Section A-Research paper
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A study of Cystatin C and Adenosine Deaminase as novel biomarkers for Chronic Kidney Disease

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Abstract

In this study was investigate thatcystatin C and ADA as novel biomarkersfor chronic kidney disease. Cystatin C 1.3kDacompound which is filtered from the blood stream through the kidneys.Adenosine deaminase is one of purine metabolic enzyme which is involved in adenosine breakdown and in development and maintenance of immune system. chronic kidney disease patients were studied in two groups namely test group of chronic kidney disease patients and normal subjects of control group; each group consists of 30 subjects.Cystatin C and Adenosine deaminase levels were significantly increased in severe kidney disease patients when compared with normal subjects.We conclude that the concentration of Cystatin C and ADA activity is a useful index for the diagnosis of chronic kidney disease

Key words: Cystatin C, ADA, Creatinine, and kidney disease

Introduction

Kidney diseases are a major public health issuearound the world. In India, over recent years, there has been a significant rise in the number of patients with chronic kidney disease (CKD), where there is increased number of patients undergoing dialysis to keep their life. The chronic kidney

diseasedcondition where the dysfunction of kidneys due to various factors resulting in accumulation of waste and toxic materials inside the body such as urea and creatinine (Cr), therefore tests of urea and Creatinine tests is routinely used to evaluate kidney function, in addition to follow-up of control and treatment of this disease [2,3].

Acute and chronic kidney diseases are the two types of kidney diseases. Based on the presence of anemia and the kidney size, these types of kidney diseases can be differentiated [4]. Chronic kidney disease (CKD) is defined as an abnormal low glomerular filtration rate (GFR) due to slowly progressive loss of kidney function over a long period [5]. In general, CKD leads to anemia and small kidney size[6]. Indeed, the kidneys lose about (85– 90) % of its function, meaning that end stage kidney disease had done, thus the dialysis is needed [5]. Dialysis is alternate to renal replacement therapies, to remove toxic, metabolic compounds and water from blood stream.[4].Ideal endogenous blood substance observed and maintained by GFR [3,4].

CysC is a basic soluble protein synthesized by nucleated cells. Cysmetabolized in tubular cells and not reabsorbed in kidney.When compared with creatinine, Cyc has a more stable rate of production with less intra volatility serum levels are also not influenced by non GFR determinants[6].

Adenosine deaminase (ADA) is one of the purine metabolic enzyme which plays a major role in irreparable hydrolytic deamination of both adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively, the step which is essential in the adenosine cycle, and a part of the purine salvage pathway[7,8].

ADA is widely present in many tissues, especially in small intestine, liver, and kidney.Many studies had reported the effect of this enzyme deficiency on the immune system and it is used as a marker for assessment of cell-mediated immunity in man[9,10]. There are two major principled isoenzymes: ADA1 and ADA2, which differ in optimal pH, Michaelis constant as well as relative substrate specificity frames. ADA2 is the predominant isoenzyme in the sera in all infectious diseases. High total activity of ADA that is reported in many pathological states is due to high activity of ADA2 isoenzyme.ADA is echo-enzyme it is found on the cell surface of many cell types. It has important role in different metabolic and pathological conditions, such as the intra renal metabolic regulation of kidney function [8].

Materials and methods:

chemicals: Cystatin C, ADA and creatinine levels were measured in morningevery day. Creatinine was determined by enzymatic method creatinine para-amino phenazone (PAP) assay. Cystatin C concentrations were measured by using immunoturbidimetry method. Adenosine Deaminase kits (EPI023)were purchased from sigma Aldrich. All the other chemicals used were of analytical grade purchased from SRL chemicals, Chennai.

Experimental design: Out of sixty samples divided into two groups, each group contains thirty samples which include,

Group I – Control (Normal Subjects)

Group-II – Chronic Kidney Disease patients.

Sample size was selected by using formula. The present study was planned and executed between Aug 2019 to Aug 2020 in the Department of clinical biochemistryand Central Research laboratory, Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu, India.

Ethical Concern: Ethical clearancenumber(IRB/005/2019)was obtained from the Institutional Ethical committee meeting conducted at Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu, India.

Statistical Analysis: Statistical analysis was done using SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA). p values <0.05 consider as highly significant

Results

Table.1. shows that the concentration of Creatinine levels was significantly (P<0.001) increased in chronic kidney diseases when compared with normal subjects but in gender wise distribution, there was significant difference in serum Creatinine levels between male and female of control and chronic kidney disease patients.

Particulars	Group –I Healthy Individuals		Group-II Chronic Kidney Disease		P Values
	Female	Male	Female	Male	
Creatinine	0.79±0.08	1.07± 0.1	3.98±0.33	5.22±0.53	<0.001
Cystatin C	0.72±0.07	0.89±0.09	4.76±0.41	4.99±0.48	

Figure 1 shows that the concentration of Cystatin C levels was significantly (P<0.001) increased in chronic kidney diseases when compared with normal subjects but in gender wise distribution there was no significant difference in Cystatin C levels between male and female of control and chronic kidney disease patients.

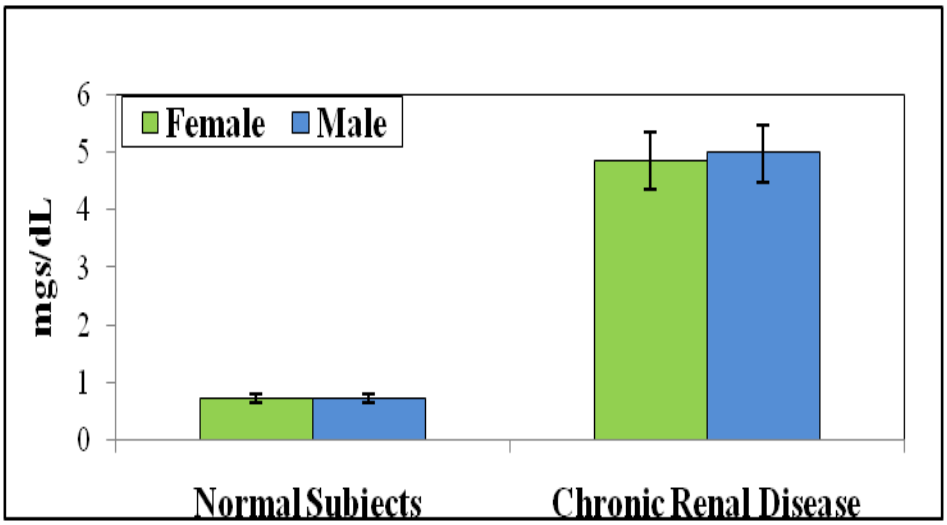

Figure -1. Serum Cystatin C Concentrations in Normal subject and Chronic Renal Disease patient

Role of Adenosine Deaminase in chronic renal disease

Table. 1. Showed that the Adenosine Deaminase activities in chronic renal disease patients. Adenosine deaminase activities were significantly (P<0.001) increased in chronic renal disease patients when compared with normal subjects.

Particulars	Group –I Healthy Individuals		Group-II Chronic Renal Disease		P Values
	Female	Male	Female	Male	
ADA (U/L/min)	16.25 ±1.5	16.67 ± 1.6	26.88 ± 2.3	26.83 ± 2.3	<0.001
Creatinine	0.79±0.08	1.07± 0.1	3.98±0.33	5.22±0.53	

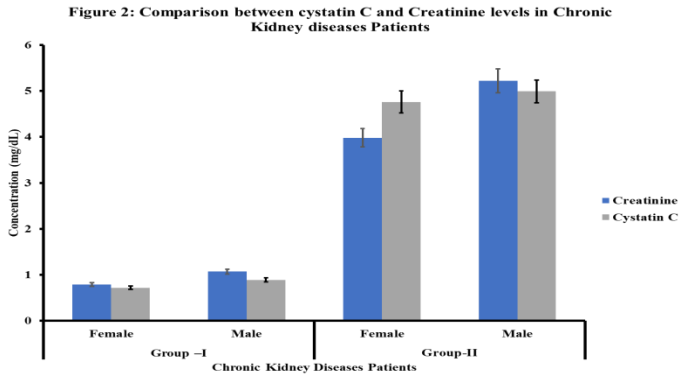

Figure 2: Comparison between cystatin C and Creatinine levels in Chronic Kidney diseases Patients

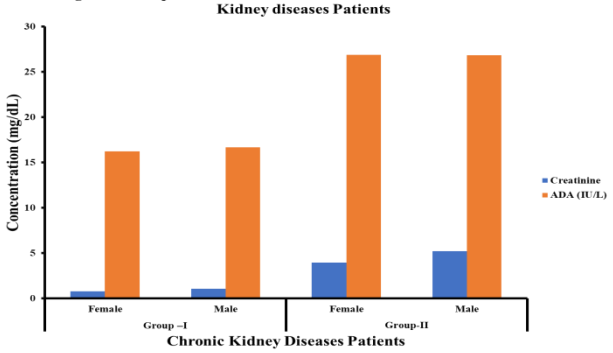

Figure 3: Comparison between ADA and Creatinine levels in Chronic Kidney diseases Patients

Discussion

Cystatin C and ADA was estimated in chronic kidney disease patients. Glomerular Filtration Rate is defined as the volume of plasma completely cleared of aparticular substance by the kidneys in aappropriatetime. GFR is routinely assessed by measuring thequantification of serum markers such as blood urea nitrogen and serum creatinine [5]. The otherparameter for determining GFR is to measure the clearance of exogenous substances such as inulin, iohexol, 51Cr – EDTA, 99mTc labelled diethylenetriaminepenta acetic acid (DTPA) or 125I – labelled iothalamate.These techniques are time

consuming, expensive, labor – intensive and require administration ofsubstances that make them incompatible with routine monitoring.

Urea and Creatinine estimation in urine has become the most commonly used for renal function. However, urea concentration in the blood can vary based on diet, hepatic function and numerous disease states [2]. Furthermore, concentration of serum creatinine in the blood stream is related to muscle mass, age and gender. Serum creatine and tubular creatine concentrations are direct proportional, it leadsto an over estimation of GFR in patients with moderate to severe decrease in GFR (<50 mL / min)[6].

plasma cystatin C (Cys C) may be a better marker for GFR estimation than serum creatinine, because it is low molecular weight (1.3kDa).

Cys C added an advantage over creatinine because of itshave age and gender independence. Cystatin C is a non-glycosylated protein produced by all nucleated cells. Cystatin C contains 122 amino acids and also acts as a cystein proteinase inhibitors. It expressed in all nucleated cells and is produced at constant rate. Cystatin C levels were significantly ($P<0.001$) increased when compared with normal subjects due to Cystatin-C is a potent inhibitor of lysosomal proteinases and cysteine proteases.The use of serum Cys C to estimate GFR is based on the same logic as the use of blood urea nitrogen and creatinine, but as is not secreted by renal tubules and also it does not return to the blood stream, so, it can be considered as an ideal endogenous marker.

Adenosine deaminase (ADA) is aenzyme, irreversibly catalyzes the hydrolytic cleavage of adenosine into inosine. This enzyme regulated the formation of some defense cells, hence, as a marker of inflammation, being generally associated with processes of infectious origin.

The present study showed that the ADA activities are significantly increased in chronic kidney disease patients when compared with normal subjects. The reasons for these findings can be speculated that the stage of the disease and the immune response of the host may have a positive influence in the release of ADA from the tissue cells. Accelerated degradation of adenine nucleotides (adenosine) in erythrocytes of CKD patients when compared with erythrocytes of healthy volunteers of control groups; adenine nucleotide catabolism was faster in CKD patients than cells of healthy control groups ADA as a marker of cellular immunity, plasma ADA activity is found to be elevated in chronic renal diseases in which there is a cell-mediated immunity.

Conclusion: In the present study we conclude that the concentration of Cystatin C and ADA activity is a useful index for the diagnosis of chronic kidney disease compared with urea and creatinine concentration because it's not dependent upon the environmental factors and sex.

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Conflict Of Interest: The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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