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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Serum Visfatin – an early diagnostic marker of diabetic nephropathy

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**ABSTRACT**

*Type 2 DM is becoming common in subjects who crossed the age 40yrs. As the main reasons includes sedentary life style, high fat diet, stress etc., as per the American Diabetes Association, USA fasting plasma glucose 126 mg% , diagnosed as DM .The main cause for this is insulin resistance. When insulin resistance is established in the cells, hyperglycemia results. Every raise of 5mg of blood glucose level there is a proportionate damage of kidney. Diabetic nephropathy (DN) is one of the microvascular complications of DM, the incidence of DN in DM is 13%. As there is a constant presence of hyperglycemia there is a more risk of renal damage. We need a biomarker which is produced or synthesized in response with the degree of renal damage, hence there is a need of an indicator which will give the sign of renal damage, so that we can save the vital organ kidney. Whenever there is a damage to the particular cell will be manifested by the release of inflammatory marker. Visfatin is protein is an inflammatory marker, which is significantly raised in nephropathy. In our present study, to estimate the levels of serum visfatin in patients with DN and to correlate with the HbA1c , Lipid profile parameters, HOMA-IR in different stages of nephropathy. In this research study we included 150 DN patients who are having history of DM more than 5yrs, and we segregated the 150 under 3 sub categories based on their urinary albumin creatinine ratio as normoalbuminuria, microalbuminuria and macroalbuminuria. 50 individuals selected under control group, who are age, sex matched. From both test and control groups consent was obtained. The study was carried out after the approval of institutional ethical committee. All the routine biochemical parameters was assessed by EM-200 fully automated analyzer. By ELISA method Serum visfatin and Insulin were assessed. Microalbumin was quantitated by turbilatex method. HbA1c was estimated by immuno turbidimetric method. Serum Visfatin levels were significantly elevated in co-ordination with different stages of DN in correlation with urinary albumin creatinine ratio and HOMA - IR. Serum visfatin levels were significantly increased in patients with different stages of DN*

***Keywords:*** *Diabetes Mellitus (DM), Insulin resistance (IR); HOMA-IR; diabetic nephropathy (DN), Visfatin.*

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## INTRODUCTION

Visfatin is an adipokine, 53KD protein is released by visceral fat. It functions as a mimic of Insulin. It influences the cells to take up the glucose and continue the glycolysis to decrease the blood glucose level, not only the visceral fat even other organs like skeletal muscle, bone marrow, hepatocytes, and mesangial cells also produce visfatin. Obese people and in type 2 diabetes mellitus people shown the higher levels of serum visfatin [1-3]. Visfatin majorly secreted by visceral fat [4]. Visfatin synthesis was markedly increased not by angiotensin II, but by high glucose stimuli, visfatin increases induced glucose uptake. Visfatin initiates cystolic GLUT-1 into cellular membranes after visfatin treatment. Visfatin had a great effect on homeostasis of insulin resistance of other tissues [5-7]. Among several adipocytes TNF alpha, leptin, resistin, visfatin are biologically impact which are marked as active biomolecules into development of insulin resistance[8,9]. Visfatin is an pro inflammatory adipokine raised in all organ or cellular injuries and also raised in metabolic syndrome including obesity, glucose tolerance and dislipidemia. Visfatin is raised in chronic DM specifically in type 2 DM. on the other hand visfatin levels were found lower in

IDDM. Serum visfatin was also raised in gestational DM in females. In Kidney glomerular cells produces the visfatin especially mesangial cells which was positively affected by hyperglycemia condition, due to DM and in lack of insulin or due to insulin resistance visceral exergonic fat synthesizes the visfatin in greater quantities. This visfatin influences the production of TGF (Transforming Growth Factor), plasmalogen, type 1 collagen, with this biochemical changes it is more evident that raised visfatin levels are the indicators for the renal damage in the DN.

## MATERIAL AND METHODS

In this study age, sex matched healthy individuals were selected under control group and a total of 150 who are aged between 35-55 years , known diabetic history of minimum five years were included in this study under test group and they were on oral hypoglycemic drugs. The test group samples were collected from who are admitted in OP/IP of department of General Medicine , Konaseema Institute of Medical Sciences, Amalapuram, Andhra Pradesh and they were informed for the consent. Patient's history and information were recorded through a proper questionnaire, according to Helsinki declaration of 1975.

Control and test groups (total of 200 individulas) were divided as four groups

50 healthy age and gender matched subjects –Control - Group I

50 patients with normoalbuminuria(<30 mg/g creatinine) - Group II

50 patients with microalbuminuria(30–299 mg/g creatinine) - Group III:

50 patients with macroalbuminuria(≥300 mg/g creatinine) - Group IV:

Research study has been approved by an institutional human ethical committee of Konaseema Institute of Medical Sciences, Amalapuram, where the research work was carried out.

As per the exclusion Criteria, we excluded the known cases of urinary tract infection, abnormal urinary sediment, history of other renal disease, active or chronic persistent infection or inflamatory disorders, neoplastic disorders ,history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease, liver dysfunction, thyroid disorders, known cases of hypertension , tobacco chewers , smokers , alcoholics.

As per the Inclusion Criteria we included type 2 diabetic patients aged between 35 to 55years and patients who are on oral antidiabetic drugs

### Methodology

From each of control and test group , 8 ml of venous blood was collected and as per the objectives all the routine biochemical parameters were performed immediately and then the samples were stored at −80 °C for further analyses of specific parameter insulin and visfatin . For the estimation of microalbumin and creatinine estimations patient first morning urine samples were collected in sterile container and used . All the routine biochemical laboratory investigations includes plasma glucose, total cholesterol, TAG, LDL-C, HDL-C were estimated by EM-200 (Transasia) fully automated analyzer. Glucose was quantitated by GOD-POD method, urinary creatinine is estimated by Jaffe's kinetic method. Serum cholesterol was estimated by CHOD-PAP method, TAG was estimated based on Glycerol phosphate oxidase/ Peroxidase (GOD/POD) method. Direct enzymatic method was used for the estimation of HDL cholesterol and Friedwald's formula was applied for LDL cholesterol quantitation. HbA1C was estimated by immune turbidometric method and microalbumin was quantitated by turbilatex method. Visfatin and insulin were assessed by using ELISA method(ELISA kits used - DiaMetra, Spello, Italy and Sincere Biotech Ltd, Beijing, China for insulin and visfatin respectively [10, 11.

Insulin resistance (HOMA-IR) was calculated from an standard formula [12]:

HOMA – IR = Fasting venous plasma insulin (mIU/L) X Fasting glucose (mM/L)/22.5 .

Statistical analysis : carried out by SPSS software, version-22.

## RESULTS

**Table - 1** showed the Comparison of groups for 'p' values and 2 way ANOVA - DMRT - depicts the data for visfatin and other biochemical parameters possessing different magnitude of statistical significance when examined under the following groups:

Control vs Normoalbuminuria, Control vs Microalbuminuria, Control vs Macroalbuminuria, Normoalbuminuria vs Microalbuminuria, Normoalbuminuria vs Macroalbuminuria, Microalbuminuria vs Macroalbuminuria.

**Table- 1: Comparison of all groups by 2 way ANOVA - DMRT for determining statistical significance of Biochemical parameters**

| S.No. | Parameters | 'p'values for comparison of control and test groups | | | | | | ANOVA |
|---|---|---|---|---|---|---|---|---|
| | | I vs. II | I vs. III | I vs. IV | II vs. III | II vs. IV | III vs. IV | |
| 1 | Visfatin (ng/ml) | 0.0034 | 0.0059 | 0.0018 | 0.0022 | 0.0011 | 0.0018 | 0.0015 |
| 2 | FBS (mg/dl) | 0.0010 | 0.0011 | 0.0030 | 0.0066 | 0.0093 | 0.0047 | 0.0039 |
| 3 | PPBS (mg/dl) | 0.0039 | 0.0011 | 0.0025 | 0.0093 | 0.0066 | 0.0017 | 0.0048 |
| 4 | HbA1c (%) | 0.0018 | 0.0027 | 0.0071 | 0.0088 | 0.0010 | 0.0050 | 0.0031 |
| 5 | Insulin(µlu/ml) | 0.0045 | 0.0040 | 0.0013 | 0.0035 | 0.0022 | 0.0023 | 0.0032 |
| 6 | HOMA-IR | 0.0031 | 0.0092 | 0.0011 | 0.0039 | 0.0021 | 0.0010 | 0.0014 |
| 7 | Cholesterol (mg/dl) | 0.0728 | 0.0027 | 0.0057 | 0.0048 | 0.0090 | 0.0037 | 0.0543 |
| 8 | TAG (mg/dl) | 0.0208 | 0.0122 | 0.0972 | 0.0761 | 0.0867 | 0.0241 | 0.0297 |
| 9 | HDL (mg/dl | 0.0451 | 0.0115 | 0.0614 | 0.0136 | 0.0118 | 0.0418 | 0.0522 |
| 10 | LDL (mg/dl) | 0.0959 | 0.0190 | 0.0152 | 0.0249 | 0.0794 | 0.0136 | 0.0412 |
| 11 | Microalbumin (mg/dl) | 0.0073 | 0.0043 | 0.0098 | 0.0018 | 0.0020 | 0.0053 | 0.0014 |
| 12 | Urinary Creatinine mg/ml | 0.0060 | 0.0087 | 0.0015 | 0.0055 | 0.0024 | 0.0010 | 0.0029 |
| 13 | ACR (mg/g of creatinine) | 0.0028 | 0.0049 | 0.0016 | 0.0071 | 0.0023 | 0.0015 | 0.0027 |

P<0.05 significant ; P<0.001 – highly significant

I – Control group; II – Normoalbuminuria T2 DM patients; III – Microalbuminuria T2 DM patients; IV – Macroalbuminuria T2 DM patients ; FBS- Fasting blood sugar; PPBS-Post prandial blood sugar(glucose); HbA1c-Glycosylated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; TAG –Triacylglycerol; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein; ACR-Albumin -Creatinine ratio

**Table 2: Correlation data between visfatin and other measured parameters**

| Parameters | Correlation Coefficient(r) |
|---|---|
| FBS | 0.769** |
| PPBS | 0.812** |
| HbA1C | 0.893** |
| HOMA-IR | 0.822** |
| Cholesterol | 0.386* |
| TAG | 0.563* |
| HDL | -0.497* |
| LDL | 0.412* |
| ACR | 0.886** |

**Correlation is significant at the 0.01 level (2-tailed).*Correlation is significant at the 0.05 level (2-tailed)

Table- 2: Indicates the correlation between Visfatin and measured parameters FBS, PPBS, HbA1C,HOMA-IR, TAG, HDL cholesterol, ACR,

From the above results it is clearly noticeable that visfatin positively correlates strongly with ACR, and thereby confirms that visfatin can be used as an early marker of diabetic nephropathy, also good correlation was observed between visfatin and insulin resistance ( HOMA-IR).

## DISCUSSION

As the previous studies of Axelessor [13] stated that serum visfatin was raised in the Diabetic nephropathy. Bessass *et al* [14] concluded that upon chronic diabetes mellitus serum visfatin level was significantly elevated which is associated with Nephropathy. Eveleston J *et al* in 2010 explained about the raise of serum visfatin levels significance in the Diabetic nephropathy [15]. Ayo *et al* [16], Ayo S Radmik *et al* [17], proved that visfatin was secreted significantly and elevated in Diabetic Nephropathy [16, 17]. Due to hyperglycemia there is an increased uptake of cells of glucose will alter the metabolism which is the route cause of metabolic disorder and this is the underlying cause of the pathology of micro vascular disease [18, 19]. Accumulation of extra cellular matrix in glomerular cells is the key factor for DN [20]. Heilig *et al* stated that over expression of GLUT 1 which stimulates more glucose increases uptake by cells further supports link between glucose uptake and ECM protein system in mesangial cells [21]. Hye Kyoung *et al*, stated that , high glucose could continuously stimulates the increased secretion of visfatin , and states that visfatin damages the mesangial cells injury in DN. Increased visfatin synthesis from

mesagial cells under high glucose condition was hypothesized to possibly contribute to increased glucose influx into mesagial cells there by accelerating the DN by alterations of major metabolism. Visfatin has a role of proinflammatory which promote angiogenesis by activation of MAPK pathway and suggesting a new physiological role of visfatin [22, 23]. In the present study we have an evidence that free circulating visfatin levels are associated with endothelial dysfunction in patients with diabetic nephropathy and that visfatin itself activates various proinflammatory cytokines [24].

## CONCLUSION

In this study Serum visfatin levels are significantly increased in patients with different stages of diabetic nephropathy and positive significant correlation of visfatin with HOMA-IR is observed.

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