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The Comparison and Correlation of Serum Levels of Insulin and Na+/ K+ Atpase In Type 2 Diabetes Mellitus (T2DM) And Non-Diabetes Mellitus Individuals

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Abstract

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic condition characterized by hyperglycemia that persists. Diabetes mellitus can cause damage to multiple organ systems, leading to the development of severe and life-threatening health issues, due to decreased insulin secretion, resistance to peripheral insulin effects, or both.
Objective: the comparison and correlation of serum levels of insulin and Na+/ K+ ATPase in T2DM and Non-Diabetic patients.
Material and methods: A case-control study was carried out in the max cure diabetes center and Al-Zahra Teaching Hospital in the city of AL-Kut, Iraq. Including 114 patients divided into 54 patients of T2DM in addition to 60 individuals as control (male and female), ages ranging from (20-70 years) from November 2021 –to March 2022. It was the measurement of serum Na+/K+ATPase and insulin level.
Results: In the comparison of insulin levels in Type 2 Diabetes Mellitus (T2DM) and healthy control group, serum insulin was found to be significantly decreased (p value<0.005) and Na+/K+ ATPase significantly decrease with a (p value<0.001) in diabetics as compared to control group. The correlation was positive and significant between the serum Na+/K+ ATPase and insulin.
Conclusions: serum insulin and Na+/K+ ATPase showed a significant decrease in diabetics as compared to the control group. Whereas a significant positive correlation is obtained between the parameters.

Publisher : Middle Technical University

Keywords: Type 2 Diabetes Mellitus (T2DM); Insulin; Na+/K+ ATPase; Hyperglycemia; Beta-cell.

1. Introduction

Diabetes Mellitus type 2 is one of the most prevalent metabolic disorders around the world, it may have resulted from a combination of two main factors: insufficient insulin production by the beta cell in the pancreatic or failure of insulin-sensitive tissues to respond to insulin [1]. Diabetes mellitus is characterized by high plasma glucose (hyperglycemia) [2]. Insulin is a hormone that is generated by beta cells in the pancreatic islets of Langerhans. It lets glucose from the bloodstream reach the cells of the body, where it is transformed into energy. Insulin is also essential for the metabolism of protein, fat, and carbohydrate [3]. In a healthy person, insulin release is highly precise to balance metabolic requirements. Specifically, beta-cell sense change in glucose level and responds by releasing equal level of insulin [4]. To sense nutritional state, beta cells are localized in an islet that is connected to vasculature to sense nutritional condition. Islets have a rich network of tiny blood capillaries and get 10 times as much blood as cells in the surrounding exocrine region. Capillaries that surround islets have an unusually high number of tiny pores known as fenestrae, which allow for better nutrition exchanges between surrounding tissues and circulation. This structure increases permeability, allowing unfettered nutrient access and allowing beta-cells to rapidly detect nutritional status. Fenestrations also allow insulin to enter the bloodstream quickly [5]. Additionally to glucose, certain amino acid and fatty acid also regulates insulin production and secretion. A deficiency of insulin, or the inability of cells to respond to it, results in high blood glucose levels (hyperglycemia), which is a clinical sign of diabetes [6].

Type 2 Diabetes Mellitus accounted to be approximately 90% of DM cases, this syndrome is usually characterized by inadequate insulin secretion in pancreatic islets cells, "tissues insulin resistance, and inadequate compensatory-insulin secretory responses [7]. As this condition progresses, insulin production is usually insufficient to maintain glucose regulation, which might result in a hyperglycemic state. Patients with Type 2 Diabetes Mellitus are usually overweight or have great body fat percentages, which was concentrated typically in the abdomin[8]. Adipose tissues induce insulin resistance in such situation via a variability of inflammatory mechanisms, including high free fatty acid releasing in addition to adipokine dysregulation. Globally growth in sedentary lifestyle, obesity, high-calorie diet, and population aging were the primary causes of Type 2 Diabetes Mellitus epidemic, which had increased the occurrence and prevalence of Type 2 Diabetes Mellitus [9]. Patients must maintain good blood glucose control to avoid or delay diabetes complications such as nephropathy, retinopathy, and neuropathy [10]. Several human investigations have found that uncontrolled diabetes mellitus produces changes in membrane protein structure, organization, and function, all of which play a significant role in the pathogenesis of diabetic complications [6]. The sodium-potassium pump, like other membrane proteins, is physically and functionally altered in diabetes mellitus [7]. The sodium-potassium pump,

Nomenclature			
T2DM	Type 2 Diabetes Mellitus	ELISA	enzyme-linked immunosorbent assay
IR	insulin resistance	SPSS	Statistical Packages for Social Sciences
FFA	free fatty acid	α	alpha subunit
Na+ /K + ATPase	sodium-potassium adenosine triphosphatase	β	beta subunit

also known as sodium-potassium adenosine triphosphatase (Na+ /K + ATPase), is a membrane enzyme (protein in nature) that is found in almost all eukaryotic cells [8]. It uses ATP as an energy source to catalyze the counter-transport of sodium and potassium across the cell membrane [9]. For every ATP consumed, the Na/K ATPase pumps three sodium ions out of the cell and two potassium ions into it [10]. The plasma membrane is an asymmetrically structured lipid bilayer that contains cholesterol, phospholipids, glycolipids, sphingolipids, and proteins. The Na/K-ATPase pump contributes to the maintenance of osmotic balance and membrane potential in cells [11]. Na/K-ATPase is a membrane protein that is essential for the maintenance of intracellular sodium and potassium concentrations, cell volume, membrane potential, and electrochemical gradients [12] The Na+K+ATPase is mainly composed of catalytic α subunits and auxiliary β subunits. Some Na K ATPase also has a tissue-specific component that belongs to FXYDs proteins family. The α subunits include trans-membrane regions made up of ten helices known as MA1M10. Ion-binding sites, especially three binding sites that bind to Na+ in the E1 state and two binding sites that bind K+ in the E 2 states, are found inside these 10 helices [13]. Exercise training, hypokalemia, type 1 and type 2 diabetes mellitus all have varied effects on Na+-K+-ATPase protein expression [14]. Physical activity and exercise training lower the chance of developing type 2 diabetes and obesity, in part by increasing the protein expression of key genes involved in glucose absorption and utilization in skeletal muscle [15]. Endurance exercise training also elevates the content of Na+/K+-ATPase [16]." In diabetic patients decrease in Na/K ATPase enzyme activity is due to defects in insulin action or secretion where insulin is responsible for stimulating sodium-potassium pump activity [17]. The study aimed to evaluate, compare and correlate the serum insulin and Na+-K+-ATPase in diabetic type 2 patients and non-diabetic individuals as the control group.

2. Materials and methods

It was a case-control study for which subjects were selected from the max cure diabetes center and Al-Zahra Teaching Hospital in the city of AL-Kut, Iraq. Includes 114 patients divided into 54 patients of T2DM and 60 individuals as control (male and female) with ages ranging from 20 to 70 years had registered in the study from November 2021 –to March 2022 and all information about each patient filled questioner sheet. Included in the study are patients who have type 2 diabetes according to WHO criteria. Excluded patients suffering from diabetes mellitus Type1, pregnant females suffering from Diabetes Mellitus, thyroid dysfunction, and patients taking medicine like insulin injection.
For estimation of serum insulin and serum Na/K ATPase level, 5 ml of venous blood was taken after 8 hours of fasting. Measurement of insulin and Na+/K+ ATPase make by utilizing "enzyme-linked immunosorbent assay (ELISA) "(sandwich method) and reading results in ELISA reader (Huma reader).

3. Statistical Analysis

All data were present as mean ± SD. Statistically significant and the differences among test value and control worked by "Student t-test" were done by using the available statistical package of SPSS. Statistical significance was considered whenever the P-value was equal to or less than 0.05. Correlation coefficients were used to describe the effects of one variable on the other.

4. Results

In the present study, we have compared the serum levels of insulin and serum Na+/K+ ATPase among diabetic type 2 and the control groups. It was observed that serum insulin significantly varies between-group (1) and group (2) with a p value<0.005 and Na+/K+ ATPase significantly varies between group 1 and group 2 with a p value<0.001 (Table 1 and Fig. 1 & 2).
In our study, it was revealed strong positive correllation between the groups with serum insulin, and Na+/K+ ATPase was found to be significant (Table 2 and Fig. 3 & 4).

Table 1 Comparison of serum insulin and Na+/K+ ATPase in diabetic type 2 patients and healthy under Fasting Conditions

Parameters	Diabetes patients group 1 N=54			Normal Control group 2 N=60			P-value¥
	Mean	±	SD	Mean	±	SD	
insulin	16.11	±	8.09	19.00	±	3.82	0.019*
Na/K ATPase	2.40	±	0.49	4.19	±	1.29	0.001**

All data are presented as mean ± SD (Standard Deviation), * significant, ** highly significant, t-test

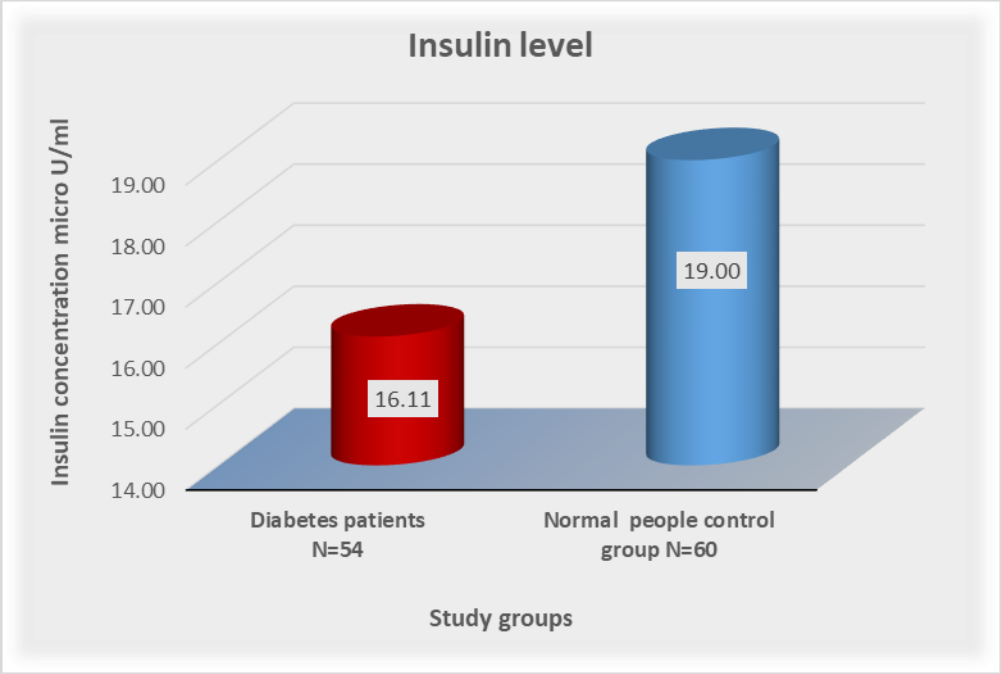

Fig 1. Comparison of serum insulin in diabetic type 2 patients and healthy people (control group)

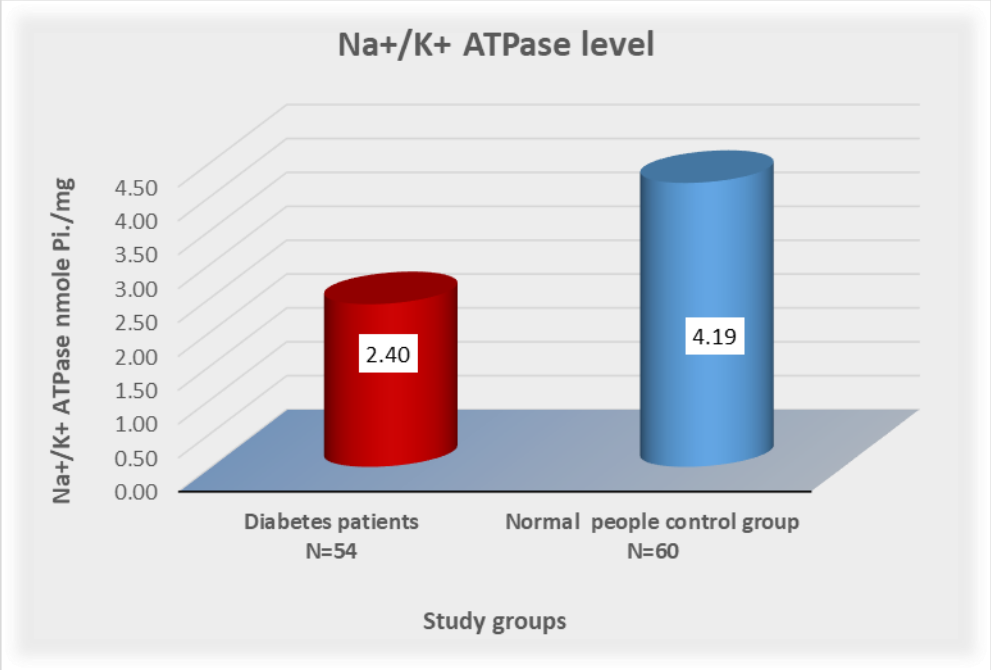

Fig 2. Comparison of serum Na+/K+ ATPase level in diabetic type 2 patients and healthy people

Table 2 Correlation of serum insulin and Na+/K+ ATPase with groups (1, 2)

Study groups		Correlation coefficient (r)
		Na/K ATPase
Diabetes patients group 1	Insulin	0.48**
Normal-control group 2	Insulin	0.41**

Correlation coefficient

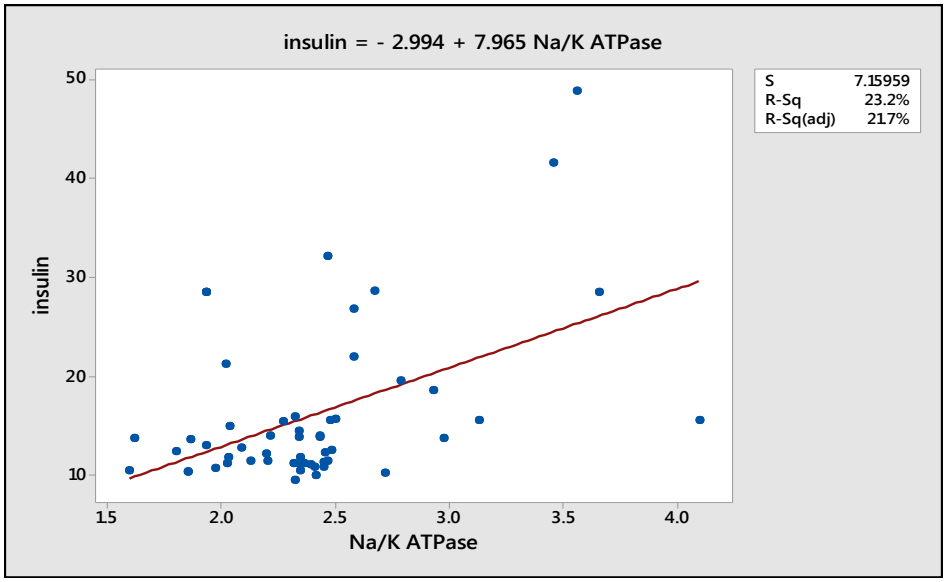

Fig 3. Correlation between serum insulin and Na/K ATPase in diabetic type 2 patients

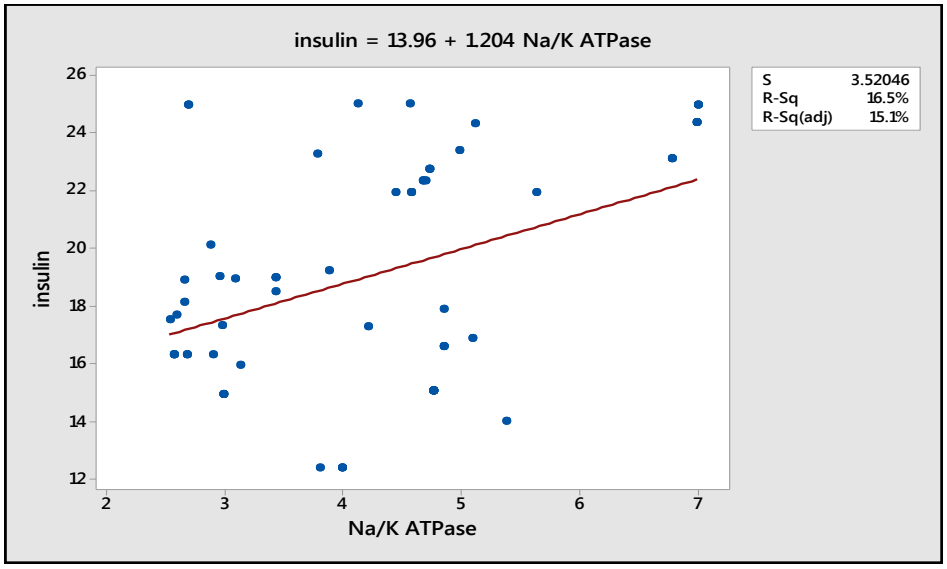

Fig 4. Correlation between serum insulin and Na/K ATPase in the healthy control group

5. Discussion

The present study is mainly focused on the comparison of serum insulin and Na+/K+ ATPase between the groups and correlating the parameters between the groups. The subjects were divided into two groups as explained in the study subjects of materials and methods. Our study includes patients with type 2 diabetes, and as we know that diabetics suffer from high blood sugar due to a defect in the action and/or secretion of insulin [18]. Insulin is an essential hormone for regulating the metabolic processes of carbohydrates, fats, and proteins. Insulin is secreted from the beta cells of the pancreas. In some cases of diabetes, the insulin secretion is high, but there is no response by the target cells. The beta cells continue to secrete insulin and this condition is called insulin resistance [19]. In other cases, especially in patients with uncontrol diabetes, the consumption of insulin due to the constant high sugar works to exhaust the beta cells and decrease their efficiency and production, or the decrease mass and their destruction, and thus the low level of insulin and the lack of its secretion in insufficient quantities [20]. This, in turn, causes complications of diabetes and an imbalance of many electrolytes, some of which are considered As an activator for the action of some enzymes, for example, magnesium acts as a co-factor of certain enzymes, and this could be explained as a reason for the decreased activity of the Na+/K+ ATPase in diabetic patients [21].

The present study in group 1 shows a significant decrease ($P \leq 0.05$) in serum insulin (16.11±8.09) as compared with the control group (19.00±3.82) The possible explanation for the decrease in insulin level is that it may support the hypothesis that the number is reduced of beta cells rather than impaired Beta-cell function is a major factor in T2DM. The reduction in beta-cell mass up to 60% in T2DM, is equivalent to the extent of the decrease in insulin secretion caused by glucose [22]. which agrees with the results of RosemaryCTemple et al., who found lower plasma insulin concentrations in diabetic patients compared to the matched controls [19]. This Agrees with another study by Louise bennet et al. the research invited citizens of Malmo and Sweden between the ages of 30 and 75 who were born in Iraq or Sweden to participate in this study. Insulin secretion (insulin response) and action (insulin sensitivity index) were assessed by oral glucose tolerance tests

and shows that insulin secretion was further decreased in Iraqi immigrants and native Swedes [20]. While disagreement with other studies show fasting insulin level was very high in patients with diabetes mellitus than in those in the healthy control group [21-23] This high insulin concentration reflects the degree of Insulin resistance as indicated by an average HOMA-IR value till then Patients had varying degrees of beta-cell.

The current study found statistically significant differences when comparing group (1) and group (2) in the level of Na+/K+ ATPase in diabetic patients (group 1) a significant decrease with a p value<0.001 (2.40±0.49) compare with the healthy (control group 2) (4.19±1.29) in (Table 1). This agrees with the study by Mohamed A. Abosheasha *et al*., which shows that Na+/K+ ATPase activity is significantly decreased in T2DM [24]. In agreement with P. Vague *et al*., this study found a decrease in Na-K ATPase in T2DM [25]. The altered Na+/K+ ATPase activity may be due to C-peptide and insulin deficiency that may alter the long-term regulation of enzyme units expressed on cell surfaces. Studies have presented significant decreases in m RNA level of four subunits in diabetes induction [14] or The diabetes-induced impairment in Na+, K+-ATPase activity could be related to hyperglycemia via increased glycosylation of its β subunit involved in the maturation of the enzyme and its localization and stabilization in the plasma membrane [26].

In the current study, in Table (2) and Fig.s (3 & 4), the correlation between the groups with serum insulin and Na+/K+ ATPase was observed to have a significant positive correlation between them. This is compatible with the study by Xu-Peng Wen, which explains how insulin regulates and stimulates Na+/K+ ATPase activity [22]. Generally, it was understood that Na+ controls Na+/K+-ATPase in the majority of mammals cells. Increasing Na+ inflow in response to insulin stimulation may activate Na+/K+-ATPase. Such mechanisms might be a receptor-mediated process, that exhibits tissue-specific variations. In terms of the mechanism by which insulin regulates Na+/K+-ATPase, it was observed that it promotes a relative abundance of Na+/K+-ATPase and restores transport efficiency in different systems [23]. Insulin's action on Na+/K+-ATPase is mostly seen as a short-term regulation, in which only Na+/K+-ATPase 1 is translocated to the plasma membrane and internalized into the cytoplasm, and insulin modifies its molecular shape and regulates transport efficiency [24]. However, Na+/K+-ATPase activity may be susceptible to long-term modulation, i.e., insulin may induce transcription and translation of the α1 subunit or decrease its degradation. [22].

6. Conclusion

It was observed that serum insulin and Na+/K+ ATPase activity was significantly decreased in patients suffering from type 2 diabetes mellitus as compared to controls. the Correlation between the groups with serum insulin and Na+/K+ ATPase was observing a significant positive correlation between them.

7. Recommendation

The researchers need to develop more comprehensive and systematic research with advanced diagnostic.
Advances in the detection of the risk factor for the development of diabetic complications will improve comprehensive management of the disease.

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