

Original Research Article

Association between albumin creatinine ratio and e-GFR in type 2 diabetes mellitus patients in Oredo Local Government Area, Benin City, South-South Nigeria.

ABSTRACT

BACKGROUND: Diabetes mellitus is the commonest endocrine disease in Nigeria.

Nephropathy is one of the complications of type 2 diabetes mellitus that could lead to end stage renal disease. Persistent microalbuminuria is a predictor of high risk of developing diabetic nephropathy. Early detection of kidney disease and intervention will prevent progression to end stage renal disease.

OBJECTIVE: To determine the association between albumin creatinine ratio and eGFR in type 2 diabetic patients, their usefulness as early predictors of diabetic nephropathy and progression of disease.

MATERIALS AND METHODS: This was a cross sectional study conducted in the State Specialist Hospital, Benin City, Edo State, Nigeria. Forty six type 2 diabetes mellitus patients, both male and female within the age range of 30 – 85 years were recruited for the study, after meeting the inclusion criteria. Twenty age and gender matched healthy subjects were selected as controls. Fasting plasma glucose, glycosylated haemoglobin, C-Reactive protein, serum creatinine were assayed. Urine albumin was estimated and albumin creatinine ratio determined, eGFR was estimated according to the Modification of Diet in Renal Disease (MDRD) formula.

RESULTS:

There was a significant difference between means of glycosylated haemoglobin, C-Reactive protein, Albumin creatinine ratio, eGFR of subjects which was $(8.3 \pm 2.1\%, 17.$

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30 9±1.3mg/l, 247.7±22.2mg/g, 88±5.9ml/min) respectively and that of controls which
31 was (4.5±1.1%, 10.2±2mg/l, 22.7±5.1mg/g, 93.1±2.3ml/min) p<0.05 .ACR showed
32 a strong and linear negative correlation with eGFR (r=-.682, p<0.05). A higher per
33 centage of patients (64.7%) with e-GFR<60 ml/min had microalbuminuria, compare
34 d with (57.7%) with microalbuminuria in the subjects with eGFR>60 ml/min. **Micr**
35 **eAa** Albuminuria worsened with increased duration of disease, as 55.6% of patient
36 s with diabetes mellitus of 5 years and above had macro albuminuria compared w
37 ith 25% of patients with disease less than 5 years. Decline in eGFR increased with i
38 ncreasing duration of disease, 55.6% with disease duration of 5 years and above h
39 ad eGFR<60ml/min, compared with 45.5% in those with disease less than 5 years.

40 **Conclusion:** Albumin creatinine ratio and eGFR had a significant and linear negativ
41 e correlation in this study. Therefore, they could be useful as predictors of early ki
42 dney disease in type 2 diabetes mellitus in this local government area. Increase in
43 severity of **micro**albuminuria, coupled with higher prevalence of decline in eGFR
44 with increasing duration of disease may be suggestive of their usefulness as predi
45 ctors of disease progression.

46 **Key words:** Albumin creatinine ratio(ACR), eGFR, diabetes mellitus type 2.

48 Introduction

49 Diabetes mellitus is a chronic disease with an increasing prevalence worldwide. Th
50 e prevalence in Nigeria varies from 0.65% in rural areas and villages to 11.0% in ur
51 ban Lagos¹. Good glycaemic control and control of other cardiovascular risks in dia
52 betic patients lead to reduced morbidity and mortality².

53 Diabetic nephropathy is the leading cause of end stage renal disease worldwide³. I
54 t is said to affect 25-40% of diabetic patients⁴. The incidence of end stage renal fai
55 lure due to diabetes has increased during the past two decades⁵. Roughly 50% of t
56 he end stage renal disease due to diabetes mellitus is associated with type 2 diabe
57 tes mellitus⁶. To prevent this increase, screening for chronic kidney disease and ea
58 rly intervention is necessary⁵. In diabetic patients, the early detection of diabetic n
59 ephropathy has been focused on measurement of urinary albumin excretion rate⁵

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60 . The detection of elevated urinary albumin excretion rate identifies patients with
61 an increased risk for progression of diabetic nephropathy. The course of kidney fu
62 nction in type 2 diabetic patients with albuminuria is characterized by a highly vari
63 | able but relentless decline in glomerular filtration rate and a concomitant rise in
64 albuminuria and arterial pressure.^{6,7,8} Previous studies have suggested that arteria
65 l blood pressure and albuminuria both act as progression promoters⁷. The Nation
66 al Kidney Foundation has recommended that diabetics should be screened annual
67 ly for kidney disease⁸.

68 The aim of this study was to determine the association between eGFR and albumi
69 n creatinine ratio in type 2 diabetic patients, and its usefulness as markers for earl
70 y detection of renal impairment in diabetics and its possible use as predictors of di
71 sease progression.

72 **Subjects, Materials and Methods**

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73 This was a cross sectional study carried out in the State Specialist hospital Benin Ci
74 ty, Edo State, Nigeria. Study was carried out between September 2012 and April 2
75 013. Consent for the study was obtained from the ethical committee of the hospit
76 al and informed consent obtained from the patients and controls.

77 Forty-six type 2 diabetic patients both male and female aged 38 to 83 years were
78 recruited for the study. Every 3rd consenting patient was selected from those atte
79 nding the diabetic outpatient clinic was selected for the study. They were patient
80 s who were already diagnosed and being managed as type 2 diabetic patients. The
81 y have been diabetic for a mean duration of 5 years and 3 months. Structured que
82 stionnaires were used to obtain vital information from the patients, which include
83 d duration of disease, type of medications they are on, history suggestive of comp
84 lications. General physical examination was performed on the patients and param
85 eters such as height and weight were measured with a stadiometer and weighing
86 scale respectively, blood pressure was measured with a sphygmomanometer.

87 **Exclusion criteria**

88 Subjects with proven urinary tract infection, diagnosed kidney disease other than
89 diabetic nephropathy, concurrent acute illnesses including infectious diseases, ma

90 lignancy, acute immunological disease, history of clinical cardiovascular disease, t
91 uberculosis and smoking were excluded from the study.

Comment [U5]: ? reason for this exclusion

92 Twenty age and gender matched controls were involved in the study. They were n
93 ot known diabetics, hypertensive nor had chronic kidney diseases.

94 **SAMPLE COLLECTION**

95 10ml of fasting venous blood was collected from the patients in the morning. 2.5
96 ml each was dispensed into lithium heparin, fluoride oxalate, ethylene diaminetetr
97 acetate (EDTA) and plain bottles. ~~The samples in fluoride oxalate, lithium heparin a
98 nd EDTA bottles were inverted 8 to 10 times to ensure proper mixture of blood an
99 d anticoagulants.~~ Samples in the EDTA bottle was immediately refrigerated at 4°C,
100 while that in the plain bottle were allowed to clot and then centrifuged at 3000 r
101 pm for 5 minutes, separated into clean plain tubes and kept in an ultradfrizer at -8
102 0°C till time of analysis. Samples in the lithium heparin and fluoride oxalate bottles
103 were also centrifuged at 3000rpm for 5 minutes and plasma separated into clean
104 plain tubes and stored in an ultradfrizer at -80°C until time of analysis. First void m
105 orning urine was collected in a universal bottle with preservative.

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106 **METHODOLOGY**

107 Plasma glucose was assayed by the glucose oxidase method. Glycated hemoglobin
108 was assayed by the boronate affinity method using the Kit (in-2-it system from Bi
109 o-rad). Marker of inflammation C-reactive protein was measured by latex enhanc
110 ed turbidimmunometric assay.

111 Urinary Albumin excretion was measured using first void morning urine sample by
112 the Lowry method⁹, serum and urinary creatinine were assayed by modified Jaffe
113 kinetic method.

Comment [U7]: Please state definition of
micro and macroalbuminuria

114 eGFR was calculated using the Modification of diet in Renal Disease (MDRD) equat
115 ion^{10,11}.

116 $GFR(ml/min/1.73m^2) = 175 \times \text{Serum creatinine}(mg/dl)^{-1.154} \times \text{Age in years}^{-0.203} \times 0.742$
117 (if female) $\times (1.212 \text{ if black})$.

118 **STATISTICAL ANALYSIS**

119 This was done using SPSS version 13, means of variables are reported as mean $\pm$ s
120 tandard deviation. Test of significance differences between means of variables wa
121 s determined using the student 'T' test and ANOVA. A value of ≤ 0.05 was conside
122 red significant. Pearson's correlation was used in analyzing correlation between v
123 ariables.

125 Results

126 A total of 46 diabetic subjects participated in the study, with 38.1% males and 61.
127 9% females, all in the age range of 38 to 83 years. Mean age was 58.3 ± 1.2 years (
128 Table 1). Twenty six of them were on angiotensin converting enzyme (ACE) inhibit
129 ors

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130 Mean fasting plasma glucose, glycatedhaemoglobin and body mass index (BMI) of
131 the subjects was 7.9 ± 0.4 mmol/l, $8.3 \pm 2.1\%$ and 27.6 ± 6.5 kg/m² respectively .(T
132 able 1)

133 Mean albumin creatinine ratio and C-reactive protein of the subjects were $247.7 \pm$
134 22.2 mg/g and 17.9 ± 1.3 mg/l respectively. There was a statistically significant diff
135 erence between the mean and controls ($p < 0.05$). (Table 1)

136 Figure 1 shows regression curve between albumin creatinine ratio and eGFR in th
137 e diabetic subjectspatients.

Comment [U9]: Use either subjects or patients consistently

138 eGFR showed a significant negative correlation with ACR in the subjects studied (r
139 $= -0.681$, $p < 0.05$) while systolic blood pressure showed a significant positive corr
140 elation with ACR, $r = .506$, $p < 0.05$. (Table 2)

141 While 57.7% of the patients with $eGFR \geq 60$ ml/min had microalbuminuria, 42.3 ha
142 d macroalbuminuria. 64.7% of patients with $eGFR < 60$ ml/min had microalbuminuri
143 a and 35.3% had macroalbuminuria. (Table 3)

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144 75% of the patients with diabetes for less than 5 years had microalbuminuria and
145 macroalbuminuria, while 25% had macroalbuminuria. 44.4% of those with diabet
146 es mellitus of 5 years and above had microalbuminuria and 55.6% macroalbumin
147 uria. 54.5% of patients with diabetes mellitus of below 5 years duration had e GFR

of ≥ 60 ml/min while 45.5% had $eGFR < 60$ ml/min. Also 44.4% of those with diabetes of 5 years and above had $eGFR \geq 60$ ml/min, and 55.6% had $eGFR < 60$ ml/min. (figures 2a and 2b) (table 43).

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DISCUSSION

Diabetic nephropathy is a common complication of type 2 diabetes mellitus. Good glycaemic control and screening for early detection of diabetic nephropathy will reduce morbidity, progression to end stage renal disease and mortality. The National kidney Foundation (NKF) and American Diabetic Association (ADA) recommends that annual screening of patients diagnosed as diabetic for albumin creatinine ratio and $eGFR$ is ideal. Also the American Diabetic Association recommends annual screening to assess urine albumin excretion in all type 2 diabetic patients, starting at diagnosis.¹²

There was a significant difference between the body mass index (BMI), glycated haemoglobin, ACR, $eGFR$ and C-reactive protein of the subjects and controls in this study. Our findings corroborate what other studies have revealed^{14,16}. The significant difference observed between mean BMI and mean CRP of subjects and controls, may be indicative of the roles they play in pathogenesis of diabetes mellitus. Hu FB¹⁷ in their study on “inflammatory markers and risk of developing type 2 diabetes in women in the USA” reported that elevated plasma levels of CRP were an independent predictor of type 2 diabetes mellitus in apparently healthy women. Wang Z¹⁸ also reported in the study on aboriginal Australians, that CRP is independently associated with development of diabetes in the aboriginal people.

We observed a significant difference between albumin creatinine ratio and $eGFR$ of subjects and controls, this may be suggestive of varying degrees of renal impairment in these patients. None of the patients in this study was normoalbuminuric, they already had varying degrees of microalbuminuria as at the time of the study. This may be a reflection of the degree of prevalence of renal impairment among t

178 | these subjects¹² patients, and it highlights the need for screening for microalbuminur
179 | ia in all newly diagnosed diabetics, and annual screening in all diabetics, for early
180 | detection of kidney disease or prevention of progression to end stage renal diseas
181 | e as recommended by the National Kidney Foundation⁸ and American Diabetic As
182 | sociation¹². Whether APOL 1 and MHY 9 genes could be contributory factors to th
183 | e prevalence of renal impairment in these subjects was not ascertained in this stu
184 | dy. However, studies have shown the roles these genes could play in the develop
185 | ment of diabetic nephropathy in Africans. Palmer ND¹⁹ et al in their study reporte
186 | d that variants in MHY 9 genes played the most significant role in the developmen
187 | t of nephropathy.

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188 | Estimated glomerular filtration rate (eGFR) showed a significant negative correla
189 | tion with albumin creatinine ratio, with decline in eGFR , ACR increased. Other stu
190 | dies have reported similar findings¹⁹²⁰. Decline in eGFR therefore may also be usef
191 | ul as a marker for early detection of renal impairment in type 2 diabetes patients,
192 | in conjunction with albuminuria just as the study by Berhane A and coauthors²⁰²¹ r
193 | eported in Pima Indians with type 2 diabetes. They reported in their study that a c
194 | ombined measure of albuminuria and eGFR is a significantly better predictor of en
195 | d stage renal disease.

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196 | Following²¹ With the National Kidney Foundation recommendation that diabetics sh
197 | ould be screened annually for kidney disease, which includes ACR, serum creatinin
198 | e and eGFR measurements, and the screening should include measurement of AC
199 | R in a spot urine sample, measurement of serum creatinine and e-GFR, the observ
200 | ation from this study shows that estimation of albumin creatinine ratio and e-GFR
201 | could be useful markers in this environment for early detection of diabetic nephro
202 | pathy, prevention of overt nephropathy and progression to end stage renal diseas
203 | e. This is very important as these tests are relatively cheaper and therefore more
204 | affordable compared with other diagnostic tests such as magnetic resonance imag
205 | ing, computerized tomography scan, which are more expensive and may not be r
206 | eadily affordable by people of lower socioeconomic group. Also they serve as che
207 | aper screening tests for early detection of renal impairment, and therefore preve
208 | nt increased incidence of chronic kidney disease or end stage renal disease. Chron
209 | ic kidney disease which is still largely managed in this environment by dialysis is n

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210 ot easily affordable for patients of low socioeconomic group, therefore simple scr
211 eening tests like this will go a long way in early detection and intervention to prev
212 ent chronic kidney disease. Moreover, it was observed that while 57.7% of the p
213 atients with $\text{eGFR} \geq 60 \text{ ml / min}$ had microalbuminuria, a larger percentage (64.7%
214) had microalbuminuria in those with $\text{eGFR} < 60 \text{ ml/min}$, prevalence of albuminuria
215 increased with duration of disease. This still shows the roles the two factors coul
216 d play in the detection of kidney disease and its progression. Per K⁶ and coauthors
217 reported a more rapid decline in GFR and progressive rise in albuminuria in type
218 2 diabetes mellitus with glomerulopathy, compared with those without glomerulo
219 pathy, indicating the roles microalbuminuria and eGFR could play as progression
220 promoters.

221 When a comparison was done between patients with diabetes of below 5 years, a
222 nd those of 5 years and above, the role these two factors play was better highligh
223 ted. A higher percentage of patients with diabetes of 5 years and above had macr
224 oalbuminuria, compared with those below 5 years, indicating that with increasing
225 duration of disease the degree of albuminuria worsened. J.K Lutale^{21,22} and coauth
226 ors in Tanzania reported the same finding that abnormal albumin excretion rate w
227 as significantly related to duration of diabetes. Similarly, a larger percentage of th
228 e subjects with diabetes of 5 years and above had $\text{eGFR} < 60 \text{ ml / min}$ compared wi
229 th those with diabetes of less than 5 years duration, showing a decline in e GFR wi
230 th increasing duration of disease.

231 CONCLUSION

232 Albumin creatinine ratio showed a significant negative and linear correlation with
233 eGFR in this study. They may be useful tools for screening and early detection of k
234 idney disease in type 2 diabetes mellitus patient in this environment. Increased pr
235 evalence and severity of microalbuminuria and increased prevalence of decline in
236 eGFR with increasing duration of disease, is suggestive that ACR measurements a
237 nd eGFR estimation may be useful as predictors of disease progression in type 2 di
238 abetes mellitus. It is recommended that type 2 diabetes mellitus patients should b
239 e screened annually for microalbuminuria and eGFR determined, for early detecti
240 on and prevalence of kidney disease.

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Comment [U12]: Please also highlight that a low percentage of diabetic subjects were on ACEi or ARB, please also allude to public health implications that such a prevalence of overt or incipient nephropathy

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UNDER PEER REVIEW

330 Table 1:Demographic and Clinical Characteristics of the subjects

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PARAMETER CONTROL	CONTROLS	SUBJECTS	p-VALUE
Male Population (%)	40	38.1	
Female Population (%)	60	61.9	
Age (Years)	56.1±1.0	58.3 ± 1.2	0.32
BMI (Kg/M ²)	23.2±1	27.6 ± 6.5	0.063
Systolic blood pressure(mmHg)	120.5±3	144±44	0.095
Diastolic blood pressure(mmHg)	76.8±2.5	84±10	0.630
Fasting Plasma Glucose(mmol/l)	4.6±1.1	7.9±0.4	0.001
GlycatedHaemoglobin(%)	4.5±1.1	8.3 ± 2.1	0.000
C – Reactive Protein (mg/l)	10.3 ± 2.0	17.9±1.3	0.005
Albumin Creatinine Ratio(mg/g)	22.7 ± 5.1	247.7±22.2	0.000
eGFR(ml/min)	93.1±2.3	88 ± 5.9	0.040

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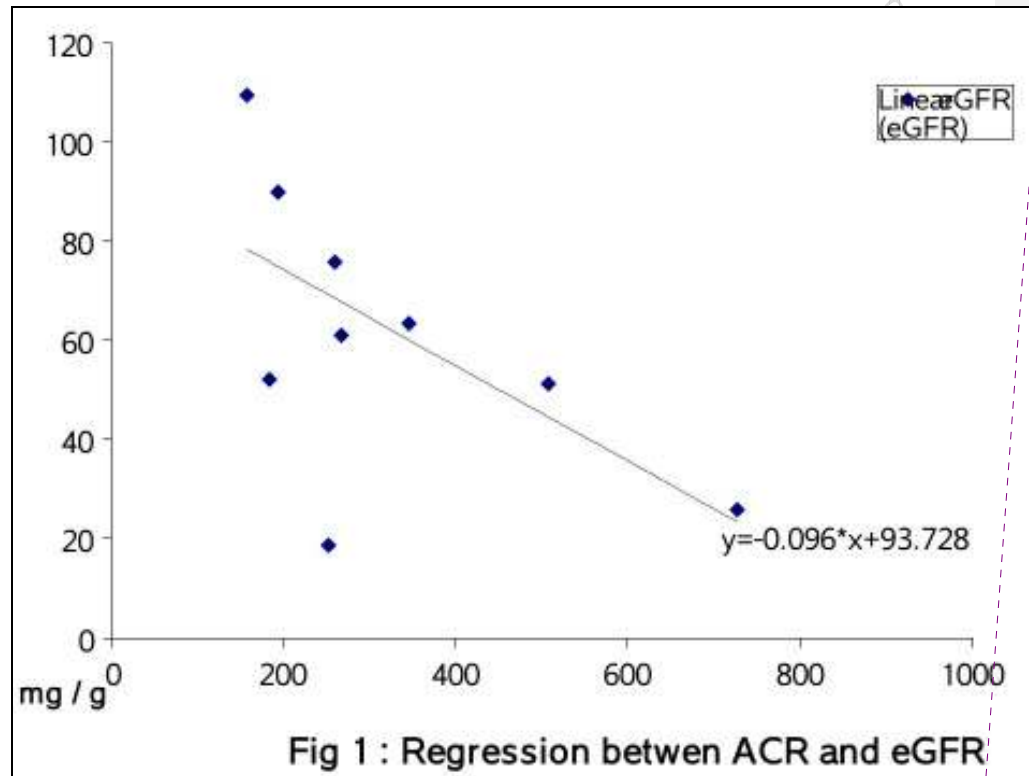
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Table 2 Correlation of eGFR and systolic blood pressure with Albumin Creatinine Ratio.

Parameter	r	P-value
eGFR	.681	.021
Systolic Blood Pressure	.506	.006

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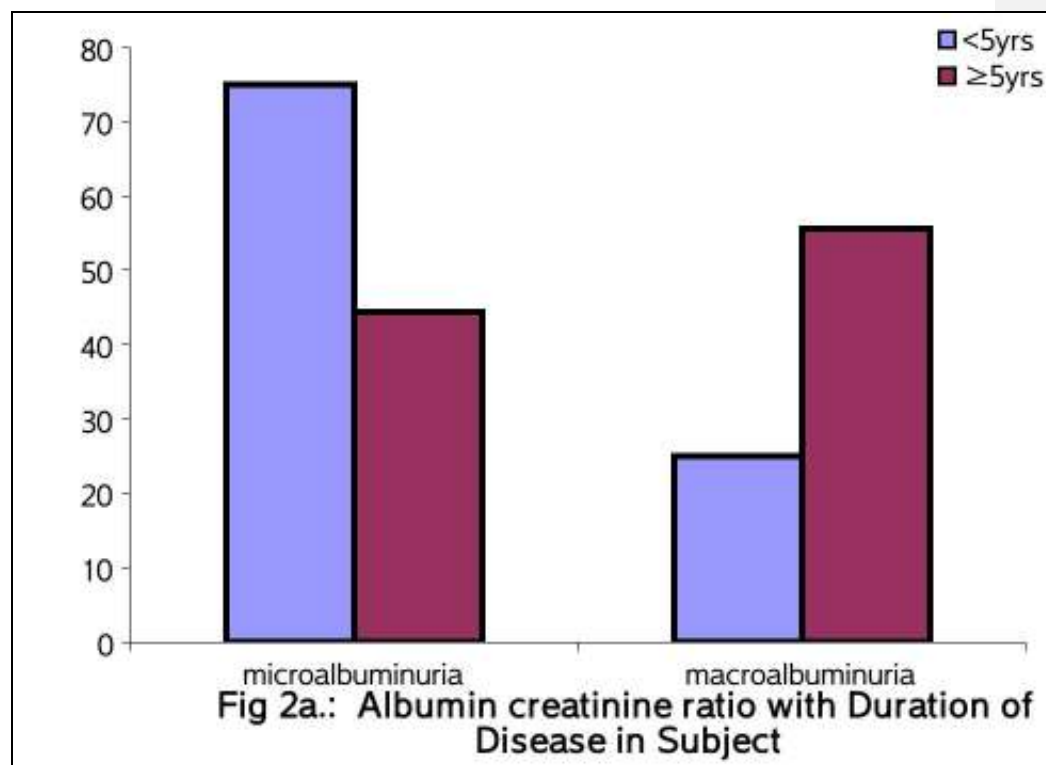
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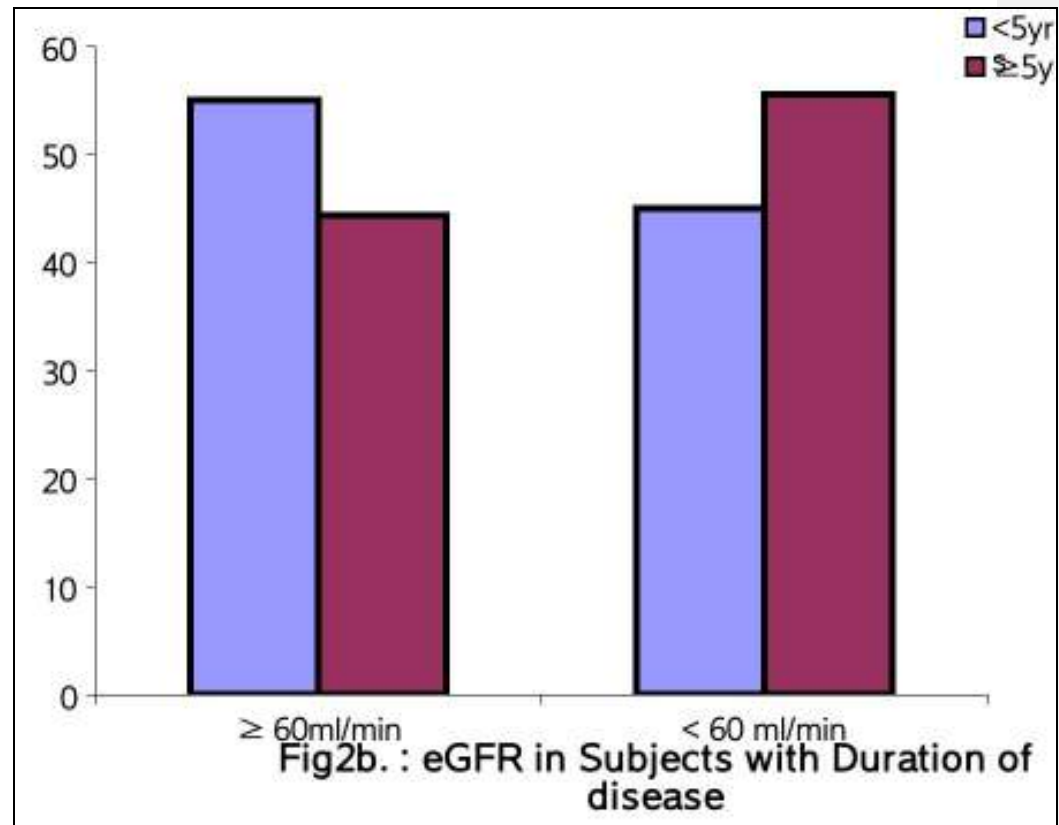
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389 Table 32

390 Association between eGFR and Urine albumin excretion rate in type 2 diabetic pat
391 ients

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	e GFR $\geq$ 60ml / min n = 26	e GFR < 60ml/min n = 17
Normoalbuminuria (<30mg/g)	0 (0%)	0 (0%)
Microalbuminuria (30 – 299 300 mg / g) %)	15(57.7	11 (64.7%)
Macroalbuminuria($\geq$ 300mg/g)	11(42.3%)	6(35.3%)

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394 | Table 43

395 | **ACR and eGFR in the Subjects based on duration of diabetes below 5 years and 5**
396 | **years and above**

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	Below 5 years N=22	5 years and above N=21
Microalbuminuria	17(75%)	10(44.4%)
Macroalbuminuria	5(25%)	11(55.6%)
eGFR $\geq$ 60	12(54.5%)	10(44.4%)
eGFR<60	10(45.5%)	11(55.6%)

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