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Serum Aldosterone Level in Patients with Diabetic Nephropathy in Relation to Vascular Calcification

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Abstract

Diabetic Nephropathy(DN) is a complex disease manifested by persistence microalbuminuria occurring due to the interaction between hemodynamic and metabolic pathway that activates the local renin-angiotensin-aldosterone system resulting in a decline in renal functions.

This study aimed to quantify the associations between serum aldosterone concentration and fetuin- A as a marker of calcification in type 2 diabetic patients with and without microalbuminuria from one side, and study the possible relationship between aldosterone and fetuin-A with glycemic indices, serum electrolyte, renal function and microalbuminuria and body mass index from the other side.

A case-control study involved eighty-six adult subjects classified into three groups after testing urine microalbumin including thirty-two diabetics type 2 patients with positive microalbuminuria and twenty-eight diabetics type 2 patients with negative microalbuminuria and 26 healthy subjects during their visit to AL kindy specialized Center for Endocrinology and Diabetes / Baghdad. Those patients were compared to control group of 26 apparently healthy subjects, fasting blood samples was obtained from each of them in one occasion only to measure: fasting serum glucose, electrolyte, aldosterone, fetuin-A, urea, and creatinine. In addition to glycoheamoglobin, glomerular filtration rate and body mass index.

Despite the presence of microalbuminuria in thirty-two of the studied diabetics, there was no positive correlation between aldosterone and fetuin- A, besides that no significant variations in serum aldosterone ,glomerular filtration rate(GFR) values, while both groups showed a significant increase in fasting serum glucose and glycaoheamoglobin ,significant decrease in serum sodium and chloride in comparison with the control group , significant increase was detected in serum fetuin-A mean values in microalbuminuric diabetics. Whereas, negative microalbuminuric diabetics measures expressed a positive correlation between both serum sodium and chloride levels and fetuin -A.

The conclusion of this study diabetic patient are prone to vascular calcification (VC) might be due to increase in aldosterone level or due to diabetic itself from this study we can conclude microalbuminuria can occur without a decline in renal function or a change in estimated GFR ,no definite correlation occur between aldosterone and fetuin- A, fetuin- A mean values are higher in diabetic patient with microalbuminuria compared to diabetic patients without microalbuminuria and control group and this referred to uncontrolled diabetes ,aldosterone show a correlation with weight and body mass index while fetuin- A does not show such correlation.

In general, electrolyte disturbances (hypernatremia) is more obvious in this study , and its occurrence is due to diabetic (osmotic diuresis) or drugs, while sodium retention which is a sign of aldosterone increment does not occur. Hypochloremia that occur in this study is due to chloride and it is in parallel with sodium level.

Keywords: Aldosterone, Fetuin A, Vascular Calcification, Type 2 Diabetes Mellitus, Glomerular Filtration Rate, Diabetic Nephropathy (DN).

مستويات الالدوستيرون في مصل الدم في مرضى السكري الذين يعانون من اعتلال الكلى السكري وعلاقتة بتكلس الاوعية الدموية.

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الخلاصة

إعتلال الكلى السكري هو مرض معقد يحدث بسبب التفاعل بين مسار الدورة الدموية والتمثيل الغذائي الذي ينشط نظام الرينين أنجيوتنسين الألدوستيرون المحلي مما يؤدي إلى انخفاض في وظائف الكلى.

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هدفت هذه الدراسة إلى تقيم العلاقة بين تركيز الالدوستيرون في المصل والفتوين أ كعلامة للتكلس عند مرضى السكري النوع الثاني الذين يطرحون او لا يطرحون زلال دقيق في الادرار من جهة ودراسة العلاقة الممكنة بين اللالدوستيرون والفتوين أ مع موشر السكري والاملاح في المصل و فحص وظائف الكلى والزلال الدقيق في الادرار ومؤشر كتلة الجسم من جهة اخرى .

تضمنت دراسة حالة - التحكم : ستة وثمانون شخصا بالغا يصنفون الى ثلاث مجاميع بعد فحص الزلال الدقيق في الادرار , يتضمنون اثنان وثلاثون مريضا مصابا بالسكري من النوع الثاني لديهم بول زلالي دقيق و ثمانية وعشرون مريضًا مصابًا بالسكري من النوع الثاني ليس لديهم بول زلالي دقيق وستة وعشرون شخصا اصحاء , أثناء زيارتهم لمركز ألكندي التخصصي لامراض الغدد الصماء والسكري / بغداد. تمت مقارنة هؤلاء المرضى بمجموعة السيطرة المكونة من ستة وعشرين شخصًا يبدو أنهم أصحاء ، تم جمع عينات الدم الصيامي و الحصول عليها من كل واحد منهم لمرة واحدة فقط لقياس: جلوكوز المصل ، الاملاح، الألدوستيرون ، فيتوين -أ ، اليوريا والكرياتينين. بالإضافة إلى الهيموجلوبين الغليكوزيلاتي وترشيح الكلى الكبيبي.

على الرغم من وجود البول الزلالي الدقيق في اثنين وثلاثين من مرضى السكري الذين خضعوا للدراسة ، لم تكن هناك اختلافات كبيرة في معدل ألدوستيرون في المصل و معدل ترشيح الكلى الكبيبي ، بينما كلا المجموعتين اظهرت تغير كبير في قيم السكر الصيامي و الهيموكلوبين الكلايكوزيلاتي وانخفاض كبير للصوديوم والكلورايد في المصل مقارنة بمجموعة السيطرة ولكن تم اكتشاف زيادة كبيرة في متوسط القيم للفتوين-أ لمجموعة المرضى الذين لديهم زلال دقيق بالمقارنة مع الذين ليس لديهم زلال في الادرار و مجموعة السيطرة وهذا يشير الى السكر الغير المسيطر عليهة . في حين أعربت مجموعة المرضى الذين ليس لديهم بول زلالي ارتباطات إيجابية بين الصوديوم والكلوريد مع الفتوين أ على حد سواء. الاستنتاج من هذه الدراسة المرضى المصابين بالسكري من النوع الثاني عرضة للإصابة بتكلس الاوعية الدموية ربما لزيادة الالدوستيرون او عن طريق مرض السكري ,من هذه الدراسة نستطيع ان نستنتج ان الادرار الزلالي الدقيق يمكن ان يحصل بدون انخفاض بوظائف الكلية او تغير بمعدل الترشيح الكبيبي كما ان لاتوجد هنالك علاقة واضحة بين الالدوستيرون والفتوين- أ ,معدل قيم الفتوين -أ اعلى في مرضى السكري الذين لديهم ا ادرار زلالي دقيق بالمقارنة مع الذين ليس لديهم ادرار زلالي دقيق ومجموعة السيطرة وهذ يشير الى السكري الغير مسيطر عليه كما ان الالدوستيرون يظهر علاقة مع الوزن ومؤشر كتلة الجسم بينما الفتوين- أ لم يظهر مثل هذه علاقة , بصورة عامة اختلال الاملاح (انخفاض الصوديوم)واضح في هذه الدراسة والذي حدث نتيجة لمرض السكري (الادرار التناضحي) او الادوية بينما انحباس الصوديوم الذي هو علامة لارتفاع مستوى الالدوستيرون لم يحدث. انخفاض في الكلورايد الذي حصل في هذه الدراسة لانة الكلورايد متوازي مع مستوى الصوديوم.

الكلمات المفتاحية: الالدوستيرون ، الفتوين – أ , تكلس الاوعية ، السكري النوع الثاني ، معدل الترشيح الكبيبي،أعتلال الكلية السكري.

Introduction

Diabetic nephropathy (DN) is one of the microvascular complications that develops in about 30% of patients with type1 diabetes mellitus and about 40% in those with Type2 diabetes mellitus (T2DM) [(1)]. It's characterized by albuminuria, irreversible decrease in glomerular filtration rate (GFR) and arterial hypertension[(2)].

Microalbuminuria is an earlier sign of general vascular dysfunction and nowadays is considered a predictor of worse outcomes for both kidney and heart [(3)]. Hence, patients with T2DM should be screened for microalbuminuria from the date of diagnosis [(4)]. With diagnostic reference standard of 30 to 300 mg of albumin in a 24-hour urine sample [(5)]. Persons with type 1 or 2 diabetes and microalbuminuria should continue to be tested for albuminuria annually to observe disease progression and response to therapy [(4)].

Essential causes in the pathogenesis of diabetic nephropathy are metabolic and hemodynamic pathways [(6)]. Heamodynamic pathway had been reported to include activation of local Renin Angiotensin Aldosterone System (RAAS) in proximal tubular epithelial cells, mesangial cells, and podocyte [(7)]. With consequences of reactive oxygen species(ROS) generation, inflammation, over expression of transforming growth factor-β (TGF-β), and deregulations of different vascular growth factors such as the vascular endothelial growth factor-A (VEGF-A)[(8)]. Activating local (RAAS) results in increased angiotensin II (Ang II**)**, in addition to action Adrenoctincorticotrpic hormone (ACTH), and potassium are three principal factors that adjusted aldosterone secretion [(9,10)].

Classical effects of aldosterone are to promote sodium retention and potassium loss by the kidney, although it exerts similar but lesser effects on the colon, sweat, and salivary glands [(11)]. Aldosterone/mineralocorticoid receptor (MR) system plays an important role in cardiovascular and renal diseases, particularly in the presence of excessive salt intake. In individuals with metabolic syndrome, adipocyte-derived aldosterone-releasing factors cause inappropriate release of aldosterone in the adrenal glands during salt loading, resulting in the development of salt-induced hypertension, cardiac and renal damage [(12)]. Aldosterone also plays a definitive role on systemic and vascular insulin resistance, as the vascular insulin resistance is considered an early cause to vascular damage. Accordingly, aldosterone impairs insulin receptor (IR) signaling by changing in the phosphatidylinositol 3-kinase (PI3K) / nitric oxide (NO) pathway and by inducing oxidative stress and crosstalk between the IR and the insulin-like growth factor-1 receptor (IGF-1R) leading to proliferation, oxidative stress and inflammation. Meanwhile, aldosterone exerts negative effects on structural and functional integrity of the pancreatic β-cell by encouraging inflammatory and oxidative stress conditions, which lead to decreased insulin release and actions, including actions in the vasculature [(13)].

Aldosterone is a new and axial factor that causes vascular calcification (VC) [(14)]. Also, it has a detrimental effect in the vasculature (enhances

vascular oxidative stress), promote vessel inflammation and apoptosis [15]. One of hemodynamic pathway factors is aldosterone hormone, aldosterone can promote vascular change and calcification in patients with diabetes through several mechanisms [16].

A wide range of biomarkers has been studied for identification of type 2 diabetes patients at microvascular and macrovascular risk. Fetuin-A is a novel biomarker that is used with metabolic complication to understand the causes that lead to microvascular or macrovascular changes that occur in diabetic nephropathy[17] .Fetuin-A: is a 60 kDa (kilo Dalton) glycoprotein produced exclusively by the liver and secreted into serum in relatively high concentrations in humans[18]. Fetuin-A is known to inhibit ectopic calcium deposition and protect from vascular calcification [19]. Epidemiological studies suggested that higher serum fetuin-A levels are associated with insulin resistance (IR), metabolic syndrome (MS) and Type2DM [20].

Subjects and Methods

The study was conducted on patients with type2 diabetes mellitus in AL kindy Specialized Center for Endocrinology and Diabetes. For the period From November/2017 to February 2018. A total number of 60 diabetic patients (30 males and 30 females) were included in this study, 32 patients were positive for microalbuminuria and 28 were negative for microalbuminuria ,and their age ranged between (40) and (65) with a mean ±SD of (53.28±7.20 years). Patients were selected after excluding those on insulin therapy, hypertensive patients, or those with thyroid disorder or other endocrinopathies, or those with active liver diseases, pregnant females and smokers. Those patients were compared to 26 apparently healthy subjects (13 males&13 females). The study was approved by The Local Research Ethics Committee and all subjects were signed on a written informed consent to participate in this study.

Testing for microalbuminuria was performed by using (Combina 13) urine test strip [21]. purchased by Human Diagnostic worldwide, Germany .While, fasting glucose, creatinine and urea were measured by COBAS C 311 ROCH analyzer [22-24] Glycoheamoglobin(HbA_{1c}) was measured by spectrophotometer Apel PD-303[25] .Serum electrolytes (Sodium, Potassium, and Chloride)were estimated based on the potentiometric difference between the sample (electrodes) and reference standard, using Fuji Dri-chem NX 500 Electrolyte analyzer[26]. Specific competitive binding ELISA kit for human aldosterone, purchased by Demiditic, Germany [27]. Serum fetuin A levels were measured by utilizing quantitative sandwich ELISA kit from Cusabio, Shanghai /China[28].

Glomerular Filtration Rate Determination :

1. The Cockcroft-Gault Formula CCr={((140-age) x weight)/(72 SCr)} x 0.85 if female , where CCr is expressed in milliliters per minute, age in years, weight in kilograms, and serum creatinine (SCr) in milligrams per deciliter[29].
2. Modification of Diet in Renal Disease Equation (The 4-variable MDRD Study equation): GFR = 175 x (Standardized SCr)-1.154 x (age)-0.203x (0.742 if female) x (1.210 if African American**)** GFR is expressed in mL/min/1.73 M^2 Cr is serum creatinine expressed in mg/dL, and age is expressed in years [30].

Statistical analysis was performed using the SPSS statistical package for Social Sciences (version 20.0 for Windows, SPSS, Chicago, IL and USA). Data are presented as means ± SD. Significance was set at $p < 0.05$. Cases and controls were compared using either the t-test for independent samples. The Pearson coefficient test was used to test the relation between studied parameters.

Results

Subject's characteristics are listed below in Table-1.

Table (1) Subject characteristics

Parameters	Group				P value
	Patient (N=60)		Control (N=26)		
	Mean	SD	Mean	SD	
Age (years) *	53.28	7.20	47.88	6.35	0.001
Gender (M/F)	30:30		13:13		0.999
Weight (Kg) *	77.81	18.61	70.77	7.11	0.013
BMI ($Kg/m^{2)}$ *	29.46	6.18	26.32	2.14	0.001
Duration of DM (years)	7.29	5.20	.	.	-
FSG (mmol/L) *	12.20	4.64	5.12	.63	0.005
HbA1C (%) *	8.24	1.84	4.62	.88	0.005
Creatinine (µmol/L)	71.57	37.56	71.38	8.25	0.972
Urea (mmol/L)	4.21	1.13	3.87	.93	0.179
GFR MDRD ($ml/min1.73m^2$)	105.26	42.35	94.42	9.47	0.064
GFR Crock (ml/min)	117.98	45.39	106.35	10.45	0.065
Albuminuria(mg) *	34.00	46.07	.00	.00	0.005
Sodium (mEq/L)	119.5	15.0	140.7	3.9	0.005
Potassium(mEq/L)	3.9	.5	4.1	.1	0.199
chloride(mEq/L)	82.5	11.0	99.6	4.3	0.005
SBP (mmHg) *	13.43	1.80	12.15	.54	0.005
DBP(mmHg)*	8.47	1.19	8.08	.39	0.026
Aldosterone (Pg/ml)	145.63	68.51	116.03	134.14	0.179
Fetuin-A	628.76	829.14	254.83	151.00	0.001

***= significantly different from control, Body Mass Index (BMI), Fast Serum Glucose (FSG), GlycoHemoglobin (HbA1C),Glomerular Filtration Rate (GFR).Systolic Blood Pressure(SBP), Diastolic Blood Pressure (DBP)**

As shown in (Figure -1), the mean values of fasting serum glucose (FSG) and glyco hemoglobin (HbA_{1c}) of group A (diabetic patients with positive +ve microalbuminuria) and group B (diabetic patients with negative –ve microalbuminuria) were significantly higher than group C(control).

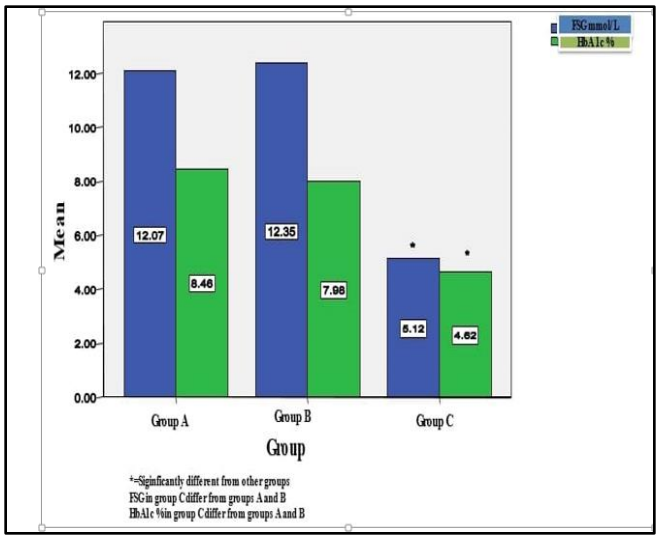

Figure (1) Mean values of fasting serum glucose and HbA_{1c} among groups.

Furthermore, mean values of GFR (**Cockcroft-Gault Formula)** for groups A and B were not significantly different from that of group C (the control), although these values were elevated as compared to that of group C .as shown in (Figure -2).

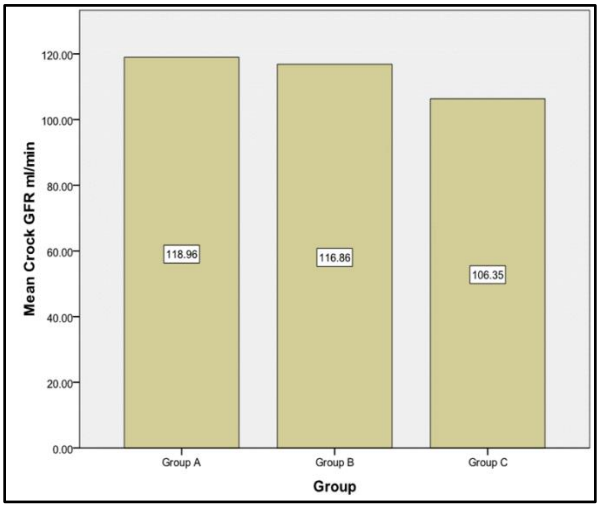

Figure (2) Mean of GFR (ml/min) (Cockcroft-Gault Formula) In:
Group A (Diabetic Positive microalbuminuria).
Group B (Diabetic Negative microalbuminuria) .
Group C (Control).

Mean values of serum creatinine level for groups A and B were not significantly different from that of group C (the control)

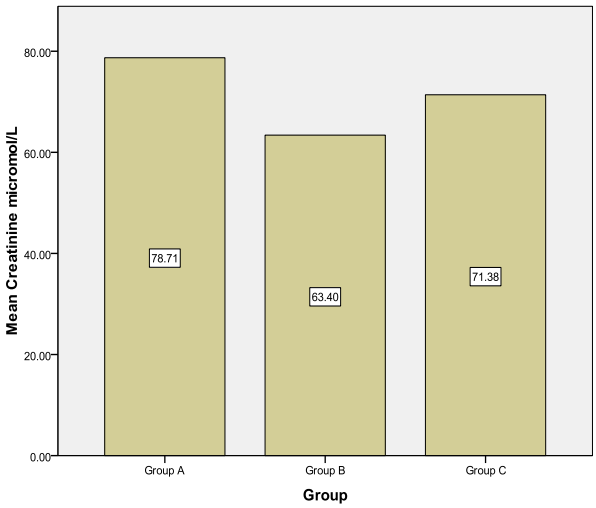

Figure (3) Mean values of serum creatinine level in:
Group A =Diabetic positive microalbuminuria.
Group B =Diabetic Negative microalbuminuria.
Group C =Control**.**

Data analysis of the estimated serum values of aldosterone among studied groups indicate a non significant difference between diabetics (with and without microalbuminuria) and the control subjects (figure-4) .

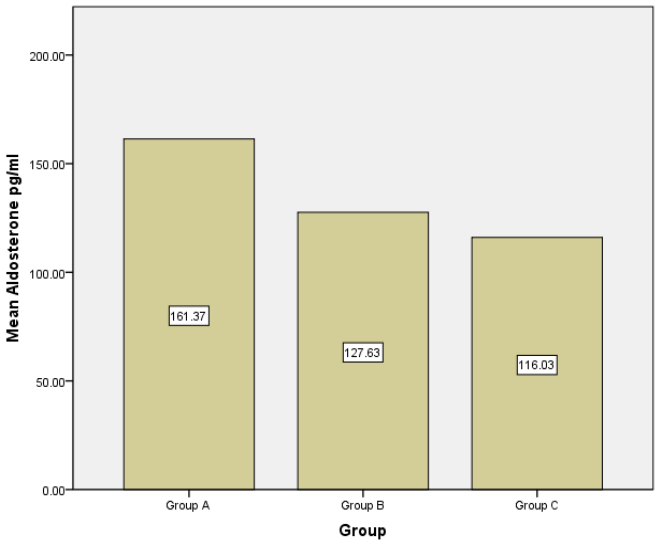

Figure (4) Mean values of serum aldosterone levels

As illustrated in figure-5 serum fetuin-A levels were significantly elevated in group A (diabetic positive microalbuminuria) when compared to group B (diabetic negative microalbuminuria) as well as when compared to group C (Control).

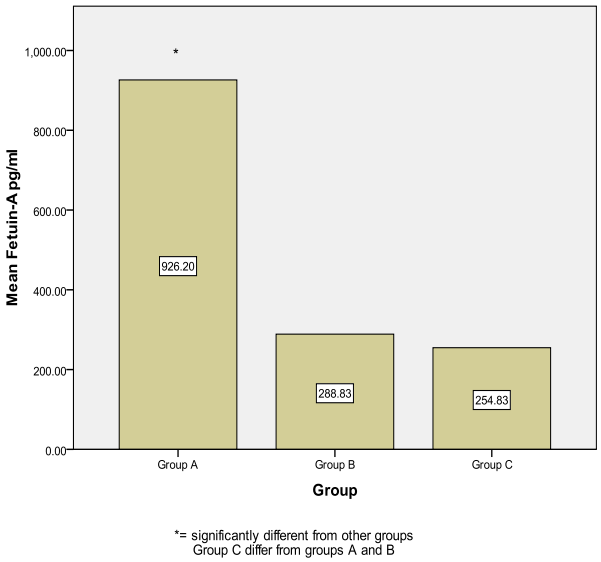

Figure (5) Mean values of Serum fetuin-A levels

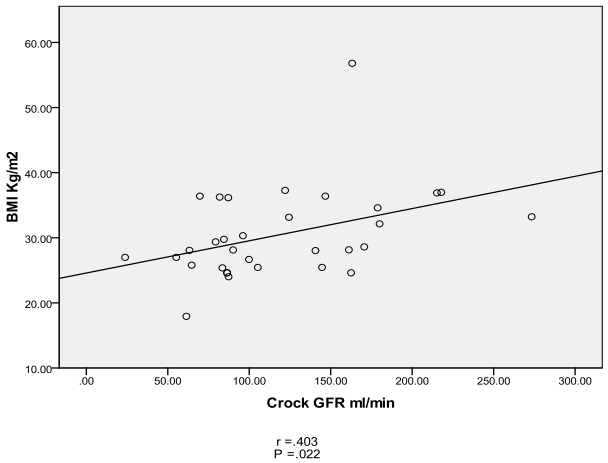

Figure (6) Significant correlation between BMI and GFR (Crock-Gault) in group A

Whereas, in group B (diabetic negative microalbuminuria) Pearson's correlation coefficient values of 0.388 and P value 0.041 indicating significant correlation between weight and aldosterone levels (Figure-7).

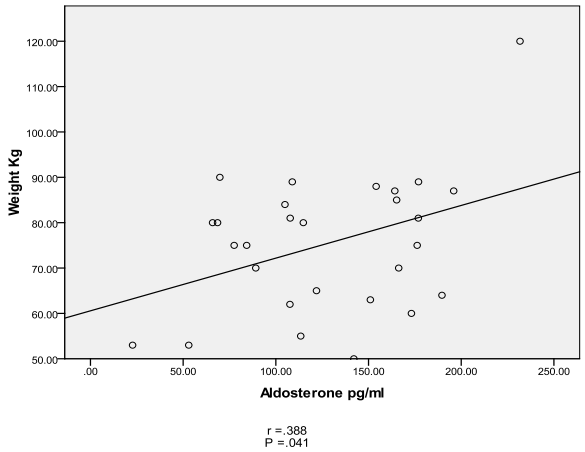

Figure (7) Significant correlation between weight and aldosterone in group B.

As shown in (figure-8) Pearson's correlation coefficient values of 0.390 and P value 0.040 indicating significant correlation between serum chloride (Cl) and fetuin-A levels in diabetics without microalbuminuria.

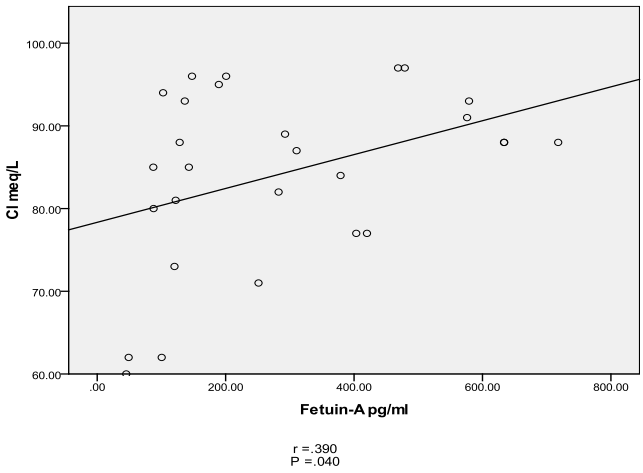

Figure (8) Significant correlation between chloride and fetuin-A in group B.

Whereas, Pearson's correlation coefficient values of 0.431and P value 0.022 indicating significant correlation between serum sodium and fetuin-A in non-albuminuric diabetics, as presented in (figure-9).

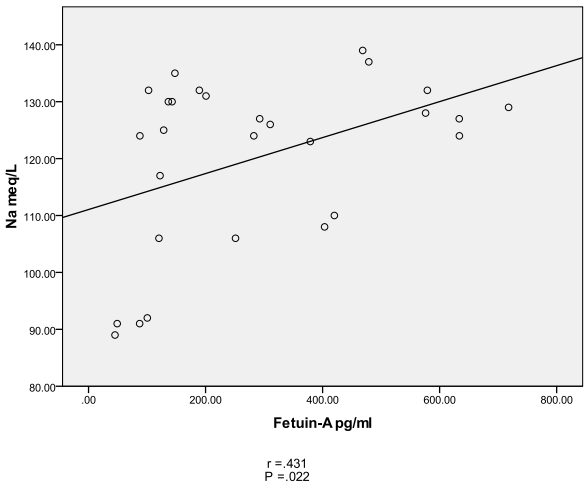

Figure (9) Significant correlation between sodium and fetuin-A in group B.

Discussion

The level of glycemic control seems to be the strongest factor influencing transition from normoalbuminuria to microalbuminuria[31]. Glycemic control plays as vital role in diabetic microvascular complications. It was shown that for each 1% reduction in updated mean of HbA1c there is a 37% reduction in microvascular complication risks [32].

The FSG in diabetic patients mean value was (12.20±4.64 m mol/L) and HbA_{1C} mean was (8.24±1.84 %) which is significantly higher than the control group (mean FSG 5.12±0.63 m mol/L, HbA_{1C} mean 4.62±0.88%), as summarized in (figure-1). There was no positive correlation between (FSG, HbA1c) and microalbuminuria, in contrast to a study by Chen et al [31].

Considering renal function assessment; the mean values of serum creatinine and estimated GFR were within normal range for both groups (Group A diabetics with positive microalbuminuria and Group B diabetics with negative microalbuminuria as shown in (figure-2) and (figure-3). Estimated GFR and serum creatinine depend on the renal hemodynamics, systemic blood pressure, urinary findings, and susceptibility to therapeutic intervention. On the basis of these findings, it is concluded that microalbuminuria may not be associated with abnormal creatinine or creatinine clearance. [33]

Among the important parameters that had been shown to directly affect renal haemodynamics and alter the afferent/efferent balance, is BMI which could result in glomerular hypertension , hyperfiltration and ultimately, renal injury[34]. The present study shows a positive correlation between (BMI, weight) and estimated GFR (Crock-Gault) in diabetic patient with positive microalbuminuria as in (figure-6).

Studies had explained the assessment of GFR in relation to body dimensions, however, it is important to be aware that renal function equations are subjected to BMI-associated bias, for example: Kwakernaak et al compared between Cockcroft–Gault and Modification of Diet in Renal Disease (MDRD) in healthy subject, and found that the effect of BMI on overestimation of GFR was the largest for Cockcroft–Gault and less for MDRD [35]. The renal haemodynamic profile in overweight and obesity, and in subjects with a central body fat distribution, may be affected by other factors which are sodium intake and volume homeostasis, as well as long-term susceptibility to renal damage. [36, 37]. Interestingly, in young normotensive subjects, overweight is associated with a rise in filtration fraction (FF) in response to high salt intake, whereas in lean subjects GFR increases without a rise in FF. In overweight subjects, moreover,a high salt intake is associated with a larger increase in the extra cellular volume (ECV) than in lean subjects, supporting the impact of subtle changes in renal haemodynamics on volume homeostasis[38], as being presented in this study with a positive correlation between body weight and serum aldosterone (figure-7) in group B diabetic patients.The long-term consequences of this unfavorable renal haemodynamic profile, elicited by the combination of overweight and excess sodium intake may well contribute to the development of salt-sensitive hypertension and renal damage later in life[39].

Diabetic patients are more prone to develop electrolyte disturbances because of disease state itself and associated disruption of blood glucose homeostasis. The Use of antidiabetic drugs also leads to the development of electrolyte disturbances[40].In the present study, the mean values of serum Na+ level in both groups of diabetic patients were significantly reduced compared to control group (Table-1) . Similar findings were observed by Alaka Das [41].

There were individual variations in sodium level in both groups of diabetic patients some of them were within normal range, but others were under normal range .Hyponatremia is the most common electrolyte abnormality in clinical practice and is associated with increased morbidity and mortality and even small decreases of serum sodium are associated with increased probability for adverse outcomes [42]. Drugs represent a common cause of hyponatremia in individuals with diabetes [42]. Such as the first generation sulphonylureas (tolbutamide and chlorpropamide) [43, 44]. Other drugs of common utilizations include NSAIDs, angiotensin converting enzyme inhibitors, rosiglitazone or even amlodipine. Patients with central nervous system disorders,

pulmonary disorders including lung infections, and malignancies may exhibit hyponatremia due to the syndrome of inappropriate antidiuretics. Patients with diabetic nephropathy and chronic renal failure are very prone to the development of hyponatremia due to decreased water excretion [42].

Furthermore, potassium levels in both groups of diabetic patients were near to normal range , with no significant change in serum K+ level had been observed between the diabetic group and control group. Similar findings were reported by Alaka Das [41].

Although there were elevated serum aldosterone levels in microalbuminuric diabetics but the wide variation of these values when analyzed statistically were not of enough significance when compared with mean value of diabetic negative microalbuminuria and control group (Figure- 4) which are similar to the result obtained by Hollenberg et al [45]. Meanwhile, a positive correlation between weight and serum aldosterone level was observed in group B patients (figure-7), similarly Tuck et al. demonstrated that weight loss is accompanied by reductions in PRA (plasma renin activity) and aldosterone, irrespective of sodium intake, and this affects the decline in BP in obese patients [46]. Interestingly, high levels of PRA, ACE, aldosterone, and insulin with sodium retention and potassium loss were found in patients with visceral obesity, but all of these tended to disappear upon weight reduction and were not found in patients with peripheral obesity [47]. Another study demonstrated that the PAC (plasma aldosterone concentration) is positively correlated with the amount of visceral adipose tissue and is inversely correlated with insulin sensitivity, independent of the PRA level. This suggested that a fat derived- substance contributes to aldosterone excess in patients with visceral obesity [48].

Fetuin -A had been reported to have a role in causing vascular calcification in diabetic patient, fetuin-A gives a picture if the patient has calcium deposition in vascular (calcification occurs or not), it is a multifunctional glycoprotein predominantly secreted by the liver and mainly involved in promoting insulin resistance[49]. Accumulating experimental and epidemiological studies reported that it was associated with a spectrum of cardiometabolic disorders, such as metabolic syndrome [50], nonalcoholic fatty liver disease [51], T2DM [52], and cardiovascular diseases (CVD) [53].A study by LV X et al showed a positive association between serum fetuin-A levels and albuminuria in patients with metabolic syndrome or T2DM [54], however, no such correlation had been detected in this study.

Furthermore, the mean value of serum fetuin-A in diabetics with positive microalbuminuria was higher than that in diabetics with negative microalbuminuria and control group (Figure-5) and this indicated to uncontrolled DM .

In this study, we evaluated the associations of some parameters related to microvascular diseases in patients with T2DM and early diabetic nephropathy as the degree of albumin excretion, renal function (GFR and serum creatinine). All of these parameters showed no significant correlation with fetuin A, nor to correlate with some clinical and metabolic parameters as BMI, blood pressure which are similar to the study by Ayman Ramadan et al [55].

However, it had been found that fetuin-A levels seem to be associated with prevalent macrovascular disease (as coronary artery disease, stroke and peripheral artery disease) in T2DM, and fetuin-A serum levels are not associated with microvascular complications (24 h urinary albumin excretion) in patients with early diabetic nephropathy [56] which almost in agreement with our study in some extent in that fetuin-A levels do not correlate with metabolic parameters in T2DM patients with prevalent late complications.

On the other hand, serum fetuin -A showed a positive correlation with serum chloride in group B as shown in (figure-8) and this is obvious that chloride is most commonly associated with proportionate changes in sodium concentration so therefore, the concentration of Cl usually parallels to that of sodium and may correlate with fetuin A in same mechanism that control serum level of sodium through (RAAS activation, higher insulin concentration) causing sodium retention with a parallel increase in chloride level[57]. Similarly, a positive correlation between serum sodium level and fetuin- A is present as shown in (figure-9). In diabetic patient, there are 2 mechanisms that cause sodium retention, first one is the activation of (RAAS), whereas the secon, T2DM, have a high circulating insulin level but lack its functional role in regulating glucose level,and insulin thought to have a role in increasing sodium level by its antidiuretic effect. High concentration of fetuin A in patients with type 2 diabetes is associated with increasing insulin level; these two mechanism may counteract the osmotic diuresis that occur due to hyperglycemia [58].

According to ther result of this study, it is concluded that in diabetic patient with microalbuminuria it may not necessary to have high serum level of aldosterone besides that aldosterone hormone is not the only factor that cause calcification. Calcification may occur due to diabetes itself .Microalbumiuria in diabetics may not associate with the decline in renal function or a change in estimated GFR. In this study diabetic patients with microalbuminuria are characterized with high level of

(FSG &HbA_{1c}) in comparison with control group and this increment may have a relationship with the increment of fetuin A .

In the current study, metabolic parameter like weight and body mass index showed a correlation with aldosterone level but lack this relation with fetuin- A.

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Limitation

Lack of individual variation (ethnic and race).

This study conducted on small scale of patients.

This study need longer duration of diabetes more than 7 years.

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