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COMPARATIVE EVALUATION OF CONVENTIONAL AND NEW BIOMARKERS OF RENAL INVOLVEMENT IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND HYPERTENSION – A PILOT STUDY.

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ABSTRACT

Transient kidney damage is identified in type-2 Diabetes Mellitus(DM) and Hypertension(HT). Most of the Indian studies that evaluate the association between renal damage and DM and HT were carried out with the conventional markers. In this study renal involvement in patients with DM, HT and both were assessed by conventional biomarkers (urea, creatinine, uricacid, urine protein-creatinine ratio(PC-Ratio) and compared with newer markers (cystatinC and microalbuminuria). Results of 41 patients are compared with 19 age and sex matched controls. Among the conventional markers creatinine and PC-ratio were found to be better markers than urea. Creatinine based estimated glomerular filtration rate(eGFR) was unable to predict the early renal dysfunction. Among new markers cystatinC, microalbuminuria and cystatinC based eGFR were found to be more reliable as predictive markers. So from this study it can be concluded that the newer markers are better to assess the early renal damage in HT and in DM.

KEYWORDS: **DiabetesMellitus,ChronicKidneyDisease,Hypertension,Microalbuminuria,Estimated Glomerular filtration rate.**

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INTRODUCTION

Chronic Kidney Disease (CKD) is a global health problem[1]. DM and HT contribute to a major cause of the early renal damage which is evidenced by proteinuria. Several epidemiological studies have demonstrated that diabetes and hypertension increases the risk of kidney disease and proteinuria[2]. Proteinuria itself intensifies the renal damage[3]. Control of the primary disease is essential in preventing the renal damage[4]. Detection of early renal damage and control of the primary disease like DM and HT is required for the prevention progression of the CKD to end stage renal disease (ESRD) which will lead to dialysis and renal transplant which are expensive and often limits the active life span[5]. The conventional renal function tests like urea, creatinine and creatinine based eGFR are unable to detect the early renal damage[6,7]. This study was undertaken to compare the conventional and newer renal biomarkers in predicting the onset of CKD. An attempt is also made to correlate microalbuminuria with Glycation status in diabetes and blood pressure in hypertension. A correlation between eGFR based on creatinine and cystatinC using different formulae were also studied.

MATERIALS AND METHODS

Forty One patients (20 diabetic, 12 hypertensive and 9 diabetics with hypertension) were selected at random from the outpatient department of Educre Institute, Malappuram. Nineteen age and sex matched controls were selected from the siblings of the patients. DM was confirmed using fasting blood sugar (FBS), postprandial blood glucose (PPBS) and the HbA1c values using the American Diabetes Association criteria[8]. Hypertension was confirmed by the repeated measurements of blood pressure by the aesthetic technique and Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure (JNC) 7 criteria[9]. Subjects with any present or previous sign or symptom of any other chronic or acute illness and autoimmune diseases were excluded from the study. Five ml of fasting venous blood and Random urine samples were collected from all the subjects after getting their informed consent. Two ml postprandial blood samples were also collected from all the subjects for the estimation of postprandial blood sugar (PPBS). Fasting blood sugar (FBS) and PPBS were estimated using the hexokinase method. Blood urea, serum creatinine, cystatinC and uric acid were estimated in the serum samples. Urine sample was used for the estimation of microalbumin and protein creatinine ratio. All assays were carried out in the AU 480 fully automated analyser (Beckman, India). Controls and calibrators were obtained from M/s Bio-rad. eGFR were calculated using the following four different formulae.

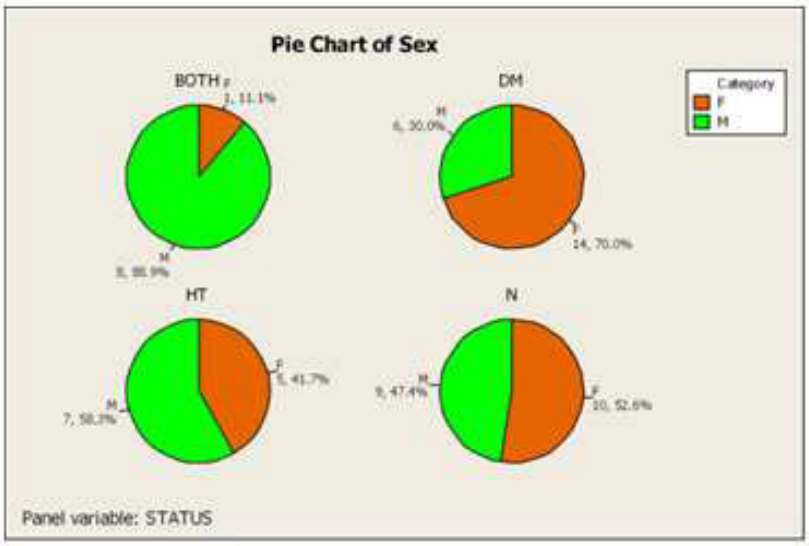
1. MDRD formula:[10]
   $170 \times SCr^{-0.999} \times age^{-0.176} \times BUN^{-0.170} \times albumin^{0.318} \times 0.762$ (if female)
2. Abbreviated MDRD formula:[11]
   $186 \times [SCr\ (mg/dl)]^{-1.154} \times [age]^{-0.203}$ × [0.742 if patient is female] × [1.21 if patient is African-American]
3. Cockcroft and Gault equation:[12]
   [(140 - age) × Total Body Weight] / (SCr × 72) (× 0.85 for females)
4. Cystatin C based formula:[13]
   $76.7 \times CysC^{-1.19}$

Statistical analysis was performed using SPSS and Minitab. T test, correlation and ANOVA were used. Statistical significance was defined as $P>0.05$.

RESULTS

Age and sex distribution of all the subjects of the present study are given in Figure 1. The histogram of the Systolic and Diastolic blood pressure of the study subjects are given in Figure 2 and Glycemic markers are given in Figure 3. Descriptive statistics of all the physical and biochemical data of the study populations (Mean ± SD) are given in the Table 1. Histogram - Normal Curve of Gycimic markers are given in Figure 3 and that of renal biomarkers of the study population is given in Figure 4.

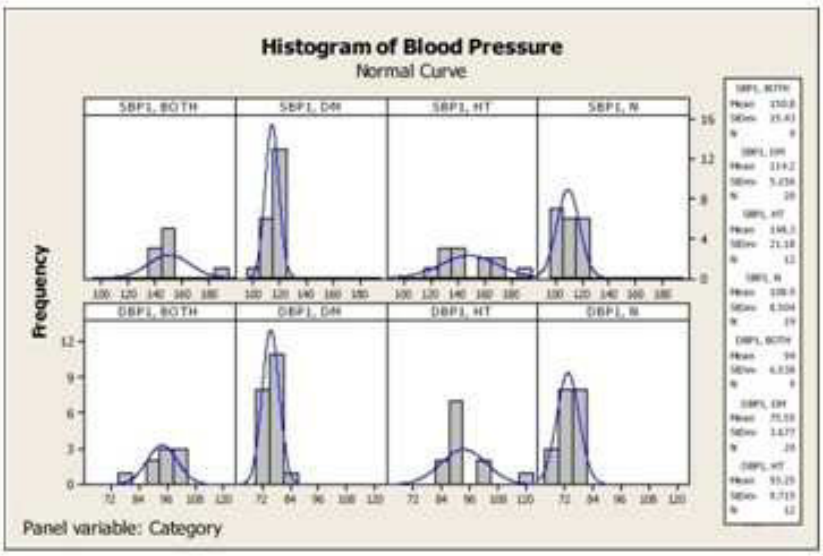
Figure 1
Category wise Pie Chart of Age and Sex Distribution of the study population.



N : Normal, HT : Hypertension
DM : Type 2 Diabetes Mellitus
BOTH : Both DM and HT

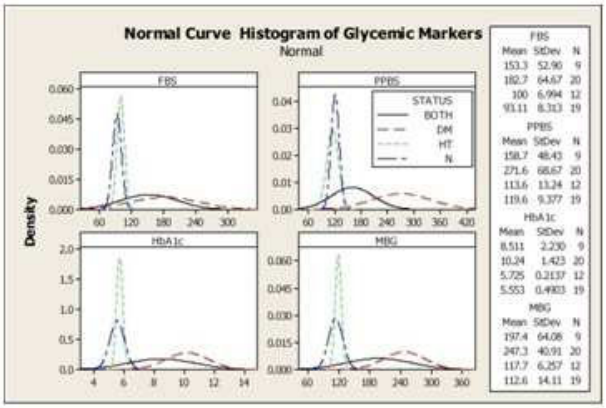
Out of the 19 Control Subjects 10 (53%) were Females and 9(47%) were male subjects. In 20 Diabetic Subjects 14(70%) were Female and 6 (30%) were male while in 12 hypertensive 5 (42%) were Female and 7 (58%) were male. Among the both hypertensive and diabetic category there were one (11%) female and 8 (89%) male subjects.

Figure 2
Normal Distribution Histogram of systolic and Diastolic blood pressure of the study population.



SBP1 : Systolic Blood Pressure in mmHg
DBP1 : Diastolic Blood Pressure in mmHg
DM : Type 2 Diabetes Mellitus
HT : Hypertension
BOTH : Hypertensive subjects who are Type 2 diabetic
N : Normal Controls

Figure 3
Normal curve Histogram of glycemic Markers of the study population.



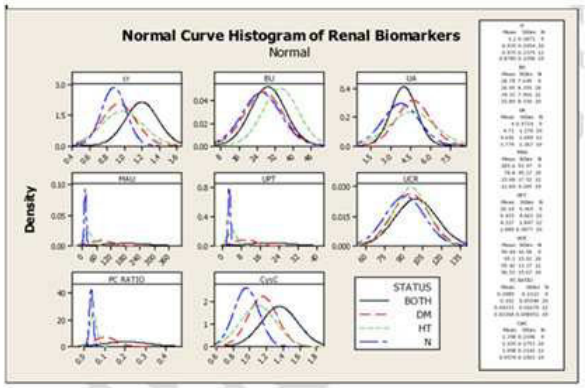
FBS : Fasting Blood Glucose in mg/dL
PPBS : Postprandial Blood Glucose in mg/dL
HbA1c : Glycated Hemoglobin (%)
MBG : Mean blood glucose in mg/dL calculated from HbA1c

Table 1
Descriptive statistics of the study population

	Category			
	Normal (n=19)	**Type 2 Diabetes Mellitus** (n=20)	**Hypertension** (n=12)	**Both DM & HT** (n=9)
Parameters	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Age	54.05±8.07	53.8±9.87	54.42±8.45	59.22±6.59
Systolic BP (mmHg)	108.89±8.5	114.2±5.16	148.33±21.18	150.78±15.43
Diastolic BP (mmHg)	73±4.85	75.55±3.68	93.25±9.72	94±6.54
Height (cm)	168.4±7.96	164±7.69	160.92±7.72	160.67±10.76
Weight (Kg)	69.63±9.47	63.6±7.56	65.5±9.76	71.33±8.76
BMI (Kg/m^2)	24.46±2.01	23.6±1.68	25.12±1.31	27.64±2.34
PPBS (mg/dL)	119.5±9.38	271.6±68.67	113.58±13.24	158.67±48.43
FBS (mg/dL)	93.11±8.31	182.7±64.67	100±6.99	153.33±52.9
HbA1c (%)	5.55±0.49	10.24±1.42	5.73±0.21	8.51±2.23
MBG (mg/dL)	112.6±14.1	247.3±40.91	117.67±6.26	197.44±64.08

Key: BP: Blood Pressure, BMI: Body Mass Index, PPBS: Postprandial Blood Glucose,
FBG: Fasting Blood Glucose, MBG: Mean Blood Glucose calculated from HbA1c, SD: Standard Deviation

Figure 4
Normal Curve Histogram of Renal Biomarkers of the study population.



Cr : Creatinine in mg/dL
BU: Blood Urea in mg/dL
UA: Uric acid in mg/dL
MAU : Microalbuminuria in mg of microalbumin per gram of creatinine
UPT: Urine protein in mg/dL
PC Ratio : Urine Protein Creatinine Ratio.
CysC: Cystatin C in mg/L

Comparison of conventional and novel biomarkers is made between diabetics, hypertensive, both diabetics and hypertensive and the normal are done using the ANOVA and the significance is tabulated in Table 2. Significant difference in mean observed in serum creatinine, blood urea, microalbuminuria, cystatin C, and eGFR using cystatin C.

Table 2
ANOVA results of the conventional and novel biomarkers among the different categories

		Sum of Squares	df	Mean Square	F	Sig.
Creatinine in mg/dL	Between Groups	0.66	3	0.22	6.195	0.001
	Within Groups	1.98	56	0.04		(HS)
	Total	2.64	59			
Blood Urea in mg/dL	Between Groups	584.97	3	194.99	2.871	0.044
	Within Groups	3802.96	56	67.91		(Sig)
	Total	4387.93	59			
Uric Acid in mg/dL	Between Groups	10.73	3	3.58	1.933	0.135
	Within Groups	103.64	56	1.85		(NS)
	Total	114.37	59			
Microalbuminuria in mg/G of Creatinine	Between Groups	205222.12	3	68407.37	33.87	0.000
	Within Groups	113114.87	56	2019.91		(VHS)
	Total	318336.98	59			
Urine Protein in mg/dL	Between Groups	2045.23	3	681.74	33.29	0.000
	Within Groups	1146.80	56	20.48		(VHS)
	Total	3192.03	59			
Urine Protein Creatinine Ratio	Between Groups	0.22	3	0.07	24.76	0.000
	Within Groups	0.16	56	0.00		(VHS)
	Total	0.38	59			
Cystatin C	Between Groups	1.03	3	0.35	10.26	0.000
	Within Groups	1.88	56	0.03		(VHS)
	Total	2.92	59			
eGFR Using Cocroft Gault	Between Groups	3377.05	3	1125.68	2.765	0.050
	Within Groups	22799.13	56	407.13		(NS)
	Total	26176.18	59			
eGFR using MDRD	Between Groups	2353.02	3	784.34	2.302	0.087
	Within Groups	19079.96	56	340.71		(NS)
	Total	21432.98	59			
eGFR using abbreviated MDRD	Between Groups	2033.18	3	677.73	2.108	0.109
	Within Groups	18001.55	56	321.46		(NS)
	Total	20034.73	59			
eGFR cystatin C	Between Groups	5501.60	3	1833.87	12.4	0.000
	Within Groups	8280.59	56	147.87		(VHS)
	Total	13782.18	59			

eGFR: Estimated Glomerular Filtration Rate in ml/min, MDRD: Modification of Diet in Renal Disease, Sig: Significant, HS: Highly Significant, VHS: Very Highly Significant, NS Not Significant

A significant correlation was observed between the glycemic status (better expressed by the fasting Glucose and the HbA1c) level and renal markers cystatin C, microalbuminuria, Urine protein, the urine protein creatinine ratio and eGFR using cystatin C. Correlation of biomarkers against glycimic markers and blood pressure are done using Pearson correlation and is given in Table 3, Figure 5 and Table 4, Figure 6 respectively.

Table 3
Comparison of different biomarkers against glycemic markers

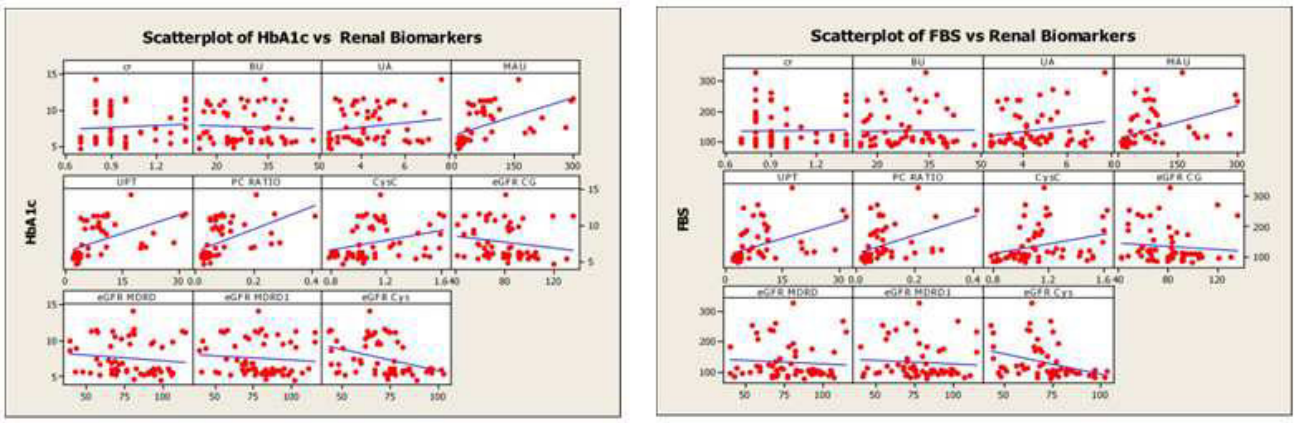
Correlation table				
N=60		FBS	HbA1c	PPBS
Blood Urea (BU) in mg/dL	r	0.010	-0.050	-0.190
	p	0.950	0.680	0.140
Serum Creatinine (Cr) in mg/dL	r	0.030	0.070	-0.180
	p	0.830	0.610	0.180
Cystatin C (CysC) in mg/L	r	0.310	0.310	0.090
	p	0.020	0.010	0.520
eGFR by Cockroft Gault Formula	r	-0.110	-0.190	-0.040

	p	0.390	0.140	0.740
eGFR cystatin C in mg/L	r	-0.360	-0.370	-0.180
	p	0.010	0.000	0.170
eGFR Modification of Diet in Renal Disease(eGFR MDRD)	r	-0.080	-0.140	0.050
	p	0.530	0.300	0.700
eGFR by Abbreviate MDRD formula (eGFR MDRD1)	r	-0.090	-0.100	0.100
	p	0.510	0.470	0.460
Fasting Blood Sugar (FBS) in mg/d	r		0.840	0.820
	p		<0.0001	<0.0001
HbA1c in %	r	0.840		0.860
	p	<0.0001		<0.0001
Microalbuminuria (MAU) in mg per Gm of Creatinine	r	0.460	0.530	0.220
	p	0.000	<0.0001	0.090
Protein Creatinine RATIO (PC Ratio)	r	0.430	0.510	0.190
	p	0.000	<0.0001	0.150
Post Prandial Blood Sugar (PPBS) in mg/dL	r	0.820	0.860	
	p	<0.0001	<0.0001	
Urine Creatinine (UCR) in mg/dL	r	0.180	0.090	0.190
	p	0.170	0.510	0.150
Urine Protein (UPT) in mg/dL	r	0.450	0.520	0.220
	p	0.000	<0.0001	0.100

r=Pearson correlation coefficient p=significance level

Microalbuminuria was found to be correlating well with FBS, PPBS and HbA1c indicating that it is the marker of choice for the detection of renal involvement with patients with diabetes. A similar significant correlation was observed between Blood pressure with cystatin C, microalbuminuria, Urine protein, the urine protein creatinine ratio and eGFR using cystatin C. In all significantly correlation tests microalbuminuria, cystatin C and cystatin C based GFR were better correlating (by higher r value).

Figure 5
Scatter plot of Glycemic markers vs Renal biomarkers



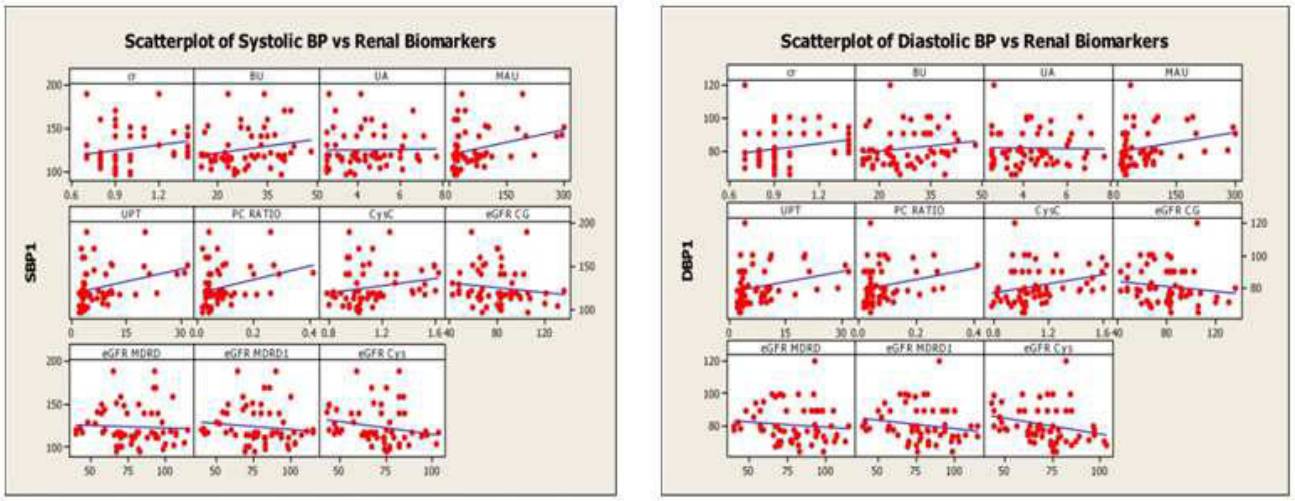
A significant positive correlation was observed for both Fasting Blood Sugar (FBS) and HbA1c with Microalbuminuria (MAU), Urine Protein (UPT), Urine Protein Creatinine Ratio (PC Ratio) and cystatin C. A Significant Negative correlation was observed with eGFR using cystatin C.

Table 4
Correlation of different biomarkers against Systolic and diastolic blood pressure.

N=60		DBP1	SBP1
Blood Urea in mg/dL	r	0.173	0.214
	p	0.187	0.101
Serum Creatinine in mg/dL	r	0.226	0.192
	p	0.083	0.142
CysstatinC in mg/L	r	0.28	0.214
	p	0.03	0.1
eGFR by Cockroft Gault Formula	r	-0.152	-0.142
	p	0.245	0.28
eGFR cystatin C	r	-0.275	-0.212
	p	0.033	0.103
eGFR MDRD formula	r	-0.127	-0.063
	p	0.334	0.635
eGFR MDRD abbreviated formula	r	-0.176	-0.126
	p	0.178	0.336
Microalbuminuria in mg per gm of creatinine	r	0.275	0.318
	p	0.034	0.013
Urine Protein Creatinine RATIO	r	0.257	0.291
	p	0.048	0.024
Urine creatinine in mg/dL	p	0.11	0.155
	r	0.403	0.239
Urine Protein in mg/dL	p	0.276	0.316
	r	0.033	0.014

r=Pearson correlation coefficient p=significance level, DBP1: Diastolic Blood Pressure, SBP1: Systolic Blood Pressure.

Figure 6
Scatter plot of Blood Pressure vs Renal biomarkers



A significant positive correlation was observed for both Systolic and Diastolic blood pressure with microalbuminuria (MAU), Urine Protein (UPT), Urine Protein Creatinine Ratio (PC Ratio) and cystatinC. A Significant Negative correlation was observed for Systolic Blood pressure against eGFR using cystatin C.

DISCUSSION

Creatinine measurement using the conventional Jaffe method has been used for years and the clinical utility of it questionable[14]. Creatinine based eGFR is considered to be a better marker for the early detection[15,16] of CKD but both creatinine and creatinine based eGFR cannot be used as markers of renal injury especially in patients with borderline renal damage[6,7]. So these methods were being replaced by cystatin and cystatin Based eGFR[17,18,19]. Similarly microalbumin is also considered as a marker for the early detection of renal damage especially in patients with DM and HT. In the Present Study an attempt is

being made to evaluate all the conventional and newer biomarkers for the early detection of renal damage. Even though creatinine is cheaper it lacks sensitivity and specificity and is affected by many variables such as muscle mass, age, sex etc. cystatin C is far superior as a biomarker as the serum levels are not influenced by any of the above variables[20]. But the method is not cost effective and each time venipuncture is essential which may predispose the patient to infections. Urinary microalbumin is found to be a cheaper, reliable and a non invasive marker for the prediction of renal involvement[21]. In this study it was observed that urinary microalbumin is correlating well with glycimic indices in diabetes and blood pressure in hypertension. Our findings were in agreement with the findings of (Yoo, 2011 and Chae, 2012) [22],[23]. This is a pilot study and hence the study population is small. More elaborate studies with large number of subjects in each group will help in a clear understanding of the problem.

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