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Biochemical Study on Diabetic Nephropathy Patients

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Abstract

This study deals with thirty non-insulin dependent diabetes mellitus patients suffering from diabetic nephropathy in addition to twenty five healthy control.Some biochemical parameters were determined in the serum of all subjects enrolled in the study.These parameters are serum glucose,serum urea,serum creatinine,total serum protein and serum albumin.The aim of the present study was to estimate these parameters in diabetic nephropathy patients.

The results of the present study revealed a significant increase in glucose,urea and creatinine in patients as compared to controls . Also a significant decrease was found in total serum protein, serum albumin and albumin to globulin ratio (A/G) in patients compared to controls,while a significant increase in serum globulin in patients compared to control was found ($P<0.001$).In conclusion no statistical differences were found in the study parameters between gender and between groups with different ages.

Key words:Diabetes mellitus,total serum protein,albumin,chronic renal failure,end stage renal disease.

Introduction

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized by the body tissues,producing hyperglycemia.The diabetic patients are at increasing risk of developing specific complications such as renal failure nephropathy [1].Diabetic nephropathy is the most common cause of end-stage renal failure in patients starting dialysis in the developed world [2].In individual cases,it is often difficult to establish the duration of renal failure[3].Chronic kidney disease (CKD) is associated with an advers effect on prognosis from cardiovascular disease.This includes increased mortality after an acute coronary syndrome and after precutaneous coronary intervention (PCI) with or without stenting [4],[5].Chronic renal failure (CRF) is a pathophysiologic process with multiple etiologies, resulting in the alteration of nephrone number and function,and frequently leading to end-stage renal disease.In turn,end stage renal disease (ESRD) represents a clinical state or a condition in which there has been irreversible loss of endogenous renal function,a degree sufficient to render the patient permanently dependent upon renal replacement therapy (dialysis or transplantation) in order to avoid life-threatening uremia.The main causes of CRF are glomerulonephritis, polycystic kidney, accelerated hypertension, calculi disease,analgesic nephropathy, diabetes mellitus, amyloid, hereditary nephritis and interstitial nephritis[5].Hypoalbuminemia although not synonymous of malnutrition, is highly related to it.Poor nutrient, frequently observed in uremia ,may cause malnutrition and subsequently hypoalbuminemia. In addition ,it has been recently reported that a systemic inflammatory response may participate in developing hypoalbuminemia in CRF.Other conditions non-associated to inflammatory response,such as the protein losses through dialysis,may cause and increase malnutrition. Overhydration ,frequently present in patient with renal failure, on the one hand causes dilution of serum albumin concentration,and on the other hand ,is a cause of onset and/or enhancement of congestive cardiac failure,which in turn may be associated with malnutrition[6].Many symptoms of uremia result from accumulation of urea,creatinine,and other nitrogenous end products of amino acid and protein metabolism in blood.Among the

nutritional parameters,serum albumin level has been identified as the most powerful laboratory predicator of mortality and as an independent risk factor for death [6]. The aim of the present work is to evaluate urea, creatinine,total serum protein,serum albumin and globulin in diabetic patients suffering from renal failure according to genders.

Experimental Part:

-Selection of subjects and blood sampling:

This study was conducted during the period from December 2008 until the end of March 2009.The blood samples were taken from patients whose ages ranged from (38-72) year admitted to Medical City in Baghdad.Ten ml of venous blood was obtained after a 12 hour fast from thirty patients with non insulin dependent diabetes mellitus and non-hepatitus, these patients were on hemodialysis treatment for different durations, and twenty five healthy individuals as control group and their ages ranged from (31-65) Year. Blood samples were transferred into plain tube, allowed to stand for 15 minutes at room temperature, centrifuged at 3500 rpm for 10 minutes.The resulting serum was separated and used for the estimation of glucose, urea, creatinine, total serum protein, serum albumin, and globulin.

Methods

-Determination of serum glucose:

Glucose was determined by using the enzymatic method (GOD-POD) according to the Triender P.method [7]. Glucose in the fasting serum is oxidized to gluconic acid by glucose oxidase. Hydrogen peroxide, which is also formed in the reaction, reacts with the indicator reagents 4-aminoantipyrine in the presence of peroxides enzyme to form a stable red quinine compound which absorbs at 500 nm.

-Determination of serum urea:

Urea was determined by using coloremetric method (Urease-Berthelot Modified) according to the Fawcet and Scot method [8],which urease catalyses the convertion of urea to ammonia ion and bicarbonate ,then ammonia hydrolysed to form ammonium ion,which reacts with salicylate and hypochlorite to give colored complex absorbance at 580 nm

Determination of serum creatinine:

Creatinine was measured by using kinetic method [9].Creatinine reacts with alkaline picrate yielding an orange yellow complex which absorbs at 462nm.It is measured within one minute thereby avoiding interferences of other compounds which also react with picrate,but more slowly than creatinine.

-Determination of total serum protein:

Total serum protein was measured by (Coloremetric Biuret Method).Copper salts in an alkaline medium reacts with the peptide bonds of the protein producing a violet colour which is proportional to the amount of protein present [10].Absorbance at 546nm

-Determination of serum albumin:

Serum albumin was measured by bromo cresol green (BCG) [11].The measurement of serum albumin is based on its quantitative binding to the indicator (3,3',5,5'-tetra bromo-m-cresol sulphonphthalein).The albumin-BCG-complex absorbs maximamlly at 578nm.

-Globulin was calculated using the relation:

(Total Serum Protein=Albumin+Globulin).

Statistical Analysis:

All values were expressed as mean ± standard deviation (M±SD). Statistical analysis were performed using student 's T-Test ($p \leq 0.01$) the lowest limit of significance difference between the studied groups [12].

Rsults and Discussion

Table (1) shows a marked significant increase in blood glucose,urea,and creatinine in diabetic nephropathy patients as compared to controls.

The mean (±SD) of serum glucose in the control group was (85.36±10.22) mg/dl,while in patient group was (218.60±59.21) mg/dl which exhibited a significant elevation ($P<10^{-5}$) from the control level.Fasting glucose increases per decade throught life.Renal threshold (the point at which glucose spills in the urine) also increases with age; there is also an altered insulin response to glucose [13].

Table (1) also showed elevation in urea and creatinine.Increases in urea in renal failure are caused by impaired ability to excrete protein aceous catabolites because of marked reduction in glomerular filtration rate(GFR).Urea is directly related to the protein content of the diet.Urea can also be increased by gastrointestinal hemorrhage,enhanced protein catabolism.Increases in creatinine are a result of decreased renal excretion.Because many external factors may influence urea concentration ,creatinine is often used as a more reliable indicator of glomerular filtration rate in patients with renal disease [14].

Table (2) showed a marked significant decrease in total serum protein,serum albumin and A/G ratio in patients group,while a significant increase was found in serum globulin as compared with their controls.Indicate that those patients have a sever malnutrition state nephropathies and controls. Patients with CRF develop hypoalbuminemia to a complex setting of conditions,with systemic inflammatory response as a major cause, not with standing ,other factors such as malnutrition and overhydration can also play a relevant role [15].

It has been postulated that increased incidence of renal disease and a reduced ability to handle the excretion of drugs.Problems affecting renal function are related to damage from infections or drugs disorders such as diabetes mellitus [16],[17].The sex dependent and gender of diabetic nephropathy patients and control group are shown in table (3) ,and table(4).

It has been concluded from the results of this study that no statistical differences were found in parameters(serum glucose, serum urea, serum creatinine, total serum protein and serum albumin) between gender and between groups with different ages.

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Table (1): Serum glucose,urea and creatinine in patients group and their controls (mean±SD) in mg/dl.

Clinical data	Patients	Healthy control
Number	30	25
Age	54.37±11.35	47.20±9.25
Serum glucose	218.60±59.21***	85.36±10.22
Serum urea	174.83±38.81***	23.36±6.58
Serum creatinine	8.03±1.34***	0.98±0.51

***$P<10^{-5}$

Table (2): Total serum protein,serum albumin & globulin in (g/l) and (A/G) ratio in patients group and their controls (mean±SD).

Clinical data	Patients	Healthy control
Total serum protein (g/l)	63.23±0.70***	69.04±0.71
Serum albumin (g/l)	30.83±46.8***	41.36±0.44
Serum globulin (g/l)	32.40±0.80**	27.68±0.80
(A/G) ratio	1.04±0.38***	1.64±0.56

***$P<10^{-5}$, **$P < 0.001$

Table (3):Distribution of diabetic nephropathies patients and healthy control groups according to gender.

			Studied groups		
			Healthy control	Patients	Total
Gender	Male	N	12	16	28
		%	48.0%	53.3%	50.9%
	Female	N	13	14	27
		%	52.0%	46.7%	49.1%
Total		N	25	30	55
		%	100.0%	100.0%	100.0%

Table (4):The (mean±SD) in diabetic nephropathies patients and healthy control groups according to gender

Clinical data	Patients	Healthy control
Age/years (mean±SD)	54.37±11.35	47.20±9.25
Minimum	38	31
Maximum	72	65
t-test (P-value)	0.014	

دراسة كيموحيوية لمرضى الإعتلال الكلوي السكري

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الخلاصة

تتعامل هذه الدراسة مع ثلاثين مريضاً مصاباً بداء السكري غير المعتمد على الإنسولين ممن يعانون من الإعتلال الكلوي السكري فضلا عن خمسة وعشرين شخصاً من الأصحاء.

قيست بعض الدوال الكيموحيوية لجميع الأشخاص المساهمين في الدراسة و كانت هذه الدوال الكلوكوز ،اليوريا ،الكرياتنين،البروتين الكلي و الألبومين في أمصال المساهمين في الدراسة. الهدف من هذه الدراسة تقدير هذه المتغيرات لدى مرضى الإعتلال الكلوي.

نتائج الدراسة الحالية تشير الى أن هناك زيادة معنوية في تركيز الكلوكوز و اليوريا و الكرياتنين في أمصال المرضى مقارنة مع الأصحاء، اذ كانت($P< 10^{-5}$) كذلك هناك نقصان معنوي ملحوظ في البروتين الكلي و الألبومين و نسبة الألبومين الى الكلوبيولين في مصل الدم ، بينما وجد إرتفاع معنوي في تركيز الكلوبيولين في أمصال المرضى مقارنة مع الأصحاء ،اذ كانت ($P< 0.001$).

يمكن الإستنتاج من هذه الدراسة بأنه لا يوجد أختلاف معنوي في الدوال التي تم دراستها(الكلكوز ،اليوريا ،الكرياتينين،البروتين الكلي،والالبومين) بين المرضى من حيث الجنس ،وكذلك بين المرضى من حيث العمر.

كلمات مفتاحية: مرض السكري،البروتين الكلي،الألبومين،العجز الكلوي،المراحل النهائية لعجز الكلية.