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RESEARCH ARTICLE

NOVEL DOUBLE MARKER APPROACH IN CHRONIC KIDNEY DISEASE AND ASSOCIATION WITH PROGRESSION TO END-STAGE RENAL DISORDER

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Abstract

Introduction:-

"The importance of renal disease has been recognized since Hippocrates made the association between bubbles in the urine and disease of the kidneys in 400 BC"

- Chadwick J, Mann WN[1].

Chronic Kidney Disease (also known as chronic renal failure, chronic renal disease, or chronic kidney failure) is a slow and progressive loss of kidney function over a period of several years and is much more widespread than people realize; it often goes undetected and undiagnosed until the disease is well advanced **[2].** In its early stages, symptoms of chronic kidney disease (CKD) are usually not apparent. Characteristic features of chronic kidney disease (CKD) involve progressive destruction of the renal parenchyma and the loss of functional nephrons.

As kidney failure advances and the organ's function is severely impaired, dangerous levels of waste and fluid can rapidly build up in the body. It is not usually until the disease is progressively advanced and the condition has become severe that its symptoms can be noticed; by which time most of the damage is irreversible that is beyond 50%. Chronic kidney disease (CKD) is a global health burden with a high economic cost to health systems and is an independent risk factor for cardiovascular disease (CVD). CKD affected 753 million people globally in 2016, including 417 million females and 336 million males**[2].**

Causes of chronic kidney disease include diabetes and hypertension -one of the most common risk factors.Age (chronic kidney disease is much more common among people over 60), a family history of kidney disease, atherosclerosis, chronic glomerulonephritis and congenital kidney disease (kidney disease which is present at birth) are linked to a higher risk of developing kidney disease.**[3]**Chronic kidney disease is associated with increased risk of adverse outcomes, including death, cardiovascular events, and the development of end-stage renal disease.**[4]**

Currently, the diagnosis of CKD is made usually on the levels of blood urea and serum creatinine (Sr.Cr).However, Sr.Cr has been shown to be lacking high predictive value. Moreover, Steubl et al. **[5]** suggested that, creatinine concentrations were increased in serum only when approximately 40–50% of renal parenchyma was reversibly or irreversibly damaged. Serum creatinine levels are affected by muscle mass, age, and race. Therefore, current practice and staging systems based primarily on serum creatinine may misclassify individuals while assessing these risks. Despite these advances in kidney disease evaluation, a double-marker approach for the detection and classification of CKD using eGFR creatinine and ACR was not well evaluated. Use of double marker approach can accurately reflect prognosis for CKD complications. We designed this study to evaluate the yield of adding ACR and eGFR to forecast risk compared with a CKD definition using creatinine-based estimates alone. We hypothesized

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that eGFR and ACR would add complementary risk information among persons with and without CKD, as defined by creatinine-based estimated GFR ($eGFR_{creatinine}$).

Glomerular filtration rate (GFR) is a test used to check the functional status of the kidneys. Specifically, it estimates how much blood passes through the glomeruli each minute. All individuals with a glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for 3 months are classified as having chronic kidney disease**[6]**, irrespective of the presence or absence of kidney damage**[7].**Classification of CKD can be done on the basis of GFR into five stages, Stage 1 to Stage 5, where Stage-1 (eGFR>90), Stage-2 (eGFR 60–89), Stage-3 (eGFR 30–59), Stage-4 (eGFR 29–15) and Stage-5 (eGFR<15).**[8]**

It is an established fact that albuminuria is an early marker for renal diseases. It is defined as urinary excretion of protein albumin in range of 30-300 mg%; the increased levels of urinary albumin secretion may represent a renal injury. Renal injury causes disorders of the albumin excretion rate by damaging the podocytes from basal membrane and their loss with urine, therefore the determination of the presence of some structural proteins connected with glomerular barrier may be helpful in the diagnosis of renal disease.

Alternative methods have been suggested to improve detection and classification of CKD. Instead serum creatinine, novel factors such as Urine Albumin Values and **eGFR** (Estimated Glomerular Filtration Rate) may play a far more important role in **ESRD** (End-Stage Renal Disease)**[9].**

At present, certain research reflects the revision of guidelines for evaluation and staging of CKD on the basis of eGFR with the explicit objective of developing staging systems that accurately reflect prognosis for CKD complications.[**10, 17-23**] In India, however, the diagnosis of CKD is done predominantly on the basis of serum creatinine and urea estimation but sCr has been shown to be lacking high predictive value. Hence, there is need to introduce new approach which can diagnose the CKD at early stages (Stage 1 and 2).

In the view of above, the present study was thus conducted to evaluate a simple and clinically applicable tool to detect and risk stratify CKD in practice with available markers. Keeping this in mind, an attempt had been made to do a cross-sectional study and test whether the depth of the double marker approach is effective than just creatinine-based tests alone in early stages of CKD, among the patients attending OPD of Government Medical College and Hospital, Akola.

Review of Literature:-

The initial recognition of kidney disease as independent from other medical conditions is widely attributed to **Richard Bright's(1827)book** "Reports of Medical Cases," which detailed the features and consequences of kidney disease. For the next 100 years or so, the term "Bright's disease" was used to refer to any type of kidney disease. Bright's findings led to the widespread practice of testing urine for protein — one of the first diagnostic tests in medicine**[10]**.The study of kidney disease was furthered by **William Howship Dickinson's** description of acute nephritis in 1875 and **Frederick Akbar Mahomed's** discovery of the link between kidney disease and hypertension in the 1870s**[3]**. In the twentieth century, **Homer Smith's** findings led to important medical therapies for multiple kidney diseases.

Kidney Disease: Improving Global Outcomes (KDIGO 2013) suggested the diagnosis of chronic kidney disease should be revised and rather than relying on serum creatinine diagnosis, results should be confirmed with the use of more advanced and efficient markers having high sensitivity and specificity **[11] .Andrew S. Levey**, on the other hand took a step further and classified the chronic kidney disease patients in different stages which were based on the stratification by GFR. **[8]**

Chronic kidney diseases have become a major public health problem and are leading cause of morbidity and mortality in India and other low- and middle-income countries. The chronic diseases account for 60% of all deaths worldwide. In India, the projected number of deaths due to chronic diseases was around 5.21 million in 2008 and is expected to rise to 7.63 million in 2020 (66.7% of all deaths).**[12]**

Diabetes and hypertension is known to be the established risk factor for CKD. **As per the Diabetes Atlas** in **2006**, the number of patients with DM in India (currently around 40.9 million) is expected to rise to 69.9 million by 2025 unless urgent preventive measures are taken**.[13]** With increasing prevalence of CKD, CKD related excess CVD

(Cardiovascular disease), ESRD and the consequent financial burden of renal replacement therapy (RRT), the importance of CKD and its risk factors has to be realized. The prevalence of ESRD and patients on RRT has increased over last two decades**.[14] .** These individuals who are at increased risk of developing CKD - DM, hypertension, age more than 60 years, CVD, families' history CKD, hyperlipidemia, obesity, metabolic syndrome, smokers and patients treated with potentially nephrotoxic drugs should be screened with tests including a urine test for proteinuria or albuminuria and a blood test for serum creatinine to estimate glomerular filtration rate (GFR). **[15]**

MacIssac et al. in a single-center outpatient population (n = 301) showed influence of the degree of proteinuria on progression, demonstrating a decline in GFR at 2.8 mL/min/year in people with diabetes and microalbuminuria, compared with 3.0 mL/ min/year in those with macroalbuminuria.**[16]**.There has since been broad consensus and supportive observational evidence that the diagnosis and staging of CKD should be based on both an evaluation of kidney function (i.e. estimated GFR) and the presence or absence of kidney damage (persistent albuminuria, persistent proteinuria, persistent hematuria after exclusion of urological causes, or structural abnormalities on kidney imaging tests) **[17-21]**.

Interestingly, it has been suggested by **Nosadini R. et al** in **2003[22]** that hyperglycemia, hypertension and renal injury can cause disorders of the albumin excretion rate by damaging the podocytes from basal membrane and their loss with urine, therefore the determination of the presence of some structural proteins connected with glomerular barrier may be helpful in the diagnosis of renal disease. The preferred test for urinary albumin detection is **UACR** test. This test can be performed on a single sample of spot urine. **[22]**.It has been found that Diabetic nephropathy is the most common cause of ESRD. And the earliest evidence of diabetic nephropathy is microalbuminuria. **[23]**

Dr. Carmen A. Peralta et al. in**2011** April carried out a cohort study includingoverall, 2904 participants (11%) and classified them on the basis estimated eGFR Cystatin C.He found that, Among them, 701 participants (24%) had CKD defined by estimated $GFR_{creatinine}$ alone and 148 participants (5%) had CKD defined by estimated $GFR_{creatinine}$ and ACR**.**Chronic kidney disease (CKD) is much better if defined as a creatinine-based estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m2 or a urine albumin-to-creatinine ratio (**ACR**) of 30 mg/g or higher. Clinical laboratories are routinely reporting estimated GFR, and electronic medical records often alert clinicians to the presence of CKD on this basis alone. But routine assessment of the ACR is only recommended for persons with diabetes, initial CKD detection in routine practice is primarily limited to serum creatinine testing.[**23**]

Care Process Model [CPM] published in **May 2018**, developed by multidisciplinary team of clinical experts from Select Health And Intermountain Health Care using guidelines for international, the National Kidney Foundation, U.S Veterans Administrations / Department Of Defense. This CPM represents screening diagnosis and treatment processes to improve care and outcomes for patients with CKD in primary are along with nephrology consult/ referral criteria to enhance quality outcomes. [https://intermountainhealthcare.org/ext/Dcmnt?ncid=521395847 as of 10/06/2018]

Today, clinical nephrology continues to advance with many forms of renal replacement therapy — both acute and chronic — including hemodialysis, peritoneal dialysis, hemofiltration and hemodiafiltration, the use of erythropoietin for anemia in chronic kidney disease, treatment of renal osteodystrophy, ongoing improvements in immunosuppression for transplantation, and specific treatments for many nephropathies.**Joseph A. Vasseloti et al**. introduced a practical approach to detect and manage CKD for primary care and clinicians. They demonstrated that diabetes and high bp present the dominant factors for CKD. Testing risk stratification and treatment plans differ acc to eGFR and ACR. They suggested that the major approach to CKD therapy should include avoidance of exacerbating drugs, tests and interventions and expected treatment of CKD to slow down the progression and underlying complications including CVD.

Epidemology:

Global prevalence:

According to **Nathan R. Hill** et al. **[8]**, global prevalence of CKD (by stage) is-Stage 1 (3.5%), Stage 2 (3.9%), Stage 3 (7.6%), Stage 4 (0.4%) and Stage 5 (0.1%).CKD has a high global prevalence with a consistent estimated global CKD prevalence of between 11 to 13% with the majority Stage 3**[2]**.

Prevalence in India:
In India, according to **A. K. Singh et al**the prevalence of CKD in India reports of Stage 1 (7%), Stage 2 (4.3%), Stage 3 (4.3%), Stage 4 (0.8%) and Stage 5 (0.8%)**[24]** .

Classification of CKD:
Changes in the GFR gives an idea about what percent the kidney is damaged and can be used to access the Stage of CKD. Classification is done as follows.
Table 4.1:- Staging and Stratification of chronic kidney disease with the help of GFR.**[22].**

CKD Stage	GFR	Kidney damage
G1	>105 ml/min	No damage
G2	60-90 ml/min	Minimal damage
G3a	45-59 ml/min	Mild damage
G3b	35-44 ml/min	Moderate damage
G4	16-34 ml/min	Severe damage
G5	<15 ml/min	Kidney failure

Aim And Objectives:-

Aim:-

To evaluate whether combining **$eGFR_{creatinine}$** (Creatinine based estimated Glomerular Filtration Rate) and **uACR** (urine Albumin-Creatinine Ratio) would improve identification of risks associated with CKD compared with **Creatinine** alone.

Objectives:-

1. To evaluate the use of Serum Creatinine, as a marker for CKD and divide the test subjects on basis of absence [Group 1] or presence [Group 2] of chronic kidney disease.
2. To compare both estimated Glomerular Filtration Rate (eGFR) and Urine Albumin/Creatinine Ratio (ACR) in Group 1 and Group 2.
3. To discern whether this double marker approach for staging the CKD patients is beneficial and reliable than using just Creatinine based results**.**

Matreial and Methods:-

Study Design:
1. Study Type: A hospital based cross-sectional, prospective study.
2. Study Site: In-Patient and Out-Patient Department of Medicine atGovernment Medical College and Hospital Akola
3. Study Duration: 1 Jun. 2018 to 31 Jul. 2018.

Sample Size:
1. The study was commenced after obtaining Institutional Ethical Committee Clearance Certificate N0.: GMCA/EC/186/2018 dated 13/06/2018.
2. A total of 67 subjects of age group >30 years visiting indoor and outdoor patients in Medicine department at Government Medical College and Hospital, Akola were included in this study.

Selection Criteria:
A patient to be selected as a subject must follow a certain kind of selection criteria.

Inclusion Criteria:
Some factors makes the patient susceptible to Chronic Kidney Disease and may complicate to End Stage Renal Problems.
a) Age group: >30 years
b) Diabetic
c) Hypertensive
d) Patients with CVD
e) Family history of Kidney Disease

Exclusion Criteria:
Certain parameters may interpret false results due to non-specificity of the markers of CKD. Furthermore, calculations based on imperfect or missing baseline data may result into wrong findings. These parameters must be excluded.

a) Subjects with age <30 years.
b) Subjects who were missing baseline data for serum creatinine.
c) Subjects who had received a renal transplant in their medical history.

Grouping:
In the first step, we screened the total subjects (n=67) for CKD on the basis of serum creatinine only and divided them into 2 groups – **Group 1** and **Group 2.**
Group 1: (n=27) Patients who had normal levels of **serum creatinine (0.2-1.2 mg/dl**). These subjects had negative diagnosis of CKD and labeled as Non -CKD).
Group 2: (n=40) Patients who had elevated levels of **serum creatinine (>1.2 mg/dl)** and These subjects had positive diagnosis of CKD and labeled as subjects with CKD. **(n = no. of subjects)**

Sub-Grouping:
To confirm the diagnosis, both group were screened for eGFR and ACR on the basis of the data collected earlier.

In a second step, we calculated the frequency of CKD, defined by the use of eGFR and ACR and divided the subjects into different stages of CKD. Both the groups were assessed for **$eGFR_{creatinine}$** and **ACR** (Albumin-Creatinine Ratio) and were divided into subgroups on the basis of **eGFR** in reference to the staging of CKD. (Please refere **Table 4.2** in **Annexure 1**.)

Sample Collection And Processing:
Method Of Collection, Processing And Storage:
After obtaining written/informed consent from subject or subject's legal representative, attending the IPD/OPD at Government Medical College and Hospital Akola, following samples were collected.

1. **Blood sample:**
   Approximately 5 ml of blood sample was collected from antecubital vein and stored in plain bulb. The samples were allowed to clot and then centrifuged at 3000 rpm for at least 10 minutes so as to separate serum and plasma. The serum was then stored at 2-8° C until use after addition of 5% sodium azide and analyzed for estimation of serum creatinine.
2. **Urine sample:**
   Spot urine was collected and immediately analyzed for urine albumin and urine creatinine and ACR was calculated.

Estimation of Biochemical Parameters:
All biochemical parameters were assessed at Clinical Biochemistry Laboratory, Department of Biochemistry, Govt.Medical College Akola.

Estimation Of Serum Creatinineby Jaffe's Method [28]:
Creatinine is a waste product that comes from the normal wear and tear on muscles of the body. Serum creatinine values are not the best way to determine kidney health. The kidneys maintain the blood creatinine in a normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine level signifies impaired kidney function or kidney disease. Serum creatinine is part of a routine lab report. Creatinine was estimated by Jaffe's Method using ERBA XL-640 auto analyzing as per the SOP (standard operating procedure).

Principle:
Creatinine reacts with alkaline picrate to produce a orange-red colored complexintensity of which is directly proportional to the amount of Creatinine present in the sample (Jaffe's reaction). This is given by many other substances. Specificity of the assay has been improved by the introduction of a kinetic method.

Reagent Composition:

Active Ingredients	Concentration
R1 Sodium hydroxide	240 mmol/L

R2 Picric acid	26 mmol/L

Calculations:
Reagents were pipetted into 2ml glass vials labelled as 'Blank', 'Standard' and 'Test'. The contents were mixed well and incubated for 10 minutes at 37 degree Celsius and studied at the absorbance at 505 nm and the levels of creatinine in the blood (serum creatinine) were estimated and recorded.

Estimation of eGFR [29] :
We measured the serum creatinine levels and used the result in a formula to calculate a number that reflects the overall kidney health, called the estimated GFR or eGFR. Measuring GFR directly is still considered the most accurate way to detect changes in kidney status, but measuring the GFR directly was complicated, and required experienced personnel, and can be performed only in research settings and transplant centers. Because of this, the estimated GFR was preferred.

The following two are most common and require a person's blood creatinine result, age, and assigned values based upon sex and race**[30] .**

- Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2009) recommended eGFR in adults.
- Modification of Diet in Renal Disease Study (MDRD) equation:

$$\mathbf{eGFR = 175 \times (S_{Cr})^{-1.154} \times (age)^{-0.203} \times 0.742 \text{ [if female]} \times 1.212 \text{ [if Black]}}$$

To make the tedious process easy, the $eGFR_{creatinine}$ was calculated with the help of online eGFR calculator. **[30][**http://www.calculator.net/gfr-calculator.html**]**

Estimation Of ACR[27]
Albuminuria is increased excretion of urinary albumin and a marker of kidney damage. Normal individuals excrete very negligible amounts of protein in the urine. Albumin is the most common type of protein excreted in the urine. All patients with CKD were be screened for albuminuria. Persistent increased protein in the urine represents kidney damage, acting as an early and sensitive marker in many types of kidney disease**.**

Urine microalbumin:[**31-34**]
Urinary albumin concentration (UAC): It is concentration of albumin present in 1 L of urine or albumin excreted per L of urine. It is expressed as (mg/L).
UAC (mg/L) = Albumin (mg/dl) x 10.

Reagents:
Final concentration of reactive ingredients:
1. Tris buffer 95 mmol/L
2. Goat anti-human Albumin antiserum
3. Also contains preservatives.
4. Microalbumin Calibrator (ODR3024)
5. 0.9% Saline

Principle:
Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Turbidimeters measure the reduction of incident light due to reflection, absorption, or scatter. In the procedure, the measurement of the decrease in light transmitted (increase in absorbance) through particles suspended in solution as a result of complexes formed during the antigen-antibody reaction, is the basis of this assay.

Procedure:-
The urine albumin assay is an automated immunoturbidimetric assay. Microalbumin is reported as a normalized ratio to urinary creatinine in order to account for variations in urine flow rate. Therefore microalbumin and urine creatinine are preferably tested from the same aliquot.[**35**]

Albumin is stable in untreated urine for at least one week when stored at either 4 or 20 °C.**[36].**Samples should ideally be analyzed fresh. If prolonged storage is required, freezing at −20 °C appears to preserve albumin integrity when measured by immunoassay**[37].**

Urine creatinineby Jaffe's Method[**28**] :
Creatinine is also stable in urine for at least one week at either 4 or 20 °C and is unaffected by freezing at either −20 °C. Prior to analysis, urine should be inspected for clarity and centrifuged if visibly cloudy.

1. Urine albumin and urine creatinine were converted so that both values are expressed as mg/L or mg/ml to convert them into useful ratio.
2. Urine albumin concentration was divided by the creatinine concentration.
3. The resulting ratio was multiplied by 1,000 to get mg albumin over grams creatinine.

$$\text{ACR (mg/g)} = \frac{\text{Albumin (mg/dl)}}{\text{Creatinine (mg/dl)}} \times 1000. \text{ [31]}$$

The online calculator was used to speed up the process [**38**].

Statistical Analysis:
Qualitative analysis was done using the **Fisher Exact Probability Test** to discern the effectiveness of GFR and ACR over sr. creatinine. To calculate the average of different parameters, mean and Standard deviation was measured and expressed in percentage.

Observation and Results:-

A hospital based cross sectional study was carried out at Government Medical College and Hospital Akola, from 1 Jun. 2018 to 31. Jul. 2018.

Present study includes total 67 subjects and serum creatinine was estimated. Based on the results obtained subjects were grouped as follows:
Group 1: (n =27) labelled as non-CKD. (i.e.: sr. creatinine was within range 0.2-1.2 mg/dl).
Group 2: (n =40) labelled as CKD (i.e.: sr. creatinine levels>1.2 mg/dL)
Further, both the groups(i.e.: Group 1 and 2) were screened for eGFR and ACR.

Gender And Agewise Distribution:
Table 7.1a:- Gender Distribution In Both The Groups.

Gender	Group 1 (n=27)	Group 2 (n=40)
Male	16	25
Female	11	15

Table 7.1b:- Mean Age Wise Distribution.

	Group 1 (n=27)	Group 2 (n=40)
Mean Age (years)	39.72 ± 0.20	44.85 ± 0.25

Table 7.1a and 7.1b shows gender and age wise distribution among Group 1 and group 2 subjects. Group 1 out of 27 subjects, 16 were male and 11 were Female, whereasmean age for the same group was 39.72 ± 0.20 years. Similarly, in Group 2, out of 40, 25 were male and 15 were female with mean age in years was found to be 44.85 ± 0.25. This shows inclination of the test subjects towards males more than females. Group 1 subjects had a lower mean age than Group 2 reflecting the fact that the burden of CKD is over the elderly.

Biochemical Parameters Obtained In Group 1 And Group 2:
Table 7.2a:- Distribution and Stagingof group 1 (n=27) (on the basis of eGFR):

Group 1 subjects (non-CKD) (n=27)				
Staging (eGFR)	**No. of subjects**	**Sr. Creatinine**	**eGFR**	**ACR**
		Mean ± SD	**Mean ± SD**	**Mean ± SD**
Normal Subjects	n=10	0.42 ± 0.01	114.62 ± 0.29	2.56 ± 0.12

Stage 1	n=10	0.74 ± 0.02	91.33 ± 0.43	**5.33 ± 0.34**
Stage 2	n=7	0.73 ± 0.06	**84.20 ± 0.43**	**16.80 ± 0.42**
Stage 3	n=0	-	-	-
Stage 4	n=0	-	-	-
Stage 5	n=0	-	-	-

Sr. Cr = Serum creatinine (unit = mg/dl), **eGFR** = Estimated glomerular filtration rate (unit = ml/min/1.73m^2) **, ACR** = albumin creatinine ratio (unit = mg/g), Stage 1 – 5 (as per table 4.1)

Table 7.2a depicts –different parameters -**Sr. creatinine**, **eGFR** (estimated Glomerular Filtration Rate) and **ACR** (Albumin-Creatinine ratio) studied in both Group 1 and Group 2**.** Group 1 subjects (n=27) distributed in Stage 1 to 5. Out of 27, 10 subjects had a completely normal levels of sr. creatinine, eGFR and ACR with a mean value of 0.42 ± 0.01, 114.62 ± 0.29 and 2.56 ± 0.12respectively. Out of remaining 17 subjects, 10 had a normal sr. creatinine and eGFR with mean of 0.74 ± 0.02 and 91.33 ± 0.43, but had slightly elevated ACR values (**5.33 ± 0.34**). In last 7 subjects, even though the sr. creatinine level were normal (0.73 ± 0.06), they had a decreased eGFR with mean of **84.20 ± 0.43** and elevated ACR with mean of **16.80 ± 0.42.** No subject was found in Stage 3, 4 and 5.

Table 7.2b:- Distribution and Staging of group 2 (on the basis of eGFR):

Group 2 subjects (CKD) (n=40)				
Staging (Using eGFR)	**No. of subjects**	**Sr. Cr (mg/dl)**	**eGFR (ml/min)**	**ACR (mg/g)**
		Mean ± SD	**Mean ± SD**	**Mean ± SD**
Normal Subjects	n=0	-	-	-
Stage 1	n=0	-	-	-
Stage 2	n=0	-	-	-
Stage 3	n=27	**1.58 ± 0.10**	**53.71 ± 0.54**	**52.23 ± 0.59**
Stage 4	n=12	**1.90 ± 0.12**	**22.12 ± 0.19**	**103.75 ± 0.9**
Stage 5	n=1	**2.50**	**14**	**210**

Sr. Cr = serum creatinine (unit = mg/dl), **eGFR** = Estimated glomerular filtration rate (unit = ml/min/1.73m^2) , **ACR** = albumin creatinine ratio (unit = mg/g), Stage 1 – 5 (as per table 4.1)

Table 7.2b depicts group 2 subjects (n=40) distributed in Stage 1 to 5. No subject was found out to be of Stage 1 and 2. Out of 40 subjects, 27 subjects showed raised levels of sr. creatinine and ACR with mean values of **1.58 ± 0.10**and**52.23 ± 0.59**respectively. However, eGFR was found to be low with mean of **53.71 ± 0.54** in Stage 3. Similar results were reflected in 12 subjects with elevated sr. creatinine and ACR levels with mean **1.90 ± 0.12** of **103.75 ± 0.9** and respectively, but decreased eGFR with mean of **22.12 ± 0.19**of Stage 4 subjects. However, in Stage 5 (n=1) the subject had a sr. creatinine level of 2.5, eGFR less than 15 and ACR of 210.

Table 7.3:- Fisher Exact Probability Test in all the test subjects:

Serum Creatinine			
	Subjects with Normal Values (in %)	**Subjects with Abnormal Values (in %)**	**Total subjects (in %)**
CKD absent	10(14.92%)	0(0%)	10 (14.92%)
CKD present	18(26.86%)	39(58.2%)	57 (85.07%)
Total %	**28 (41.79%)**	**39 (58.2%)**	**67 (100%%)**
Estimated GFR (eGFR)			
	Subjects with Normal Values (in %)	**Subjects with Abnormal Values (in %)**	**Total subjects (in %)**
CKD absent	10(14.92%)	0(0%)	10 (14.92%)
CKD present	10(14.92%)	47(70.14%)	57 (85.07%)
Total %	**20 (29.85%)**	**47 (70.14%)**	**67 (100%)**
Urinary Albumin Creatinine Ratio (uACR)			
	Subjects with Normal Values (in %)	**Subjects with Abnormal Values (in %)**	**Total subjects (In%)**
CKD absent	10(14.92%)	0(0%)	10 (14.92%)

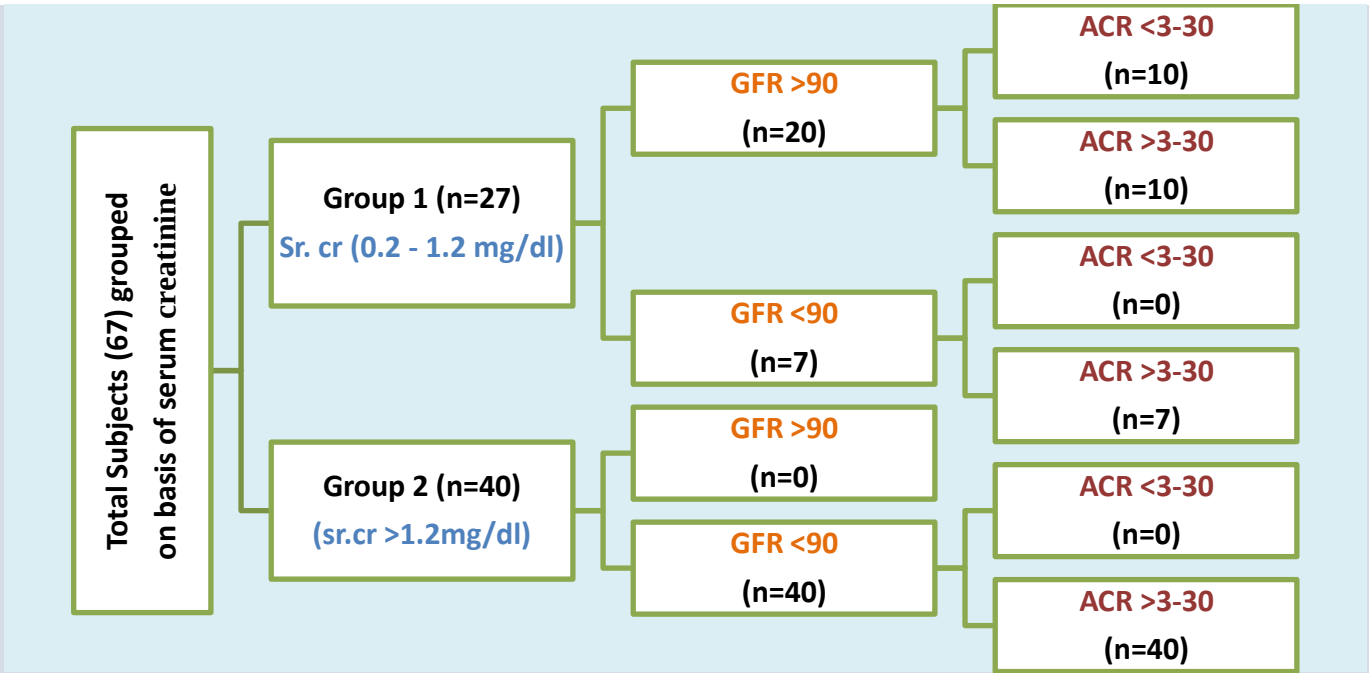
CKD present	0(0%)	57(85.07%)	57 (85.07%)
Total %	**10 (14.92%)**	**57 (85.07%)**	**67 (100%)**

Table 7.3 Fisher Exact Probability Test showing the percentage (%) of each marker and its association with presence or absence of chronic kidney disease.

Above table shows that : out of total 67 subjects, 28 (41.79%)subjects had normal whereas, 39 subjects (52%) had abnormal I,e raised level of serum creatinine .When studied estimated GFR in , 20 subjects (29.85%) had shown normal and 47 subjects (70.14%) on the other contrary shoed lowered eGFR level.

When compared ACR levels in total 67 subjects . It was observed that 10 subjects (14.9%) were normal ACR wheras about 57 (85.1%) found to be abnormally high albumin creatinine ratio.

Observations At Glance:



The above tree chart shows the overall distribution of test subjects into groups and subgroups. Grouping was done on the basis of serum creatinine values as mentioned in table 5.3. Similarly staging was done on the basis of eGFR and ACR values obtained in both the groups.

N.B.: Units for sr. creatinine is mg/dl, for eGFR is ml/min/1.73m^{2}and ACR is mg/g.

Discussion:-

Chronic kidney disease (CKD) is a very common clinical problem in elderly patients and is associated with increased morbidity and mortality. As life expectancy continues to improve worldwide, there is a rising prevalence of co-morbidities and risk factors such as hypertension and diabetes predisposing to a high burden of CKD in this population. The prevalence of CKD rises dramatically with age. **(39 a)**

(Refrence :39 a Suma Prakash[1] and Ann M. O'Hare[2] Interaction of Aging and CKD, Semin Nephrol. 2009 Sep; 29(5): 497–503.)

In its early stages, symptoms of chronic kidney disease (CKD) are usually not apparent. Characteristic features of chronic kidney disease (CKD) involve progressive destruction of the renal parenchyma and the loss of functional nephrons.

In comparison, the epidemiological study of early kidney disease is a recent area of interest. The prevalence of Chronic Kidney Disease (CKD) has reached epidemic proportions. CKD contributes to around a tenth of all deaths,

which is a similar proportion to that from smoking or obesity. On average, CKD was the underlying and/or associated cause of death in around 12,900 deaths per year between 2007 and 2017 [**39]** Risk factors and therapeutic targets in the general population do not seem to explain the CKD patient's prognosis **[39]**.

Present Cross-Sectional study was conducted on 67 subjects visiting Medicine OPD/IPD at GMC, Akola and was categorized into two groups on the basis of serum creatinine values:
Group 1: Included **27** subjects with serum creatinine within normal range (0.2-1.2 mg/dl)
Group 2: Included **40** subjects with elevated serum creatinine (>1.2 mg/dl)

The samples were analyzed for estimated GFR from serum creatinine values and assessed the ACR from the data we extracted from urine sample. On the basis of GFR staging, both the groups were divided into Stages 1 to 5.

Present study showed the Normal distribution and mean age of the total subjects (**Table 7.1a**, **and Table 7.1 b)** . It has been indeed clear that Group 1 had 16 males and 11 females, whereas group 2 had 25 males and 15 females. And the mean age of Group 1 subjects was 39.72 and that of Group 2 had a mean age of 44.85 which showed that elder population are predominately affected with renal dysfunction with preponderance towards male population. Similar results were observed by thePo-Ya Chang, in 2016 where he found that male patients show a substantially higher prevalence of CKD and incidence rate of ESRD than those observed in female patients which was consistent with our findings. [**51**]. The high prevalence of CKD in the elderly no doubt reflects the presence of a variety of different risk factors for CKD such as diabetes and hypertension in older individuals. However, high rates of CKD in the elderly may also occur because of an age-associated decline in kidney function that is not explained by other known risk factors. **[39]**

In present study -Table 7.2a, shows the distribution of subjects according to the staging system classified on the basis of eGFR. The group 1 subjects which were labeled and diagnosed as non-CKD on the basis of normal sr .creatinine (n=27), We noticed that ,out of these 27 subjects; 10 subjects were completely normal (no CKD diagnosed by any of the biomarker Sr Cr, eGFR & ACR).However, 10 subjects experienced normal creatinine and eGFR values but elevated ACR and hence were stratified into Stage 1 of CKD . Remaining 7 subjects had decreased eGFR and increased ACR and were stratified as Stage 2 of CKD as per the guidelines suggested (Please refer **Annexure 1, Table 1)**. No subject observed to be of Stage 3, 4 or 5.

The present finding **(Table 7.2 a)** indeed confirms that creatinine alone is not a predictive markers to diagnose the early cases of CKD. Accumulating evidence has demonstrated that these biomarkers like creatinine and urea estimation are not optimal to detect kidney disease in early stages, which are in line with our results observed in present study **[52**]. On the contrary, in (**Table 7.2b**) we found increased level of serum creatinine in all group 2 subjects (n=40). And when staging of CKD was on the basis of GFR was done of these patients .It was observed that all these patients were land up into stage 3 (n=27), stage 4 (n=12)and stage 5 (n=01) since no subject had a eGFR values of Stage 1 and 2 were observed.

In present study **(Table 7.3)** we measured biochemical parameters (Sr Cr. eGFR and ACR) in all test subjects and probability was calculated to identify the predictive value of each variables.

With a long latent period of CKD when the disease is clinically silent and therefore diagnosis, evaluation and treatment is based mainly on biomarkers that assess kidney function. The present study showed an inverse relationship between eGFR and ACR which is well in accordance with the earlier studies (29, 35 ,39,42 ,51). Present finding showed that ACR was stand out to be even more valuable tool to assess and predict the early progression of CKD along with eGFR . Ample of evidences [40,41,51,52]available suggesting the role of GFR and ACR in staging ,sreening and predicting the progression CKD to ESRD. Present findings i.e.: eGFR and ACR being better marker in CKD is consistent with several previous works done till date **[40,41,42]**.National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002 also suggested the use of eGFR and ACR in diagnosis of CKD over serum creatinine **[40]**. Our findings were similar to the study done by Matsushita K and Astor BC, et al. on Chronic Kidney Disease Prognosis Consortium. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. **[41]**. Dr. Carmen A. Peralta, in his research papers published showed that in overall, 2904 participants studied (11%),he observed and classified them as having CKD based on estimated $GFRc_{reatinine}$. Among them, 701 participants (24%) had CKD

defined by estimated $GFR_{creatinine}$ alone and 148 participants (5%) had CKD defined by estimated $GFR_{creatinine}$ and ACR. Thus, Glomerular filtration rate (GFR) remains the ideal marker of kidney function. In addition albuminuria may precede kidney function decline and have demonstrated to have strong associations with disease progression and outcomes. [39, 51].

Results discussed above suggested that **double marker approach** can more accurately stratify chronic kidney disease patients into proper respective stages from 1 to 5 rather than relying on diagnosis by serum creatinine and falsely interpreting them as a non-CKD. The current results extend prior findings to highlight that measurements of both eGFR and albuminuria are complementary to identify individuals with CKD who have an increased risk of end-stage renal disease. . Several groups are currently advocating new international guidelines that more accurately reflect prognosis of CKD **[45-47]** and have proposed adding ACR to staging of CKD.**[42]**

The statistics show that since ACR was found to be elevated since Stage 1 itself, it stood out to be the sensitive marker for early stages of chronic kidney disease. While eGFR was used to stratify the subjects accordingly, it was useful marker as well. Creatinine had a 95% accuracy after Stage 3 but failed before Stage 3 at accuracy of 41.79% only.

Conclusion:-

Nowadays it is observed that the chronic kidney disease (CKD) is a growing health problem worldwide and the most com-mon illness associated with this is hypertension (HTN) and diabetes mellitus (DM).Most kidney diseases do not have symptoms or findings until later in their course and are detected only when they are chronic. Most of the causes of CKD are irreversible, and treatment aimed at slowing progression to kidney failure

The combination of $eGFR_{creatinine}$ and ACR measures improved the diagnosis of chronic kidney disease and end-stage renal disease. Early diagnosis of CKD and identification of those likely to progress to end-stage renal disease (ESRD) has become highly important. Existing measures including creatinine level seem to be insufficient. Therefore, new validated biomarkers are required for CKD progression and cardiovascular disease (CVD) risk. Complications of CKD development and progression cannot be assessed only on the basis of a single marker but their combination in order to mirror all types of alterations occurring in the course of this disease.

On the basis of aforementioned studies, it can be concluded that a panel of biomarkers rather a single marker is required to diagnose CKD with high sensitivity and specificity and to identify persons at high risk of progression. Moreover, it seems that in not so distant future, conventional markers may be exchanged for new ones, however, the confirmation of their efficacy, sensitivity and specificity as well as the reduction in analysis costs is required. The increasing number of studies concerning the search for new, sensitive and selective biomarkers useful for the diagnosis and quantitative assessment of mechanisms occurring in diseased kidneys confirms the importance of this issue **[28,43,44,48]. Cut**

Summary:

We evaluated a double-marker approach for the assessment of kidney disease in a small cohort of Akola District. Using $eGFR_{creatinine}$ and uACR resulted in an increased ability to discriminate risk of death and end-stage renal disease. eGFR and ACR were both strongly and independently associated among persons with or without CKD defined by creatinine-based estimated GFR. The risk of future end-stage renal disease was concentrated within the subset of participants who had CKD defined by both the markers.

Early detection of CKD by screening for kidney disease in high-risk patients (hypertension,family history of CKD and DM), Early referral to nephrologist, appropriate treatment of hypertension, DM and other risk factors, lifestyle modification with specific emphasis on reduction in salt intake, physical exercise, abstinence from smoking, will retard progression of kidney disease to an advanced stage. There is an urgent need for a national program to control the epidemic of NCDs like hypertension, DM, chronic respiratory and CKD in India. The current dependence on the private sector for treatment of kidney patients with severe renal disease needs to be reduced with infrastructure upgradation in government run hospitals to facilitate accessibility of treatment for the majority of our population who cannot afford treatment in private hospitals.

In present we observed:

1. Total 67 subjects when investigated for serum creatinine ,we found raised level >1.3 mg/dl in 40 patients and labeled as CKD (group 2) and 27 found normal serum creatinine level marked as Non-CKD.(group 1).
2. When screened both groups for estimated GFR and Urine Albumin creatinine ratio:-

- In group 1: Out of 27, 17showed abnormal results and staged as 1 (n=10) and stage 2 (07) based on the classification proposed by (KDIGO) in 2013 (Annexure- 1).
- In group 2: Out of 40 subjects, all the subjects were found n=27were found in stage 3, n= 12 were in stage 4, and only o1 subjects was found in stage 5 with decreased GFR and raised uACR .(Annexure -1)

3. Qualitative analysis was done by applying Fisher's probability test :

- We observed that uACR stood out to be the earliest marker above GFR to predict the risk of CKD.
- Because in **Group 1** (Non CKD subjects - diagnosed by normal serum Creatinine) showed altered (raised) uACR (n=17) however, same patient when screened for eGFR , GFR was found to be normal. This indicates utility of uACR in early diagnosis and staging of CKD even before GFR and serum creatine shows any alteration.
- Thus Combining all these parameters will definitely improve the diagnosis and helps staging and eventually modifying the management modalities in subjects with CKD.

Limitations:

Future studies are needed using the double-marker approach to evaluate clinicals strategies that may reduce these risks.

- ✓ It remains unclear whether early detection of CKD by this approach would be cost effective [**49**].
- ✓ In addition, we relied on a one-time measure of biomarkers including albuminuria, which is known to be variable, particularly at lower ranges
- ✓ The period of time for research limited the follow-up studies
- ✓ The sample size we used for research was small and the results may differ in large cohorts.
- ✓ Our study is limited by the lack of direct GFR measurements. Unfortunately measuring GFR is time consuming and therefore GFR is usually estimated from equations that take into account endogenous filtration markers like serum creatinine (SCr). Added

Suggestions:-

The global epidemic of chronic kidney disease (CKD) is a significant public health issue affecting up to 10% of the adult population and account for 60% of all deaths worldwide. 80% of chronic disease deaths worldwide occur in low- and middle-income countries. In India, the projected number of deaths due to chronic disease was around 5.21 million in 2008 and is expected to rise to 7.63 million in 2020 (66.7% of all deaths).

The progression of CKD is often slow, and there are few specific symptoms until the disease is very advanced. Changes in albuminuria (ACR) may serve as early indicators of CKD progression and complications beyond eGFR, but the risk implications of these changes are not well documented, with existing studies predominantly limited to subjects with kidney disease.

Many previous current recommendations, which rely only on eGFR changes to define CKD progression, our study clearly showed how a change in ACR adds important prognostic information to initial ACR and eGFR changes. In our study, the prognostic association between ACR changes and eGFR staging was strong and its screening in such patient would definitely add on importance in our real-life health care setting.

Notably, the association was not materially modified by baseline albuminuria, which suggested that reducing albuminuria both in the moderately and severely increased range might slow kidney disease progression. This provided additional support for the notion that albuminuria and eGFR represent interconnected but distinct measures of pathways leading to kidney failure.

Thus , present study suggests that patients representing symptoms or risk factors that could lead to chronic renal disease should be screen for eGFR and ACR along with serum creatinine to identify the early stages of CKD like Stage 1 or 2 .

Final Recommendation:-

We recommend that the stages of CKD should be based in the combined indices of kidney function (eGFR and kidney damage and ACR, irrespective of underlying diagnosis).

1. Calculate eGFR from stable serum creatinine levels at least once a year in all patients showing symptoms of CKD to stratify the staging of CKD .
2. Changes in albuminuria (ACR) may serve as early indicators of CKD progression and complications beyond eGFR, but the risk implications of these changes are not well documented, with existing studies predominantly limited to subjects with kidney disease.
3. The screening tests should include a urine test for proteinuria or albuminuria and a blood test for serum creatinine to estimate glomerular filtration rate (GFR).

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Annexure 1:

Classification Of CKD on the basis of eGFR and ACR:

Composite ranking for relative risks by GFR and ACR				ACR stages, description and range (mg/g)					
				A1		A2		A3	
				Optimal and high normal		High		Very high and nephrotic	
				N	>3	3-14	15-30	>30	A
eGFR stages, description and range (ml/mon per 1.73 m2)	G1	High and optimal	90-105						
	G2	Mild	60-89						
	G3a	Moderate	30-44						
	G3b	Moderate	45-59						
	G4	Severe	15-29						
	G5	Kidney failure	<15						

Table 1:- Classification of chronic kidney disease using GFR and ACR. Adapted: Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group (2013) KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney International (Suppl. 3: 1-150) [**22**]

Annexure 2:

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Government Medical College and Hospital, Akola

Topic: NOVEL DOUBLE MARKER APPROACH IN CHRONIC KIDNEY DISEASES AND ASSOSIATION WITH PROGRESSION TO END STAGE RENAL DISORDER

Consent Form

I ……..…….. son/daughter/wife of …… residing at ……

exercising my free will, hereby give my full consent to be included as a subject for the biochemical study entitled as above, the nature and effect of which have been explained to me to the best of my understanding and to my full satisfaction.

Signature/Thumb impression
(Subject)

	Signature/Thumb impression (Guardian)
Name:	Name:
Date:	Date:

जीवरसायनशास्त्रविभाग

जिल्हा रुग्णालय , अकोला

संमतीपत्र

मी .....राहणार .....उपरनिर्देशितसंस्थेमधील अभ्यासातसहभागीहोण्यासमाझ्याअनुमतीनेतयारआहे . अभ्यासाचाप्रकारआणिहोणारेपरिणाममलासमजतीलआणिमाझेशंकानिरसनहोईलअश्याप्रकरेसमजावूनसांगण्यातआलेलेआहेत .

सही / अंगठ्याचाठसा

सही / अंगठ्याचाठसा

नाव :	नाव ::
तारीख :	तारीख :

जीवरासायानशास्त्रविभाग

जिलाअस्पताल, अकोला हस्ताक्षर / अंगूठेकी छाप

संमतीपत्र

मैं ..... **रहनेवाला/ ली** ..... **उपरनिर्देशितसंस्थामेंहोनेवालेअभ्यासमेंसहभागीहोनेकेलिएमेरीअनुमतिहै।अभ्यासकाप्रकारऔरहोनेवालेपरिणाममुझेसमझाएगएहैऔरमेरीसभीशंकाओकासमाधानकियाहै।**

हस्ताक्षर / अंगूठेकीछाप

नाम:	**नाम:**
दिनांक:	**दिनांक:**