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

# Significance of serum Apelin-13 levels as an early marker of diabetic nephropathy and its correlation with insulin resistance.

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**ABSTRACT**

*Apelin-13 is a peptide encoded by the APLN gene. It belongs to G - protein, function as signaling the receptor for coupling of G-protein. Apelin 13 level has significance in the various stages of diabetic nephropathy. Till now there was very few and short studies were done to establish the correlation of Apelin-13 level in diabetic nephropathy in accordance with insulin resistance. Hence the aim of our study was oriented towards the role of Apelin-13 as an early predictive marker for the diagnosis of diabetic nephropathy. A total of 200 individual were included in the research study. Age 35-55 year and age, sex matched individuals were informed for a consent through a proper questionnaire. Among this 200 participants, fifty are healthy individuals are categorized as control group. Age sex matched 150 individulas who are known diabetic and are on oral hypoglycemic drugs included under test group . Based on their urinary protein creatinine ratio we subdivided the 150 patients into three sub groups as normoalbuminuria (50), microalbuminuraia (50), macroalbuminuria (50). Serum Apelin-13 and Insulin were assessed by ELISA method . HbA1c was quantitated by immune turbidimetric method and microalbumin was assessed by turbilatex method. Routine biochemistry was enabled through ERBA EM-200 fully automated analyzer. Quality control was run. The study was carried out after the approval of institutional ethical committee. Serum Apelin -13 levels were significantly elevated in different stages of diabetic nephropathy and were positively correlated with albumin-creatinine ratio and insulin resistance (HOMA-IR). Apelin-13 as novel parameter could be used as an early diagnostic marker of diabetic nephropathy.*

***Keywords:*** *Apelin-13; Insulin resistance (IR); HOMA-IR; diabetic nephropathy*

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## INTRODUCTION

Apelin is an adipokine secreted by all most all the cells of the body. Major function of Apelin is signaling the receptors for coupling G-protein. Apelin has a significant role in the positive regulation of corticotropine release. Apelin pre protein has constituting 77 amino acids [1]. After entry into ER, it exists in its active form peptide, containing 55 amino acids. This 55 amino acid peptide may further split into different form of Apein peptide , namely apelin 36(36 amino acids), apelin17(17 amino acids) and Apelin-13(13amino acids), corresponding to the sequence of 65-77 gene [2].. Apelin-13 initiates the formation of new blood vessel i.e angiogenesis [3,4] and initiates the vaso-dilatation and controls the raise of blood pressure [5]. This is achieved by Apelin-13 by the activation of the receptors opened at the surface of endothelial cells. This stimulates the release of nitric oxide, and this produced nitric oxide dilates the smooth muscle of the walls of the arteries [6]. Apelin-13 also activates the mechanisms of transduction cascades like ERKS, AT phosphorylations [7,8], which leads to the proliferation of endothelial cells for formation of new blood vessels [9].. Apelin-13 which is secreted from adipocytes is elevated in the plasma in the process of differentiation of adipocytes. Insulin has a great effect on the adipocyte moderation. Hence the obese people who are having more insulin may be having the more Apelin-13 levels in their plasma [10]. In type-2 DM generally insulin resistance is seen ,because of the high circulating insulin levels, stimulating the Apelin-13 production. In pancreas Apelin-13 inhibits the

secretion of insulin [11]. This inhibition is due to the mutual dependency of insulin and Apelin-13 signals. Diabetic nephropathy is one of the commonest condition of microvascular disease of type-2 DM. Apelin is an adipokine which has a beneficial role in early prediction of diabetic nephropathy, which is the significant cause for renal failure. The aim of our study was oriented towards the role of Apelin-13 as an early predictive marker for the diagnosis of diabetic nephropathy

## MATERIAL AND METHODS

A total of 150 patients of age ,sex matched with a known diabetic history of minimum five years or more and aged between 35-55 years were included in this study under test group, who are admitted in General Medicine department(OP/IP), KIMS & RF, Amalapuram, East Godavari dist., A.P. They were on oral hypoglycaemic drugs. Age, sex matched fifty normal, healthy individuals were selected under control group. According to Helsinki declaration of 1975, the groups were informed for the consent. Patient's history and information were recorded through a proper questionnaire. This research study has been approved by an institutional human ethical committee of KIMS & RF, where the research work was carried out.

The above 200 individuals were divided as four groups.

The groups were divided as, 50 patients with normoalbuminuria (<30 mg/g creatinine), 50 patients with microalbuminuria (30–299 mg/g creatinine), and 50 patients with macroalbuminuria (≥300 mg/g creatinine). Fifty healthy age, gender matched subjects constituted the controls.

*Control and Test groups:*

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

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

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

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

**Inclusion Criteria:**

Type 2 diabetic patients aged between 35 to 55years

Patients on oral antidiabetic drugs.

**Exclusion Criteria:**

Known hypertension

Smokers ,alcoholics, tobacco chewers

Abnormal urinary sediment, urinary tract infection, history of other renal disease

Active or chronic persistent infection or inflammatory disorders, neoplastic disorders

Thyroid disorders, liver dysfunction, history of acute myocardial infarction, stroke, and occlusive peripheral vascular disease.

**Biochemical analysis*:***

8 ml of venous blood was collected from each of control and test group and routine laboratory investigations were performed immediately and samples were stored at −80 °C for further analyses of Apelin-13 and insulin. Patient first morning urine samples were collected in sterile container and used for microalbumin and creatinine estimations. Routine laboratory investigations like glucose, urea, creatinine, lipid profile parameters were estimated by ERBA- EM-200(Transasia) fully automated analyzer. Glucose was estimated by Glucose oxidase-Peroxidase method. Urea is estimated by urease-GLDH method. Serum creatinine is estimated by Jaffe's kinetic method. Serum cholesterol was estimated by Cholesterol oxidase/ Peroxidase(CHOD/POD). Estimation of triacylglycerides was based on Glycerol phosphate oxidase/ Peroxidase (GPO/POD) method. HDL cholesterol was quantitated by direct enzymatic method and LDL cholesterol was calculated by using Friedwald's formula. Glycated Hemoglobin (HbA1C)was estimated by immunoturbidimetric method. Microalbumin was assessed by turbilatex method. Insulin and Apelin-13 were estimated using ELISA kits supplied by DiaMetra, Spello, Italy and Sincere Biotech Ltd, Beijing, China respectively [12,13]..

HOMA- IR, of insulin resistance (IR) was calculated from the fasting glucose and insulin values by using the formula:

HOMA – IR = Fasting venous plasma insulin (mIU/L) X Fasting glucose (mM/L)/22.5 [14].

**Statistical analysis :** carried out by SPSS software, version-22.,using two way ANOVA- DMRT

## RESULTS

**Table - 1** : Comparison of groups for 'p' values and 2-way ANOVA - DMRT - depicts the data for Apelin-13 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- 2**: Indicates the correlation between Apelin-13 and ACR, FBS, PPBS, HbA1C,HOMA-IR,Urea, creatinine, TAG, HDL cholesterol.

From the results depicted in Table-2, it is very noticeable that Apelin-13 correlates strongly with ACR, thereby confirming the diagnostic significance of Apelin-13 as an early marker of diabetic nephropathy, as observed in this study on insulin resistant T2DM. Also, good correlation was observed between Apelin-13 and insulin resistance ( HOMA-IR).

**Table- 1: Comparison of groups for 'p'values and 2-way ANOVA – DMRT**

| S.No. | Parameters | Comparison of Groups for obtaining 'p'values | | | | | | All Groups For the purpose of computing ANOVA |
|---|---|---|---|---|---|---|---|---|
| | | I vs. II | I vs. III | I vs. IV | II vs. III | II vs. IV | III vs. IV | |
| 1. | Microalbumin (mg/dl) | 0.0073 | 0.0043 | 0.0098 | 0.0018 | 0.0020 | 0.0053 | 0.0012 |
| 2. | Urinary Creatinine(mg/dl) | 0.0060 | 0.0087 | 0.0015 | 0.0055 | 0.0024 | 0.0010 | 0.0036 |
| 3. | Urinary Creatinine mg/ml | 0.0060 | 0.0087 | 0.0015 | 0.0055 | 0.0024 | 0.0010 | 0.0036 |
| 4. | ACR (mg/g of creatinine) | 0.0028 | 0.0049 | 0.0016 | 0.0071 | 0.0023 | 0.0015 | 0.0024 |
| 5. | FBS (mg/dl) | 0.0010 | 0.0011 | 0.0030 | 0.0066 | 0.0093 | 0.0047 | 0.0043 |
| 6. | PPBS (mg/dl) | 0.0039 | 0.0011 | 0.0025 | 0.0093 | 0.0066 | 0.0017 | 0.0051 |
| 7. | HbA1c (%) | 0.0018 | 0.0027 | 0.0071 | 0.0088 | 0.0010 | 0.0050 | 0.0037 |
| 8. | Insulin(µIu/ml) | 0.0045 | 0.0040 | 0.0013 | 0.0035 | 0.0022 | 0.0023 | 0.0029 |
| 9. | HOMA-IR | 0.0031 | 0.0092 | 0.0011 | 0.0039 | 0.0021 | 0.0010 | 0.0011 |
| 10. | Urea mg/dl | 0.0183 | 0.0330 | 0.0162 | 0.0285 | 0.0173 | 0.0845 | 0.0473 |
| 11. | Creatinine mg/dl | 0.0246 | 0.0640 | 0.0443 | 0.0299 | 0.0407 | 0.0213 | 0.0112 |
| 12. | Cholesterol (mg/dl) | 0.0728 | 0.0027 | 0.0057 | 0.0048 | 0.0090 | 0.0037 | 0.0487 |
| 13. | TAG (mg/dl) | 0.0208 | 0.0122 | 0.0972 | 0.0761 | 0.0867 | 0.0241 | 0.0361 |
| 14. | HDL (mg/dl) | 0.0451 | 0.0115 | 0.0614 | 0.0136 | 0.0118 | 0.0418 | 0.0493 |
| 15. | LDL (mg/dl) | 0.0959 | 0.0190 | 0.0152 | 0.0249 | 0.0794 | 0.0136 | 0.0396 |
| 16. | Apelin-13 ng/ml | 0.0079 | 0.0011 | 0.0086 | 0.0039 | 0.0027 | 0.0010 | 0.0012 |

I – Control; II – Normoalbuminuric T2 DM ; III – Microalbuminuric T2 DM; IV – Macroalbuminuric T2 DM ; ACR-Albumin -Creatinine ratio ; FBS- Fasting blood sugar(glucose); PPBS-Post prandial blood sugar(glucose); HbA1c-Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment-Insulin resistance; TAG –Triacylglycerol; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein; NT-Nitrotyrosine

$P<0.05$ significant ; $P<0.001$ – highly significant

**Table 2: Correlation data among Apelin-13 and other biochemical parameters**

| Parameters | Correlation Coefficient(r) |
|---|---|
| ACR | 0.805** |
| FBS | 0.827** |
| PPBS | 0.780** |
| HbA1C | 0.815** |
| HOMA-IR | 0.830** |
| Urea | 0.314* |
| Creatinine | 0.491* |
| Cholesterol | 0.400* |
| TAG | 0.689* |
| HDL | -0.531* |
| LDL | 0.304* |

ACR-Albumin- creatinine ratio FBS-Fasting blood sugar (glucose); PPBS- Post prandial blood sugar(glucose); HbA1c-Glycated haemoglobin; HOMA-IR- Homeostatic model assessment-Insulin resistance; TAG –Triacylglycerol; HDL-High Density Lipoprotein; LDL-Low Density Lipoprotein

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

## DISCUSSION

As micro vascular damage is one of the common damage due to chronic diabetes mellitus, namely diabetic retinopathy, diabetic nephropathy, diabetic neuropathy and cardio vascular disease. As diabetic nephropathy is one of the second common microvascular disease of DM, leading to the end renal disease and further leads to the renal failure. [15]. The major clinical symptoms of the renal damage is

proteinuria. Physical examination of the urine is white in color and small albumin particles are excreted in urine will be frequently expressed to the clinician by the patient. This condition is termed as albuminuria/proteinuria [16]. This leads to the decrease of protein content in plasma, further leads to the development of cardio vascular disease [17].

In the present study, serum Apelin-13 was significantly elevated in chronic diabetic nephropathy. Generally Apelin-13 which is an adipokine functions as a receptor for G-protein and increases sensitivity of insulin. But as the cells are insulin resistant, it can't take up by the cells, this leads to the more and more adipocyte moderation to secrete more Apelin-13 to improve the sensitization of insulin to the cells. This process of secretion of Apelin-13 is continuous as hyperglycemia is prolonged, further the Apelin-13 which is shorter 13 amino acids biologically active being secreted [18]. Previous studies of Senthill *et al.*, stated that Apelin-13 levels improves the sensitivity for insulin and reduces the hyperglycemia [19]. But this mechanism applies only when the hyperglycemia is shorter in duration. As the heperglycemia persistent constantly the sensitization of Apelin-13 on insulin decreases. Again adipokines starts producing Apelin-13.

Zhuo Gao *et al*, 2021, conducted the animal study and concluded that Apelin-13 was decreased in diabetic nephropathy and administration of Apelin-13 improves the diabetic nephropathy [20]. Zhang *et al*, 2013, conducted research study in humans who are suffering from diabetic nephropathy and estimathed their serum Apelin-13 levels. They concluded that there is a high positive correlation between diabetic nephropathy with the stage of renal damage [21].

Significance of elevation of serum Apelin-13 in DN patients is quite controversial. This may be due to the selection of the sample size for Apelin -13 estimation may be small and duration of DM in those patients is also may be shorter. Hence the significant correlation was not obtained and they suggested that, it needs a larger sample size and long duration of DM gives a better clarity of the significance of the Apelin-13 levels in DN. Hence we have conducted our research study by keeping the above two important points i.e good sample size and longer the duration of DM. Very carefully we estimated the serum Apelin-13 with high accuracy and sensitivity. In our study as we selected the test group patients with minimum of five years and maximum of 20 years of history of DM and who are on oral hypo glycemic drugs , we are able to specify the raise of serum Apelin-13 levels as per the stages the renal damage. Apelin-13 has a beneficial role in DN as a novel biomarker of early renal damage.

Alaa Dawood *et al.,* 2017 concluded in their study that there was a significant raise of serum Apelin-13 levels in DN . Our study also well correlating with this. Serum-13 levels are increased as the abnormal changes occurred in the glomerular cell membrane increased thickness, lack of aneurysm formation, mesangial nodule formation [22, 23]. Afferent arterioles appear more dilated in the early stages of DN . This may be due to glomerular hyper perfusion and hyper filtration. This type of hemodynamic modifications may affect the albumin to pass through the glomerular capillaries and further may appears in urine [25]. In the study Zhan et al [24], they detected the up regulation of VEGFR-2 BY Apelin-13. In addition to this Thurston & Ward et.al ,2002, also stated that Apelin-13 contributes to the glomerular hyper perfusion and hyper filtration that often happens in the early stages of the DN [26,27]. The results of the present study supports the raise of Apelin-3 starts in the early stages of DN.

## CONCLUSION

From this study we can conclude that serum Apelin-13 levels rationally elevated in with the different stages of diabetic nephropathy

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