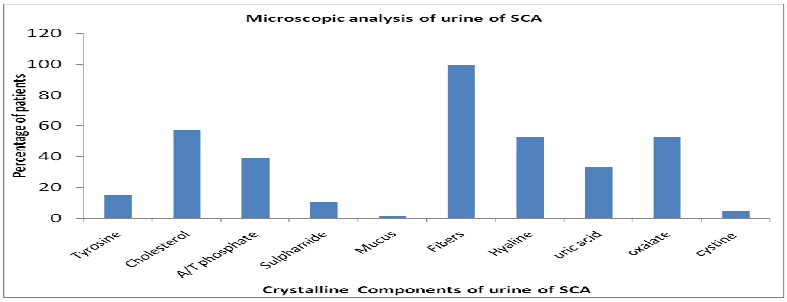


Fig.1: Cellular Components in Urine of SCA



A: Amorphous, T: Triple

Fig. 2 : Non- Cellular Components in Urine of SCA

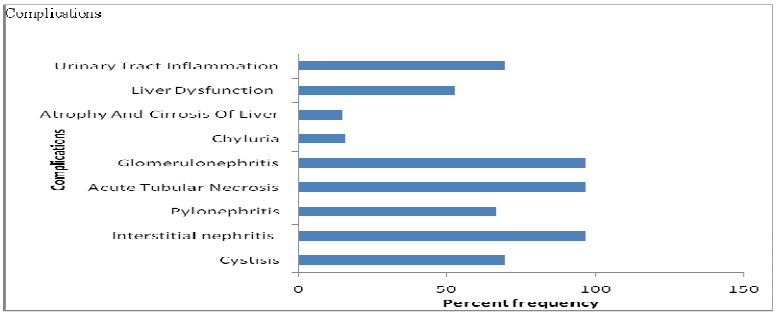


Fig.3: Various Probable Complications seen in SCA of District Amravati as Viewed in Urine

X axis represents percent frequency of complications of excretory system in Sickle cell anemic patients and Y axis represents possible complications in the excretory system of Sickle cell anemic patients