Original Article

Association of visfatin with chronic kidney disease in a cohort of patients with and without diabetes

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Abstract

Objective: To evaluate association of serum visfatin with CKD secondary to diabetic nephropathy and to compare it with patients of CKD secondary to other risk factors.

Methods: Seventy eight individuals including 28 healthy controls and 50 patients of CKD were included in this study. Patients with CKD were further grouped based on etiology of CKD into diabetics and non-diabetics. Patients with type 1 diabetes mellitus, urinary tract infection, urolithiasis, liver cirrhosis, stroke, ischaemic heart disease, and rheumatoid arthritis were excluded. Measurement of Serum visfatin was done through EIA Kit (Phoenix pharmaceuticals Burlingame CA)

Results: Visfatin concentration was significantly high in patients with CKD compared to controls (8.7 ± 4.7 vs. 5.2 ± 3.3 p = 0 .001). No significant difference in Visfatin concentrations between patients of CKD with and without diabetes was detected (9.2± 5.5 vs. 8.3± 3.2 p = 0.694). A significant negative correlation of visfatin with estimated GFR (r^2= -0.383, p=0.01) and a positive correlation with degree of proteinuria (p=0.01) was observed.

Conclusion: The present study confirms the association of visfatin with CKD, however further studies at molecular level to check its expression within renal tissue may clarify its definitive role in CKD (JPMA 60:922; 2010).

Introduction

Chronic kidney disease has become a global public health threat. The prevalence of CKD in United States has significantly increased from 14.5% (1988-1994) to 16.8% (1999-2004).[1] Community based data from urban settings in Karachi, Pakistan shows that 29.9% persons above 40 years of age have reduced estimated GFR.[2] The irreversible nature of disease, association with significant morbidity and mortality as well as the cost of renal replacement therapy leads to a large burden for health care systems, particularly in developing countries like Pakistan. Diabetic nephropathy ranks top amongst the causes of end stage renal disease in patients older than 40 years old accounting for 44% of new cases of chronic kidney disease in United States,[3] 20% cases of end stage renal disease in England[4] and is the leading known cause of chronic renal failure in Pakistan.[5]

One of the most interesting features of research done in last decade is emergence of adipose tissue as an endocrine organ. It is no more taken as an inert site of nutrient storage but rather a metabolically active organ capable of producing soluble factors termed adipokines,[6] visfatin being one of them. Moreover, research is throwing light on the association of adiposity with CKD and this emerging connection has led to the generation of several hypotheses.[7] Much interest is focused to disclose the possible novel mediators expressed within adipose tissue, which might play some role in the pathogenesis of CKD.

Visfatin also known as B-cell colony-enhancing factor

(PBEF) and Nicotinamide phosphoribosyl transferase (Nampt), was first identified by Fukuhara et al in 2005.[8] By using differential display of gene expression in samples of subcutaneous and visceral fat and analyzing 8800 genes using CDNA probes researchers found one product had much greater expression in visceral fat and thus named it Visfatin.[9]

This exciting adipocytokine has been the subject of intense research because of its pleiotropic actions. Among the diverse effects of visfatin, most striking is its insulin mimetic activity, Fukuhara et al have proposed it to act as insulin mimetic and it binds to insulin receptor at a site different from insulin binding site and increases glucose uptake.[8] It also acts as a Nicotinamide phosphoribosyl transferase and is thus involved in production of reactive oxygen species.[10] Even more interestingly it acts as an inflammatory cytokine and its levels are elevated in a number of acute and chronic inflammatory diseases including sepsis, acute lung injury, rheumatoid arthritis, inflammatory bowel disease levels. Several studies have found a relation of visfatin with diabetes mellitus and visceral obesity whereas many researchers failed to replicate such findings.[11] Axelson et al,[12] in 2007 for the first time reported an increased serum level of visfatin in CKD and later on, several other studies reproduced similar relationship between visfatin and CKD.[13-15] Moreover Axelson et al also found Visfatin to be associated with Svcam-1(Soluble Vascular Adhesion Molecule 1) which is a biomarker of endothelial damage in chronic kidney disease.[12] Proteinuria is an important predictor of endothelial dysfunction in early diabetic nephropathy and an association is observed between proteinuria and visfatin level.[16]

Song et al analyzed these clinical results at molecular level in 2008. Treatment of cultured mesangial cells with recombinant visfatin led to activation of protein kinase b and a dramatic increase in the synthesis of profibrotic molecules including TGF ß(Transforming growth factor beta), PAI 1 (Plasminogen activation inhibiting factor 1) and type 1 collagen which are well known to contribute to pathogenesis of diabetic nephropathy.[17]

Thus the fibrotic buildup observed by Song et al and possibility of reactive oxygen species via its activity as a Nicotinamide phosphoribosyl transferase (Nampt) strongly supports the concept that visfatin could be one of the cytokines responsible for renal damage in diabetic nephropathy.

Considerable progress is made in identifying association of visfatin with visceral adipose tissue, diabetes and inflammation but its role in renal damage in CKD secondary to diabetes mellitus has not been fully assessed. In this study, we investigated whether visfatin serum concentration is associated with renal damage in type 2 diabetes and compared it with patients of CKD secondary to causes other than diabetes.

Patients and Methods

A total of 78 patients (40-60 years old) were studied between January 2009 to October 2009. There were 28 normal healthy controls and 50 patients with chronic kidney disease. Patients having CKD were registered at Department of Nephrology, JPMC. Non randomized purposive sampling was done for recruiting patients. CKD was defined as an estimated GFR < 60 ml/min/1.73 m^2 for more than 3 months (NFK KDOQI™ National Kidney Foundation Kidney Disease Outcomes Quality Initiative).[18] They were sub grouped into diabetics and non-diabetics according to etiology of CKD. Patients with type 1 diabetes mellitus, history of ischaemic heart disease or vascular intervention, urinary tract infection, urolithiasis, liver cirrhosis, stroke, and rheumatoid arthritis were not included in this study.

Age matched controls were selected among general population of same socioeconomic group through convenient sampling and some of the controls were healthy attendants of the diseased subjects. They were included if they met the following inclusion criteria, 40-60 years old having no clinical evidence of hypertension, liver disease, joint disease, acute or chronic inflammation or a recent febrile illness and on lab investigations had a FBS<100 mg/dl[19] and estimated GFR > 90 ml/min/1.73 m^2 (K/DOQI).[18]

History of diabetes, hypertension, duration of nephropathy and smoking was asked through a structured questionnaire. Those who had stopped smoking for more than 1 year were taken as non-smokers. In diabetic group, only those patients in whom nephropathy developed 8-10 years after the onset of diabetes were selected. Anthropometric and blood pressure measurement was done according to standard methods in all subjects. Waist circumference was measured using soft inch tape from the point midway between lowest rib and uppermost lateral border of right iliac crest just above the umbilicus. Twenty-four hours urinary protein was analyzed in all subjects after proper urine sample collection.

Calculations

BMI was calculated as weight in kilograms divided by height in meter square, and obesity was defined as a BMI $\geq$ 25kg/m^2 according to WHO recommendations for Asian Indians, Asia pacific criteria (APC- BMI $\geq$ 25kg/m^2).[20] We defined visceral obesity as a waist circumference > 90 cm in males and >80 cm in female (APC-WC) WHO recommendations for Asian Indians.[21] To segregate the patients according to the degree of renal dysfunction estimated GFR was calculated using MDRD (Modification of Diet in renal disease) described in the MDRD study: eGFR = 186.3 x serum creatinine$^{-1.154}$x age$^{-0.203}$x 0.742 (if female) x 1.21 (if black). The renal dysfunction was grouped as

Moderate (MDRD GFR 60-30 ml/min/73m^2) and severe (MDRD GFR<30 ml/min/73m^2) according to Chronic Kidney Disease classification by National Kidney Foundation.[22]

Urinary protein < 3gm/day was defined as Non Nephrotic protienuria; between 3- 5 gm/day was defined as Nephrotic protienuria and > 5gm/day as massive protienuria.

All participants gave written informed consent and ethical committee of Ziauddin University approved the study.

All blood draws were performed at the Nephrology Laboratory, Jinnah Post Graduate Medical Centre. Venous blood samples were taken after overnight fast. Samples were centrifuged within 30 minutes of collection and separated serum was immediately frozen at -70 C. Serum Visfatin was measured using EIA kit from Phoenix Pharmaceuticals, Burlingame, CA Catalog No: EK-003-80 LOT No.: 601344. The performance characteristics of this assay were intraassay coefficient of variant <10% interassay coefficient of variant <15%. The minimum detectable concentration with this method was 2.13ng/ml.

Statistical Analysis:

All statistical analysis was done using SPSS version 11. Baseline characteristics were compared between the CKD and control groups using the χ^2 tests to assess differences between qualitative variables among different groups and one way ANOVA was used to assess quantitative differences between the groups followed by post hoc Tukey Krammer test for multiple comparisons. Pearson's correlation was used to analyze linear correlation between continuous variables. A p value of <0.05 was considered as significant.

Results

Baseline Characteristics

The demographic and clinical characteristics of subjects are shown in Table.

Among the entire study population, there were 19 subjects without visceral obesity and 59 subjects with visceral obesity and the level was not different between the two groups. (n=19, 7.3 ± 2.9 vs. n=59, 7.6 ± 4.9 p=0.728). Similarly the levels were not different between obese and non obese subjects (n=39, 6.8 ±3.14 vs. n=39, 8.3 ± 5.4 p=0.163).

Patients with CKD had a higher concentration of visfatin than controls (8.7± 4.7 vs. 5.2 ± 3.3 p=0.001). Among patients with CKD, there was no significant difference in serum creatinine, estimated GFR, and uric acid between the two groups. Moreover, no statistically significant difference was observed in serum visfatin level between diabetics and non diabetics (9.2 ± 5.5 vs. 8.3 ± 3.2 p =0 .694).

There was a positive correlation between serum creatinine and serum visfatin (r^2= +0.322, p=0.001) and an inverse correlation between estimated GFR and serum visfatin (r^2= -0.383, p=0.01).

Table: Characteristics of subjects and controls.

	Control	CKD (DN)	CKD (NDN)	
N	28	28	22	
Sex (M/F)	16/12	16-Dec	10-Dec	
Age (years)	48.4 ± 6.1	52.2 ± 6.9	49.5 ± 5.7	P=0.07 C vs. DN 0.823 C vs. NDN 0.292 DN vs. NDN
Smoking (n)	9	4	9	
BMI (kg/m)^2	25.7 ± 4.5	26.1 ± 5.8	26.9 ± 4.9	P=0.991 C vs. DN 0.678 C vs. NDN 0.750 DN vs. NDN
Visfatin (ng /ml)	5.2 ± 3.3	9.2 ± 5.5	8.3 ± 3.2	0.002 C vs. DN** 0.039 C vs. NDN* 0.694 DN vs. NDN

ns, not significant; C, controls; DN, ckd due to diabetes mellitus; NDN, ckd due to causes other than diabetes mellitus; BMI, body mass index; WHR, waist hip ratio;*, p<0.05,**, p<0.01.

Visfatin concentration in CKD patients was not significantly different between patients with moderate and severe renal dysfunction. There were 12 patients having moderate renal dysfunction and 38 having severe renal dysfunction with a mean serum visfatin (7.1 ± 3.48 vs. 9.4 ± 4.9, p=0.705)

Average 24-hour urinary protein excretion was 4.16 ± 1.68 gm/day. We found no significant differences between these subgroups in terms of age, sex, BMI, WHR, but a significant direct correlation was observed between serum visfatin and proteinuria (r^2=+ 0.533 =0.01). The serum visfatin in non-nephrotic proteinuria range was (n=14, 5.8 ± 2.2), in nephrotic range of proteinuria was (n=17, 8.15 ± 2.53) and in massive proteinuria was (n=19, 11.7 ± 5.8). Serum visfatin level was significantly high in patients with massive proteinuria than those in range of non-nephrotic proteinuria and nephrotic proteinuria (p =0.001 and 0.036 respectively). However no significant difference was observed between patients with nephrotic and non nephrotic proteinuria (p=0.181).

Discussion

Our results show a high level of visfatin in patients with chronic kidney disease compared to controls. Different researchers have studied association of visfatin with different stages of CKD but interestingly a positive association was observed by all suggesting a possible role of visfatin in CKD.[12-16] In this study we included patients who were in CKD stage 3-5, Axelsons et al[12] also recruited CKD patients of stage 3-5. Yilmaz et al[13] studied patients of all CKD stages from stage1 to 5, and they found a higher level of visfatin in stage 3-5 as compared to subjects with stage 1-2 and controls

but no significant difference was observed between controls and stage 1-2 CKD subjects. Moreover, they also studied subjects with early diabetic nephropathy having microalbuminuria but no renal dysfunction and found a significant association of visfatin with proteinuria. Nuksen et al[14] did their work on patients receiving haemodialysis, J Malayszko et al[15] did their research on Kidney Allograft recipients and both these groups reported a higher level of visfatin. The reason for such observation is not clear as we have no specific data about its renal metabolism but decreased clearance secondary to low glomerular filtration in renal damage could be one explanation another reason could be excessive release from progressively damaging renal cells in advance stages as it is proposed to be located intracellularly and releases upon cell lyses.[23]

There is a great body of contrasting evidence concerning the association of visfatin with diabetes mellitus as some studies have observed an association while others have not observed any association[11] but it has been proposed as insulin mimetic acting on insulin receptor at a site different from insulin binding site.[8] Due to above, possible role and findings of Song et al who observed a profibrotic buildup by treatment of cultured mesangial cells with recombinant visfatin we expected a higher level in Diabetic group of CKD patients which we failed to observe. This directs us to the fact that visfatin is primarily an inflammatory cytokine rather than insulin mimetic[24] and the role of low-grade inflammation in CKD is no more a hidden fact.[25] Another reason for lack of such association could be that some of our patients were on insulin that might have suppressed visfatin leading to spuriously low level, as insulin is known to suppress visfatin level.[11]

In our study, a strong association of visfatin with protienuria was observed irrespective of the cause of renal dysfunction. Protienuria is a characteristic feature of diabetic nephropathy and an important indicator of endothelial dysfunction in CKD. Endothelial dysfunction also increases the risk of cardiovascular diseases, which is an important predictor of survival in these patients. Inflammation may be a common trigger to endothelial dysfunction in both CKD and CVD. Association of visfatin with protienuria observed by Yilmaz et al[13] and SVCAM (Soluble Vascular cell adhesion molecule 1) observed by Axelsons et al[12] is a solid contribution towards possible role of visfatin in endothelial damage, as both of these are important predictors of endothelial dysfunction. Even more interestingly, Yilmaz and colleagues[13] in a recent publication have disclosed that improvement in endothelial function after renal transplantation is associated with a decrease in visfatin level in their patients.[26]

Role of visfatin as a Nampt is overlooked; a detailed review of NAD (Nicotinamide Adenine Dinucleotide) biology shows that as a Nampt it is capable of producing reactive oxygen species, which are known to be involved in pathogenesis of CKD. As observed by Song et al[17] there was a significant increase in NAD level on visfatin treatment and such rise was significantly reduced after treatment with FK866 which is an anticancer agent and a Nampt inhibitor.[17] Novel therapeutic approaches targeting Visfatin is the goal of future research.

Inflammation and endothelial dysfunction is a common pathological event in many diseases including cerebrovascular disease, ischaemic heart disease, peripheral vascular disease, and chronic kidney disease blocking such novel inflammatory cytokine may be a breakthrough in preventing or at least retardating progression of such diseases.

Limitations:

A number of limitations of this study need to be considered. First, the study population was enrolled in disease management programme of a tertiary care hospital thus because of the sampling bias it is probably not fair to assume that results are reasonably representative of the Pakistani population. Further population based studies involving larger cohorts are needed to generalize the results.

Next, its cross sectional design, allowed to pickup association but the causal relationship between visfatin and CKD could not be determined. Moreover serial measurements at onset of CKD and then during progressively declining stages of renal dysfunction would have been more informative.

Also all of our diabetic patients were on ACE inhibitor, which has shown to improve endothelial function in diabetic nephropathy, thus their use might have led to some unknown confounding effect.

Conclusion

The study concluded that multifunctional adipokine is up regulated in patients with chronic kidney disease with and without diabetes. Moreover, its association with increasing degree of proteinuria signifies its role as a marker of endothelial damage in CKD.

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