

Assessment Of Urinary Cystatin C In Type1 Diabetic Children

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Abstract

Background: Diabetes is the disease of this century; its incidence had progressively increased in the last few years especially in young ages. Cystatin C is a single chain non glycosylated basic protein produced by all nucleated cells at a constant rate freely filtered by the renal glomeruli and almost entirely reabsorbed in the proximal tubule and its level in urine increases in cases with renal tubular dysfunction.

Aim: The aim of the present study was to assess the urinary Cystatin C excretion in diabetic children attending the pediatric department of the National Institute of Diabetes and Endocrinology (NIDE) in comparison with apparently healthy children with comparable age and sex.

Methods: We studied 60 children and adolescents (38 Females and 22 Males) with type 1 diabetes. They had a mean age of 13.93 ± 2.92 years ranging from (10- 18) years and a mean duration of 6.67 ± 3.35 years of diabetes. In addition to 30 healthy children and adolescents included as a control group, fourteen of them were females and 16 were males having a mean age of 14.73 ± 2.13 years with the age range of (10- 18) years. All subjects were subjected to full history taking and thorough clinical examination with special emphasis on diabetic details and complications and an investigations including Microalbuminuria detected by measuring the albumin in urine by turbid metric method and urine creatinine by colorimetric measure and represented by [(A/C) ratio] also urinary Cystatin C excretion by ELISA from a morning urine sample was tested.

Results&Conclusion: High urinary Cystatin C levels were present in 41diabetic patients and none of the control group and were positively correlated to HbA1c and microalbuminuria (MA).

Key Words: Urinary Cystatin C in type 1diabetes: assessment, and correlations.

دراسة السيستاتين سي البولي في اطفال السكر من النوع الأول

الخلفية: إن مرض السكرى هو مرض العصر فقد زادت نسبة حدوثه بطريقة مطردة خلال السنوات الأخيرة وخاصة في الأعمار الصغيرة. سيستاتين سي هو سلسلة أحادية من البروتين القاعدي غير النشوي ينتج من كل خلايا الجسم بمعدل ثابت ويتم خروجه من خلايا الكلى بحرية تامة ثم يتم إعادة امتصاصه كاملاً في الأنابيب الكلوية القريبة ويزيد معدل إخراجها في البول في حالات اختلال عمل هذه الأنابيب.

الغرض: هو بحث زيادة إفراز السيستاتين سي في الأطفال مرضى السكرى الذين يترددون على قسم الأطفال بالمعهد القومي لأمراض السكر والغدد الصماء مقارنة بالأطفال الأصحاء المقارنين لهم في السن والجنس.

الأسلوب: اشتملت هذه الدراسة على 60 طفلاً ومراهقاً (38 أنثى و22 ذكر) من مرضى داء السكرى من النوع الأول والذين تراوحت أعمارهم بين (10- 18) سنة بمتوسط أعمار 13,91 سنة $\pm 2,92$ ومتوسط مدة حدوث السكرى 6,67 سنة $\pm 3,35$ بالإضافة إلى 30 طفلاً ومراهقاً من الأصحاء كمجموعة مقارنة (14 أنثى و16 ذكر) والذين تراوحت أعمارهم أيضاً بين (10- 18) سنة بمتوسط أعمار 14,73 سنة $\pm 2,13$. وقد تم أخذ التاريخ المرضي المفصل لهم وعمل الفحص الإكلينيكي مع التركيز على تفاصيل مرض السكرى كما تم عمل بعض الفحوصات المعملية والتي اشتملت على الفحص الدقيق في البول عن طريقة قياس الزلال البولي بطريقة قياس معامل التكتير والكرباتينين البولي بطريقة قياس معامل التلون وتم التعبير عنه بنسبة الزلال إلى الكرباتينين في البول كما تم قياس معامل إخراج السيستاتين سي في البول بطريقة الأليزا من عينة بول الصباحية.

النتائج: خلصت النتائج إلى وجود معدلات زائدة للسيستاتين سي في البول في 41 من مرضى داء السكرى الخاضعين للدراسة وعدم وجوده في أى من الأطفال الأصحاء في المجموعة المقارنة. كما تبين ارتباطه طردياً بنسبة السكر التراكمي والزلال الدقيق في هؤلاء الأطفال.

الكلمات المفتاحية: سيستاتين سي في مرضى السكرى من النوع الأول بحث وجوده وارتباطه.

Introduction:

Type 1 diabetes is the most common endocrinal and metabolic disease in children and adolescence. Its incidence and prevalence has been increased largely in the last decade.⁽⁵⁾ It causes great burden on patients and their families either economic or psychological. Diabetes mellitus affects the renal tissues initiating several structural and functional abnormalities of the tubular interstitium up to glomerular changes resulting in higher excretion of tubular injury markers.⁽⁶⁾

An increased urinary low molecular weight protein (tubular proteins) indicates proximal tubular injury as N. acetyl B glucosaminidase, retinol binding protein, B2 microglobulin and Cystatin C⁽⁸⁾. Cystatin C is a basic protease inhibitor that is produced constantly by all nucleated cells. It's freely filtered by renal glomeruli and primarily catabolized by the tubules. Its level doesn't depend on age, gender and body mass.⁽³⁾ Increased urinary Cystatin C can detect tubular dysfunction in pure and mixed nephropathies. In this study we assessed the urinary Cystatin C excretion in type 1 diabetic children and adolescents and its correlation to microalbuminuria.⁽⁹⁾

Aim Of The Work

The aim of this study was to assess the urinary Cystatin C excretion in type 1 diabetic children and adolescents and its correlation with microalbuminuria.

Ethical Aspect:

- ✧ Ethical Consent: This study was accepted by the ethical committee of the institute of postgraduate childhood studies and ethical committee of NIDE. Written informed consent was obtained from care givers after explanation of the nature and aims of study, its benefits for their children and for all the community and expected risks for their children in this study.
- ✧ Verbal Assent: Informed verbal assent was being taken from children over 8 years after a simplified explanation of the aim and benefits of the study.

Type Of Study:

Case Control Study.

Subjects And Methods**Subjects:**

Sixty children and adolescents suffering from type 1 diabetes for more than 3 years were included in this prospective study as the patient group. They were attending the pediatric department and outpatient clinic at National Institute of Diabetes & Endocrinology (NIDE) during year 2015 from 1/ 1/ 2015 to 31/ 12/ 2015. They had a mean age of 13.93 ± 2.92 years ranging from (10- 18) years and a mean duration of 6.67 ± 3.35 years of diabetes.

Additionally 30 apparently healthy children and adolescent with comparable sex and age without type1 diabetes were included as a control group. Fourteen of them were females and 16 were males having a mean age of 14.73 ± 2.13 years with the age range of 10:18 years

1. Inclusion Criteria:

- a. Type I diabetic children and adolescents diagnosed according to American Diabetes Association (ADA) criteria.
 - ✧ Age: (6- 18) years old.
 - ✧ Disease duration more than 3 years of diabetes.
 - b. For the Control Group:
 - ✧ Non diabetic children and adolescents.
 - ✧ Age: (6- 18) years old.
2. Exclusion Criteria:
- a. Diabetics with any renal affection other than microalbuminuria (based on past medical data).
 - b. Presence of metabolic or any chronic disease other than diabetes (based on past medical data).

Methods:

Each subject was subjected to the following:

- ✧ Full history taking with emphasis on the important relevant data.
- ✧ Thorough clinical examination including both general and systemic one with special emphasis on the following:
 1. Blood Pressure.
 2. Growth assessment by performing anthropometric measurements: including height, weight, and comparison of measures with the same age and sex using standard Egyptian charts and pubertal staging according to Tanner's White House (1973), body mass index was calculated using the equation: $(BMI = wt (kg) / height (m^2))$.
- ✧ Investigations Included:
 1. Assessment of glycemic control by assessment of HbA1c using liquid chromatographic assay method (in addition to 2 readings from the medical records of patients).
 2. Assessment Of Albuminuria: Morning urine sample was processed and albumin in urine was measured by turbidimetric measure and creatinine by enzyme colourimetric measure and A/ C ratio was calculated. A/ C ratio between (30 mg/ gm- 300 mg/ gm) is considered microalbuminuria or positive result and below this range is normoalbuminuria or negative result. If the microalbuminuria is positive, it is repeated and considered positive if it is positive for 2 times in a period of 3 months with negative urinary culture.⁽¹⁾
 3. Assessment of Urinary Cystatin C by ELISA (Sunredbio. com): Morning urine sample was collected, centrifugated and frozen at - 20°C till the time of analysis samples were processed after melting by ELISA technique to detect Cystatin C urine sample using Shanghai Sunred Biological Technology Co. Ltd kit.

Statistical Methods:

SPSS version 17.0 was used for data analysis. Mean and standard deviation described quantitative data and median with range when appropriate. Parametric and non- parametric t- test was used for comparing means of independent groups. Pearson correlation analysis was used to show magnitude of association between quantitative variables. P-

value is two tailed and significant at 0.05 levels.⁽⁷⁾

Results:

Table (1) Clinical and laboratory characteristics of the studied groups.

		Control Group	Patients Group	Independent T- Test	
				T	P- Value
Age	Mean±SD	14.73± 2.13	13.93± 2.92	1.346	0.182
	Range	11- 18	10- 18		
Wt	Mean±SD	46.23± 11.90	46.61± 15.59	- 0.116	0.908
	Range	31- 86	25- 82		
Ht	Mean±SD	147.07± 13.53	146.43± 15.45	0.193	0.847
	Range	109- 167	106- 174		
BMI	Mean±SD	21.21± 3.61	21.05± 4.21	0.170	0.865
	Range	15.7- 33.5	14.2- 33.3		
Systolic BP	Mean±SD	109.00± 9.23	110.50± 12.13	- 0.596	0.553
	Range	90- 130	90- 130		
Diastolic BP	Mean±SD	69.67± 8.09	72.67± 7.56	- 1.734	0.086
	Range	50- 80	60- 80		
Cystatin C	Mean±SD	81.66± 8.99	91.82± 22.49	- 2.375	0.020
	Range	65.9- 102.5	3- 140		
HbA1C	Mean±SD	5.39±0.29	11.61± 2.70	- 12.537	0.000
	Range	4.8- 5.8	4.4- 16.5		
CHOL	Mean±SD	172.60± 37.51	187.62± 45.27	- 1.567	0.121
	Range	15- 224	103- 348		
T.G	Mean±SD	109.70± 49.55	113.33± 64.11	- 0.272	0.786
	Range	43- 261	45- 279		
HDL	Mean±SD	47.70± 8.21	47.33± 8.86	0.190	0.850
	Range	31- 61	35- 66		
LDL	Mean±SD	107.63± 18.72	119.33± 38.09	- 1.586	0.116
	Range	79- 147	38- 237		
Urea	Mean±SD	24.37± 6.29	25.48± 6.77	- 0.755	0.452
	Range	13- 39	12- 40		
Creat	Mean±SD	0.82±0.15	0.76±0.12	2.103	0.038
	Range	0.5- 1.2	0.5- 1.1		
Micro	Mean±SD	29.25± 66.23	43.09± 64.69	- 0.950	0.345
	Range	2.36- 364	1.43- 285		
A/C	Mean±SD	17.51± 20.46	43.33± 48.94	- 2.765	0.007
	Range	1.21- 116	3.61- 253		
ALT	Mean±SD	16.00± 3.69	29.52± 28.16	- 2.611	0.011
	Range	9- 22	8- 200		
AST	Mean±SD	17.33± 3.18	30.90± 32.11	- 2.302	0.024
	Range	12- 27	9- 222		

*: Independent t- test BMI: Body mass index, P >0.05: Non significant Wt: weight, P <0.05: Significant Ht: height, P <0.01: Highly significant, Hba1c: Glycosylated Hemoglobin, Chol: Cholesterol, T.G: Triglycerides, HDL: High Density Lipoproteins, LDL: Low Density Lipoproteins, Micro:Microalbuminuric, A/C: Albumin/creatinine ratio, Alt: Alanine Transaminase, Ast: Aspartate Transaminase

No significant difference was found between the two groups as regards Age, Sex, BMI, Wt, and Ht.

There was a significant difference between the patient and control groups as regards Cystatin C, HbA1c, A/C ratio, and liver enzymes which were higher in the patient group. Also there was a significant difference in creatinine which was on to be lower in patient group.

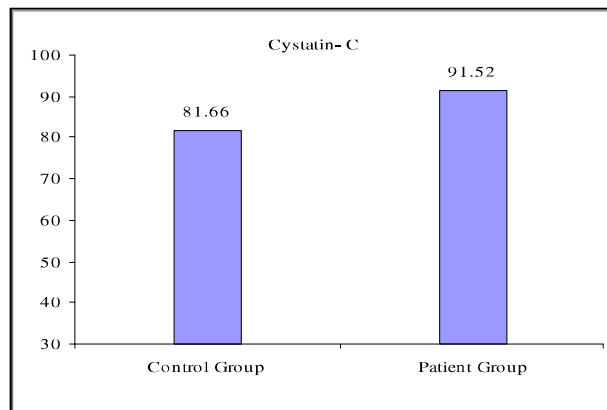


Fig (1) Urinary Cystatin C in patient and control groups. P value= 0.02 significant difference

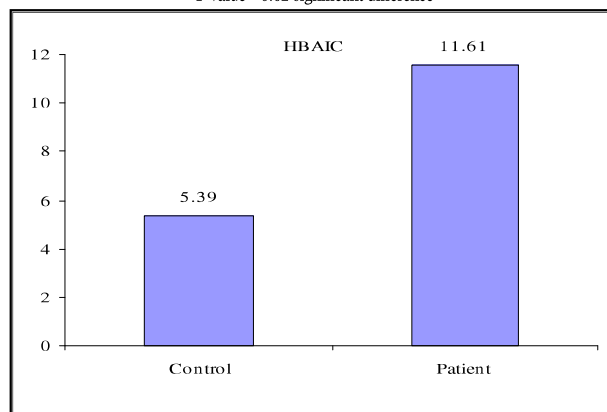


Fig (2) Glycosylated hemoglobin in patient and control groups. P value= 0.000 significant difference

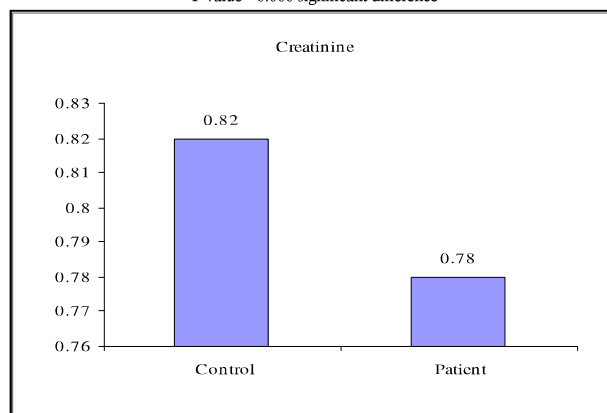


Fig (3) Serum creatinine in patient and control groups. P value= 0.038 significant difference

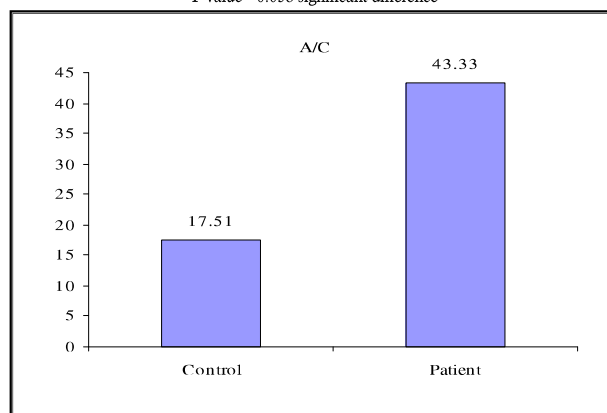


Fig (4) Albumin/creatinine ratio in patient and control groups. P value= 0.007 significant difference

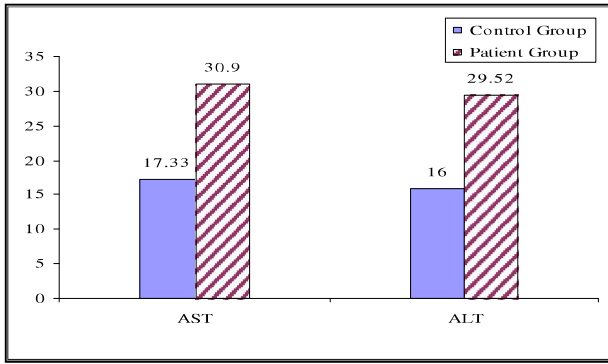


Fig (5) Liver enzymes in patient and control groups.

P value= 0.011 significant difference, P value= 0.024 significant difference

Table (2) Correlation of Cystatin C with the other studied parameters in each group.

	Cystatin C			
	Control Group		Patients Group	
	r	P- Value	r	P- Value
A/C	0.346	0.061	0.501**	0.000
Age	0.125	0.509	0.151	0.251
Wt	0.177	0.350	0.089	0.498
Ht	0.260	0.166	0.104	0.430
BMI	-0.085	0.657	0.071	0.589
Systolic BP	0.060	0.755	0.303*	0.019
Diastolic BP	-0.223	0.236	0.196	0.132
Insulin/ Kg	-	-	0.223	0.087
HBA1C	0.263	0.160	0.422**	0.001
CHOL	0.042	0.824	0.070	0.597
T. G	-0.169	0.373	0.174	0.183
HDL	0.213	0.257	-0.191	0.145
LDL	0.297	0.111	0.041	0.755
Urea	0.103	0.590	-0.080	0.545
Creat	0.156	0.410	-0.160	0.221
Micro	0.233	0.215	0.219	0.093
ALT	0.035	0.855	-0.016	0.902
AST	-0.078	0.681	0.036	0.783
DD	-	-	0.106	0.422

*: Independent t- test BMI: Body mass index, P >0.05: Non significant Wt: weight, P <0.05: Significant Ht: height, P <0.01: Highly significant, Hba1c: Glycosylated Hemoglobin, Chol: Cholesterol, T.G: Triglycerides, HDL: High Density Lipoproteins, LDL: Low Density Lipoproteins, Micro: Microalbuminuric, A/C: Albumin/creatinine ratio, Alt: Alanine Transaminase, Ast: Aspartate Transaminase

Studying the correlation between Cystatin C and different clinical and laboratory parameters in the control group revealed no correlation with any of these parameters while it revealed that Cystatin C had a significant positive correlation with A/ C ratio, HbA1c, and systolic blood pressure in the patient group.

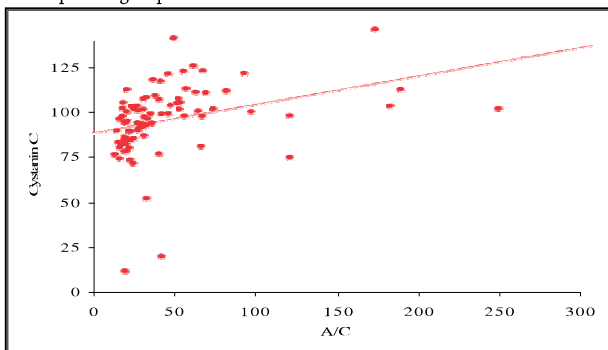


Fig (6) Correlation between Urinary Cystatin C and A/C ratio in the patient group.

P value= 0.000 positive correlation

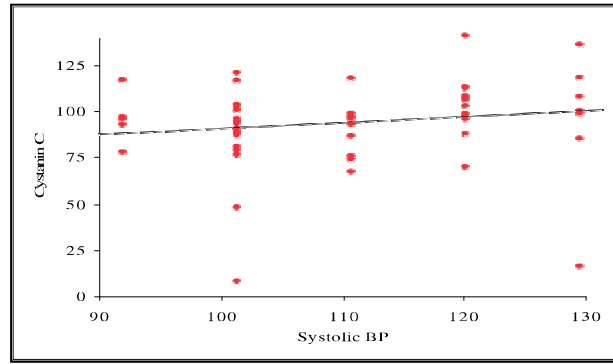


Fig (7) Correlation between Urinary Cystatin C and systolic BP in the patient group.

P value= 0.019 positive correlation

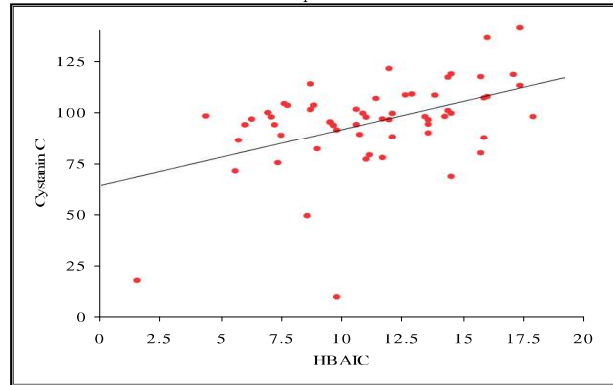


Fig (8) Correlation between Urinary Cystatin C and HbA1c in the patient group.

P value= 0.001 positive correlation

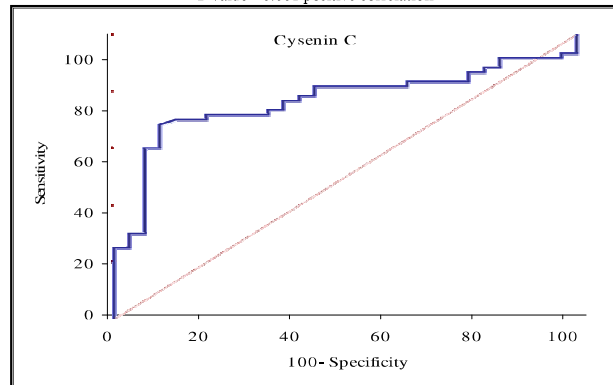


Fig (9) ROC curve between patients group and control group

Cut Off Point	AUC	Sensitivity	Specificity	+Pv	- Pv
>90.4	0.766	68.33	90.00	93.2	58.7

On doing a rock curve the cutoff point of Urinary Cystatin C was found to be 90.4 and it was moderately sensitive (sensitivity 68.33) and highly specific (specificity 90.0).

Table (3) Correlation of Cystatin C with the other studied parameters in all studied subjects.

	Cystatin C	
	r	P- Value
A/C	0.556**	0.000
Age	0.043	0.685
W	0.052	0.627
H	0.085	0.426
BMI	-0.006	0.955
Systolic BP	0.221*	0.036
Diastolic BP	0.151	0.154
Insulin/ Kg	0.223	0.087
HBA1C	0.559**	0.000

P >0.05: Non significant, P <0.05: Significant, P <0.01: Highly significant

As regards studying the relation between Cystatin C and the different parameters in all subjects, it revealed highly significant positive relation with A/ C ratio, HbA1c, systolic blood pressure.

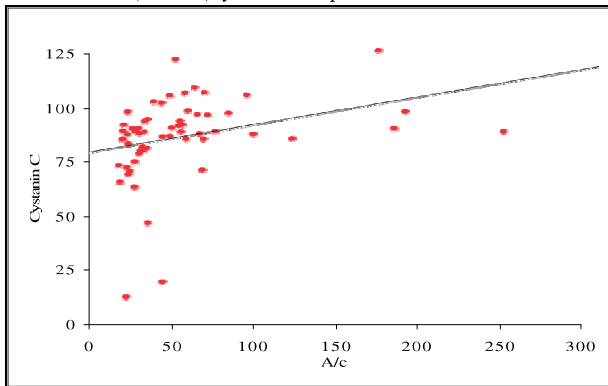


Fig (10) Correlation of Cystatin C with A/C ratio uric in studied patients.
P value= 0.000 highly significant

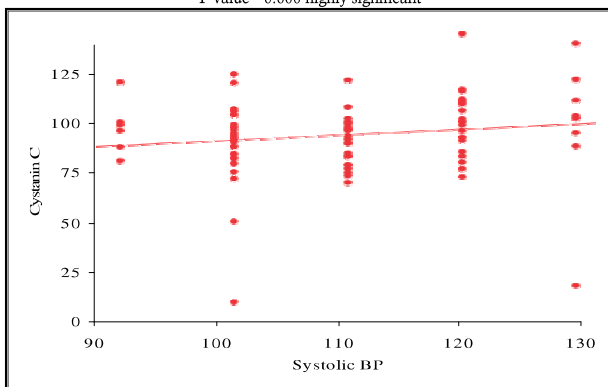


Fig (11) Correlation of cystatin C with systolic blood pressure in the studied subjects.
P value= 0.036 significant

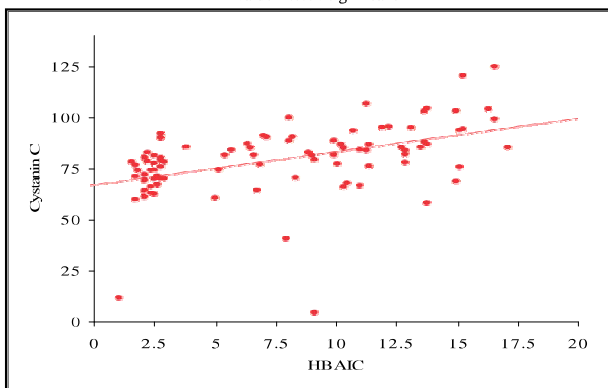


Fig (12) Correlation of cystatin C with HbA1c in the studied subjects.
P value= 0.000 highly significant

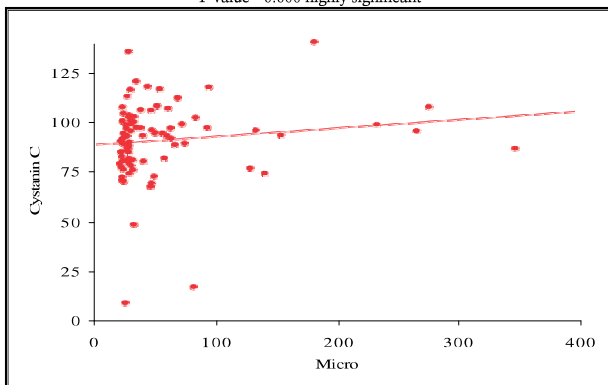


Fig (13) Correlation of cystatin C with micro albumin in the studied subjects.
P value= 0.008 highly significant

Discussion:

In our study urinary Cystatin C was found to be significantly high in 41 of the 60 diabetic patients and in none of the control group and was positively correlated to urinary albumin excretion and glycosylated hemoglobin.

Our results are in accordance with Ibrahim et.al.⁽⁴⁾ Who conducted a prospective observational study on 42 patients in addition to 6 healthy controls to assess urinary Cystatin C excretion and its correlation with microalbuminuria.

They found that there is statistical difference between the 2 groups in urinary Cystatin C which was higher in the patient group than the control one, and they found a positive correlation between urinary Cystatin C and A/ C.

Our results were also in accordance with the results of Lee and Choi⁽⁵⁾ who conducted a prospective study on 91 subjects (61 diabetics and 30 prediabetics) aiming to assess the level of novel urinary Biomarkers as Cystatin C, NGAL (Neutrophil Gelatinase Associated Lipocalin) in diabetes and prediabetes and their correlation with microalbuminuria and they found that urinary level of Cystatin C was significantly higher in microalbuminuric compared to normoalbuminuric patients.

Also our results are in accordance with Fiseha 2015.⁽²⁾ who reviewed the recent literatures to determine if serum and urine Cystatin C measurements differ between diabetics and non diabetics, their search included 23 studies that have been published to date and all studies concluded that serum and urinary Cystatin C were elevated in type 2 diabetic patients compared to non diabetic controls including patients who had no signs indicating nephropathy, also these studies found a significant positive correlation between Cystatin C levels and albuminuria.

Conclusion& Recommendation:

Urinary Cystatin C increased in type 1 diabetic children with more than three years duration. It was significantly correlated to microalbuminuria and glycosylated hemoglobin. It's recommended to search its role as a marker of nephropathy in diabetic patients.

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