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Screening of the renal diseases in the subjects belonging to the urban area with serum urea and serum iron

Priyanka Sharma[1], Reena Chaurasiya[1], Rashmi Arnold[2], Santosh Agnihotri[2], Arti Saxena[3]and Seema Tiwari[1*]

1, Study centre for Biochemistry, A.P.S.U., Rewa, (MP) - India
2, Department of Botany, Model Science College, Rewa, (MP) - India
3, Department of Zoology, Model Science College, Rewa, (MP) - India

Abstract

Urea and iron is the strong prediction for the occurrence of both for renal diseases and its severity and complication. Iron is important predictor for understanding to the severity in renal diseases and its complication. The kidney has a bean-shaped structure; each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The amount of urea nitrogen released and the amount reincorporated into albumin has been measured in healthy and uremic individuals on both normal and low-protein diets. The importance of iron in renal injury is derived from the ease with which iron is reversibly oxidized or reduced, enabling it to participate in the production of free radicals. Experimental evidence for the role of oxidants and iron in progressive renal disease falls into two broad categories. Oxidants derived either from leukocytes in proliferate glomerulonephritis or from resident glomerular cells in nonproliferative glomerulonephritis have been shown to have several biological effects relevant to chronic kidney disease. This study was aimed to study the blood serum iron, urea in the subjects from Rewa district, to see if any correlation exists between iron and other parameters and to evaluate the risk factors for other complicated diseases.

Key-Words: Urea, serum, Kidney, chronic kidney disease (CKD), Glomerular filtration rate (GFR)

Introduction

The Latin term renes is related to the English word "reins", a synonym for the kidneys in Shakespearean English (e.g. Merry Wives of Windsor 3.5), which was also the time the King James Version was translated. The kidney has a bean-shaped structure; each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal fascia (of Gerota) and paranephric fat. The anterior (front) border of these tissues is the peritoneum, while the posterior (rear) border is the transversalis fascia. The superior border of the right kidney is adjacent to the liver; and the spleen, for the left kidney. Therefore, both move down on inhalation. The kidney is approximately 11–14 cm in length, 6 cm wide and 4 cm thick. The substance, or parenchyma, of the kidney is divided into two major structures: superficial is the renal cortex and deep is the renal medulla 1.

*** Corresponding Author**
E.mail: seemat452@gmail.com

The tip, or papilla, of each pyramid empties urine into a minor calyx; minor calyces empty into major calyces, and major calyces empty into the renal pelvis, which becomes the ureter. The kidneys receive blood from the renal arteries, left and right, which branch directly from the abdominal aorta. Despite their relatively small size, the kidneys receive approximately 20% of the cardiac output 2.

Each renal artery branches into segmental arteries, dividing further into interlobar arteries which penetrate the renal capsule and extend through the renal columns between the renal pyramids. The interlobar arteries then supply blood to the arcuate arteries that run through the boundary of the cortex and the medulla. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply the glomeruli. The interstitum (or interstitium) is the functional space in the kidney beneath the individual filters (glomeruli) which are rich in blood vessels. The interstitum absorbs fluid recovered from urine. Various conditions can lead to scarring and congestion of this area, which can cause kidney dysfunction and failure. After filtration occurs the blood moves through a small

network of venules that converge into interlobular veins. As with the arteriole distribution the veins follow the same pattern, the interlobular provide blood to the arcuate veins then back to the interlobar veins which come to form the renal vein exiting the kidney for transfusion for blood. The asymmetry within the abdominal cavity caused by the liver typically results in the right kidney being slightly lower than the left, and left kidney being located slightly more medial than the right.3, 4. The left kidney is approximately at the vertebral level T12 to L3. And the right slightly lower. The right kidney sits just below the diaphragm and posterior to the liver, the left below the diaphragm and posterior to the spleen. Resting on top of each kidney is an adrenal gland. The upper (cranial) parts of the kidneys are partially protected by the eleventh and twelfth ribs, and each whole kidney and adrenal gland are surrounded by two layers of fat (the perirenal and pararenal fat) and the renal fascia. Each adult kidney weighs between 125 and 170 grams in males and between 115 and 155 grams in females. The left kidney is typically slightly larger than the right kidney 5.

The amount of urea nitrogen released and the amount reincorporated into albumin has been measured in healthy and uremic individuals on both normal and low-protein diets. The albumin synthesis rate was measured simultaneously. Gut urea breakdown was only 50% higher in renal failure than in healthy, but the efficiency of utilization of the nitrogen thus released was increased more than 6-fold in renal failure and was higher on a low protein than on a normal protein diet. The lower albumin synthetic rate, the greater was the efficiency of incorporation of urea nitrogen into albumin. The rate of urea nitrogen incorporation into albumin increased on average 14-fold in chronic renal failure. The absolute rate of utilization (84 mumole/hr) was, however, small and comprised on average only 2.4% of the nitrogen used in albumin synthesis. These findings suggest that although some urea derived nitrogen is incorporated into albumin, the amount is not nutritionally significant even under conditions of protein deprivation and high urea availability 6. The importance of iron in renal injury is derived from the ease with which iron is reversibly oxidized or reduced, enabling it to participate in the production of free radicals. Experimental evidence for the role of oxidants and iron in progressive renal disease falls into two broad categories 7.

Anemia is a universal problem among children with chronic kidney disease (CKD). Lower levels of glomerular filtration rate (GFR) are associated with lower levels of hemoglobin, and in adults the latter is most pronounced when the GFR falls below 60 mL/min per 1.73 m^2 .10 In children, the relationship between GFR and anemia is less clear. However, treatment of anemia in both adults and children has improved dramatically with the advent of regular erythropoietin (EPO) and iron therapy, and it has become possible to avoid routine transfusions to maintain a patient's hemoglobin. As well, the many studies performed in adults and relatively fewer studies carried out in children have demonstrated that improved hemoglobin levels are associated with benefits in quality of life, cognitive function, exercise capacity and cardiovascular function 8,9,10. Management guidelines for anemia in pediatric CKD patients have been developed from reported studies in both adults and children, from clinical experience and from expert opinion. The revised National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) clinical practice guidelines for the management of anemia specifically for children have been recently published 11.

Patients with chronic kidney disease (CKD) need regular monitoring, usually by blood urea and creatinine measurements, needing venepuncture, frequent attendances and a healthcare professional, with significant inconvenience. Noninvasive monitoring will potentially simplify and improve monitoring. We tested the potential of transdermal reverse iontophoresis of urea in patients with CKD and healthy controls. Reverse iontophoresis is safe, can potentially discriminate patients with CKD and healthy subjects and is able to track blood urea changes on dialysis. Further development of the technology for routine use can lead to an exciting opportunity for its use in diagnostics and monitoring 12.

Anemia resulting from iron and erythropoietin deficiencies is a common complication of advanced chronic kidney disease (CKD). This article covers major advances in our understanding of anemia in patients with CKD, including newly discovered regulatory molecules, such as hepcidin, to innovative intravenous iron therapies. The use of erythropoiesis-stimulating agents (ESA) in the treatment of anemia has undergone seismic shift in the past 3 years as a result of adverse outcomes associated with targeting higher hemoglobin levels with these agents. Potential mechanisms for adverse outcomes, such as higher mortality, are discussed. Despite the disappointing experience with erythropoiesis-stimulating agents, there is a tremendous interest in other novel agents to treat anemia in CKD 13,14,15. Lastly, while awaiting updated guidelines, the authors outline their recommendations on how to best manage patients who are anemic and have CKD.16 Anemia remains an early

and common complication of chronic kidney disease that causes troubling symptoms and reduced quality of life. Recent literature has raised concern about the safety of erythropoiesis-stimulating agent treatment to higher hemoglobin targets, making this an ideal time to review this subject. In addition, new drugs are being developed in both the ESA and intravenous (i.v.) iron classes. A more cautious era of anemia therapy in chronic kidney disease has emerged. As the mechanisms of safety problems are being worked out, new ESA and iron drugs continue to be developed in an attempt to improve treatment options 16, 17.

Various biochemical parameters that are presently determine in serum /plasma. Total iron and urea for the screening and diagnosis of liver disease as well as to alter mine the change that occurs in the metabolic process associated with the renal disease complication. The purpose of this research is to establish biochemical parameters for the screening and diagnosis of renal diseases and its complication with risk factors of iron and urea parameter and o determine the interrelationship of iron and other diagnosed parameter through them.

Material and Methods

Place of study

Present work was completed in Dept of Biochemistry A.P.S. University Rewa in collaboration with Dept of Biochemistry S.S. Medical College Rewa, (M.P.).

Source of data

The study group compared subjects from 0-90 years of age in screening programming of renal diseases conducted by Dept. of Biochemistry S.S. Medical College Rewa (M.P.). Subjects were screened in C.P.L. of Biochemistry section of S.G.M.H. & S.S. Medical college Rewa (M.P.) in the year 2012 (Month-May, June and July 2012).

Collection of samples

Venous blood was collected from all subjects after 12 hours over night fasting. Fasting venous blood were drawn from all 3 ml of venous blood was collected and stored in a sterile vial . The blood was allowed to clot of room temperature .The clot was rimmed, centrifugation serum was separated by low speed centrifugation and the serum was stored in a sterile vial, urea and iron were estimated hemolyzed and lipemic samples were rejected.

Biochemical analysis

Serum Urea and Serum iron were estimated by colorimetric method . And all the laboratory investigation were performed in subjects. All the results were expressed as mean + / - sd student test was used to assess statistical significance of the results within age and sex.

Estimation of urea (DAM Method)

Urea reacts with DAM in an acetic medium to produce a colored complex. The color is intensified by using thiosemicorbazide and a cadmium salt. The absorbance of the colored complex is proportional to the urea concentration.

Reagents:

1. Diacetyl monoxime; Dissolve 1.56 gm diacetyl monoxime in 250ml water.
2. Ferric chloride dissolves 324mg of ferric chloride in 10ml of 56 percent orthophosphoric acid.store in a brown bottle.
3. Thiosemicarbazide: Dissolve 41 mg of thiosemicarbazide in 50ml of water.
4. Sulfuric acid 20%: Add 200 ml of concentrated sulphuric acid to 800ml of water in a beaker slowly with stirring and cooling.
5. Acid reagent: Mix 1 litre of 20% sulphuric acid (Reagent4) with 1 ml of ferric chloride reagent (Reagent2).
6. Trichloroacetic acid, 10% dissolve 10gm of TCA in water and make up to 100ml.
7. Preservative diluents for standard: boil 250 ml water and add 40 mg of phenyl mercuric acetate, mix to dissolve .transfer to 1 litre graduated cylinder. Add 0.3 ml concentrated sulphuric acid and make up the volume to 1 litre and mix. The use of preservative diluents is optional.
8. Stock standard urea: 0.5mg per ml.
   Dissolve 50 mg urea (GR Grade) in 100 ml of preservative diluents (Reagent 7) or can be dissolved in deionized water it is stable for a week if it is refrigerated.
9. Standard urea for use: 0.01 mg per ml
   Dilute 1ml of stock standard (Reagent 8) solution to 50 ml with deionized water.

Procedure

Pipette into three clean dry tubes labeled blank (B) standard (S) and test (T) as shown below**:**

Reagents	**B**	**S**	**T**
Water	-	-	3.4
Serum	-	-	0.1
TCA, 10%	-	-	1.5
Mix wait for 10minutes, centrifuge			
Supernatant	-	-	1
Water (ml)	1	-	-
Standard urea for use	-	1	1
Diacetyl monoxime	1	1	1
Thiosemicarbazide	1	1	1
Acid reagent	3	3	3
All in ml, Place in a boiling water bath exactly for 15 minutes and cool and Read the absorbance at 540 nm or blue filter			

Estimation of serum iron

Nearly two third of the body's iron (4 to 5 gram) is present in hemoglobin (338mg iron per 100 gram) of which about 90 present in red cells hemoglobin the rest muscles hemoglobin .The greater part of the remaining iron is stored apparently combined with protein as ferritin. The liver being the tissue richest in iron. This iron readily available when required in addition iron is present in most tissue in protein such as cytochrome and catalase which also contain heme . Iron is thus concerned with the transport of oxygen by the blood and in cellular activation. The blood iron all most entirely present in the red cell hemoglobin.

Dipyridyl method (Ramsay, 1954 and 1958)

Ferrous iron gives a pink color with 2-2,dipyridyl .A solution of Dipyridyl in acetic acid is add to serum followed by reducing agent, protein is removed by heating in boiling water and then centrifuging or filtering . Mix equal volume of serum 0.1ml sodium sulphate and Dipyridyl reagent in a glass stopper tube which can be centrifuged heat in boiling water for five minute cool add 1ml of chloroform stopper and shake vigorously for thirty second remove the stopper and centrifuge for five minute at 300 r.p.m. if the supernatant fluid is not completely clear repeat the shaking and centrifuging read at 520 millimicrons or using a green filter .As blank use water instead of serum for the standard put through the working standard in the same way clean the tubes used by placing them in boiling 5N hydrochloric acid, then wash with glass distilled water and keep for this determination only.

Interpretation

The normal range for serum has been variously given. There is general agreement that it is a little higher in men then in women . The following ranges for normal have been obtained made , 80 – 175 mg per 100 ml female , 60 – 160 per 100 ml, and although both higher and lower limits have been suggested most workers have give ranges hear to those above .Ramsay et al (1958) can conclude that the over all range extends from 60 -200 mg per 100 ml and that the average for adult males is about 130 , Dahl. et al showed that the serum iron is a little lower during menstruation then at other time . The serum iron at birth in most infant is in the range 150 to 220 mg per 100 ml and so is a good deal higher then that of the mother . However it falls quickly in a few hours to below 100 and does not region adult value for three to seven years . There is a diurnal variation in the serum iron which is highest on rising and fall during the day by an average of 20% .In addition, appreciable random, fluctuation appears to occur in normal person so that result covering most of the normal range can be obtained in a single individual at different times. It is best to take specimen for determination serum iron at the same time of day . Preferably between 9-10 am.

Results and Discussion

The present study was done with an aim to screen the subjects 0-90 years of age in Rewa city for renal disease. The serum iron level obtained was then correlated with other parameter with determined.

Table 1: The level of Serum urea and iron in the age group of 0-30 years

Variables	Male (n=80)	Female (n=29)
Serum urea	23·20±10·44	26·97±10·08
Serum iron	74·30±60·46	70·44±53·33

Table 2: The level of Serum urea and iron in the age group of 31-60 yrs

Variables	Male (n=42)	Female (n=60)
Serum urea	54·92±28·51	48·25±18·24
Serum iron	70·28±27·11	62·60±41·21

Table 3: The level of Serum urea and iron in the age group of 61-90 yrs

Variables	Male (n=38)	Female (n=34)
Serum urea	70·99±14·62	65·88±20·12
Serum iron	65·57±32·13	56·18±17·48

Correlation coefficient and significance in the study group

Parameter	Correlation coefficient	P value
Serum urea and Serum iron	-0·65	P < 0·0001

The kidney and nervous system communicate via the renal plexus, whose fibers course along the renal arteries to reach each kidney. Input from the sympathetic nervous system triggers vasoconstriction in the kidney, thereby reducing renal blood flow. The kidney also receives input from the parasympathetic nervous system, by way of the renal branches of the vagus nerve (cranial nerve X); the function of this is yet unclear. Sensory input from the kidney travels to the T10-11 levels of the spinal cord and is sensed in the corresponding dermatome. Thus, pain in the flank region may be referred from corresponding kidney.

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and regulation of blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions; these include renin, angiotensin II, aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others 18, 19. Erythropoietin (Epo) and iron therapy plays a major role in the management of renal anaemia. Iron sucrose (IS) has been used to treat iron deficiency anaemia (IDA) and to maintain adequate iron store in chronic kidney disease (CKD). Iron sucrose was effective in improving IDA in CKD without significant side effects. Iron sucrose may be used to treat IDA with monitoring for iron overload 20.

Iron deficiency is the most common cause of hyporesponsiveness to erythropoiesis-stimulating agents (ESAs) in end-stage renal disease (ESRD) patients. Iron deficiency can easily be corrected by intravenous iron administration, which is more effective than oral iron supplementation, at least in adult patients with chronic kidney disease (CKD). Iron status can be monitored by different parameters such as ferritin, transferrin saturation, percentage of hypochromic red blood cells, and/or the reticulocyte hemoglobin content, but an increased erythropoietic response to iron supplementation is the most widely accepted reference standard of iron-deficient erythropoiesis. Parenteral iron therapy is not without acute and chronic adverse events. While provocative animal and in vitro studies suggest induction of inflammation, oxidative stress, and kidney damage by available parenteral iron preparations, several recent clinical studies showed the opposite effects as long as intravenous iron was adequately dosed. Thus, within the recommended international guidelines, parenteral iron administration is safe. Intravenous iron therapy should be withheld during acute infection but not during inflammation. The integration of ESA and intravenous iron therapy into anemia management allowed attainment of target hemoglobin values in the majority of pediatric and adult CKD and ESRD patients 21, 22.

Conclusion

In the present study in patients at the acute phase of the disease shows that decreased iron may lead to abnormal synthesis of urea and other disease on the other hand prolonged deficiency of iron may lead to abnormal production of urea. Urea and iron is the strong predictor for the occurrence of both for renal disease and its severity and complication. Iron is important predictor for understanding to the severity in renal disease and its complications. A well accepted fact the increasing incidence of disease with advancing age a possible explanation for the association of age and disease is based on the implication of iron deficiency in the pathogenesis of several disorders. Long term follow up in a large number of patients would be necessary to confirm these results.

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