SERUM LEVELS OF ADIPOCYTE FATTY ACID BINDING PROTEIN 4 AND RETINOL BINDING PROTEIN 4 AS BIOMARKERS FOR EARLY DETECTION OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES

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ABSTRACT

Background: Adipocyte fatty acid binding protein 4(A-FABP4) and retinol binding protein 4(RBP4) are recently discovered adipokines, which are members of lipocalin family. Both adipokines have been proposed to be important markers for metabolic syndrome and diabetes mellitus **.** Diabetic nephropathy is a leading cause of chronic kidney disease in patient starting renal replacement therapy and is associated with increased cardiovascular mortality **.**

Objective: To study serum A-FABP4 and RBP4 levels in patients with type 2 DM with different stages of diabetic nephropathy and to investigate whether serum A-FABP4 and RBP4 could be used as biomarkers-in single or combination-for early detection of diabetic nephropathy.

Subjects and methods: 60 subjects were included in this study ,they were divided into six groups according urinary albumin excretion(UAE) and glomerular filtration rate (GFR) ***Group 1*** (Control group) consists of 10 patients who are normo-albuminuric with normal GFR . ***Group 2*** consists of 10 patients who are normoalbuminuric with increased GFR≥120 ***Group 3*** consists of 10 patients who are microalbuminuric i.e. UAE 30-300 mg/day. ***Group 4*** consists of 10 patients who are macroalbuminuric i.e. UAE ≥ 300 mg/day without renal impairment (normal creatinine and GFR > 90 ml/min/1.73m²). ***Group 5*** consists of 10 patients who are macroalbuminuric with renal impairment and declining GFR <90 ml/min/1.73m². ***Group 6*** consists of 10 patients who are end-stage renal disease (GFR <15 ml/min/1.73m²). Measurement of serum AFABP4 , serum RBP4 , UAE, GFR were done for every subject

Results: There was significant increase in the serum level of AFABP4 and RBP4 among different stages of diabetic nephropathy and there was significant difference between microalbuminuric group and normoalbuminuric group so both biomarkers can be used for early detection of diabetic nephropathy. Both AFABP4 and RBP4 correlated positively with UAE and negatively with GFR.

Conclusion: High circulating AFABP4 and RBP4 concentrations were demonstrated in early diabetic nephropathy in type 2 DM. AFABP4 and RBP4 increased significantly with the progression of diabetic nephropathy. Large scale multicenter and prospective studies are necessary to gather a definitive support that these adipokines might be directly involved in early detection of diabetic nephropathy and in impairment of kidney function in type 2 DM.

Key words : AFABP4 ,RBP4 ,diabetic nephropathy ,type 2 diabetes

INTRODUCTION

In recent years it has been shown that adipokines may play important roles in pathogenesis of insulin resistance and related disorders[(1)].

Adipocyte fatty acid binding protein 4(A-FABP4) and retinol binding protein 4(RBP4) are recently discovered adipokines, which are members of lipocalin family[(2)]. Both adipokines have been proposed to be important markers for metabolic syndrome and diabetes mellitus[(3)].

A-FABP4 has been regarded as an adipocyte and macrophage specific proteins and demonstrated as an important lipid chaperone related to type 2 DM in mice models[(4)]. It is one of the most abundant proteins in mature adipocytes[(5)]. It belongs to a family of fatty acid binding proteins, which are small cytoplasmic proteins expressed into a highly tissue specific manner, thought to be important in mediating intracellular fatty acid trafficking and energy metabolism[(6)].

Recent studies in animal models suggested that A-FABP gene protected mice from insulin resistance and hyperinsulinemia associated with both diet induced obesity[(7)] and genetic obesity[(8)].

In a recent study, high circulating A-FABP4 concentration was associated with high plasma creatinine level in patients with type 2 DM[(9)].

Retinol binding protein 4 is a transport protein for retinol (vitamin A) in the circulation and a previous study showed that RBP4 might affect insulin responsive glucose transporter 4 (GLUT-4) in adipocytes which is associated with insulin sensitivity[(10)].

Elevated circulating RBP4 have been found in subjects with insulin resistance impaired glucose tolerance and type 2 DM[(11)].

Mechanistic studies have suggested that RBP-4 impaired insulin sensitivity by inhibition of insulin receptor substrate-1 phosphorylation and

phosphatidylinositol 3-kinase activation in muscle by induction of glucose production in liver[10].

The association of RBP4 level with decreased flow mediated vasodilatation and retinopathy suggest that the serum RBP4 level is predictive of and/or contributory to the vascular complications in type 2 DM[12].

Diabetic nephropathy is a leading cause of chronic kidney disease in patient starting renal replacement therapy and is associated with increased cardiovascular mortality[13].

There are accumulating evidence suggesting that risk of diabetic nephropathy starts when urine albumin excretion values are still within normoalbuminuric range[14].

In 2010 Toruner and his associate examined serum A-FABP4 and and RBP4 in 87 patients with type 2 diabetes and they found that high serum A-FABP4 was demonstrated in type 2 diabetes patients with early diabetic nephropathy and also it was associated with impaired renal function and increased albumin excretion rate and although RBP4 was not changed in early diabetic nephropathy there was a clear relationship between it and impaired renal function[15].

Whether these adipokines could be used as biomarkers for early detection of diabetic nephropathy as single or in combination was the motive beyond this study.

SUBJECTS AND METHODS

This study has been conducted on 60 patients with type 2 DM from the outpatient clinic and inpatient of Internal Medicine Department, Faculty of Medicine, Zagazig University Hospitals from the period of May 2011 to May 2013.

Subjects:

The study included a total number of 60 subjects (44 males and 16 females). Studied subjects were sub divided into six groups: according to urinary Albumin excretion (UAE) and glomerular filtration rate (GFR).

(1) Group 1 (Control group) consists of 10 patients (8 males and 2 females) who are normo-albuminuric i.e. urinary albumin excretion < 30 mg/day with normal glomerular filtration rate and normal kidneys in abdominal ultrasound.

Their age ranged from 42- 57 years with a mean value ± SD of 49.3 ±4.9 years. Their BMI ranged from 29.2- 30.8 kg/m² with a mean±SD of 30.2± 1.1 kg/m².Duration of diabetes in years ranged from 2.5 – 9 years with a mean value ±SD of 6.3 ±2 years and associated hypertension was found in 4 of them.

(2) Group 2 consists of 10 patients (8 males and 2 females) who are normoalbuminuric i.e. UAE < 30 mg/day with increased GFR≥120 (16) with a mean value ±SD 123 ± 3.2 ml/min/1.73m² ranged from 120-129 ml/min/1.73m² and increased kidney size in ultrasound (normal kidney size in ultrasound 9-12 cm in length and 4-5 cm in width) (17) .Their age ranged from 41-50 years with a mean value ±SD 45.7 ±2.7 years .Their BMI ranged from 29.4-32.1 kg/m² with a mean value ±SD 30.6±1 kg/m² .Duration of DM ranged from 3 to 8.5years with a mean value kg/m² ±SD of 6.4 ±2.1years. Associated hypertension was found in 2 of them.

(3) Group 3 consists of 10 patients (6 males and 4 females) who are microalbuminuric i.e. UAE 30-300 mg/day mean ± SD of 160 ± 64mg/day ranged from 75-275mg/day with normal GFR. Mean ± SD 100.3 ±5.9 ml/min/1.73m² ranged from 95-113 ml/min/1.73m².

Their age ranged from 42- 57 years with mean ±SD 49 ±4.7years . Their BMI mean ±SD of 31.6 ±1.3 kg/m² ranged from 30 -33.5 kg/m². Duration of DM ranged from 3.5- 14.5years with a mean of 9.5 ± 3.2years. Associated hypertension was found on 4 of them.

(4) Group 4 consists of 10 patients (7 males and 3 females) who are macroalbuminuric i.e. UAE ≥ 300 mg/day with a mean ±SD of 418 ±63mg/day and ranged from 345 to 520mg/day without renal impairment (normal creatinine and GFR > 90 ml/min/1.73m² mean ± SD of 94.4 ± 4.02 ranged from 90 to 103 ml/min/1.73m².

Their age ranged from 41-54 years with a mean ± SD 48.8 ±4.7 years .Their BMI ranged from 29.9 to 33.5 kg/m² with a mean ±SD value of 31.8 ± 1.35 kg/m² .Duration of DM mean ±SD of 12.5± 4 years ranged from 5.5-18years . Associated hypertension was found in 3 of them.

(5) Group 5 consists of 10 patients (8 males and 2 females) who are macroalbuminuric , their UAE ranged from 390 – 800mg/day with mean ±SD 642± 142mg/day with renal impairment and declining GFR (<90) ranged from 35-80 ml/min/1.73m² with a mean ±SD of 55.1 ± 14 ml/min/1.73m².

Age ranged from 42-63 years with a mean ±SD 59±6.8years. BMI ranged from 29.2-33 kg/m² mean ±SD 31.3 ± 1.3 kg/m² .Duration of DM ranged from 9-20years with a mean ±SD 14 ± 3.8 years .Associated hypertension was found on 6 of them.

(6) Group 6 consists of 10 patients (7 males and 3 females) who are end-stage renal disease (GFR <15) ranged from 8-14 ml/min/1.73m² with mean ±SD 11.1 ±2.1 ml/min/1.73m²

Age ranged from 48-63years with a mean ±SD 54±4.8 years .BMI from 29.1-32.3 kg/m² mean ±SD 31 ± 1.8 kg/m². Duration of DM ranged from 7.5-22 with a mean ±SD 15.6 ± 4.1. Associated hypertension was found on 6 of them.

UAE were not done in this group as most of them are oliguric.

Exclusion criteria:

Patients with proteinuria from causes other than diabetes e.g: other glomerulopathies, secondary DM, malignancy or obstructive uropathy were not included in the study.

Methods:

Every subject was subjected to:

- Medical history taking.
- Clinical examination.
- Blood samples.
- Abdominal U/S and special comment on kidneys.
- Twenty four hour urine collection and detection of urinary albumin excretion.
- Measurement of glomerular filtration rate. (MDRD-GFR) **(18).**

Full history and thorough clinical examination:

- Age and sex.
- The Body mass index weight in kg/(height in meters)^2
- Arterial blood pressure (systolic and diastolic blood pressure) was measured on 2 separate occasions.
- Duration of DM, associated hypertension and other complications.
- Symptoms and signs of chronic renal failure (Pallor, Earthy Look, Oedema, dyspnea, oliguria……)
- Past history of diseases including renal stons, T.B ,obstructive uropathy and types of medications received with special attention for nephrotoxic agents.

Laboratory investigations :

The following were measured:

- Complete urine analysis (uriscan)
- Complete blood count (by automated blood counter)(Sysmex KX 21).
- Fasting blood glucose and two-hour postprandial blood glucose by Hexokinase methods (Intgra 400 plus Roche diagnostic).
- Glycosylated haemoglobin (Cobas 600 C501 Roche diagnostic).
- Liver function tests (ALT,AST,serum albumin and serum total protein) using (Intgra 400 plus) Roche diagnostic.
- Kidney function tests including serum creatinine and blood urea (Intgra 400 plus) Roche diagnostic. .
- Serum total cholesterol level and Serum triglycerides by (Intgra 400 plus) Roche diagnostic.
- Serum A-$FABP_4$ by a human ELISA kit (www.eiaab.com)
- Serum RBP_4 by a Quantikine human RBP_4 immunoassay (www.RnD systems.com)

Urinary Albumin Excretion (UAE):

Urinary albumin excretion was estimated form the albumin content of 24-hour urine samples. Normal UAE was defined as < 30 mg/day, microalbuminuria as from 30 to 300 mg/day using Biosystem Kit **(19).**

Retinol binding protein 4

Principle of the assay

This assay employs the quantitative sandwich enzyme immunoassay technique .A monoclonal antibody specific to RBP4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any RBP4 present is bound by the immobilized antibody. After washing away any unbound substance, an enzyme linked monoclonal antibody specific to RBP4 is added to the wells. Following a wash to remove any unbound antibody enzyme reagent a substrate solution is added to the wells and color develop in proportion to the amount of RBP4 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Adipocyte fatty acid binding protein4

Test principle

The microtiter plate in the kit has been pre-coated with an antibody specific to fatty acid binding protein ,adipocyte.Standard are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for fatty acid binding protein , adipocyte and Avidin conjugated to Horseradish Peroxidase is added to each microplate well and incubated. Then a TMB substrate is added to each well. Only this wells that contain AFABP4 will exhibit a change in colour .The enzyme substrate reaction is terminated by the addition of a sulphuric acid solution and the change is measured spectrophotometrically at a wave length of 450nm-or +2.the concentration of AFABP4 was determined by comparing the optical denisity of the sample to the standard curve.

Written informed consent from every subject was obtained. The approval of medical ethics committe was obtained.

Statistical Method:

- Data were checked, entered and analysed by using SPSS version 19 software computer package.
- Data were expressed as mean ± SD for quantative variables, number and percentage for categorical variables.
- ANOVA (F test), and post hoc test, chi-squared and correlation analysis were done.

- Multiple regression analysis to find the predictors that related to the RBP4 and AFABP4.
- ROC (receiver operator characteristics) curve was used to find the cut off value for RBP4 and AFABP4.
- Validity was assessed $p < 0.05$ was considered statistically significant.

RESULTS

Table (1): Comparison of the mean values ± SD of AFABP4 and RBP4 among different groups of the study using ANOVA test

	I	II	III	IV	V	VI	F	p
AFABP ng/ml								
Mean ± SD	6.3±1.7	6.2±1.6	12.1±2.1	21 ± 2.7	29.6±2.8	40.4±2.8	338.9	<0.001 (HS)
Range	3.5-9.8	3.7-8.9	8.5-15.4	17-25	25.7-35.7	35.3-45		
RBP uglml								
Mean ± SD	47.4±1.9	47.1±3.1	67.6 ± 3	75.5± 3.4	84.9 ±3.1	94.1±5.2	317.03	<0.001 (HS)
Range	44.9-50.1	41.2-50.9	64-71.8	68.2-29.3	79.4-90.5	84.7-103		

This table revealed that there was highly significant differences among the different groups as regard to AFABP 4and RBP 4($F = 338.9$ and 317.03, $p < 0.001$).

Table (2): Comparison of the mean values ± SD of demographic and laboratory data among different groups of the study using ANOVA test:

	I	II	III	IV	V	VI	F	p
Age (years)								
Mean ± SD	49.3±4.9	45.7±2.7	49 ± 4.7	48.8±4.7	59 ± 6.8	54.1± .8	2.2	0.06 (NS)
Range	42-57	41-50	42-57	41-54	42-63	48-63		
Gender								
Male	8 (80%)	8 (80%)	6 (60%)	7 (70%)	8 (80%)	7 ((70%)	X^2 = 1.7	0.8 (NS)
Female	2 (20%)	2 (20%)	4 (40%)	3 (30%)	2 (20%)	3 (30%)		
BMI								
Mean ± SD	30.2±1.1	30.6 ± 1	31.6±1.3	31.8±1.35	31.3±1.3	31 ± 1.8	2.75	0.027 (S)
Range	29.2-32.8	29.4-32.1	30-33.5	29.9-33.5	29.2-33	29.1-32.3		
Duration of DM in years	6.3±2	6.4±2.1	9.5±3.2	12.5±4	14±3.8	15.6±4.1	14.2	<0.001 (HS)
	2.5-9	3-8.5	3.5-14.5	5.5-18	9-20	7.5-22		
Associated hyeprtension	4 40.0	2 20.0	4 40.0	4 40.0	6 60.0	6 60.0	X^2 4.62	0.46 (NS)
Hb								
Mean±SD	13.4±0.7	13.6 ± 1	13.5±0.7	13.6 ± 0.7	11.2±0.9	10.2 ± 0.9	30.4	<0.001 (HS)
Range	12.5-14.5	12.2-15.4	12.8-14.5	12.4-14.7	9.8-12.5	8.7-11.5		
FBS								
Mean ± SD	98.7±9.8	122.8±7.3	127.2±12.3	133.7±12.1	132±10.4	106.3±12	17.7	<0.001 (HS)
Range	81-111	113-135	105-145	115-155	115-148	89-119		
PBS								
Mean ± SD	201.6±8.3	226.1±116	224.5±10.9	271±28.9	264.7±33	216±19.2	17.6	<0.001 (HS)
Range	185-210	209-245	210-247	230-315	230-330	194-245		
HbA1c								
Mean ± SD	6.3±0.2	7.5±0.4	7.6±0.4	8.6±0.44	8.3±0.6	7.1±0.6	23.6	<0.001 (HS)
Range	5.9-6.5	6.9-8.2	7.2-8.5	7.5-8.7	7.5-9.5	6.3-8.1		
Urea								
Mean ± SD	19±2.4	20 ± 4.1	20.1±3.9	20.2±4.1	46.3±4.5	136±25.5	180.5	<0.001 (HS)
Range	15-22	15-28	15-27	15-28	40-54	94-170		
Creatinine								

Mean ± SD	0.8±0.07	0.85±0.09	0.96±0.1	1 ±0.1	1.98±0.6	7.9 ±1.4	200	<0.001 (HS)
Range	0.7-0.9	0.7-1	0.8-1.1	0.9-1.2	1.3-3	5.4-9.7		
GFR								
Mean ± SD	105.4±7.3	123 ± 3.2	100.3±5.9	94.4±4.04	55.1±14	11.1±2.1	53.6	<0.001 (HS)
Range	95-116	120-129	95-113	90-103	35-80	8-14		
Urinary albumin								
Mean ± SD	14 ± 6	15.9 ± 5.8	160.5±64	418 ± 63	642±142	-	132.5	<0.001 (HS)
Range	5-23	7-24	75-275	345-520	390-800			
TC								
Mean±SD	163.2±15.6	174.7±20.7	202 ± 45	204±29.9	208±34	209±27.5	4.03	0.003 (S)
Range	135-180	145-210	160-285	174-270	175-263	185-285		
TG								
Mean±SD	112.3±12	113.7 ± 8.7	113.9±8.7	114 ± 7.6	118±12	119.1 ± 10.2	0.75	0.58 (NS)
Range	95-135	98-125	100-125	105-125	105-135	105-135		

This table shows no significant difference among different groups of the study as regard to age, gender, associated hypertension and triglycerides, while there was statistically significant difference as regard to BMI and total cholesterol .There was highly significant difference regarding duration of diabetes ,Hb ,fasting blood glucose ,post prandial blood glucose , HbA1C ,blood urea ,serum creatinine ,GFR and urinary albumin excretion .

Table (3): Simple Pearson's Correlation between serum A FABP 4 and each of the studied parameters

	r	**P**
Age	0.38	< 0.001 (HS)
BMI	0.28	< 0.05 (S)
WBC	-0.02	> 0.05 (NS)
Hb	-0.8	< 0.001 (HS)
Platelets	-0.2	> 0.05 (NS)
FBG	0.06	> 0.05 (NS)
PPBG	0.28	< 0.05 (S)
HbA1c	0.32	< 0.05 (S)
Urea	0.82	< 0.001 (HS)
Creatinine	0.82	< 0.001 (HS)
Total cholesterol	0.45	< 0.001 (HS)
Triglycerides	0.27	< 0.05 (S)
GFR	-0.93	< 0.001 (HS)
UAE	0.94	< 0.001 (HS)
Total protein	-0.56	< 0.001 (HS)
Serum albumin	-0.87	< 0.001 (HS)
ALT	-0.1	> 0.05 (NS)
AST	-0.2	> 0.05 (NS)

This table shows statistically high significant positive correlations of AFABP with age ($r = 0.38$, $p<0.001$), urea ($r = 0.82$, $p<0.001$), creatinine ($r= 0.8$, $p < 0.001$), total cholesterol ($r = 0.45$, $p < 0.001$) and UAE ($r=0.94$, $p < 0.001$). There were statistically high significant negative correlations of AFABP with hemoglobin ($r = -0.8$, $p < 0.001$), glomerular filtration rate ($r=-0.93$, $p < 0.001$), serum total protein ($r = -0.56$, $p < 0.001$) and serum albumin ($r = -0.87$, $p < 0.001$). While there were significant positive correlations of AFABP with BMI ($r = 0.28$, $p < 0.05$), triglycerides ($r=0.27$, $p < 0.05$), PPBG ($r = 0.28$, $p < 0.05$) and glycosylated hemoglobin ($r=0.32$, $p< 0.05$)

This table shows also a non-significant positive correlations of AFABP with fasting blood glucose ($r = 0.06$, $p > 0.05$), non-significant

negative correlations of AFABP with white blood cells (r = -0.02, p> 0.05), platelets (r = -0.2, p > 0.05), ALT (r = -0.1, p > 0.05) and AST (r = -0.2, p > 0.05).

Table (4): Simple Pearson's Correlation between serum RBP4 and each of the studied parameters

	r	P
Age	0.35	< 0.001 (HS)
BMI	0.38	< 0.001 (HS)
WBC	-0.04	> 0.05 (NS)
Hb	-0.7	< 0.001 (HS)
Platelets	-0.19	> 0.05 (NS)
FBG	0.19	> 0.05 (NS)
PPBG	0.37	< 0.001 (HS)
HbA1c	0.46	< 0.001 (HS)
Urea	0.71	< 0.001 (HS)
Creatinine	0.7	< 0.001 (HS)
Total cholesterol	0.53	< 0.001 (HS)
Triglycerides	0.23	> 0.05 (NS)
GFR	-0.86	< 0.001 (HS)
Albumin (urine)	0.93	< 0.001 (HS)
Total protein	-0.52	< 0.001 (HS)
Serum albumin	-0.86	< 0.001 (HS)
ALT	-0.09	> 0.05 (NS)
AST	-0.21	> 0.05 (NS)

This table shows statistically high significant positive correlations of RBP4 with age (r = 0.35, p < 0.001), BMI (r = 0.38, p < 0.001), PPBG (r=0.37, p < 0.001), glycosylated hemoglobin (r = 0.46, p < 0.001), urea (r=0.71, p < 0.001), creatinine (r = 0.7, p < 0.001), total cholesterol (r=0.53, p < 0.001) and UAE (r = 0.93, p < 0.001). There was statistically high significant negative correlations of RBP4 with hemoglobin (r = -0.7, p<0.001), glomerular filtration rate (r = -0.86, p < 0.001), serum total protein (r = -0.52, p < 0.001) and serum albumin (r = -0.86, p < 0.001).

This table shows non-significant positive correlation of RBP4 with total triglycerides (r = 0.23, p > 0.05) and fasting blood glucose (r = 0.19, p>0.05). While there were non-significant negative correlations of RBP with white blood cells (r = -0.04, p > 0.05), platelets (r = -0.19, p > 0.05, ALT (r = -0.09, p > 0.05) and AST (r = -0.21, p > 0.05).

Table (5): Multiple regression Analysis for factors predicting the RBP

	B ± SE	P	95% C1
HBA_1C	0.32 ± 1.16	0.001	5.05 – 9.7
HB	0.233 ± 0.84	0.003	1.0 - 4.39
GFR	0.89 ± 0.06	0.001	0.54 – 0.299

Each factor with a significant correlation with RBP4 were studied and the only significant factors were HB, HBA1C and GFR

Table (6): Multiple regression analysis for factors predicting the AFABP

	B ± SE	P	95% C1
HBA_1C	0.132 ± 0.64	0.001	1.0 – 3.45
GFR	0.527 ± 0.032	0.001	0.24 – 0.108
UAE	0.302 ± 1.6	0.001	4.4 – 10.99

Each factor with a significant correlation with AFABP4 were studied and The only significant factors that add significant to the model were HBA1C, GFR and UAE.

Correlations between $AFABP_4$ & RBP_4

r = 0.75 P < 0.001
Highly significant correlation between both biomarkers.

Table (7): Validity of A FABP4 and RBP4 in diagnosis of DN.

Biomarker	Sensitivity %	Specificity %	Predictive value +ve	Predictive value -ve	Accuracy %
AFABP	96.7	93.3	93.5	96.6	95.0
RBP4	90.0	96.7	96.4	90.6	93.3
Both	96.7	96.7	96.7	96.7	96.7

AFABP4 was more sensitive while RBP4 was more specific.AFABP4 was more accurate in diagnosis. the sensitivity ,the specificity and the accuracy were increased when both factors were used together than if everyone was used in a single.

DISCUSSION

Diabetic nephropathy is a common microvascular complication among patients with type 2 diabetes mellitus and a major cause of kidney failure. Detection of diabetic nephropathy during its initial stages provides the opportunity for early therapeutic interventions to prevent or delay the onset of complications & improve outcomes[(20)].

Adipocyte fatty acid binding protein (A-FABP4) is one of the most abundant proteins in mature adipocytes[(5)].

There were studies in animal models suggested that A-FABP may be important in glucose homeostasis. Deletion of the A-FABP gene is associated with protection of mice from insulin resistance and hyperinsulinemia associated with obesity[(8)].

Retinol binding protein was reported as an dipokine that impairs insulin sensitivity. Injection of recombinant RBP-4 in normal mice induced insulin resistance[(10)].

Elevated circulating RBP4 concentrations have been found in subjects with insulin resistance, impaired glucose tolerance in type 2DM[(21)].

Our study aimed at detection of serum level of adipocyte fatty acid binding protein 4 and retinol binding protein 4 at different stages of diabetic nephropathy and whether they can be used individually or in combination for early detection of diabetic nephropathy in type 2 diabetic patients.

The current study revealed a significant increase in the AFABP4 concentration among studied groups but no significant difference was observed between control group and 2nd group (group with increased GFR). There was significant difference between microalbuminuric group compared with each of control group and the group with increased GFR. AFABP4 might be considered as early marker for D N. AFABP was higher in ESRD compared to control,normo,micro and macroalbuminuric group that might be attributed to changes in glomerular filtration rate as supported by the negative correlation between AFABP4 and GFR. It also might be due to increased expression of AFABP4 in renal tubules in different stages of DN.

Tourner et al.[(15)] reported a highly significant difference in AFABP concentration between normo & microalbuminuric group of diabetic nephropathy in type 2 diabetes. They suggested that increased AFABP4 concentrations might be associated with early diabetic nephropathy.

Yeung et al.[(22)] showed highly significant difference between normo, micro & macroabbuminuric group of type 2 diabetic nephropathy regarding AFABP4 and this was attributed to impaired renal clearance and increased AFABP4 production by activated macrophage in diabetic nephropathy as macrophage accumulation in the kidney, which is the primary source of AFABP, may occur in diabetic nephropathy.

We observed that AFABP correlated positively with age, BMI, post prandial blood glucose, HbA1C, serum creatinine, total cholesterol, triglycerides and urinary albumin excretion, while AFABP4 correlated negatively with haemoglobin level, GFR and serum albumin and it didn't correlate with fasting blood glucose .The positive correlation between AFABP and BMI was supported by **Xu et al.[(23)]** who fonud that AFABP was higher in obese subjects and weight loss had a decreasing effect on it.

Yeung et al.[(22)] reported that serum AFABP4 correlated positively with serum creatinine and urinary albumin excretion and negatively with GFR .There was no significant correlations of AFABP with HbA1C after adjustment for age ,sex and waist circumference .

Tourner and his associates[(15)] reported that serum AFABP had significant positive correlation with age, BMI, serum creatinine and urinary albumin excretion & also negative correlation with GFR and this goes in harmoney with our result.

Multiple regression analysis for factors with significant association with AFABP showed that the only significant factors associated with AFABP were HbA1c, GFR and UAE. AFABP was associated with nephropathy staging as macrophage accumulation in kidneys increases with progression of nephropathy in diabetes and renal injury and also several pro-inflammatory stimuli could also induce AFABP expressions in macrophages so increase its concentration. This augmented expressions of AFABP4 and macrophage accumulation in the kidney aggravates the local inflammation and contributes to the progression of diabetic nephropathy .

In **Yeung et al.**[22] the multiple regression analysis showed that serum AFABP was independently associated with urinary albumin excretion and this goes in parallel with our result as AFABP was associated with nephropathy staging.

In **Tourner et al.**[15] study, the multiple regression analysis revealed that AFABP4 concentrations were independently associated with age sex, BMI and urinary albumin excretion. The same was observed in the current study.

Cabre et al.[24] had studied serum AFABP in healthy controls and type 2 DM subjects (who were subdivided according to GFR). They documented that AFABP concentrations were higher in type 2 diabetic patients with GFR≤ 60ml/min/1.73m², on the other hand they detected no relationship between AFABP and microalbumninuria and they related that to the number of microabluminuric patient in their study which was relatively small. There was a positive correlation between AFABP and serum creatinine and negative correlation with GFR even in those with normal GFR and normoalbuminuric and these result suggested that AFABP could be early clinical marker of renal derangement in type 2 diabetic patients.

Sommer et al.[25] had studied AFABP in healthy control subjects and chronic haemdialysis patients they documented that AFABP concentrations were increased in non diabetic subjects with end stage renal disease. They attributed that to change in GFR or tubular reabsorption as renal elimination is the major mechanism influencing AFABP concentration. AFABP correlated positively with BMI and serum triglycerides and in multiple regression analysis it remained independently associated with BMI. In accordance with these finding, the AFABP inhibitor BMS309403 is an effective therapeutic agent against sever atherosclerosis and type 2 DM in mice[4].

We observed a significant difference in RBP4 level in between each of studied groups but no significant difference between control group and 2nd group (group with increased GFR). There was significant difference between microalbuminuric group compared to each of control and group with increased GFR. RBP4 might be considered as early marker for diabetic nephropathy. RBP4 was highest in the group of ESRD compared to all other groups. There was significant difference between the group with declining GFR and each of other groups so RBP4 could be used as a biomarker for renal dysfunction in diabetic patients. The kidneys play an important role in the whole body retinol homeostasis which is regulated by glomerular filtration and subsequent reabsorption by proximal tubular cells .Increased level in early diabetic nephropathy may be attributed to the inflammatory status as RBP4 is known to be related with some markers of low grade inflammation[26].

Raila et al.[27] had studied RBP4 in nomoalbuminuric and microalbuminuric type 2DM and they supported our results as they found a significant difference in between studied groups of diabetic nephropathy regarding RBP4. This might be attributed to incipient diabetic nephropathy as its production occur not only in adipose tissue but in other organs such as kidneys In their study the GFR was not different in between studied groups so elevated RBP4 was not related to GFR.

Our results were supported by **Xu et al.**[28] who had measured RBP4 in subjects with impaired glucose tolerance and newly diagnosed type 2 DM and found that serum RBP4 is increased in type 2 DM and associated with the risk of microalbuminuria.

On the other hand, **Tourner et al.**[15] showed non significant difference in RBP4 concentration between those with diabetic nephropathy and those without diabetic nephropathy this may be attributed to different design of the studies, charchteristic of the patients or treatment used.

Ziegelmeier et al.[29] had studied serum RBP4 in chronic haemodialysis (diabetic and non diabetic subjects) and showed that the level is almost fourfold higher compared with control group. The results may be attributed to the fact that renal excretion is the primary pathway for RBP4 clearance. RBP4 correlated positively with creatinine and urea and negatively with GFR .Multiple regression analysis revealed that serum creatinine remained independently associated with RBP4.

Yang et al.[10] documented that the use of fenretinide, which is a synthetic retinoid designed

for cancer therapy, in obese mouse increased the renal excretion of RBP4 ,normalizes serum RBP4 and improve insulin sensitivity.

In the current study serum RBP4 correlated positively with age, BMI, HbA1C, post-prandial glucose, serum creatinine, total cholesterol and UAE and correlates negatively with Hb, GFR and serum albumin and this result was supported by **Chang et al.**[30] who found that RBP4 positively correlated with serum creatinine and degree of albuminuria and negatively correlated with GFR. RBP4 is a small molecular weight protein which is filtered through glomerulus and then reabsorbed by renal tubules so exceded tubular capacity and tubular dysfunction could alter its homeostasis.

Lee et al.[31] and **Balagopal et al.**[26] supported us as they found a positive correlation between RBP4 and BMI .In contrary **Jia et al.**[32] found that RBP4 correlated with visceral adiposity but not with BMI as RBP4 mRNA is elevated in the visceral compared to the subcutaneous adipose tissue.

Tourner et al.[15] had found that serum RBP4 correlated positively with triglycerides and serum creatinine but not with UAE and correlated negatively with GFR so their data in contrary to us suggested that RBP4 didn't change in early diabetic nephropathy.

Raila et al.[27], supported our as they found that RBP4 correlated positively with triglycerides, urinary albumin excretion and HbA1C.Multiple regression analysis showed that RBP4 was associated only with urinary albumin.

Multiple regression analysis showed that RBP4 were independently correlated with HbA1c, Hb and GFR.

In **Tourner et al.**[15] multiple regression analysis showed that RBP4 were independently correlated with triglycerides & serum creatinine but not with UAE.

Masaki et al.[33] had studied RBP4 in type 2 DM (normo,micro,macro and ESRD) they found non significant difference between normo and microalbuminuric patients and this in contrast to our results .On the other hand they agreed with us as they found a significant increase in the groups with macroalbuminuric and ESRD compared to the normoalbuminic group. They found that RBP had no relation to early diabetic nephropathy. They documented some limitations on their study. First, the study included a relatively small number of patients. Second, there was limitation associated with the interpretation of data in a cross sectional study.

Also Masaki et al reported that RBP4 correlated positively with serum creatinine, urea and urinary albumin & negatively with creatinine clearance. Multiple regression analysis reported that RBP4 was associated with serum creatinine and creatinine clearance and this was attributed to that impaired clearance & catabolism of RBP4 in kidneys lead to accumulation of RBP4.

In the current research we found a highly significant positive correlation between AFABP4 and RBP4 so one biomarker can be used instead of another. On the contrary, **Tourner et al.**[15] demonstrated no correlation between the two biomarker although a positive correlation was expected as both adipokines participate in lipocalins family and both were found to be associated with renal dysfunction. They attributed this result to the cross sectional design of their study.

The validity of AFABP4 and RBP4 in diagnosis of diabetic nephropathy was assessed we found out that AFABP4 is more sensitive while RBP4 is more specific .Both of them showed a better positive predictive value than negative predictive value. The sensitivity and the specificity of both was increased if used together as the accuracy of both is more than the accuracy of every one alone.

CONCLUSION AND RECOMMENDATION

High circulating AFABP4 and RBP4 concentrations were demonstrated in early diabetic nephropathy in type 2 DM. AFABP4 and RBP4 increased significantly with the progression of diabetic nephropathy.

A highly significant correlation between both adipokines was demonstrated and both were associated with renal dysfunction.

So both adipokines can be used as biomarkers in single or in combination for early detection of type 2 diabetic nephropathy and they can be used for stratifying nephropathy staging. It was demonstrated that their use together is more specific and more sensitive in the diagnosis of diabetic nephropathy than using either alone.

Early detection of diabetic nephropathy is recommended to prevent disease progression and protect the patients from developing ESRD.

Good glycemic control, control of blood pressure ,control of hyperlipidaemia and also control of anaemia are recommended to prevent the development and progression of diabetic nephropathy.

Large scale multicenter and prospective studies are necessary to gather a definitive support that these adipokines might be directly involved in early detection of diabetic nephropathy and in impairment of kidney function in type 2 DM

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الملخص العربي

ان اعتلال الكلي السكري سببا رئيسيا لمرض الفشل الكلوي المزمن في المرضي الذين يبدأون العلاج بالغسيل الكلوي ويرتبط بزيادة الوفاة من امراض القلب. توجد دلالات متراكمة ان خطر اعتلال الكلي السكري يبدأ حينما تكون كمية الألبيومين في البول لا تزال في المعدل الطبيعي.

يعمل البروتين ٤الملازم للريتينول كناقل لفيتامين أ ويتكون بشكل رئيسي في خلايا الكبد ويفرز في الدورة الدموية مرتبط بفيتامين أ ويتم افرازه أيضا في الأنسجة الدهنية.

يعتبر البروتين ٤ الملازم للحمض الدهني للخلايا الشحمية من اكثر البروتينات وفرة في الخلايا الشحمية كما أن له علاقة بزيادة الوزن وارتفاع السكر بالدم ومتلازمة الأيض.

الهدف من الدراسة:دراسة مستوي البروتين ٤ الملازم للحمض الدهني للخلايا الشحمية و البروتين ٤الملازم للريتينول في مختلف مراحل اعتلال الكلي السكري في مرضي السكر من النوع الثاني وكذلك معرفة امكانية استخدامهما معا او كل علي حدة في الاكتشاف المبكر لاعتلال الكلي السكري

وقد اجريت هذه الدراسة في جامعة الزقازيق في الفترة بين مايو ٢٠١١ الي مايو ٢٠١٣ في قسم الباطنة العامة الداخلي و كذلك في العيادات الخارجية.حيث اجريت علي ستين مريض تم تقسيمهم الي ست مجموعات تحتوي كل واحدة علي عشرة مرضى :

المجموعة الاولي:لديهم كمية افراز الالبيومين في البول طبيعية وكذلك معدل الترشيح الكبيبي المجموعة الثانية:لديهم كمية افراز الالبيومين في البول طبيعية و زيادة في معدل الترشيح الكبيبي المجموعة الثلثة:لديهم كمية افراز الالبيومين في البول من ٣٠ مج الي ٣٠٠مج في اليوم ومعدل الترشيح الكبيبي طبيعي المجموعة الرابعة:لديهم كمية افراز الالبيومين في البول اكثر من ٣٠٠مج في اليوم ومعدل الترشيح الكبيبي طبيعي المجموعة الخامسة :لديهم كمية افراز الالبيومين في البول اكثر من ٣٠٠مجو معدل الترشيح الكبيبي اقل من ٩٠ مل بالدقيقة المجموعة السادسة:المرحلة النهائية لمرضي الفشل الكلوي و معدل الترشيح الكبيبي اقل من ١٥ مل بالدقيقة

النتائج:- يوجد زيادة في مستوي البروتين ٤الملازم للريتينول و البروتين ٤ الملازم للحمض الدهني للخلايا الشحمية في الد م- توجد علاقة طردية بين كلا من البروتين ٤الملازم للريتينول و البروتين ٤ الملازم للحمض الدهني للخلايا الشحمية في الدم وكلا من العمرو مؤشر كتلة الجسم والهيموجلوبين و الهيموجلوبين المتسكر والكرياتينين بالدم والكولسترول وعلاقة عكسية مع معدل الترشيح الكبيبي والالبيومين بالدم-

وقد اشار تحليل الانحدار المتعدد لارتباط البروتين ٤الملازم للريتينول مع الهيموجلوبين و الهيموجلوبين المتسكر و معدل الترشيح الكبيبيوارتباط البروتين ٤ الملازم للحمض الدهني للخلايا الشحمية مع معدل الترشيح الكبيبي و كمية الالبيومين في البول والهيموجلوبين المتسكر

وقد وجد زيادة في دقة التشخيص عند استعمال العاملين معا كما ان بينهما علاقة ارتباط طردية

الخلاصة و التوصيات:

:ارتفاع مستوي البروتين ٤الملازم للريتينول والبروتين ٤ الملازم للحمض الدهني للخلايا الشحمية بالدم في المراحل المبكرة من اعتلال الكلي السكري وكذلك في مختلف مراحل اعتلال الكلي السكري

وينبغي اجراء دراسات كبيرة علي مستوي واسع من خلال مراكز طبية متعددة لكي تدعم امكانية استخدام البروتين ٤ الملازم للحمض الدهني للخلايا الشحمية والبروتين ٤الملازم للريتينول في الاكتشاف المبكر لاعتلال الكلي السكري.