Estimation of Urinary Vitamin D Binding Protein as a Biomarker in Type 2 Diabetic Nephropathy and its correlation with estimated Glomerular Filtration Rate

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Abstract:

Background: Diabetic nephropathy is one of the most common complications, which may lead to chronic kidney disease in diabetic patients.

Objective: The studyobjectiveis to assess the role of urinary vitamin D binding protein as biomarker before onset of nephropathy in type 2 diabetes.

Patients and Methods: The urinary vitamin D binding protein levels were measured by ELIZA , estimated glomerular filtration rate levels were measured by the Chronic Kidney Disease-Epidemiology (CKD-EPI) equation. Ninety individuals were involved in this study which comprises 20 individuals apparently healthy (group I), 40 diabetic patients with normal eGFR (≥ 90 mil/min/1.73m^2) and 30 diabetic patients with abnormal value of eGFR (< 90 mil/min/1.73m^2) (group III).

Results: The mean urinary vitamin D binding protein in all groups showed a gradual increase with a significance P values. A negative correlation was obtained between vitamin D binding proteinand estimated glomerular filtration rate in group I with non significant P-value and group IIIwith significant P-value.

Conclusion: Since the increased vitamin D binding protein levels were negatively correlated with decreased estimated glomerular filtration rate levels, so vitamin D binding protein level can be considered as a novel predictor for monitoring type 2 diabetes before onset of diabetic nephropathy.

Keywords: vitamin D binding protein, estimated glomerular filtration rate, diabetic nephropathy.

Introduction:-

Due to world population growth and other factors such as aging , obesity and decreasing physical activities, the number of diabetics is continue to increase each year. Increasing diabetics prevalence, result in increasing of death due to diabetics and their complications such as cardiovascular and nephropathies [1].The group-specific component (Gc) is the major vitamin D binding protein (VDBP) in plasma [2] with molecular weight 56 K Da [3] that consists of a 458 amino acids [4] produced in hepatic cells [2 & 5], also tissues of other organs have low concentrations of VDBP than present in the liver, such as adult kidney, testis, abdominal fat, and eighteen day fetal yolk sac as well as VDBP presence on the cell surface of immunocytes and cytotrophoblasts[5]. VDBP shows a significant role in immune response and inflammation process [6]. Bioinformatics analysis also indicated that Gc had an important action on apoptotic activity and epidermal growth factor receptor. The estimated glomerular filtration rate (eGFR) is one of the key markers responsible for chronic kidney disease (CKD).Estimated glomerular filtration rate (eGFR) is more accurate than serum creatinine alone to assess renal function, as serum creatinine is affected by muscle mass, and related factors of age, gender, and race. eGFR is not reliable for patients with rapidly changing creatinine levels, highest degree in muscle mass and body size, or altered diet patterns. The normal range of eGFR is ≥ 90 ml/min/1.73m^2[7]. Our objective is to assess the role of urinary vitamin binding protein(VDBP) for monitoring diabetes type 2 before onset of diabetic nephropathy (DN).

2. Materials and Methods:-

2.1. Patients and samples:-

This study was conducted at AL-Yarmouk Teaching Hospital and the National Diabetic Center /Baghdad from January 2013 to September 2014.The study included ninety individuals attended these centers checked by the specialist in the out patients clinic, twenty voulenteers were selected from the local community non diabetic non kidney diseases with age and sex matched apparently healthy (group I), 40 type 2 diabetic patients with normal eGFR (≥ 90 ml/min/1.73m^2) (group II) and 30 type 2 diabetic patients with abnormal eGFR (< 90ml/min/1.73m^2) (group III).eGFR was checked by using chronic kidney diseases equation depending on serum creatinine

ratio. Group I included 10 female and 10 male,with the mean age of 53.8 years ± 1.988 S.E., group II included 18 female and 22 male, with the mean age of 55years ± 1.493 S.E., group III included 15 female and 15 male, with the mean age of 58.6 years ± 1.395 S.E.. All serum samples obtained from the three groups were examined by using the routine methods such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and lipid profile. All these investigations were requested by the specialist to exclude the cases with abnormal results(Data not shown). All subjects of group I, II and III were checked to be free from any chronic diseases (hypertension, heart attack, stroke, autoimmune diseases, tumors, urinary tract diseases, pregnancy, hematological disorders) which might have an impact on the studied parameters. All clinical data were collected for each patient by filling a special form.

2.2. Methods:-

2.2.1. General Urine Examination:-

This test was done by following the conventional common method. The aim of doing this routine test was to exclude all cases with abnormal urine findings (erythrocytes, leukocytes and active sediments).About 20 ml of mid stream urine were collected and centrifuged at 3000 X for about 10 minutes then supernatants were divided and stored at -20 ˚C until used.

2.2.2. Estimated glomerular filtration rate (eGFR):-

The protocol of serum creatinine applied in this test depends on the manufacturer's instruction [7].The concentration was measured by the Chronic Kidney Disease-Epidemiology (CKD-EPI) equation [8]:-

eGFR= 141 x (SCr/k) ^{a}x0.993^{Age}x [1.018 if Female] x [1.159 if Black]

a=-0.329 if serum creatinine for female < or = 0.7 mg/dl

a=-0.411 if serum creatinine for male < or = 0.9 mg/dl

a=-1.209 if serum creatinine for male and female are > 0.9 and 0.7 respectively

k=0. 7 for female and k=0. 9 for male

SCr=Serum creatinine concentration

2.2.3. Urine VDBP, ELISA method:-

This test was done according to the manufacturer's instructions (R&D Systems) [9]. In summary, the principle is as follows:-

This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Vitamin D BP has been pre-coated onto a microplate. Standards and centrifuged urine samples were pipetted into the wells and any Vitamin D BP present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Vitamin D BP was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of Vitamin D BP bound in the initial step. The color development was stopped and the intensity of the color was measured.

2.2.4. Statistical Analysis:-

Mean and standard error were calculated for the whole data by using descriptive method, the P-value was calculated by using one-way Anova method, the correlation was calculated by simple linear regression in SPSS programs version 18.

3. Results:-

The results of urinary VDBP levels among groups I, II and III shown in table 1 were as follow: 250.5 ng/ml ± 0.526, 350.63 ng/ml ± 0.8 and 503 ng/ml ± 14.056 respectively. The difference between group I and II, group I and III and group II and III was significant (P= 0.000 for all).

Table 1:Urinary Vitamin D Binding Protein (ng/ml) in controls (group I) compared to diabetics with eGFR ≥ 90 (group II) and Diabetics with eGFR < 90 (group III)

Parameters	Groups	N	Mean	Std.Error	P-value (I &II)	P-value (I & III)	P-value (II & III)
Urinary VDBP (ng/ml)	GroupI	20	250.5	0.526	*0.000	*0.000	*0.000
	Group II	40	350.63	0.8			
	Group III	30	503	14.056			

*P ≤ 0.05 significant

The results of eGFR levels among group I, II and III were shown in table 2. The eGFR mean showed a higher value in group I followed by a decreasing value in group II and III respectively as follows: 97.16 ml/min/1.73m^2 ±1.177, 93.91 ml/ min/1.73m^2 ± 1.834 and 88.13ml/min/1.73m^2 ±1.543. The difference in eGFR between group I and II was not significant (P= 0.303). The difference between group I and III and group II and III was significant (P= 0.001 and 0.010 respectively).

Figure 1 showed that there is a negative correlation coefficient (r) between eGFR and VDBP in controls (group I) (r = -0.188) with a P-value of 0.428 which is not significant.

Table 2:Estimated glomerular filtration rate (eGFR) (ml/min/1.73m^2)in controls (group I) compared to diabetics with eGFR ≥ 90 (group II) and diabetics eGFR< 90 (group III).

Groups	N	eGFR Mean	Std.Error	P-value (I&II)	P-value (I & III)	P-value (II & III)
Group I	**20**	**97.16**	**1.177**	**0.303**	**0. 001**	**0.010**
Group II	**40**	**93.91**	**1.834**			
Group III	**30**	**88.13**	**1.543**			

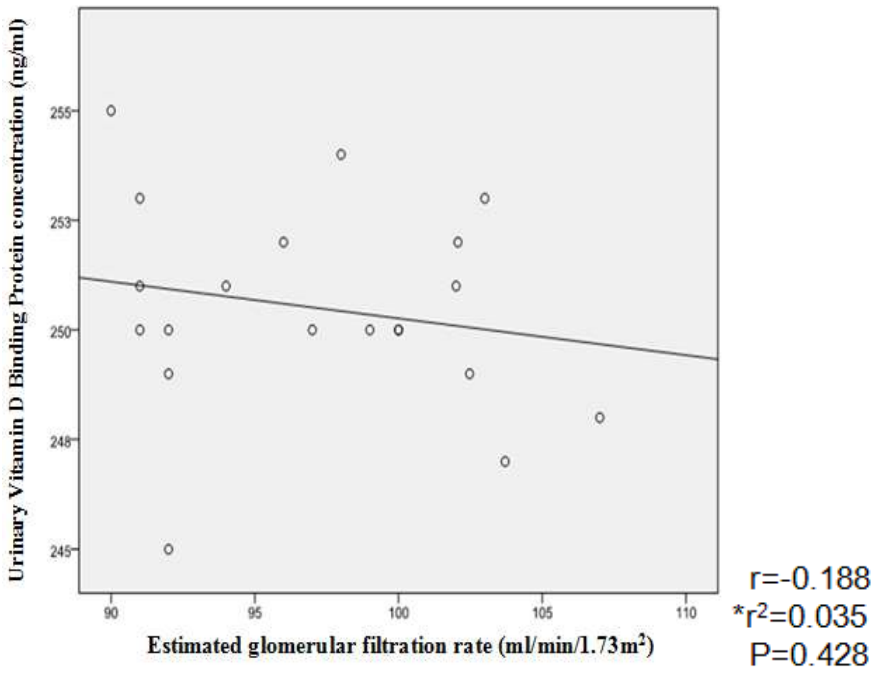

Figure 1: Correlation of VDBP concentrations with eGFR in controls (group I)

***r^2: Determination Coefficient**

Figure 2 showed that the correlation coefficient (r) between eGFR and VDBP was negatve(r = -0.524) with P-value 0.003 which is significantly among the diabetics with eGFR < 90 (group III).

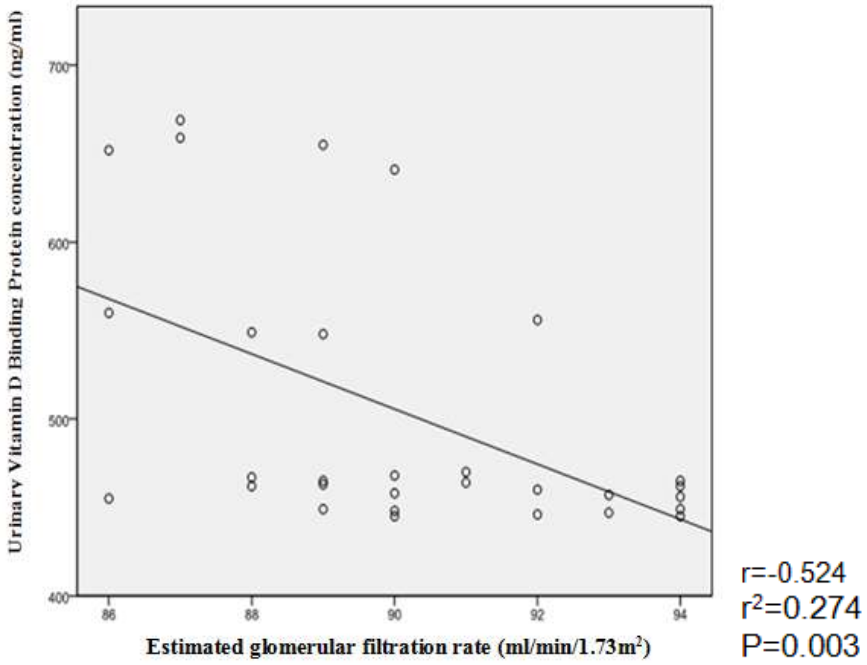

Figure 2:Correlation of Urinary VDBP Concentrations with eGFR in group III.

4. Discussion:-

Vitamin D binding protein also known as group specific component (Gc). It is a

protein that in humans is encoded by the GC gene. It belongs to the albumin gene family a multifunctional protein present in plasma, sciatic fluid, cerebro-spinal fluid and on the surface of many cell types. It combines to vitamin D and its plasma metabolites and transports them to target tissues [10].

The present study showed that VDBP level in urine increased in group II compared to group I, also this level showed a further increase in group III. Group II showed eGFR ≥90 ml/min/1.73 m^2 while group III showed eGFR of < 90 ml/min/1.73m^2. The cause of urinary VDBP elevation in group II and more elevation in group III may be attributed to dysfunction of VDBP uptake by cubilin-megalin receptors in the proximal renal tubular epithelial cells (TEC) [11] that are damaged due to elevation of inflammatory mediators [12 – 14] which are crucial factors for DN in consequence to bad glycemic control [15]. As well as, Thrikill*et al.*[16] observed that VDBP levels elevated in diabetic patients with abnormal eGFR levels than diabetics with normal range of eGFR levels and and there were further elevation than healthy controls.

The results showed that the correlation between urinary VDBP and eGFR in the group I was a weak negative with nonsignificant P- value (r=-0.188, P=0. 428) as it is expected because this is a group of apparently healthy control, but this correlation was strong negative with significant P-value (r=-0.524, P=0.003) in group III. This negative correlation may put us a light upon the necessaty of urinary VDBP as a good predicatorbefore type 2 diabetic nephropathy onset.Similarly, Thrailkill*et al.*[18]noted that there were a reciprocal correlation between VDBP andeGFR levels in diabetics with eGFR< 90 ml/min/1.73m^2.

It has been suggested that vitamin D plays an important role in the pathogenesis of diabetes and in the glucose control through several mechanisms, such as inhibiting the inflammatory responses, modulating self immune response, promoting insulin synthesis and secretion and increasing insulin sensitivity; and sincevitamin D level in the body is influenced by VDBP through activation of vitamin D[18], so VDBP is an indirectly important factor in synthesis and activation of insulin.

In conclusion, the increased VDBP levels were positively correlated with the development of the diabetic nephropathy, so the elevation of VDBP levelcan be considered as a novel predictor for monitoring type 2 diabetes before DN onset since it has a negative correlation with eGFR.

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