

# Glomerular diameter measurements on light microscopy: A new parameter available to pathologists and its utility in IgA nephropathy.



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## INTRODUCTION

- The utility of measuring glomerular diameter has been established in various renal diseases such as diabetes, FSGS etc. <sup>1</sup>
- Reduced nephron number and functional reserve, may cause glomerular enlargement, increasing susceptibility to renal injury.
- The progression of IgA Nephropathy, the most common cause of ESRD worldwide, is currently assessed using the Oxford MEST-C scores. <sup>2</sup>
- Other markers used to assess severity are serum creatinine and 24-hour urinary protein. <sup>2</sup>
- However, there are very few studies in literature assessing the predictive value of glomerular size in IgA Nephropathy (IgAN).

## AIM

- To correlate the mean and maximum glomerular diameter (GD) with serum creatinine levels, 24-hour urinary protein and the Oxford MEST-C scores in patients with Ig A Nephropathy.

## MATERIALS AND METHODS

- 100 biopsies, with  $\geq 8$  viable glomeruli, diagnosed as IgAN between 1<sup>st</sup> April 2022 to 1<sup>st</sup> January 2023, at Apollo Hospitals, Hyderabad were included.
- The serum creatinine, 24 hour urinary protein and Oxford scores at the time of biopsy were also collected.
- The glomeruli in 20 biopsies of acute tubular injury with no comorbid conditions, chronicity and with  $\geq 10$  glomeruli were taken as controls.
- The slides were scanned using the Philips Intellisite Pathology Solution- Ultra Fast Scanner.
- The glomeruli were annotated digitally for the measurements

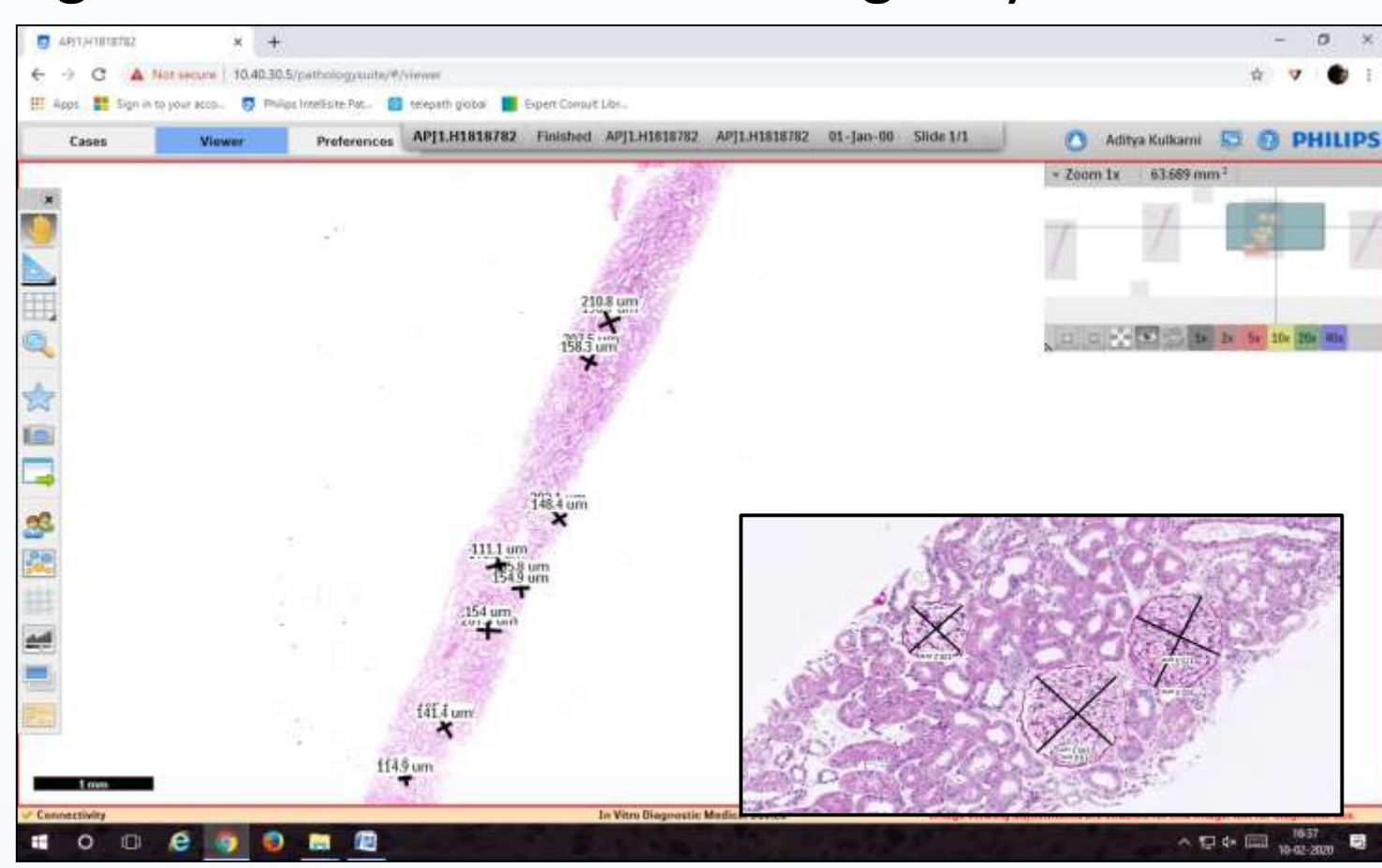


Fig. 1 Whole slide imaging (WSI) of a PAS stained section and digitally annotated glomeruli

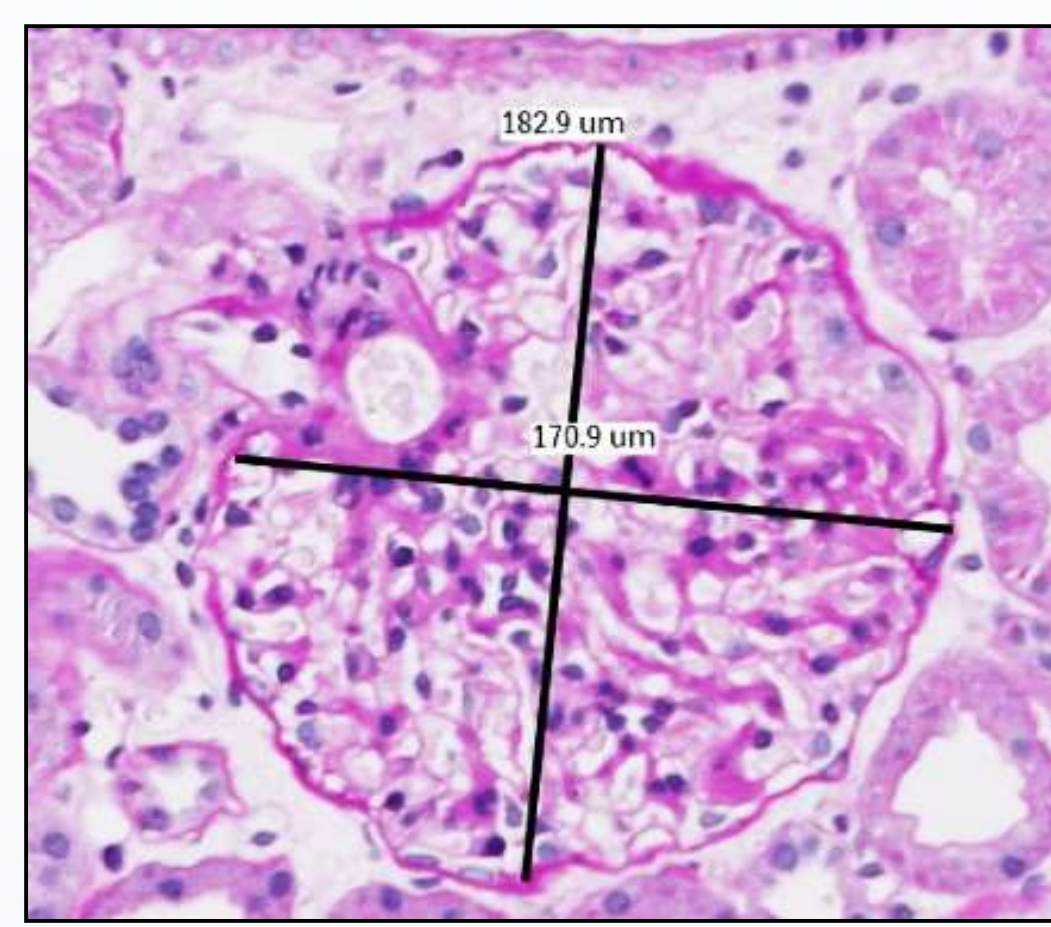


Fig. 2 Measurement of GDs

## CALCULATION OF MEAN AND MAX GD

- The maximal diameter (which passes through the geometric centre of the maximal profile of the glomerulus) and the maximal chord perpendicular to it was recorded in each viable glomerulus on a PAS stain as per the guidelines by Tsuboi and Kataoka et al. <sup>1,2</sup>
- Mean GD was calculated as the mean of these measurements.
- Max GD was taken as the mean of the maximally hypertrophied glomerulus. <sup>2</sup>
- Sclerosed or ischemic glomeruli and glomeruli with  $<40\%$  area visible were excluded. <sup>1</sup>
- The mean and max GD of the control and test biopsies was compared.
- The mean and max GD of the test cases were correlated with serum creatinine, 24 hour urinary protein and Oxford scores.

## STATISTICAL ANALYSIS

The data was analyzed using MS Excel (R) office 365, GraphPad prism 8.4.2 and SPSS version 25. Descriptive statistics were presented in the form of proportions/percentages for categorical variables and mean & SD for continuous data variables. Correlation was done using the Spearman Rho/Pearson R correlation. P value of  $<0.05$  was considered significant. A ROC analysis was done to analyze the role of mean and max GD in the assessment of E score.

## RESULTS

Table 1. The mean and the max GD were similar for control and test groups

Mean GD	Control	Cases	P Value	Max GD	Control	Case	P Value
No.	20	100	<b>0.174</b>	No.	20	100	<b>0.5279</b>
Mean	156.96	151.83		Mean	198.79	205.4	
Std Dev	15.00	28.70		Std Dev	24.89	32.76	

Table 2. Main parameters analysed in test cases

Parameters	Mean (Min-Max)
Age in years	34.67 (11-64)
Male: Female	65:35
Serum Creatinine (mg/dl)	1.82 (0.44-9)
24 hour urinary protein (gm per day)	4.01 (0.13-54.90)
Oxford scores	
• M (0/1)	34/66
• E (0/1)	57/43
• S (0/1)	42/58
• T (0/1/2)	82/15/3
• C (0/1/2)	57/33/10
Number of glomeruli	15.47 (8-36)
Number of sclerosed glomeruli	2.74 (24-0)
Mean glomerular diameter (in $\mu\text{m}$ )	<b>151.83</b> (99.9-270.67)
Maximum glomerular diameter (in $\mu\text{m}$ )	<b>205.40</b> (140.6-297.8)

Tables 3&4: No significant correlation was found between the mean or max GD and the serum creatinine, 24 hour urinary protein, sclerosed glomeruli or Oxford scores

Mean GD – Correlation univariate analysis					
Parameters	Spearman rho	95% CI	P value	Significant?	n
Creatinine	-0.054	-0.25 to 0.15	0.5967	No	100
24 hour proteinuria	-0.085	-0.28 to 0.11	0.4023	No	100
M	-0.0088	-0.21 to 0.19	0.9309	No	100
E	0.2	-0.0058 to 0.39	<b>0.05</b>	No	100
S	0.19	-0.015 to 0.38	0.0619	No	100
T	-0.11	-0.31 to 0.090	0.258	No	100
C	-0.032	-0.23 to 0.17	0.7548	No	100
No. of sclerosed glomeruli	0.04	-0.16 to 0.23	0.6912	No	100

Max GD – Correlation univariate analysis					
Parameters	Spearman rho	95% CI	P value	Significant?	n
Creatinine	0.079	-0.12 to 0.28	0.4335	No	100
24 hour proteinuria	0.015	-0.18 to 0.21	0.8831	No	100
M	-0.12	-0.32 to 0.083	0.2303	No	100
E	0.17	-0.032 to 0.36	<b>0.0883</b>	No	100
S	0.13	-0.072 to 0.33	0.1906	No	100
T	0.035	-0.17 to 0.24	0.7279	No	100
C	-0.04	-0.24 to 0.16	0.6933	No	100
No. of sclerosed glomeruli	0.091	-0.11 to 0.28	0.3678	No	100

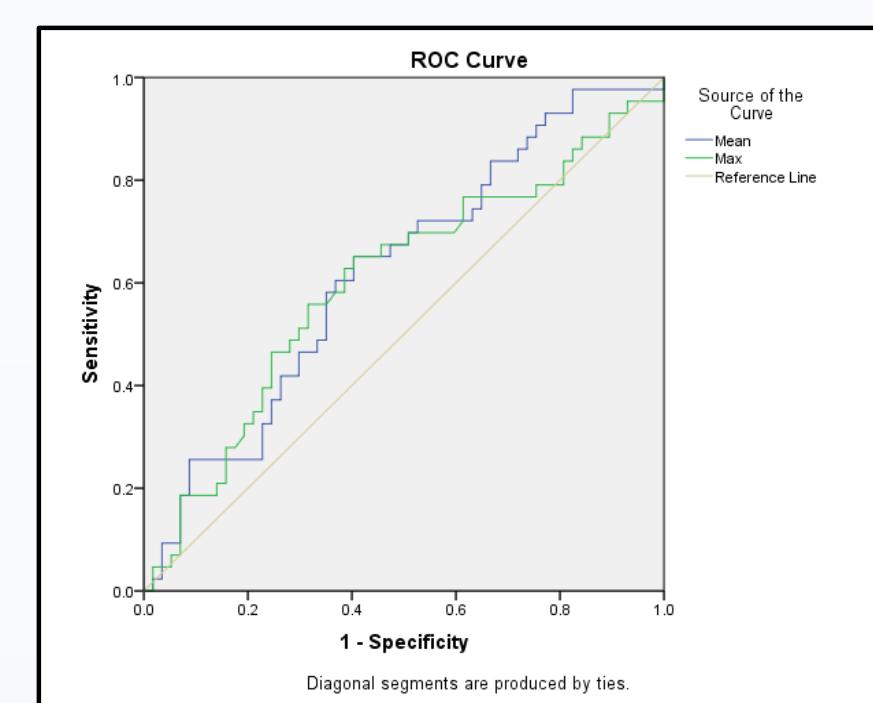


Fig 3. ROC analysis was done for E score as its p value approached significance levels

Tables 5&6: E Score and Mean/Max GD

Parameters for E score	Area under curve	Significant
Mean	<b>0.621</b>	<b>0.038</b>
Max	0.601	0.081
Cut off Value	Sensitivity	Specificity
<b>110</b>	97%	8%
<b>150</b>	<b>61%</b>	<b>62%</b>
<b>180</b>	18%	92%

## DISCUSSION

- With the advent of whole slide imaging (WSI), fairly accurate GD measurements are now possible.
- However, age and race-specific normal values need to be established before using them for disease assessment. <sup>3</sup>

	Our study (n=100)	Kataoka et al (n=97) <sup>2</sup>	Tsuboi et al (n=18) <sup>1</sup>
Age (years)	34.67 years (11-64)	34 $\pm$ 12.6	30.6 (18-51)
24 hour proteinuria(g/day)	4.01 (0.13-54.90)	0.72 (0-4.20)	1.40 $\pm$ 1.3 (0.28-5.6)
Serum Creatinine (mg/dl)	1.82 (0.44-9)	0.90 $\pm$ 0.29	0.76 $\pm$ 0.16 (0.5-1.0)
No of glomeruli per patient	15.47 (8-36)	13 (5-46)	21.0 $\pm$ 10.1 (range 10-42)
Average Max GD ( $\mu\text{m}$ )	205.40 (140.6-297.8)	218.3 $\pm$ 27	Glomerular density and glomerular volume measured

Parameters	Our study	Kataoka et al <sup>2</sup>	Tsuboi et al <sup>1</sup>
<b>Similar to our study:</b>			
24 hour proteinuria	No correlation	No correlation with Max GD at the time of biopsy. However, significant association seen at a 10 year follow up	No correlation with glomerular density and the mean glomerular volume at presentation.
MEST-C scores	No correlation	No correlation between patients with Max GD $<242 \mu\text{m}$ and $>242 \mu\text{m}$ .	No correlation
No. of sclerosed glomeruli	No correlation	No correlation	No correlation

## In contrast to our study:

Serum creatinine	No correlation with mean or max GD at the time of biopsy	Patients whose Max GD was $>242 \mu\text{m}$ had at least a 1.5-fold increase in their serum creatinine value at the 10-year follow-up examination	Glomerular density at presentation significantly correlated with creatinine clearance but mean glomerular area did not.
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## CONCLUSION

- In our study, the mean and max GD did not correlate with the serum creatinine, 24 hour urinary protein and the Oxford scores at the time of biopsy.
- At a cut off value of 150  $\mu\text{m}$  for mean GD, there is a 61% sensitivity and 62 % specificity in predicting E1 score.
- Further studies with follow up are needed to determine the utility of GD measurements in IgA nephropathy.

**Limitations:** 1. Follow up biopsies were not taken to assess the utility of GD in progression of disease. 2. Other renal disorders affecting the glomerular diameter such as obesity and hypertension were not excluded.