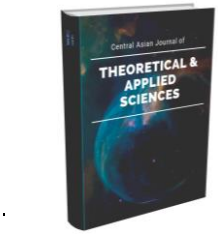

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Article

Elevated Serum Apelin, Insulin, and IL-17 Levels in Middle-Aged Patients with Type 2 Diabetes Mellitus: Associations with Hepatic Enzyme Alterations

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Abstract: The current study investigated changes in serum levels of apelin, insulin, and the pro-inflammatory cytokine interleukin 17 (IL-17) in patients with type 2 diabetes mellitus (type 2 DM). Additionally, as biomarkers for hepatic disorders, serum levels of the liver enzymes ALP, AST, and ALT were assessed in type 2 DM patients aged 40-60 years, with an equal distribution of men and women. Comparative analysis revealed a significant increase ($p<0.05$) in serum levels of apelin and insulin, with similar trends observed across both genders. Notably, IL-17 levels were also significantly elevated ($p<0.05$) in the type 2 DM group compared to control subjects. Furthermore, serum levels of ALP, AST, and ALT were significantly higher ($p<0.05$) in type 2 DM patients than in the control group. These findings, consistent with prior research, indicate that type 2 DM is associated with elevated apelin and IL-17 levels, contributing to significant insulin resistance through complex signaling pathways. Consequently, patients, irrespective of gender but likely influenced by their age (40-60 years), exhibit higher serum insulin levels compared to healthy controls.

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1. Introduction

Diabetes (DM) is a clinical endocronilogical syndrome characterized by disrupted insulin production or interaction, resulting in elevated blood glucose levels named as hyperglycemia. This condition can arise due to various factors, including psychological or organic causes, excessive intake of high glucose containing nutrients, and genetic predisposition (Galicia-Garcia et al., 2020; Genuth et al., 2021). The obesity has been identified as one of the primary causes of DM. Moreover, lack of physical activity, aging, diet, psychological stress, chemical exposure, and sudden psychological trauma have been reported as additional reasons for DM, which can elevate blood glucose levels by increasing the secretion of hormones like glucagon, catecholamines, and growth hormone, all of which counteract insulin's effects (Galicia-Garciat al., 202; El-Missiry et al., 2015; Genuth et al., 2021). As a pathological etiology for DM, the beta cells of pancreas do not produce enough insulin, or the cells do not respond effectively to the insulin produced by pancreas. As a result, glucose is not properly converted into energy, leading to an excess of glucose in the blood while cells remain deprived of energy (Cheng et al., 2021). As mentioned above, over time, this leads to hyperglycemia, which can cause severe damage

to nerves and blood vessels and result in complications such as heart disease, kidney disease, blindness, diabetic neuropathy, gum infections, diabetic foot, and even amputation (Cheng et al., 2021). Indeed, the DM is clinically divided into two types and subdivided into three subtypes. The type 2 DM is a metabolic disorder which is characterized by high blood glucose content due to insulin resistance and deficiency, accounts for approximately 90% of all DM cases (Goyal et al., 2023). However, type 1 DM and gestational DM make up the remaining 10%. Obesity is considered the primary cause of type 2 DM, especially in individuals with a genetic predisposition (Kumar et al., 2005). Apelin is a bioactive peptide and ligand for the G protein-coupled receptor and plays a crucial role in the regulation of glucose metabolism and insulin/cell interaction (Li et al., 2022). Apelin enhances the glucose uptake in skeletal muscle and adipose cells by upregulating the glucose transporter type 4 (GLUT4) translocation to the cell membrane. Therefore, it indirectly improves the insulin sensitivity (Vargas et al., 2024; Dray et al., 2008). Additionally, apelin has been shown to exert the anti-inflammatory and antioxidant effects that may protect against the endothelial dysfunction commonly associated with DM (Chen et al., 2020). These actions suggest that apelin could be a potential therapeutic target for improving metabolic control and ameliorating some of the vascular complications observed in diabetic individuals. The DM (type 1 and 2) is able to massively affect the liver histological structure and enzyme levels. DM is associated with hepatic steatosis, characterized by the accumulation of fat within hepatic cells, which can progress to non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) (Sharma and John, 2023). The hepatocyte ballooning, inflammation, and fibrosis has been reported in the liver tissue in the hyperglycemia condition (Leow et al., 2023). Furthermore, DM alters liver enzyme levels, often resulting in elevated serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of liver injury (Moriles et al., 2024). These changes are believed to be driven by insulin resistance, oxidative stress, and chronic inflammation induced by the DM, highlighting the liver as a critical organ affected by DM complications. Recently it has been shown that that hyperglycemia (in DM condition) positively associates with elevated levels of pro-inflammatory cytokines. Indeed, the elevated cytokines expression in DM condition plays a critical role in the development and progression of the disease's complications. In diabetic conditions, the cytokines such as interleukin-6 (IL-6) and 17 (IL-17), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) have been upregulated and/or increased. These cytokines contribute to insulin resistance by interfering with insulin signaling pathways and promoting chronic inflammation condition in DM patients (Garber et al., 2023; Velikova et al., 2021; Li et al., 2022). Elevated TNF-α levels, for instance, have been linked to impaired insulin receptor activity, while increased IL-6 is associated with reduced glucose uptake in tissues (Yuen et al., 2009). Furthermore, the high levels of these pro-inflammatory cytokines are not only markers of systemic inflammation but also actively participate in the pathogenesis of diabetic complications, including cardiovascular disease, neuropathy, and nephropathy (Zakir et al., 2023; Garber and Zhu, 2023). Understanding these inflammatory changes is crucial for developing targeted therapies aimed at reducing inflammation and improving metabolic control in diabetic patients. It has been shown that age significantly influences the effects of type 2 DM, with older adults being more susceptible to its complications due to several physiological changes associated with aging (Sue Kirkman et al., 2021). As individuals age, there is a natural decline in insulin sensitivity and pancreatic beta-cell function, which exacerbates the hyperglycemia characteristic of type 2 DM. In line with this issue, older adults often experience a decrease in muscle mass and an increase in adiposity, further contributing to insulin resistance. Aging also affects the body's ability to manage oxidative stress and inflammation, compounding the risk of vascular complications such as cardiovascular disease, neuropathy, and retinopathy (Assar ME et al., 2015). Cognitive decline and impaired physical function are also more common among older diabetic

patients, making DM management more challenging in this population (Aderinto et al., 2023). Thus, age is a crucial factor that worsens the effects of type 2 DM, necessitating tailored management strategies to address the unique needs of older adults. Based on recent findings, the current study aims to investigate the changes in hepatic enzyme levels (ALP, AST, ALT) and the expression level of IL-17, as well as the interplay between these parameters with serum levels of apelin and insulin in middle-aged (40-60 years) type 2 DM patients living in Hilla city, Iraq.

2. Materials and Methods

Specimen Collection

The specimens for the current study were collected from individuals with type 2 DM residing in the Babil Al-Kabir Province between January and the end of February 2024. A total of 80 blood samples were obtained from both male and female participants, aged between 40 and 60 years, who were registered with the Chronic Diseases Unit at Al-Hilla Teaching Hospital in Babylon Province. The samples were categorized into two groups:

Patient Group: This group included 40 registered patients diagnosed with type 2 DM.

Healthy Control Group: This group consisted of 40 blood samples collected from healthy individuals.

Blood samples were collected from both groups and processed using centrifugation (3000 g) to separate the serum. The biochemical parameters measured included the hormones apelin and insulin, IL-17, and liver enzymes.

Exclusion criteria included individuals outside the specified age range, those with type 1 diabetes or other forms of diabetes, chronic illnesses in the control group, pregnant women, individuals with recent infections or surgeries, those with inadequate blood samples, and those on medications that could influence insulin, apelin, IL-17 levels, or liver function, except routine T2DM treatments in the patient group.

Estimation of Apelin, Insulin, and IL-17 Levels in Serum

The concentration of apelin, insulin, and IL-17 in the serum was determined using the ELISA-Sandwich method. This method employed examination kits from reputable. Serum apelin levels were assessed using the Cusabio (Uniprot No: Q9ULZ1, USA) commercial kit following the manufacturer's instructions. The 50 µl from previously obtained serum samples were considered. Reagents were prepared by thawing and equilibrating them to room temperature (RT). The assay involved coating a microplate with the capture antibody, blocking with buffer to reduce non-specific binding, and adding serum samples and apelin standards. Absorbance was measured (450 nm) using a microplate reader (DANA, 3200, Iran), and apelin concentrations were determined by interpolating absorbance values against a standard curve.

Insulin levels in serum were determined using the My BioSource (Cat N: 90095, USA) commercial kit according to the provided protocol. Upon preparation, reagents were equilibrated to RT. For this purpose, 25 µl of the serum sample was used. The assay involved coating a microplate with the insulin-specific capture antibody, followed by blocking with buffer to minimize non-specific interactions. Serum samples and insulin standards were then added to the wells and incubated. The absorbance of each well was measured with a microplate reader (DANA, 3200, Iran), and insulin concentrations were calculated by comparing the sample absorbance (at 450 nm) to a standard curve.

The serum level of IL-17 was assessed using commercial Kit Cusabio (Uniprot No: Q16552, USA). The 100 µl from previously obtained serum samples were considered. The assay involved coating a microplate with the capture antibody, blocking with buffer to reduce non-specific binding, and adding serum samples and IL-17 standards. Absorbance was measured (450 nm) using a microplate reader (DANA, 3200, Iran), and apelin concentrations were determined by interpolating absorbance values against a standard curve.

Estimation of AST Enzyme Activity in Serum

The activity of the aspartate aminotransferase (AST) enzyme was estimated based on its ability to catalyze the transfer of an amino group between L-aspartate and 2-oxoglutarate, producing oxaloacetate and L-glutamate. Subsequently, oxaloacetate is reduced to malate by NADH, and this reaction, facilitated by malate dehydrogenase (MDH) as a cofactor, generates NAD+ and malate (Winn-Deen E et al., 1988).

Estimation of ALT Enzyme Activity in Serum

The activity of the alanine aminotransferase (ALT) enzyme was estimated by catalyzing the reaction between alanine and oxaloacetate to form pyruvate. Pyruvate is then reduced by NADH in the presence of lactate dehydrogenase (LDH) and a cofactor, resulting in the production of lactate and NAD+ (Winn-Deen E et al., 1988).

Estimation of ALP Enzyme Activity in Serum

The activity of the alkaline phosphatase (ALP) enzyme was measured by its ability to catalyze the hydrolysis of para-nitrophenyl phosphate, leading to the release of para-nitrophenol and a free phosphate group. The absorbance of the released para-nitrophenol was measured at 405 nm, with the intensity of absorption being directly proportional to the serum alkaline phosphatase activity (German Society for Clinical Chemistry, 1972).

Statistical Analysis

The results were analyzed using the Statistical Package for the Social Sciences (SPSS) software to calculate the mean and standard deviation (± SD). Comparisons between the group of type 2 DM patients and the healthy control group were made using the T-test, with statistical significance set at a probability level of $P \leq 0.05$.

3. Results

Type 2 DM significantly increased the apelin and insulin levels

Our observations showed that the patients in the type 2 DM group exhibited significantly ($p<0.05$) higher serum level of apelin versus the control subjects. In additions, the observations demonstrated a remarkable ($p<0.05$) increase in the serum level of insulin in type 2 DM group compared to the control individuals. Note Fig. 1A, 1B for the results of the apelin and insulin which are compared between type 2 DM and control subjects.

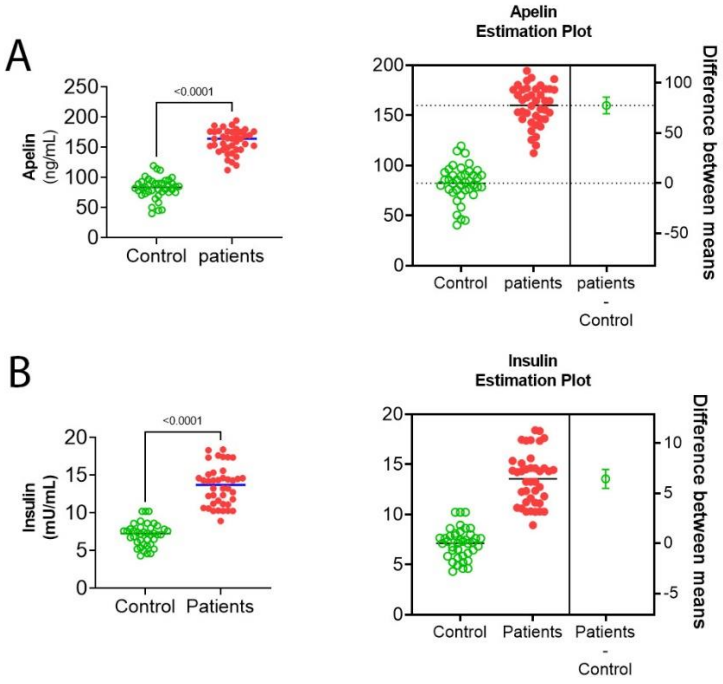

Fig. 1: Mean changes in the (A) Apelin and (B) Insulin in the control and patient groups (n=40) and the estimation plot for difference between means of data in control and patient groups.

Type 2 DM could significantly increase the IL-17 level versus the control condition

Our findings revealed that type 2 DM significantly ($p<0.05$) could increase the serum IL-17 level versus the control subjects (Fig. 2).

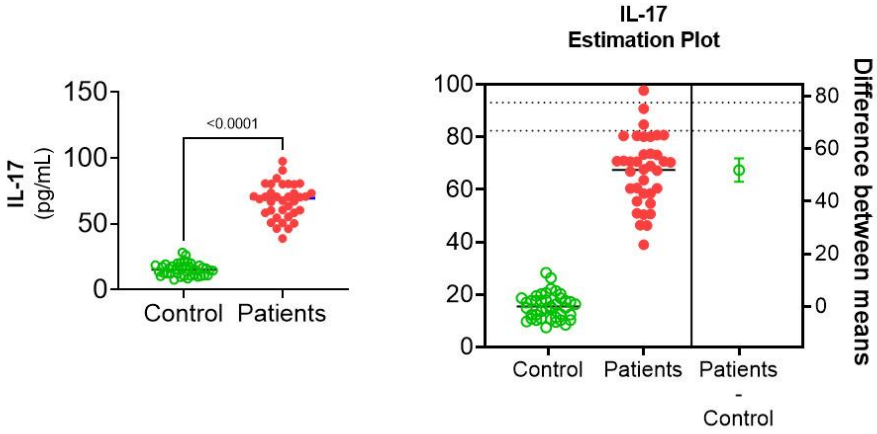

Fig. 2: Mean changes in the serum IL-17 in control and patients (n=40) and the estimation plot for difference between means of data in control and patient groups.

Type 2 DM could significantly increase hepatic enzymes level

The type 2 DM patients represented a remarkable increase in the serum levels ALP, AST, and ALT compared to the control subjects ($p<0.05$). Note Fig. 3A, 3B, 3C for the results of the changes in the hepatic enzymes level.

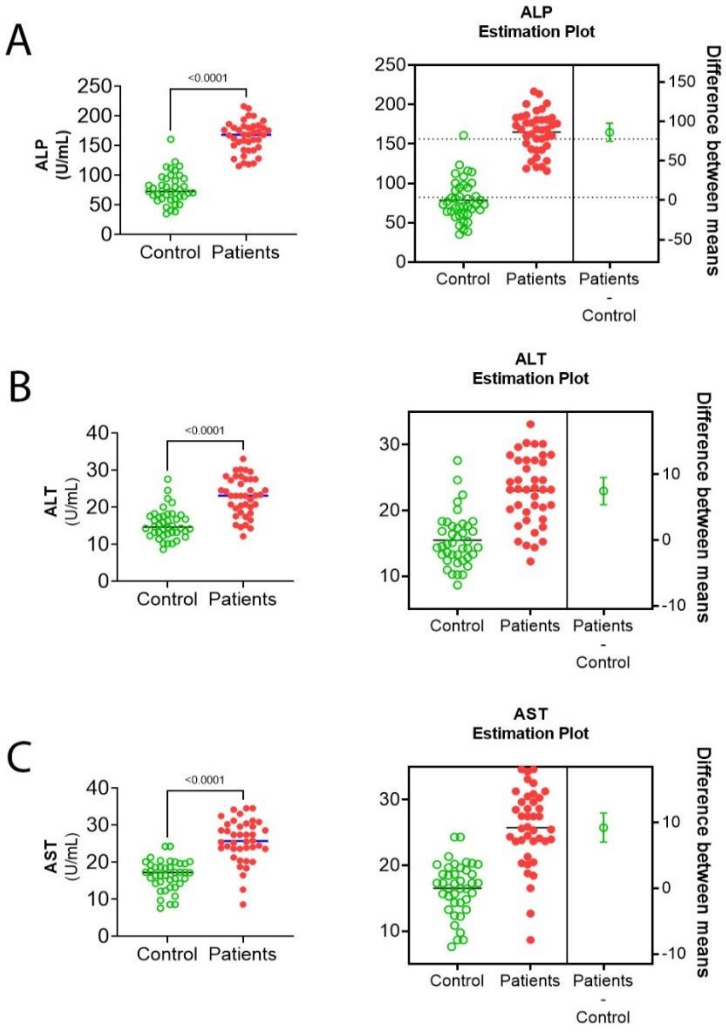

Fig. 3: Mean changes in the (A) ALP, (B) AST, (C) AST in the control and patient groups (n=40) and the estimation plot for difference between means of data in control and patient groups.

4. Discussion

The current study revealed a significant increase in serum apelin levels in patients with type 2 DM compared to the control group, consistent with the findings of Cavallo et al. (Cavallo et al., 2012). One of the theories for this situation is the presence of insulin resistance in type 2 DM condition. An increased apelin level in type 2 DM is associated with glucose imbalance and is positively correlates with an increased insulin level (Soriguer et al., 2009). Indeed, it has been shown that apelin upregulates the insulin sensitivity by directly promoting glucose uptake and modulating cellular signals to GLUT-4 translocation (Vargas et al., 2024; Dray et al., 2008) and/or indirectly by improving metabolic energy through increased mitochondrial biogenesis and balancing fatty acid oxidation with the tricarboxylic acid cycle (Krysiak et al., 2010). Additionally, apelin interacts with inflammatory markers such as TNF-α and IL-17, influencing insulin sensitivity through the activation of adenosine 5-monophosphate-activated protein kinase (AMPK), which is linked to insulin resistance in type 2 DM (Heinonen et al., 2009). Elevated serum apelin levels have also been associated with higher glucose concentrations in diabetic patients (Nishida and Hamaoka, 2013). Considering the consistent pattern of apelin levels observed in both male and female subjects, and taking into account the patients' age, we can infer that type 2 DM likely upregulates apelin concentration independently of gender. This effect appears to be more closely related to the patient's age and overall condition rather than their sex.

Our findings demonstrated a significant increase in the insulin levels of type 2 DM patients compared to the control group, in line with the results of Freedman et al. (Freedman et al., 1999). This situation may occur because of a diminished physiological response of tissues to insulin. Obesity, a common factor in type 2 DM, exacerbates insulin resistance and is associated with dyslipidemia, including elevated triglycerides and reduced HDL, poor glucose tolerance, and high blood pressure (Hamidi et al., 2006). Considering the age and weight of our cases, we suggest that obesity, in conjunction with insulin resistance, may contribute to elevated insulin levels in individuals with type 2 DM. The level of IL-17 was significantly higher in patients with type 2 DM compared to the control group, corroborating findings by Marwaha et al. (Marwaha et al., 2012). The increase in IL-17 is linked to its role in enhancing insulin resistance through the activation of the angiotensin II receptor (Ohshima et al., 2012). IL-17 activates pathways such as Jak2 and Jake, regulating genes involved in pancreatic beta-cell function, which indirectly contributes to increased insulin resistance and type 2 DM (De Morales et al., 2020; Shu et al., 2012). In summary, our findings suggest that apelin may exacerbate insulin resistance in type 2 DM by upregulating pro-inflammatory cytokines, notably IL-17, which is evidenced by elevated insulin levels in these patients. The data indicate that this relationship is likely influenced by age, with individuals between 40 and 60 years old experiencing apelin/IL-17-dependent hyperinsulinemia, independent of gender.

Serum levels of liver enzymes, including ALP, AST, and ALT, were significantly elevated in type 2 DM patients compared to the control group. The increase in ALP, as observed in the study by Al-Salihi et al. (Qiu et al., 2023), may be due to enzyme release from bones, potentially linked to bone diseases associated with DM, such as osteonecrosis, due to disrupted parathyroid hormone (PTH) secretion (Al-Salhi et al., 2011). Elevated ALP and AST levels, consistent with Abdilkarim et al. (Abdilkarim Yehia et al., 2015), suggest structural and functional abnormalities in liver cells, potentially resulting in necrosis and enzyme release into the bloodstream (Bolkent et al., 2008). Additionally, increased

glycogen production and fat accumulation in liver cells may contribute to hepatic cirrhosis (Angulo and Lindor, 2007). Moreover, it has been shown that insulin deficiency impacts liver tissue, leading to dysfunction and elevated levels of AST and ALT, markers of liver damage and chronic hepatitis related to DM (Farswan et al., 2009; Moller, 2001; Eteng et al., 2008).

5. Conclusion

In conclusion, the current study explored a significant increase in serum apelin levels in type 2 DM patients, in the corroboration with previous research by Cavallo et al. (2012). Our findings also reveal significantly elevated insulin levels and IL-17 in type 2 DM patients, indicating heightened insulin resistance exacerbated by obesity and metabolic disturbances (Freedman et al., 1999; Marwaha et al., 2012; Hamidi et al., 2006). Furthermore, increased serum levels of liver enzymes, including ALP, AST, and ALT, suggest liver dysfunction and structural abnormalities linked to type 2 DM (Al-Salihi et al., 2011; Abdilkarim Yehia et al., 2015; Angulo and Lindor, 2007). Collectively, these results underscore the complex interplay between apelin, insulin resistance, and liver function in type 2 DM, with age emerging as a critical factor influencing these relationships.

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