

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±2.1%, 17

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30 .9±1.3mg/l, 247.7±22.2 mg/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
33 percentage of patients (64.7%) with e-GFR<60 ml/min had microalbuminuria, compared
34 with (57.7%) with microalbuminuria in the subjects with eGFR>60 ml/min.
35 Microalbuminuria worsened with increased duration of disease, as 55.6% of patients
36 with diabetes mellitus of 5 years and above had macro albuminuria compared
37 with 25% of patients with disease less than 5 years. Decline in eGFR increased
38 with increasing duration of disease, 55.6% with disease duration of 5 years and above
39 had eGFR<60ml/min, compared with 45.5% in those with disease less than 5 years.
40

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

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

48 49 Introduction

50 Diabetes mellitus is a chronic disease with an increasing prevalence worldwide. The
51 prevalence in Nigeria varies from 0.65% in rural areas and villages to 11.0% in urban
52 Lagos¹. Good glycaemic control and control of other cardiovascular risks in diabetic
53 patients lead to reduced morbidity and mortality².

54 Diabetic nephropathy is the leading cause of end stage renal disease worldwide³. It
55 is said to affect 25-40% of diabetic patients⁴. The incidence of end stage renal failure
56 due to diabetes has increased during the past two decades⁵. Roughly 50% of the
57 end stage renal disease due to diabetes mellitus is associated with type 2 diabetes
58 mellitus⁶. To prevent this increase, screening for chronic kidney disease and early
59 intervention is necessary⁵. In diabetic patients, the early detection of diabetic n

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60 ephropathy has been focused on measurement of urinary albumin excretion rate⁵
61 . The detection of elevated urinary albumin excretion rate identifies patients with
62 an increased risk for progression of diabetic nephropathy. The course of kidney fu
63 nction in type 2 diabetic patients with albuminuria is characterized by a highly vari
64 | able but relentless decline in glomerular filtration rate and a concomitant rise in
65 albuminuria and arterial pressure.^{6,7,8} Previous studies have suggested that arteria
66 l blood pressure and albuminuria both act as progression promoters⁷. The Nation
67 al Kidney Foundation has recommended that diabetics should be screened annual
68 ly for kidney disease⁸.

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

73 **Subjects, Materials and Methods**

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

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

88 **Exclusion criteria**

89 Subjects with proven urinary tract infection, diagnosed kidney disease other than

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for the controls

90 diabetic nephropathy, concurrent acute illnesses including infectious diseases, ma
91 lignancy, acute immunological disease, history of clinical cardiovascular disease, t
92 uberculosis and smoking were excluded from the study.

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

95 SAMPLE COLLECTION

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

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107 METHODOLOGY

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

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

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

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

119 STATISTICAL ANALYSIS

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

125

126 Results

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

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

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

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

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

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

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

148 uria. 54.5% of patients with diabetes mellitus of below 5 years duration had e GFR
149 of ≥ 60 ml/min while 45.5% had eGFR < 60 ml / min. Also 44.4% of those with diab
150 etes of 5 years and above had e GFR ≥ 60 ml / min, and 55.6% had e GFR < 60 ml /
151 min.(figures 2a and 2b)(table 43).

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155 **DISCUSSION**

156 Diabetic nephropathy is a common complication of type 2 diabetes mellitus. Good
157 glycaemic control and screening for early detection of diabetic nephropathy will r
158 educe morbidity, progression to end stage renal disease and mortality.The Nation
159 al kidney Foundation (NKF) and American Diabetic Association (ADA) recommends
160 that annual screening of patients diagnosed as diabetic for albumin creatinine rat
161 io and eGFR is ideal. Also the American Diabetic Association recommends annual s
162 creening to assess urine albumin excretion in all type 2 diabetic patients,starting a
163 t diagnosis.¹²

164 There was a significant difference between the body mass index(BMI), glycatedha
165 emoglobin, ACR, eGFR and C-reactive protein of the subjects and controls in this s
166 tudy. Our findings corroborate what other studies have revealed^{14,16}.The signifi
167 cant difference observed between mean BMI and mean CRP of subjects and control
168 s, may be indicative of the roles they play in pathogenesis of diabetes mellitus.Hu
169 FB¹⁷ in their study on “ inflammatory markers and risk of developing type 2 diabe
170 tes in women in the USA” reported that elevated plasma levels of CRP were an in
171 dependent predictor of type 2 diabetes mellitus in apparently healthy women. W
172 ang Z¹⁸ also reported in the study on aboriginal Australians, that CRP is independ
173 ently associated with development of diabetes in the aboriginal people.

174 We observed a significant difference between albumin creatinine ratio and eGFR
175 of subjects and controls, this may be suggestive of varying degrees of renal impair
176 ment in these patients. None of the patients in this study was normoalbuminuric,
177 they already had varying degrees of microalbuminuria as at the time of the study.

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

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189 Estimated glomerular filtration rate (eGFR) showed a significant negative correla
190 tion with albumin creatinine ratio, with decline in eGFR , ACR increased. Other stu
191 dies have reported similar findings^{19,20}. Decline in eGFR therefore may also be usef
192 ul as a marker for early detection of renal impairment in type 2 diabetes patients,
193 in conjunction with albuminuria just as the study by Berhane A and coauthors²⁰²¹ r
194 eported in Pima Indians with type 2 diabetes. They reported in their study that a c
195 ombined measure of albuminuria and eGFR is a significantly better predictor of en
196 d stage renal disease.

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

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ic kidney disease which is still largely managed in this environment by dialysis is not easily affordable for patients of low socioeconomic group, therefore simple screening tests like this will go a long way in early detection and intervention to prevent chronic kidney disease. Moreover, it was observed that while 57.7% of the patients with $eGFR \geq 60$ ml / min had microalbuminuria, a larger percentage (64.7%) had microalbuminuria in those with $eGFR < 60$ ml/min, prevalence of albuminuria increased with duration of disease. This still shows the roles the two factors could play in the detection of kidney disease and its progression. Per K⁶ and coauthors reported a more rapid decline in GFR and progressive rise in albuminuria in type 2 diabetes mellitus with glomerulopathy, compared with those without glomerulopathy, indicating the roles microalbuminuria and eGFR could play as progression promoters.

When a comparison was done between patients with diabetes of below 5 years, and those of 5 years and above, the role these two factors play was better highlighted. A higher percentage of patients with diabetes of 5 years and above had macroalbuminuria, compared with those below 5 years, indicating that with increasing duration of disease the degree of albuminuria worsened. J.K Lutale^{24,22} and coauthors in Tanzania reported the same finding that abnormal albumin excretion rate was significantly related to duration of diabetes. Similarly, a larger percentage of the subjects with diabetes of 5 years and above had $eGFR < 60$ ml / min compared with those with diabetes of less than 5 years duration, showing a decline in eGFR with increasing duration of disease.

CONCLUSION

Albumin creatinine ratio showed a significant negative and linear correlation with eGFR in this study. They may be useful tools for screening and early detection of kidney disease in type 2 diabetes mellitus patient in this environment. Increased prevalence and severity of microalbuminuria and increased prevalence of decline in eGFR with increasing duration of disease, is suggestive that ACR measurements and eGFR estimation may be useful as predictors of disease progression in type 2 diabetes mellitus. It is recommended that type 2 diabetes mellitus patients should be screened annually for microalbuminuria and eGFR determined, for early detection and prevalence of kidney disease.

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UNDER PEER REVIEW

331 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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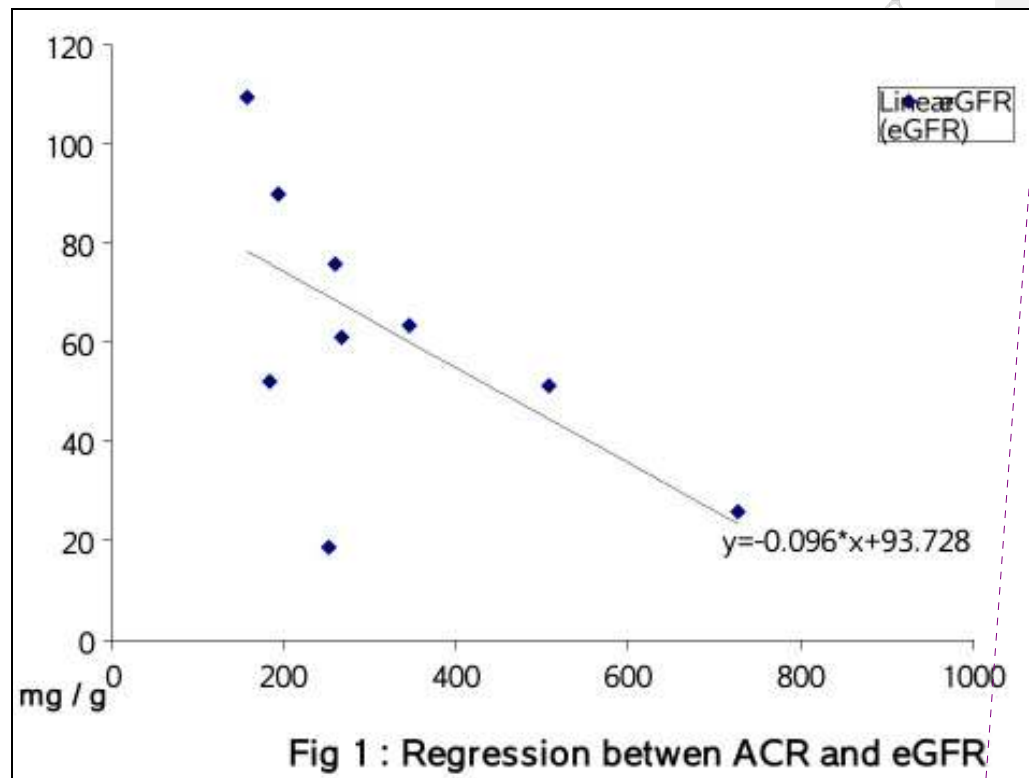
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362 **Table 2 Correlation of eGFR and systolic blood pressure with Albumin Creatinine Ra-**
363 **tio:**

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

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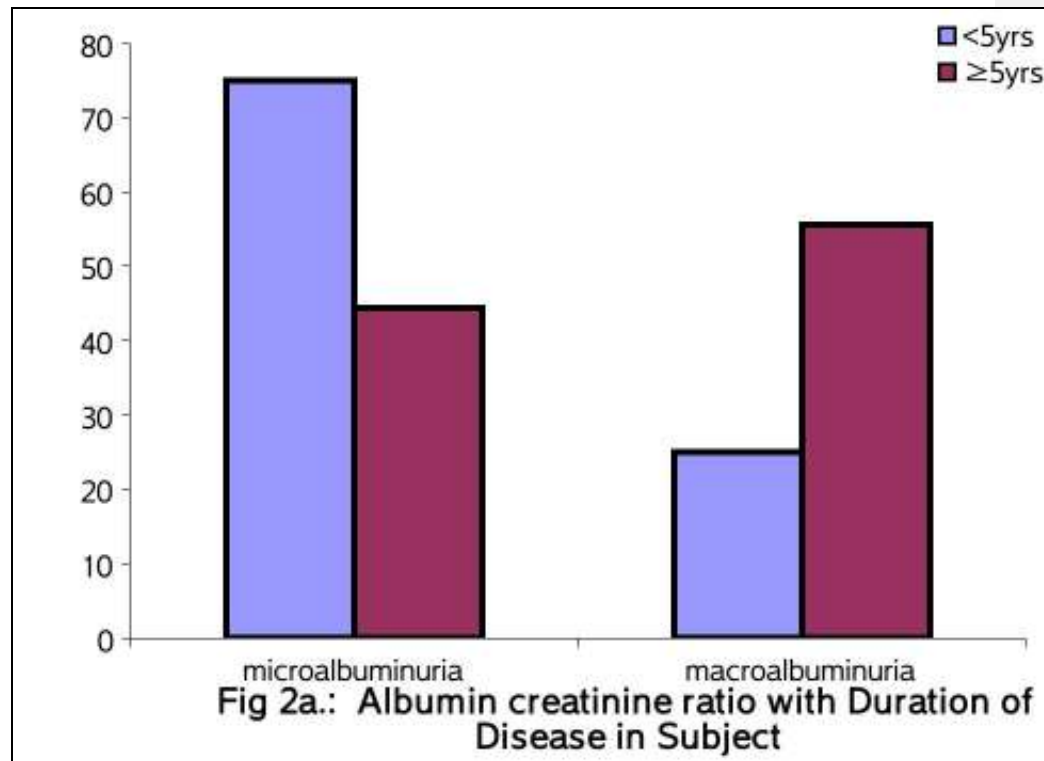
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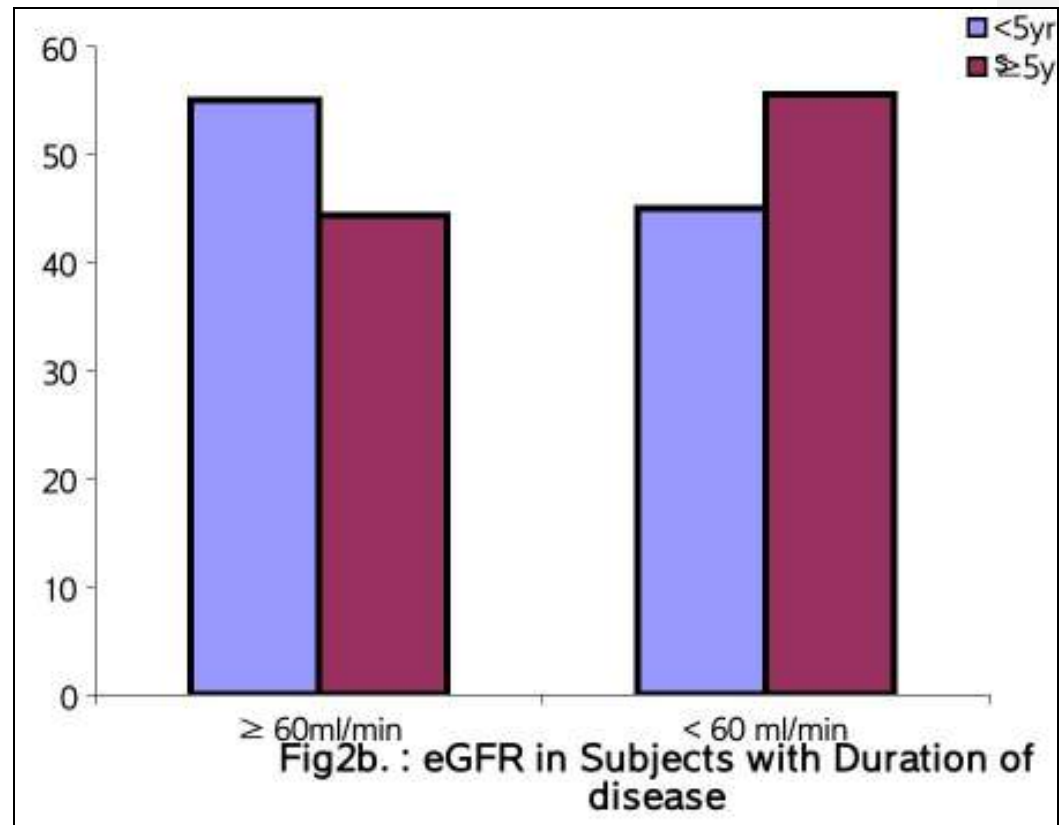
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390 Table 32

391 Association between eGFR and Urine albumin excretion rate in type 2 diabetic pat
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e GFR $\geq$ 60ml / min n = 26	e GFR < 60ml/min n = 17
Normoalbuminuria (<30mg/g)	0 (0%)
Microalbuminuria (30 – 299mg / g) %)	11 (64.7%)
Macroalbuminuria($\geq$ 300mg/g)	6(35.3%)

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Table 43

ACR and eGFR in the Subjects based on duration of diabetes below 5 years and 5 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%)