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APPRAISAL OF DIABETIC NEPHROPATHY THROUGH GFR ESTIMATION IN VIEW OF VARIOUS CYSTATIN C EQUATIONS. A RELATIVE INVESTIGATION

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ABSTRACT

BACKGROUND: Diabetes mellitus is a chronic polygenic syndrome which impairs carbohydrate metabolism. The causes for impaired carbohydrate metabolism are deficiency or decreased effectiveness of hormone insulin known as peripheral insulin resistance or due to decreased ratio between insulin and anti insulin hormones. Commonest complication Diabetic nephropathy results due to the glycation of the basement membrane protein which occurs due to longstanding hyperglycemia. Estimation of GFR is the most accurate method of detecting the initial renal impairment for which the most commonly used parameter is serum creatinine regardless of its demerits. An alternative marker is serum cystatin c which is found more advantageous. Aims and Objectives: Primary **OBJECTIVES:** - To estimate the level of cystatin c in patients with long standing type II diabetes mellitus. To compare the serum levels of cystatin c in type II diabetic patients and controls. Secondary objectives: - To compare the serum levels of creatinine in type II diabetic patients and controls. Estimation of Glomerular Filtration Rate by various equations using the parameters cystatin c and creatinine.

METHODS: Study was conducted both as a cross- sectional study involving 60 type II diabetic patients (based on elevated HbA1c levels) and a case - control study which included 60 non- diabetic controls and 60 type II diabetic patients. Serum level of both cystatin c and creatinine were measured in both groups. Serum creatinine estimated by Jaffe's kinetic method and serum cystatin c by immunoturbidimetric method.

RESULTS: Serum levels of both cystatin c and creatinine were significantly increased in cases as compared to the non- diabetic controls. The most significant equation for estimating GFR was CKD- EPI (cystatin c based).

CONCLUSION: Cystatin c is an alternative to creatinine. Cystatin c based CKD- EPI formula may be considered for estimating GFR than creatinine based MDRD formula.

KEYWORDS :

INTRODUCTION

Diabetes mellitus is a chronic polygenic syndrome which impairs carbohydrate metabolism. This is a disorder where there is decreased use of blood glucose level which can result in hyperglycemia. The causes for impaired carbohydrate metabolism are deficiency or decreased effectiveness of hormone insulin known as peripheral insulin resistance or due to decreased ratio between insulin and anti insulin hormones which may lead to hyperglycemia along with secondary changes in metabolism of proteins, lipids, electrolytes and water with grave sequences if left untreated[1]. About 5%-10% cases of diabetes mellitus is included in the category of type 1 diabetes mellitus or otherwise called as juvenile diabetes mellitus. The most common variety of diabetes mellitus is the type 2 diabetes mellitus or else known as adult- onset diabetes mellitus. This category includes 90% of total diabetic patients.

Regarding type 2 diabetes mellitus another major segment which has to be considered with importance is the complications of this disease. This can be classified into two, acute and chronic complications. Acute complications mainly involve diabetic ketoacidosis, lactic acidosis, hyperosmolar nonketotic coma and hypoglycemia. While chronic complications include diseases which affects the blood vessels. Diseases affecting blood vessels mainly include atherosclerosis of medium sized blood vessels, if this occurs in coronary artery this will result in myocardial infarction. In peripheral vessels it can lead to gangrene. If atherosclerosis occurs in small arteries it is known to be micro- angiopathy. This would lead to the most common chronic complication of diabetes called as diabetic retinopathy and diabetic nephropathy[1]. Diabetic nephropathy results due to the glycation of the basement membrane protein which occurs due to longstanding hyperglycemia[1]. This condition has a manner of very slow progression and that is why it is considered as one of the late complication of this disease. In type 2 diabetes the usual change that occurs in kidney is mainly because of the ischemia resulting in the contraction of kidney.

The presence of increased extracellular matrix in interstitium as well as in glomerulus is the main factor which links the state of hyperglycemia with cell dysfunction in diabetic nephropathy. So in diabetic nephropathy the normal structure and functional capacity of the kidney will be lost. Though microalbuminuria is the first detectable functional abnormality, estimation of Glomerular Filtration Rate (GFR) identifies the initial impairment in renal function[4]. By measuring GFR there are several merits for a doctor in treating a patient with renal disease, such as it will give an idea of how should be the treatment, for monitoring progression and also about the stage when renal replacement therapy is required[1]. . There are several methods for measuring GFR. For the early diagnosis of a renal disease the introduction of new markers as well as new methods to estimate GFR has proved really useful[1]. The gold standard for estimation of GFR is clearance of exogenous substances such as Inulin, Iohexol, 57Cr EDTA, and 99mTc DTPA or [125I] iothalamate. These techniques are time consuming, labor intensive, expensive and require administration of substances that make them incompatible with routine monitoring[5].

The most commonly used endogenous marker is creatinine. This parameter is freely filtered and is usually affected by several factors that mark its limitation to be used as a marker. The factors affecting serum creatinine level are Muscle mass, Nutritional status, Gender, Age and the most important factor which makes it unsuitable is that its serum level remains within the normal range despite of the marked loss of kidney function[2]. So Cystatin C, a cysteine protease inhibitor which is freely filtered by renal glomeruli and metabolized by proximal tubule is identified as a new promising marker for renal failure. Due to several reasons it is found to be more beneficial, as they are constantly produced by all cells, independent of age, sex, muscle mass and more significantly its concentration is almost totally dependent on GFR[6].

Hence this study is conducted to prove the significance of serum cystatin c over serum creatinine as a marker of renal impairment and to express the importance of GFR estimation using cystatin c based equation in the assessment of diabetic nephropathy.

METHODS

This study was performed as cross- sectional as well as a case control study. For cross sectional study the subjects included 60 cases who have the disease type 2 diabetes mellitus. They were selected depending on their elevated HbA1c levels. Case control study included 60 patients who were taken as cases having diagnosed with type 2 diabetes mellitus. There are 60 controls who were normal healthy individuals having all routine parameters within the reference range, thus a total of 120 subjects were involved for the case- control study. For both the study cases were the patients attending the outpatient wing of the department of Endocrinology, Amrita Institute of Medical Sciences, Kochi. Study was conducted during the year 2014- 2016. Age of the diabetic subjects and controls ranged from 30 to 70 years. Patients with cardiac diseases, thyroid dyscrasias, hypertension, malignancy and those who were on steroids or cyclosporins are excluded from the study. The study was conducted as per the guidelines and approval of the ethical committee of AIMS and since data collection is part of routine care no consent was required.

Whole blood and serum are the required samples for this study. For the estimation of HbA1c the specimen type required is whole blood which

is taken in a vacuum collection tube containing EDTA. The type of specimen used for the creatinine estimation is serum and EDTA or heparinized plasma and estimation of cystatin c is done by using standard sampling tube. Serum and EDTA or heparinized plasma is the specimen of choice. The principle used in the estimation of HbA1c is ion- exchange high performance liquid chromatography (HPLC) in the D- 10 Hemoglobin A_{1c} program. Methodology for the estimation of serum creatinine is done in BECKMAN COULTER AU 2700 PLUS. The principle of the procedure used for estimation is Jaffe method. Estimation of cystatin c was also done in BECKMAN COULTER AU 2700 PLUS by immunoturbidimetric method.

STATISTICALANALYSIS

Statistical Analysis was done using IBM SPSS statistics 20 windows (SPSS Inc., Chicago, USA). For all the continuous variables the results are given as mean ± standard deviation and for categorical variables as percentage. To compare the mean difference of numerical variable between cases and controls two sample t- test was applied. To compare the mean difference of numerical variable within the cases and control group paired t-test was applied. Probability value (p value) less than 0.05 is considered for statistical significance. To study the relationship between two variables correlation coefficient was computed. To test the statistical significance of the difference in glomerular filtration rate estimation using various equations Repeated Measure of ANOVA was used.

RESULTS

Regarding the demographic variables such as gender and age no statistically significant values were obtained. There are 34 males and 26 females in the case group and 36 males and 24 females in the control group. An association between gender and group were taken. The p value obtained for this was not considered as statistically significant (p value = 0.711). A comparison between the age of the study population and the group were taken for which the p value was 0.060 which was not below 0.05 and hence no statistical significance observed. Comparison of levels of HbA1c was done among the groups and the p value is observed to be significant (p value = < 0.001), the mean HbA1c level is higher in cases than in controls.

Mean distribution of cystatin c among gender in cross- sectional study

For the estimation of serum cystatin c level in patients with long standing type 2 diabetes mellitus the mean value of cystatin c in males was 1.14 ± 0.35 and for females it was 1.04 ± 0.23. (Table 1)

Comparison of serum cystatin c and serum creatinine between cases and controls

On comparing the serum level of cystatin c and creatinine between the cases and the controls the mean value of both serum cystatin c and creatinine was found to be higher among the case group and both were observed as statistically significant. (Table 2)

GFR estimation using various equations based on cystatin c (CKD-EPI, LEBRICON, GRUBB) and creatinine (MDRD) were compared among the group of cases, controls and between the groups. Also a pair wise comparison was done among the case group and controls. Estimated GFR using theses cystatin c based and creatinine based equations is then correlated among the case group only.

Comparison of GFR estimation by cystatin c and creatinine based equation within the case group

The mean value of GFR estimation by CKD- EPI is 74.26 ± 22.55 and for MDRD based on creatinine is 81.56 ± 20.95. The p value obtained for this comparison is 0.002 which is less than 0.05 and hence it is considered as statistically significant. (Table 3)

Comparison of GFR estimation using cystatin c and creatinine based equations are done in control group where all the four comparison gave a p value which is less than 0.05 showing all of them are statistically significant.

Comparison of GFR estimation by cystatin c and creatinine based equation between cases and controls

The comparison of GFR estimation using these different equations between the group of cases and controls were statistically significant. (Table 4)

Correlation between cystatin c based and creatinine based equations for the estimation of GFR among the case group

Correlation between GFR estimation done by using three cystatin c based and creatinine based equation is done among the case group where the cystatin c based GRUBB formula gave a negative correlation with creatinine based MDRD. (Table 5)

Comparison of GFR estimation using cystatin c and creatinine based equations between cases and controls

As the formula of GRUBB is showing a negative correlation with creatinine formula the equation of GRUBB is omitted and a further comparison is made between the GFR estimated values that are obtained by using the cystatin c based and creatinine based formula between cases and controls. (Table 6) Following this a pair wise comparison was done so as to find out among the three equations which is the most suitable for estimating GFR in order to detect the early renal impairment.

A statistically significant difference is found on comparing between the cystatin c based equations with creatinine based equation (p value = <0.05) in the control group. But in the case group on comparing between the creatinine and cystatin c based equation statistical significance is found only with the comparison between CKD- EPI (cystatin c) and MDRD (creatine) equation.

DISCUSSION

One of the commonest endocrinopathy with various etiologies is Diabetes mellitus. It is a chronic metabolic disorder where there is abnormality in protein, fat and carbohydrate metabolism resulting in defects in insulin secretion, insulin action or both[1]. Complications of Type 2 diabetes mellitus is the major part of concern in this study. While discussing about the long term complications of Type 2 diabetes mellitus, it is classified into two, Acute and chronic complications. Chronic complications include diseases that are caused due to the damage of blood vessels. Here we are considering one of the chronic complications and that is diabetic nephropathy.

Usually most often it is found associated with Type 2 diabetes mellitus, but this can also occur to patients who are suffering from Type 1 diabetes mellitus. Ideally for a patient who is diagnosed with Type 2 diabetes mellitus a regular check up on the blood pressure as well as urine and blood test must be done minimum by every six months so as to monitor the status of the renal system[2]. As the duration of the disease increases there is a high chance of developing diabetic nephropathy in around half of the patients with Type 2 diabetes mellitus. Diabetic nephropathy rarely develops before 10 years of duration in patients with type 1 diabetes mellitus. In case of type 2 diabetic patients the incidence of diabetic nephropathy is seen only in those who have had the disease for more than 10 to 20 years of duration after which the rate slowly declines[7]. Diabetic nephropathy has been classified into different stages along with the variations of GFR. GFR can be calculated using several markers, of them the most commonly used parameter is creatinine. Serum creatinine is widely used as an indirect marker as it is influenced by muscle mass and diet. Estimation of GFR using Inulin is taken into consideration but this process is time consuming as well as costly. So for calculation of GFR use of prediction equations where serum creatinine and other variables are involved is recommended by the National Kidney Foundation for the diagnosis and stratification of chronic kidney diseases[8]. Since serum creatinine is also influenced by sex, race, age other than the muscle mass and diet another alternative parameter serum cystatin c has been proposed for the estimation of GFR. Cystatin c is a protein having 122 amino acid and it is a member of the family cysteine proteinase inhibitors. It is produced by almost all nucleated cells and that too at a constant rate[9]. Serum cystatin c has been reported to be modulated by several non renal factors such as steroids, thyroid dyscrasias, smoking and malignancy. In spite of these limits there have been several studies done which suggests the dominance of serum cystatin c over serum creatinine in patients with early and moderate renal dysfunction[4,5].

In this study we have two different types of study design. One was a cross- sectional study and the other was a case control study. Statistically significant result is obtained only for the comparison of levels of HbA1c between the group of cases and controls (p Value = <0.001). For other demographic variables no statistical significant results were obtained.

The first part of the primary objective was to estimate the level of cystatin c in patients with long standing type 2 diabetes mellitus. For this the average value of cystatin c in males was 1.14 ± 0.35 and for females it was 1.04 ± 0.23. (Table 1) The second part of the primary objective of this study was to compare cystatin c between cases and

controls. This comparison is statistically significant since the p value obtained is below 0.05. (Table 2) A study done by Byung- Wan Lee et al[51] involving 320 type 2 diabetic patients also showed similar results in which an increase in the level of serum cystatin c were seen in the case group[10]. The secondary objectives in this study were mainly two. One was to compare the serum creatinine level between cases and controls and the second was to estimate glomerular filtration rate by different equations using the parameters such as creatinine and cystatin c. The p value for the comparison of serum creatinine between cases and controls is significant (p Value = <0.001). (Table 2) Similar comparison was seen in a study done by Michele Mussap et al[11]. In another study done by Ashwin Kumar A S et al[12] in which they compared the serum level of cystatin c and creatinine in patients with type 2 diabetes mellitus with the control group they got the result as both serum cystatin c as well as serum creatinine was higher among the group of patients than with that of the control group.

Present study has taken a single creatinine based MDRD equation and three cystatin c based formulas such as LEBRICON, GRUBB and CKD- EPI. We have done comparison of these cystatin c based equations with the creatinine based equation among the case group, among control group and between the case and control group. On comparison of the GFR estimations using the cystatin c based LEBRICON, GRUBB and the CKD- EPI formula with that of the creatinine based MDRD equation within the case group the only comparison which showed a statistical significance was between CKD- EPI (cystatin c) and MDRD (creatinine) since the p value obtained is 0.002. (Table 3) A similar report was obtained in a study done by S. Avinash et al[13]. In this study they did the comparison of GFR estimations between creatinine based MDRD and Cockcroft- Gault formula with the cystatin c based CKD- EPI formula. In the result they were able to show that the most accurate formula for the assessment of renal dysfunction by estimating GFR was cystatin c based CKD- EPI formula. On comparing the GFR values between cases and controls all the equations proved significant. (Table 4) Even though the equation of Grubb proved significant it could not be taken into consideration as the average value of GFR estimation in case group was higher (89.66 ± 26.33) to that of the value obtained in control group (76.95 ± 12.51), also a negative correlation with creatinine formula is found. (Table 5) Thus this equation is not considered for evaluating GFR. This result is partially supported by the study done by Grubb A et al[14] in which they got the result as this equation is only marginally better when compared to that of the creatinine based MDRD equation. Supporting articles based on study where a negative correlation is not found even though article showing that this equation is beneficial only marginally is present. So further studies must be carried out to establish this equation as a GFR calculator. After neglecting the GRUBB formula another comparison of GFR values is done between cases and controls (Table 6) where all the three proved significant. Of the three the least mean value of GFR estimation obtained for cases is by using the CKD- EPI equation and this shows that this cystatin c based CKD- EPI formula can be considered to be significant. The finding that cystatin c based equations is better to creatinine formula has been supported by several studies done by Uzun H et al[15], Min Zhang et al[16], Vishwanathan et al[17], Hany s. Elbarbary et al[18] and Ashwin Kumar AS et al[19]. Thus finally a pair wise comparison was done among the group of cases and controls where the only cystatin c based equation that gave a statistically significant result is again CKD- EPI. Our study is able to show that equations based on cystatin c can be considered first rather than creatinine based equation for measuring GFR so as to assess the early renal malfunctions. This is supported by studies done by Jeong Seon Yoo et al[20], Nabil A. El-Kafrawy et al[21] and Christensson AG et al[4].

CONCLUSION

In conclusion it has been able to prove successfully that the early assessment of any damage occurring to the kidneys can be detected well with the parameter cystatin c though creatinine also proved to be significant. Also evaluation of renal impairment done by the estimation of GFR with the cystatin c based CKD- EPI equation is more enhanced than with the other cystatin c based equations and creatinine based equation. More studies can be done using other indicator such as urine microalbumin and gold standard methods for the improved assessment of cystatin c as a marker of renal dysfunction. This may allow the early detection and better management of diabetic nephropathy.

Table 1: Mean distribution of cystatin c among gender in cross-sectional study

Gender	n = 60	Mean ± SD
Male	34	1.14 ± 0.35
Female	26	1.04 ± 0.23

Table 2: Comparison of serum cystatin c and serum creatinine between cases and controls

Parameters	Cases (n = 60)	Controls (n = 60)	P Value
Cystatin c	1.10 ± 0.31	0.94 ± 0.15	0.001
Creatinine	0.955 ± 0.207	0.82 ± 0.11	<0.001

Table 3: Comparison of GFR estimation by cystatin c and creatinine based equation within the case group

GFR estimation equations	n	Mean ± SD	p value
CKD- EPI(cys) MDRD(crea)	60	74.26 ± 22.55 81.56 ± 20.95	0.002
LEBRICON(cys) MDRD(crea)	60	79.47 ± 19.08 81.56 ± 20.95	0.328
GRUBB(cys) MDRD (crea)	60	89.66 ± 26.33 81.56 ± 20.95	0.138

Table 4: Comparison of GFR estimation by cystatin c and creatinine based equation between cases and controls

Equations	Group		P Value
	Cases	Controls	
CKD- EPI (cystatin c)	74.26 ±22.55	84.54 ± 16.97	0.006
LEBRICON (cystatin c)	79.47 ± 19.08	88.43 ± 14.15	0.004
GRUBB (cystatin c)	89.66 ± 26.33	76.95 ± 12.51	0.001
MDRD (creatinine)	81.56 ± 20.95	96.42 ± 17.64	<0.001

APPENDIX

Table5: Correlation between cystatin c based and creatinine based equations for the estimation of GFR among the case group

Cystatin c based Equations	n	Creatinine based Equation	p Value
		Correlation coefficient	
CKD- EPI	60	0.590	<0.001
LEBRICON	60	0.594	<0.001
GRUBB	60	- 0.520	<0.001

Table6: Comparison of GFR estimation using cystatin c and creatinine based equations between cases and controls

Methods	Cases		Controls		Mean difference	p Value
	Mean	SD	Mean	SD		
CKD EPI (cys)	74.26	22.56	84.55	16.98	10.29	<0.001
LEBRICON (cys)	79.47	19.08	88.44	14.15	8.97	<0.001
MDRD (crea)	81.56	20.95	96.42	17.65	14.86	<0.001

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