Extent of DNA damage and changes in eGFR in hypertensive patients

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Abstract— Hypertension(HTN) increases the risk for stroke and coronary heart disease and is a main contributor to mortality. Sustained HTN for an extended period of time can lead to DNA damage due to the oxidative stress or various other reasons. The genetic instability include mutations, chromosomal aberrations and/or unscheduled DNA synthesis. The GFR is the product of filtration rate in single nephron and the number of nephrons in both kidneys. Several formulae were developed for calculating GFR based on serum creatinine and other markers. Estimated GFR using creatinine was calculated by MDRD formula, Cockcroft – Gault formula and CKD – EPI cystatin equation. The GFR values were calculated using creatinine and compared with the eGFR using the newer biomarkers CysC. In vitro mutagen sensitivity analysis showed that the mean number of chromatid breaks per cell (b/c) was significantly higher in hypertensive patients than in controls. The mean number of chromatid breaks per cell (b/c) value correlated well with the eGFR values suggesting the influence if DNA damage in the pathogenesis in HTN.

Index Terms— Creatinine, Cystatin, DNA Damage, eGFR, Glomerular Filtration Rate, Hypertension, Mutagenesis .

1 INTRODUCTION

The HTN increases the risk for stroke and coronary heart disease and is a main contributor to premature death . When HTN is sustained for an extended period of time, actual DNA damage can occur. Most of this damage is in relation to the transference of oxygen and the reactive oxygen ions within the body, causing something called oxidative stress. However, other forms of DNA damage can occur from sustained HTN, prompting other types of damage within the system . Hypertensive patients encounter numerous metabolic and physiological changes occur in hypertensive individuals and most of these processes stem from the associated increased oxidative stress. Increased oxidants and decreased antioxidant enzymes in hypertensive patients have been reported to stimulate the synthesis of reactive oxygen species. These reactive oxygen species/free radicals thus forming an oxidant-antioxidant imbalance and cause oxidative damage to the cellular macromolecules and even the genetic material .

The genetic instability can be mutations, chromosomal aberrations and/or unscheduled DNA synthesis. High BP can also produce some DNA damage within the cells of the kidney. Again, this is a result of oxidative stress . As the reactive oxygen ions interact with the DNA, they can cause a strand break or lesion to occur within the cells of the tubule, which is part of the filtration system of the kidneys. Once the filtration is affected, waste can accumulate within the body and cause kidney failure . Just because damage has occurred within the DNA doesn't mean that the cells are permanently affected. Your body is equipped to produce DNA repairing enzymes that can alter injury or modification suffered in a cell. DNA repair is an important line of defense against mutations caused by carcinogens and by endogenous mechanisms . Inter-individual variability in DNA repair capability is an important factor influencing the risk of CAD. DNA repair capacity affords protection against genotoxic carcinogens, whereas a decrease in that capacity results in greater susceptibility to mutations and enhanced genetic instability .

The GFR is the product of filtration rate in single nephron and the number of nephrons in both kidneys. Since GFR cannot be measured directly, it is estimated from urinary clearance of filtration marker most commonly creatinine. Measurement of albuminuria is also helpful in monitoring nephron injury and response to therapy in many forms of CKD, especially chronic glomerular disease . An accurate 24-hour urine for measurement of albumin is “Gold Standard” but measurement of albumin to creatinine ratio in a spot first-morning urine sample is more practical and correlates well . Persistence of urine albumin > 17 mg per gm of creatinine in adult males and >25 mg albumin per gm of creatinine in adult females signifies chronic renal damage irrespective of GFR. Detection of MA is a good screening test for early detection of renal disease and marker for microvascular disease in general.

GFR is estimated using the clearance of substances by the kidney. The renal clearance of any substance is defined as the volume of plasma from which the substance is completely cleared by both the kidneys per unit of time. The renal clearance is measured by using the exogenous (radioisotopic and radioisotopic) and endogenous filtration markers like inulin, 125-iothalamate, 51 Cr-ethylene diamine tetra acetic acid (EDTA), 99mTc- diethylene triamine penta acetic acid (DTPA) and iohexol . Unfortunately these methods are expensive, time-consuming, cumbersome and not free of risks for patient. Above all these methods overestimate the GFR and hence they cannot be easily implemented in laboratories. In routine clinical practice, endogenous markers are commonly used.

The Kidney Disease Outcome Quality Initiative (KDOQI) recently proposed that diagnosis and monitoring of CKD should

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be based on calculate estimates of GFR. Several formulae were developed for calculating GFR based on serum creatinine and other markers . A commonly used surrogate marker for estimate of creatinine clearance is the Cockcroft – Gault (C-G) formula, that estimates GFR in ml/minute . C-G formula employs serum creatinine estimation and the patient's weight to predict the creatinine clearance. Weight measured in kilograms and creatinine measured in mg/dL is used for calculation. A factor of 0.85 is multiplied if the patient is female. This formula is useful because the calculations are simple and can often be performed without the aid of a calculator.

The most advocated formula for the GFR calculation is the one that was developed by the Modification of Diet in Renal Disease (MDRD) Study Group. The adoption of the automatic reporting of MDRD- eGFR had been widely criticized. The most commonly used MDRD formula is the "4-variable MDRD," which estimates GFR using four variables: serum creatinine, age, race, and gender. The original MDRD used six variables includes blood urea nitrogen and albumin levels in addition . The equations have been validated in patients with CKD; however both versions underestimate the GFR in healthy patients with GFRs over 60 mL/minute. The equations have not been validated in acute renal failure. A more accurate version of the MDRD equation also adds serum albumin and blood urea nitrogen (BUN) levels. These MDRD equations are to be used if serum creatinine measurements to isotope dilution mass spectrometry (IDMS). National and International organization are recommending that estimating equations that estimating equations be adopted as a way of overcoming the limitations of serum creatinine, because they incorporate known demographic and clinical variables as correction for the various physiologic factors that affect serum creatinine concentration.

To overcome the disadvantage of calculating GFR based on SCr, new biomarkers such as CysC, beta trace proteins etc. are now being used for the assessment of renal function. These biomarkers are more sensitive for the detection of renal injury and show elevation earlier in the course of disease compared to old biomarkers. CKD is a complex disease that affects multiple organ systems and coexists often with numerous associated conditions, such as CVD, diabetes mellitus, hypertension, lupus, chronic inflammation etc. There is only few CKD prevalence studies conducted in India. Hypertension is responsible for ~25 % of CKD in the United States . The progression of loss of renal function is dictated by several risk factors like hypertension, obesity, proteinuria, smoking, and dyslipidemia, which are potentially modifiable. Elevated blood pressure is a strong independent risk factor for End-stage renal disease. Progression of CKD to ESRD, when prevented, may save healthcare expense and improve quality of life. A concerted approach to manage CKD patients can effectively starts with early identification and management by multiple specialties.

There are several biomarkers in use to detect kidney damage both in the clinic and in research. These biomarkers have been used for a very long time, but are often not very specific. Standard non-invasive biomarkers, such as serum creatinine and general urinalysis provide late estimations of renal function. However, new promising biomarkers are currently being used. These biomarkers in particular are sensitive for the detection of renal injury and show elevation earlier in the course of disease compared to old biomarkers. CysC is freely filtered by the kidney glomerulus and reabsorbed by the tubules.

2 Materials and Methods

The study was conducted at Educare Institute of Dental Sciences, Malappuram, Hrithayalaya institute for preventive cardiology, Trivandrum, Kerala and MES Academy of Medical Science, Perinthalmanna, Kerala. One hundred and twenty clinically proved patients below the age of 70 years were included in the test group. Sixty subjects were consisted as control are are selected from the siblings, teaching and nonteaching staff of the institutes. Informed consents were obtained from all the study subjects included in the study. Detailed clinical, epidemiological and anthropometric characteristics were recorded using proforma.

The selection of the subjects for the study were based on the following inclusion-exclusion criteria. All the subjects included in the study were in the age group of 31 to 70 years. The subjects with the clinical diagnosis of hypertension formed the test group of the study. They had no chronic or acute illness and were not suffering from any known liver and renal diseases and had no autoimmune diseases. The control subjects were not having any diseases and they were not on any medication.

The auscultatory method of blood pressure measurement with a properly calibrated and validated instrument (Elko mercurial sphygmomanometer) is used. At least 2 measurements were made. Systolic BP is the point at which the first of 2 or more sounds is heard and diastolic BP is the point before the disappearance of sounds.

The test group was classified based on blood pressure into 4 classes based on the Seventh Report of the Joint National Committee (JNC) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

Ten ml of fasting venous blood and a random urine sample were collected from all the study subjects after getting the informed consent, as per the criteria laid down by the Institutional Ethics Committee. Three ml of blood was transferred aseptically to a heparinized vacutainer and used for in vitro mutagen sensitivity analysis.

The blood sample and urine samples collected were processed and analyzed at fully automated central laboratory. Random urine samples were used for urinalysis.

Peripheral blood was collected aseptically by venepuncture. Peripheral blood lymphocyte micro-culture was performed as described by Moorhead for determining the mutagen sensitivity . The culture medium was RPMI 1640, supplemented with 20% fetal calf serum, 0.5ml phytohaemagglutinin, 100-units/ml penicillin and 10µg/mL streptomycin. Bleomycin was used to induce chromosomal breakage according to the method of Hsu et al. For mutagen induced chromosome sensitivity analysis the mean breaks/cell (b/c) was calculated. Only frank chromatid breaks were scored.

The occurrence of breaks was expressed as b/c for comparison among subjects. Any individual expression $\geqslant$0.8 b/c was considered sensitive and that $\geqslant$1.0 b/c was considered hypersensitive. 100 metaphases per culture at least was scored and

data were analyzed. The frequency of chromatid breaks was considered as a measure of an individual's DNA repair capacity.

Estimated GFR using creatinine was calculated by MDRD formula, Cockcroft – Gault formula and CKD – EPI cystatin equation.

Results were expressed as mean ± SD. Independent sample't' test was performed using SPSS (windows version 17.0) for comparing various risk/lifestyle factors, biochemical and genetic characteristics , eGFR based on SCr and SCysC using different equations and mean break per cell value of the study subjects and the control subjects.

Parametric correlation by Pearson done to compare various risk factors with HTN among subgroups. A p value < 0.05 were considered to be statistically significant. Association between genetic characteristics, eGFR and HTN was analyzed.

3 Results and Discussion

The study was conducted to evaluate the significance of biochemical and genetic biomarkers in the assessment function of kidney in patients with hypertension and evaluate the usefulness of these parameters for the diagnosis of chronic kidney disease.

Table 1: Anthropometric data of control and test subjects.

	Male			Female	
	Group	Mean ± SD	p-value	Mean ± SD	p-value
Age in Years	Control	38.85 ± 11.63	<0.05	31.31 ± 7.5	<0.05
	Test	58.06 ± 9.56		56.5 ± 10.8	
Height, cm	Control	171.65 ± 5.08	.084	159.9 ± 5.2	0.61
	Test	169.94 ± 4.43		159.1 ± 5.5	
Weight, kg	Control	71.97 ± 6.39	<0.05	60.01± 5.11	<0.05
	Test	75.47 ± 8.64		64.5 ± 6.79	
BMI	Control	24.71 ± 1.7	<0.05	23.5 ± 1.76	<0.05
	Test	25.81 ± 2.71		24.9 ± 2.55	

Anthropometric data of control and test subjects are given in table 1. The mean age of the male test subjects was 58.06 ± 9.56 whereas that of the control subjects was 38.85 ± 11.63. The mean age of the female test subjects was 56.56 ± 10.8 whereas that of the control subjects was 31.31 ± 7.5. The mean BMI of male control subject was 24.71 ± 1.7 and that of test group was 25.81 ± 2.71. The mean BMI of female control subject was 23.52 ± 1.76 and that of test group was 24.93 ± 2.55. The BMI of both male and female test subjects was significantly higher than that of the control subjects.

Distribution of systolic (SBP) and diastolic blood pressure (DBP)of the test and control subjects is given in table 2. The systolic blood pressure and diastolic blood pressure of the patients showed significant elevation even on treatment.

The test subjects of the study were divided into 3 groups namely pre – HTN, stage 1 HTN and stage 2 HTN. The distribution of the test subjects according to the DBP and SBP are given in table 5 and figure 5. Majority of the test group subjects were in stage 1, followed by stage 2. There were only 17 test group subjects in the pre – HTN sage. All the control subjects in the present study had a normal BP of 112±7/ 74±5.

The GFR values were calculated using creatinine and compared with the eGFR using the newer biomarkers CysC. These values are given in tables 3 & 4. The eGFR calculated by both the parameters showed significant decrease in the test subjects compared to the control subjects. The eGFR using Cys C was better comparable with renal changes than the conventional eGFR.

Table 2: Distribution of BP of the control and test subjects at different stages of HTN

	Stages of BP based on SBP			
	Normal BP n = 60	Pre HTN n = 17	Stage 1 HTN n = 64	Stage 2 HTN n = 39
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
SBP (mmHg)	112 ± 7	126 ± 11	145 ± 6	181± 22
DBP (mmHg)	74 ± 5	79 ± 9	89 ± 8	105 ± 5

Table 3: eGFR using creatinine and CysC in male subjects

		Male	
	Group	Mean ± SD	p-value
eGFR MDRD	Control (n = 34)	79.19 ± 24.26	<0.05
	Test (n = 68)	55.96 ± 27.23	
eGFR CysC	Control (n = 34)	113.5 ±36.95	<0.05
	Test (n = 68)	44.99 ± 20.27	

FIGURE 1: Different stages of BP based on DBP and SBP

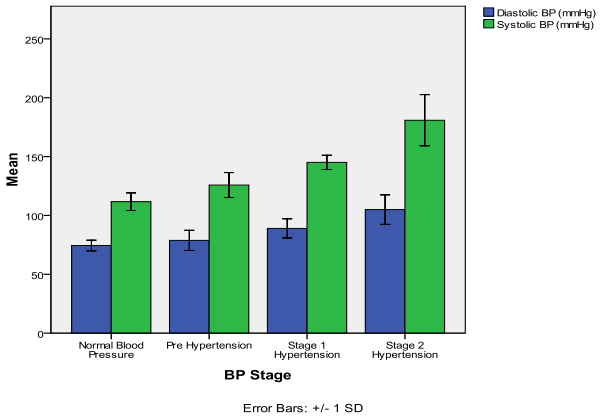

Table 4: eGFR using creatinine and CysC in female subjects

		Female	
	Group	Mean ± SD	p-value
eGFR MDRD	Control (n = 26)	162.16 ± 58.83	<0.05
	Test (n = 52)	63.84 ± 35.32	
eGFR CysC	Control (n = 26)	153.14 ± 45.76	<0.05
	Test (n = 52)	47.99 ± 23.84	

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GFR is considered to be a measure of renal function and usually measured in terms of creatinine clearance as it is not influenced by diet. But an accurate 24-hour urine measurement is not possible especially the subject is treated as outpatient. So it was universally accepted to use a calculated GFR value based on certain equations. Estimated GFR based on creatinine using MDRD equation was reported to be more reliable than the other methods. In this study eGFR was calculated based on creatinine as well as CysC. In both the cases the eGFR was significantly lower in the test group than that of the controls both in males and females. These values were found to be correlating well with the different stages of HTN. This is well in agreement with the earlier findings . In this study CysC and creatinine-based eGFR was more or less same both in the test and control subjects. This is not in agreement with the finding of Shilpak et al, who reported that CysC is superior to creatinine. This may be due to the test group subjects of the present study were not clinically diagnosed as CKD patients whereas Shlipak et al had carried out their work on patients who are already suffering from renal complications.

In vitro mutagen sensitivity analysis showed that the mean number of chromatid breaks per cell (b/c) was significantly higher in hypertensive patients than in controls (Tables 5 & 6). Increased a mean b/c value which is an indicator of increased DNA damage and decreased DNA repair pro-efficiency was found to be significantly associated HTN.

Table 5: Mean b/c value of male subjects

	Group	Male	
		Mean ± SD	p-value
Mean breaks/cell (b/c)value	Control (n = 34)	0.68 ± 0.04	<0.05
	Test (n = 68)	0.76 ± 0.07	

Table 6: Mean b/c value of female subjects)

	Group	Female	
		Mean ± SD	p-value
Mean breaks/cell (b/c)value	Control (n = 26)	0.70 ± 0.04	<0.05
	Test (n = 52)	0.77 ± 0.07	

DNA repair proficiency will help to identify the population who are at risk of developing complications. In this study b/c value was taken as a marker to identify the genetic instability. The mean b/c values of the test group subjects were significantly higher in both male and female test subjects. BP both systolic and diastolic was found to be correlating with the b/c value. This may be due to the genetic instability produced due to DNA damage within the cells of the kidney. The increased b/c value indicates decrease efficiency in DNA repair which may result in greater susceptibility to mutation and enhanced genetic instability. The present finding clearly indicates the increased morbidity and mortality in patients with HTN.

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