

## Association of Serum Apelin levels with Peripheral Neuropathy in Type-2 Diabetes Mellitus

Syeda Fouzia Mazloun, Amina Nadeem, Sidra Shauqat, Nadia Latif

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

### ABSTRACT

**Objectives:** To determine serum levels of Apelin in healthy individuals, type-2 diabetic patients and diabetic peripheral neuropathy patients (DPN) and to find the correlation with serum Apelin levels and serum fasting blood glucose, glycosylated hemoglobin.

**Study Design:** Cross-sectional analytical study.

**Place and Duration of Study:** Physiology Department and Centre for Research in Experimental and Analytical Medicine (CREAM) lab of the Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, from Jan to Dec 2021.

**Methodology:** A total of 90 individuals comprising three Groups having 30 subjects in each were recruited. Group-I included thirty healthy subjects, and Group-II consisted of thirty newly diagnosed patients having T2DM, Group-III consisted of thirty T2DM patients with DPN. We used Michigan Neuropathy Screening Instrument (MNSI) to assess neuropathy. Serum Apelin and tumour necrosis factor-alpha (TNF-alpha) levels were recorded from the blood samples of all subjects by enzyme-linked immunosorbent assay (ELISA) using human serum Apelin and TNF-alpha ELISA kit catalogue no. E2014Hu (bio-assay technology) and E0082Hu (Bio-assay technology), respectively.

**Results:** The mean values of serum Apelin were higher in Group-III compared to Group-II and Group-I, and a statistically significant difference was found ( $p$  value=0.001). The serum Apelin levels showed a strong negative correlation with Group-III serum FBG, HbA1c and TNF-alpha with  $r$  value -0.728, -0.79, and -0.95, respectively.

**Conclusion:** Serum Apelin has a beneficial role in DPN in T2DM, with reduced TNF-alpha levels as one of the possible mechanisms.

**Keywords:** Apelin, Diabetic peripheral neuropathy, TNF-alpha, Type-2 diabetes mellitus.

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### INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic health issue associated with various micro and macrovascular complications. Diabetic peripheral neuropathy is a common complication of various oxidative and inflammatory processes.<sup>1</sup> Many adipokines are proposed to be involved in the pathophysiology of diabetes mellitus and its complications. For example, *Apelin* is a novel adipokine secreted by white adipose tissue which acts on angiotensin-II protein J (APJ) receptors.<sup>2</sup>

Apelin and its receptor are widely expressed in neurons suggesting their role in the regulation of diabetic neuropathy. It exerts its anti-inflammatory effect in diabetic neuronal cells by reducing the activation of nuclear factor kappa B (NF-Kb), which inhibits the formation of pro-inflammatory cytokines like TNF-alpha. It also prevents the progression of diabetic neuropathy by clearing reactive oxygen species (ROS)

production in neurons. The anti-inflammatory and anti-oxidative properties of apelin make it a protective adipokine in diabetic neuropathy and an interesting therapeutic target in T2DM.<sup>3</sup> Many clinical studies have shown altered serum apelin levels in T2DM with and without neuropathy, but the results are controversial. It increases insulin sensitivity and glucose tolerance as well as reduces hyperinsulinemia. Therefore, it has a beneficial role in T2DM patients and emerges as an intriguing therapeutic target in diabetes.<sup>4,5</sup>

Tumour necrosis factor alpha (TNF- $\alpha$ ), a pro-inflammatory cytokine, is an independent risk factor for diabetic peripheral neuropathy in T2DM. Apelin inhibits the expression of TNF- alpha and exhibits a potent anti-inflammatory effect.

### METHODOLOGY

This was a cross-sectional analytical study conducted at Army Medical College and Pak Emirates Military Hospital (PEMH) Rawalpindi Pakistan, after formal approval from the Ethics Review Committee of Army Medical College (ERC#161). The T2DM patients admitted or visiting the outpatient department (OPD)

**Correspondence:** Dr Syeda Fouzia Mazloun, Department of Physiology, Army Medical College, Rawalpindi, Pakistan  
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from April 2021 to August 2021 were selected. We used a non-probability consecutive sampling technique. The sample size was calculated using the WHO sample size calculator, taking a confidence level of 95%, a margin of error of 5%, and a reported prevalence of type-2 diabetes of 6.28%.<sup>6</sup> The estimated sample size came out to be 90 individuals. The sample size was calculated using the WHO sample size calculator using the prevalence of type-2 diabetes as a reference parameters. Patients with diabetic peripheral neuropathy (DPN) were assessed using Michigan Neuropathy Screening Instrument (MNSI). For our study, we took 90 subjects comprising three groups having 30 subjects in each. Group-I, Group-II and Group-III had 30 healthy subjects, 30 newly diagnosed T2DM patients and 30 T2DM patients with DPN, respectively.

**Inclusion Criteria:** Age and gender-matched healthy individuals and diagnosed cases of T2DM patients with and without DPN were included in the study from Outpatient Department.

**Exclusion Criteria:** Patients with type 1 diabetes mellitus, other neuropathy causes like hypothyroidism, alcohol abuse, patients with systemic diseases like renal or cardiac diseases or cancers and subjects using any anti-inflammatory medications in the last two weeks were excluded from our study.

We took detailed written informed consent from all the participants. Their demographic details and history were recorded. Subjects underwent a detailed physical examination. A total of 5ml of blood was drawn from the antecubital vein using standard precautions of venipuncture. Samples were transferred to the centre for research in the experimental and applied medicine (CREAM) lab in the icebox. Serum separator tubes were used to separate serum after centrifugation, and serum was stored at -80°C. We analysed serum using enzyme-linked immunosorbent assay (ELISA) at CREAM Lab Army Medical College, Rawalpindi. Serum Apelin and TNF-alpha were recorded from the blood samples of all the participants by ELISA.

Data analysis was carried out using computer software IBM SPSS (Statistical Package For Social Sciences) version 25. First, one-way analysis of variance (ANOVA) was carried out, followed by the Post-Hoc Tuckey test to compare serum Apelin levels for all three groups. Finally, the Pearson correlation coefficient was applied to assess the relationship between numerical variables. The *p*-value lower than or up to 0.05 was considered as significant.

## RESULTS

The total number of subjects included was 90(30 in each Group). There was an equal number of males and females in each Group. The mean age was 41.90±8.58 years in Group-I, 48.27±8.83 years in Group-II and 51.7±10.44 years in Group-III.

We determined Apelin serum levels in healthy individuals, T2DM and DPN subjects (Table-I). The mean value of serum Apelin was higher in Group-III compared to Group-II and Group-I, and a statistically significant difference was found (*p*-value 0.001).

**Table-I: Mean values of Serum Apelin for Healthy, Type-2 Diabetes Mellitus, Peripheral Neuropathy Patients (n=90)**

Parameters	Group-I (n=30)	Group-II (n=30)	Group-III (n=30)	<i>p</i> -value
FBG(mmol/l)	4.87±0.49	5.18±0.83	4.98±1.00	0.244
HbA1c(%)	5.23±0.89	7.35±0.75	6.79±0.18	0.001
Serum Apelin(ng/l)	209.5±8.80	264.5±8.80	565.83±9.19	0.001
Serum TNF-alpha(ng/l)	8.79±1.49	7.79±1.34	5.34±0.18	0.001

We found a statistically significant difference between healthy and type-2 diabetics (*p*=0.001), type-2 diabetics and DPN subjects (*p*=0.001), and healthy and DPN subjects (*p*=0.001) (Table-II).

**Table-II: Comparison of Biochemical parameters among Healthy, Type-2 Diabetes Mellitus and Peripheral Neuropathy Patients (n=90)**

Parameters	Group-I Vs. Group-II	Group-II Vs. Group-III	Group-I Vs. Group-III
Serum HbA1c	0.001	0.005	0.001
Serum Apelin	0.001	0.001	0.001
Serum TNF-alpha	0.004	0.001	0.001

We determined the Pearson correlation of serum Apelin with FBG, HbA1c and TNF-alpha. The serum Apelin levels strongly correlated with Group-III FBG with *r*-values-0.728 (Figure-1). Similarly, serum Apelin levels showed a strong negative correlation with Group-III HbA1c with the *r*-value of -0.79 (Figure-2) and with Group-III TNF-alpha at the *r*-value of -0.951 (Figure-3). A correlation was not found in Group-I and Group-II between serum Apelin and FBG, HbA1c and TNF-alpha.

The strong negative correlation between serum Apelin and FBG (*r* value -0.728) indicated that the higher the Apelin, the lower the FBG. The similarly strong negative correlation of serum Apelin with HbA1c and TNF-alpha shows that the higher the levels of Apelin lower will be the HbA1c and TNF-alpha.

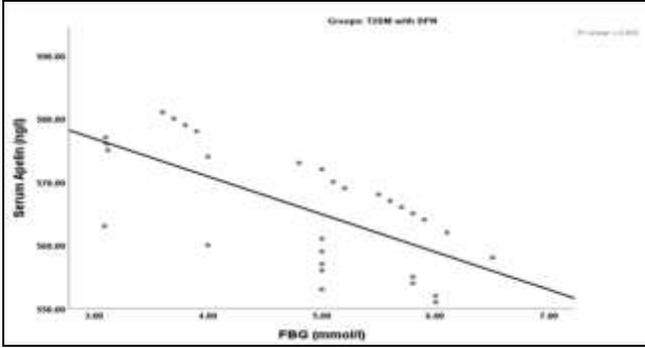


Figure-I: Correlation curve between Serum Apelin and Fasting Blood Glucose (Group-III) (n=30)

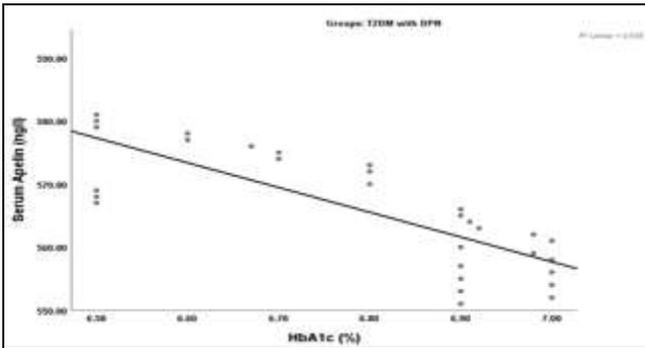


Figure-II: Correlation curve between Serum Apelin and HbA1c (Group-III) (n=30)

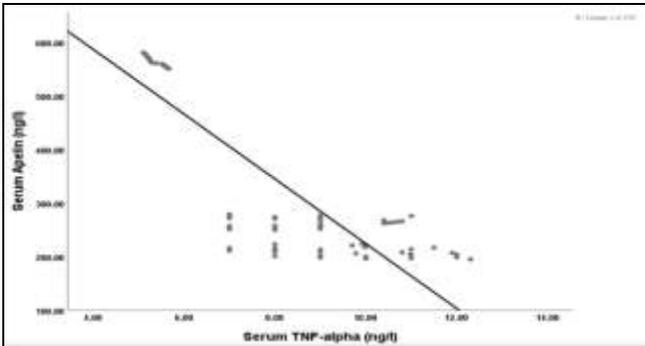


Figure-III: Correlation curve between Serum Apelin and Serum TNF-alpha(n=30)

**DISCUSSION**

Mainly our study was focused on determining the values of serum Apelin. The mean values of Apelin were highest in Group-III compared to Group-I and Group-II. We found a statistically significant difference in serum Apelin levels among Groups with a *p-value* of 0.001. This result is validated by a study conducted by Bilir *et al.* in which they noted that levels of Apelin were higher in people with diabetes than in healthy people.<sup>7</sup> He concluded that higher serum Apelin levels in DPN patients might be associated with endothelial changes, inflammatory mechanisms and angiogenesis.

However, this conclusion contradicted Another study sought to determine whether levels of plasma Apelin in T2DM patients were different regarding the presence of diabetic neuropathy. They noticed that Apelin levels were similar in diabetic and DPN patients, yet, this research partially backed up our findings that Apelin levels were greater in neuropathic patients than in healthy controls. Apelin is crucial in improving insulin sensitivity and promoting glucose utilisation in DPN.<sup>8</sup> Additionally, there is mounting evidence that Apelin may be able to remove reactive oxygen species (ROS).<sup>9,10</sup> This anti-oxidative property of apelin is produced by inhibiting the protein kinase-c (PKC) pathway and by inhibiting poly ADP ribose polymerase (PARP).<sup>11</sup>

We also determined the correlation between serum Apelin levels and TNF alpha in T2DM patients with and without neuropathy. *TNF-alpha* is a multifactorial cytokine involved in the regulation of immunity and inflammation.<sup>12</sup> Apelin inhibits ROS-induced production of TNF alpha and TNF alpha, which in turn inhibits ROS production. In this way, Apelin exhibits its anti-inflammatory and anti-oxidative role.<sup>13,14</sup> The Apelin-induced TNF-alpha reduction is one of the possible mechanisms of action which needs to be explored scientifically as serum TNF-alpha are genetically determined, and there are inter-ethnic variations in its serum levels.<sup>15</sup> In our study, we found low levels of TNF alpha in DPN patients, and there is a statistically significant difference in all three Groups (*p-value* of 0.001). We also found a strong negative correlation of serum Apelin levels with TNF alpha in DPN. This is because in patients with DPN, Apelin levels were higher, and they probably limit DPN by inhibiting ROS production and NF- $\kappa$ b.<sup>16</sup> The results of our study are validated by a study conducted by García-Díaz *et al.* on mice in which they used Apelin as a treatment option, and Apelin showed its anti-inflammatory effect in T2DM mice by lowering TNF-alpha levels.<sup>17</sup> Serum Apelin exhibited a strong negative correlation with FBG in Group-III, with the *r-value* of -0.728. This is because serum Apelin levels are higher in Group-III subjects compared to Group-II and Group-I, in which higher Apelin increases glucose uptake in target tissues such as skeletal muscle and adipose tissue.<sup>18</sup>

Our study suggests a link between Apelin levels and glycemic balance in type-2 diabetic patients. We determined a negative correlation between apelin levels and HbA1C, showing a strong negative

correlation in Group-III ( $r$ -value -0.79). The effect of apelin on insulin sensitivity could be one explanation as apelin increases insulin sensitivity. These findings are validated by a study conducted by Habchi *et al.* demonstrating that higher levels of circulating Apelin are linked to better glycemic control.<sup>19</sup>

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### LIMITATION OF STUDY

We could not track serum Apelin levels during the disease due to time and financial constraints. We could not determine the exact molecular mechanism by which serum Apelin levels reduce serum TNF-alpha. Further studies should be carried out at the genetic level in T2DM. We could not study other pro-inflammatory and anti-inflammatory adipocytokines in comparison due to time and financial constraints.

### CONCLUSION

Serum Apelin levels are increased in DPN and are associated with reduced TNF-alpha levels.

**Conflict of Interest:** None.

### Author's Contribution:

Following authors have made substantial contributions to the manuscript as under:

SFM & AN: Conception, study design, data acquisition, data analysis, critical review, approval of the final version to be published.

SS & NL: Drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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