Original article

Serum Caveolin-1 Level is Inversely Associated with Serum Vaspin, Visfatin, and HbA1c in Newly Diagnosed Men with Type-2 Diabetes

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Abstract

Background: The fluctuation in serum caveolin-1 (Cav-1) concentrations is an important indicator of many diseases. Irrespective of the actual cause, a significant reduction of serum Cav-1 is associated with a significant increase in insulin secretion and hyperinsulinemia. The aim of the current study was to evaluate the relationship between serum Cav-1, serum vaspin and visfatin in newly diagnosed men with T2DM.

Methods: Eighty-two newly diagnosed men with T2DM were matched for age and body mass indexes (BMIs) with a similar number of non-diabetic men. Serum Cav-1, vaspin and visfatin were assessed through enzyme-linked immunosorbent assay. Fasting serum glucose (FSG), glycohaemoglobin A1C (HbA1c) were both measured using automated method. In addition, waist-circumferences, waist-hip ratio, systolic (SBP), and diastolic blood pressure (DBP) were also obtained.

Results: Serum concentration of Cav-1(ng/mL) was significantly low in men newly diagnosed with T2DM, (2.334±0.7627) compared with non-diabetic controls (4.321±1.143), p< 0.0001. In contrast, patients with T2DM exhibited significantly higher serum concentrations of vaspin and visfatin (ng/mL), 142.4±60.53) and 2.99±1.091), than controls, 81.53±39.32) and 1.456±0.654), respectively, p< 0.0001. Expectedly, patients with T2DM have significantly higher FSG, HbA1c, systolic blood pressure (SBP), and diastolic blood pressure (DBP).

Conclusions: There was an inverse significant relationship between Cav-1 and vaspin, visfatin, HbA1c, FSG, and hypertension. This study suggests that serum Cav-1 can be used as a diagnostic marker to predict T2DM in individuals and families under high risk.

Keywords: Caveolin-1, HbA1c, Insulin resistance, T2DM, Vaspin, Visfatin.

Introduction

The last three decades have witnessed a sharp increase in the prevalence of obesity in developed and developing countries (1). Not surprisingly, this worldwide escalation in the prevalence of obesity was paralleled with a similar trend of escalation in the incidence/prevalence of Type 2 diabetes (T2DM) which represents more than 90% of patients with diabetes (1, 2). Most patients with T2DM are overweight or obese. Obesity, especially abdominal obesity, is an established risk factor for T2DM. Obesity and T2DM both share similar risk factors which include modifiable and non-modifiable factors (2).

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Non-modifiable risk factors include older age, ethnicity, genetic predisposition, low birth weight and family history, whilst modifiable factors include inactivity and dietary fat intake (2, 3). Furthermore, obesity and T2DM are both associated with chronic inflammation and insulin resistance (4). Although, both diseases have shared similar health consequences such as hypertension, cardiovascular disease (CVD) and certain types of cancer, however, mortality rate is much higher among patients with T2DM. It is estimated that life expectancy is seven years shorter in patients with T2DM than non-diabetic individuals of general population (2).

Classically, patients with T2DM have similar symptoms to those with Type 1 diabetes mellitus (T1DM), although blood biochemistry may vary due to differences in the pathogenesis/the pathophysiology of each disease (2, 3, 5). Whilst, a rapid, irreversible autoimmune destruction of pancreatic β-cells, usually occurs due to viral infection, genetic factors are the main causes of T1DM. However, insulin resistance and inflammatory cytokines are the main culprits for the gradual, prolonged dysfunction and progressively irreversible reduction of pancreatic β-cells in T2DM (4, 6, 7).

Visceral adipose tissue-derived Serine Protease Inhibitor (vaspin) and visfatin (nicotinamide phosphoribosyl-transferase/nampt), have both been identified to be mainly secreted from adipocytes of visceral fat depot in a rat model of abdominal obesity and T2DM (8, 9). It has been reported that serum vaspin and visfatin are associated with insulin resistance (10) and are both significantly elevated in patients with T2DM (11) compared with non-diabetics. Increased serum visfatin in patients with T2DM is associated with the deterioration of β-cells function, suggesting a pivotal role in the pathophysiology of T2DM (10, 12). In contrast high level of vaspin has been shown to have a protective effect, because it inhibits the proteases responsible for insulin resistance (13, 14). Caveolin-1 (Cav-1), on the other hand is the main structural protein of caveolae located in the cellular membrane and is shown to play a crucial role in regulating lipid metabolism (15). Cav-1 activation is associated with cell migration and signaling, and Cav-1 knockout mouse displayed impairment of peroxisome proliferator activated receptor-α (PPAR-α) -dependent oxidative fatty acid metabolism and ketogenesis (16). A significant decrease in Cav-1 is associated with many diseases, such as cancer, liver, and lung diseases (17, 18, 19). The current study was designed to assess the relationship between serum Cav-1 level and serum vaspin and visfatin in newly diagnosed patients with T2DM in a large public hospital in the city of Ramdi, the capital of Al-Anbar province, Iraq.

Materials and Methods
This study consisted of one-hundred and sixty-four men (age range 40 to 70 years), including eighty-two patients newly diagnosed with T2DM and eighty-two, age and BMI's matched controls. The study protocol was approved ethically by the Scientific Research Ethics Committee at the University of Anbar (No: 159SC-2021), and all the participants have willingly signed a written consent form.

Collection of the study participants
The current study was performed during the time from February 2021 to July 2021. All T2DM patients were evaluated before their treatment was initiated. Patients and most of the controls were collected from the same hospital. We further, requested from our patients to bring relatives and/ or friends to join the study as controls.

Clinical examination
Previously scheduled interviews were performed at the main general hospital by well trained nurses. In order to collect demographic, socioeconomic and physiological details including anthropometrics, medical history, blood pressure, blood pressure was obtained using a fully automated machine. Each participant of the current study was asked to rest for five minutes in a sitting position, before taking the first measurement. We obtained three measurements for each participant, between each measurement and the one following it, there was a period of five minutes resting in a

sitting position. The average of three readings then was considered as blood pressure target.

Collection and handling of blood samples

Approximately 10 ml of blood was drawn from each participant after an overnight fast. Well trained nurses have briefly explained the procedure to each participant before blood was withdrawal. All blood samples were collected using two type of tubes, purple and yellow. Blood collection started early at the morning between 7.30 to 8.30 Am, blood samples then were rested for 30 minutes in a cold room before samples of the yellow tubes separated from cells by centrifugation at 3000 x g for 10 minutes. The plasma was then stored at deep freezer at -20 °C until testing. Purple tubes were left on a blood laboratory mixer, before processing to get glycohaemoglobin A1C (HbA1c) results which were reported as a percentage of total hemoglobin.

Data analysis

Data analysis was carried out using GraphPad prism version 7.04 and Software Excel 2016. Descriptive statistics for each parameter consisted of the standard error (SE), the mean (M) and the standard deviation (SD). The statistical significance level was placed if p value is< 0.05, the relationship between Cav-1 and vaspin, visfatin concentrations in patients with diabetes and their controls were evaluated using Pearson's correlation (r=−1 to 1). Logistic regression model was carried out to validate the relationship between Cav-1 and selected variables, in order to eliminate any possible interruption of other variables.

Results

One-hundred and sixty-four men have participated willingly in the current study. This included eighty-two newly diagnosed men with T2DM; mean age (54.63±6.02 years), and mean BMI (29.49±3.26 kg/m^2) were matched with a similar number of non-diabetic men; mean age (51.54±5,49 years), mean BMI (27.44±3.81 kg/m^2). The current study has shown a significant reduction of serum Cav-1 in patients with T2DM compared with their counterparts in the control group (p< 0.001). Serum vaspin, serum visfatin were both increased significantly (p< 0.001) in patients with T2DM compared with controls (Figs. 1 and 2). In addition, the significant decrease in serum Cav-1 concentrations was associated with a significant increase in serum vaspin (p< 0.001) and serum visfatin (p< 0.001). Compared with controls, fasting serum glucose (FSG) levels and HbA1c were both increased significantly (p< 0.001) in patients with T2DM (Figs. 3 and 4). In addition, fasting serum glucose (FSG) levels were significantly increased among patients with T2DM (p< 0.001) compared with controls.
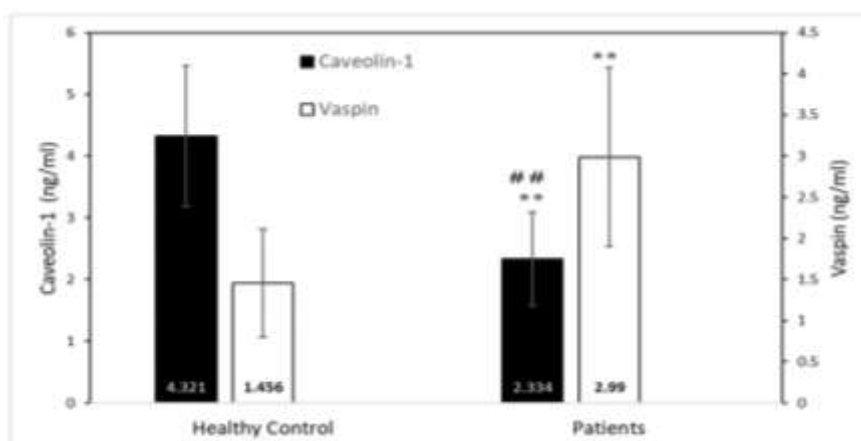
Fig. 1. The relationship between serum Caveolin-1 levels and serum vaspin. Compared with controls, a significant decrease in Caveolin-1 levels (*p <0.001) was correlated with a significant reduction in serum vaspin (**p< 0.001). Significant differences in serum vaspin concentrations have been detected between cases and controls ($^{###}$p< 0.001). Each bar is a mean ±SEM. **p< 0.001 and ##p< 0.001.
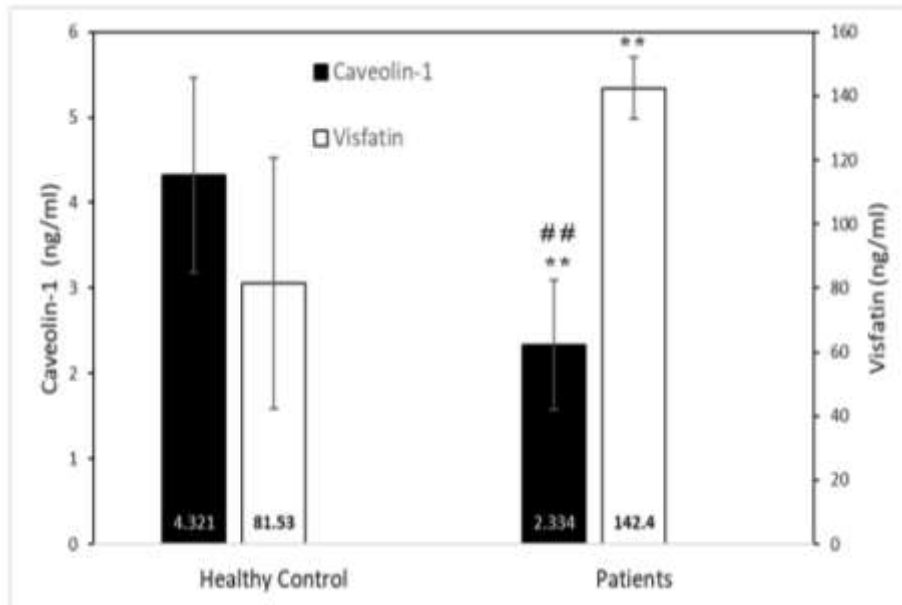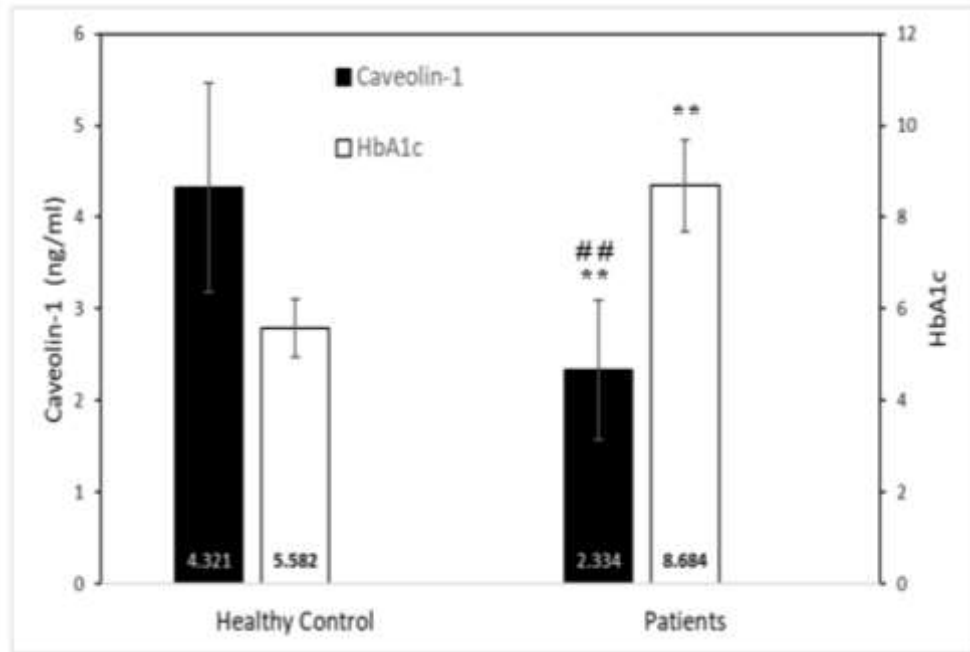
Fig. 2. The relationship between serum Caveolin-1 levels and serum visfatin. Compared with controls, a significant decrease in serum Caveolin-1 levels (**p< 0.001) was correlated with a significant reduction in serum visfatin (**p< 0.001). Significant differences in serum visfatin levels have been detected between cases and controls ($^{##}$p< 0.001). Each bar is a mean ±SEM.
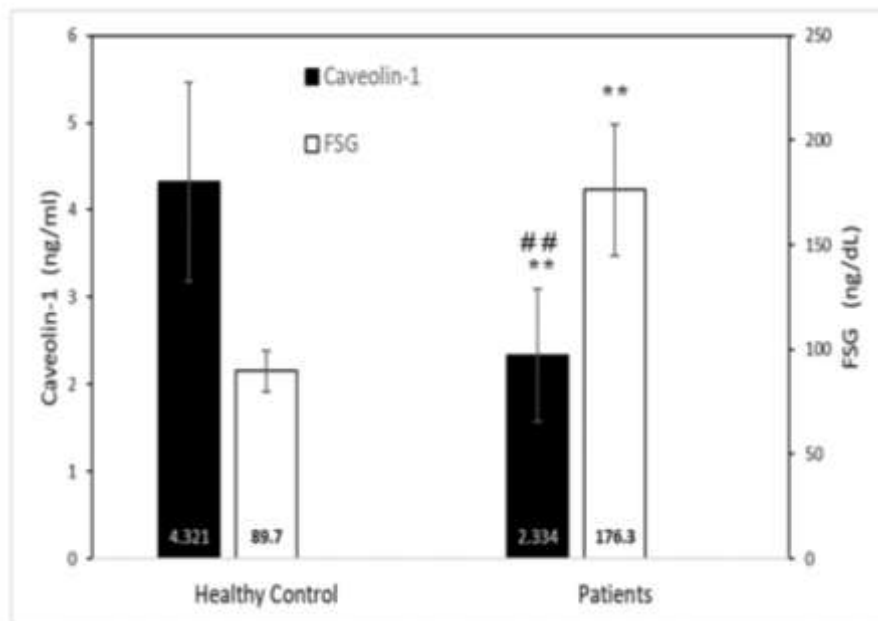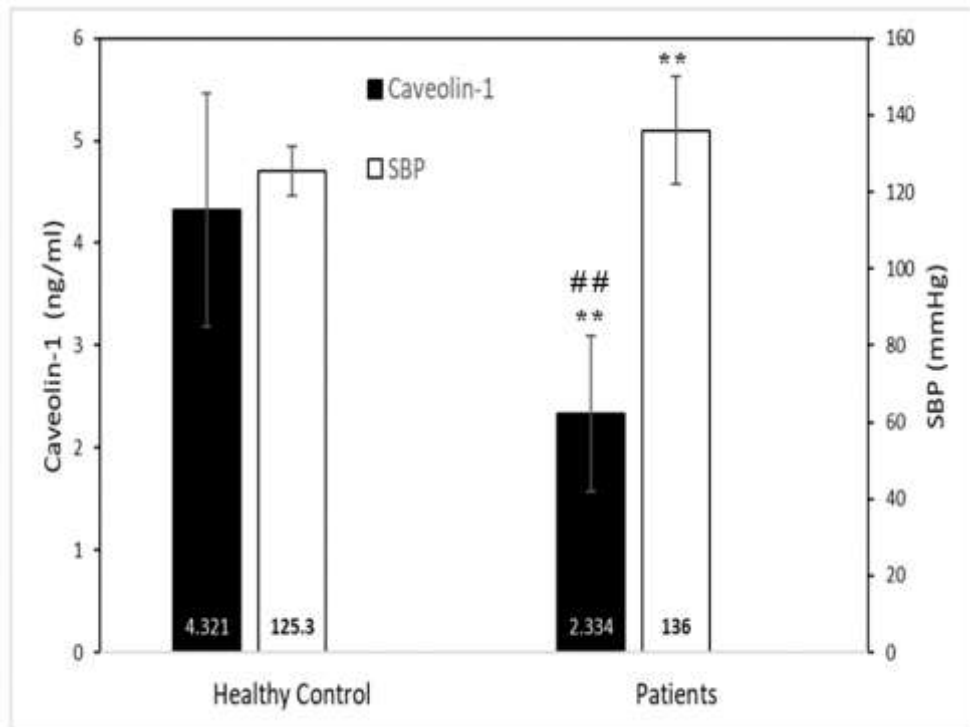

Fig. 3. The relationship between serum Caveolin-1 levels and fasting serum glucose (FSG). Compared with controls, a significant decrease in serum Caveolin-1 levels (**p< 0.001) was correlated with a significant increase in FSG (**p< 0.001). Significant differences in FSG levels have been detected between cases and controls ($^{##}$p <0.001). Each bar is a mean ±SEM.

An inverse significant relationship between serum Cav-1 and FSG were detected (Fig. 3). Expectedly, the significant increase in FSG levels in patients with T2DM have severe impact on HbA1c. Resulting in a significant increase in HbA1c levels (p< 0.001) compared wi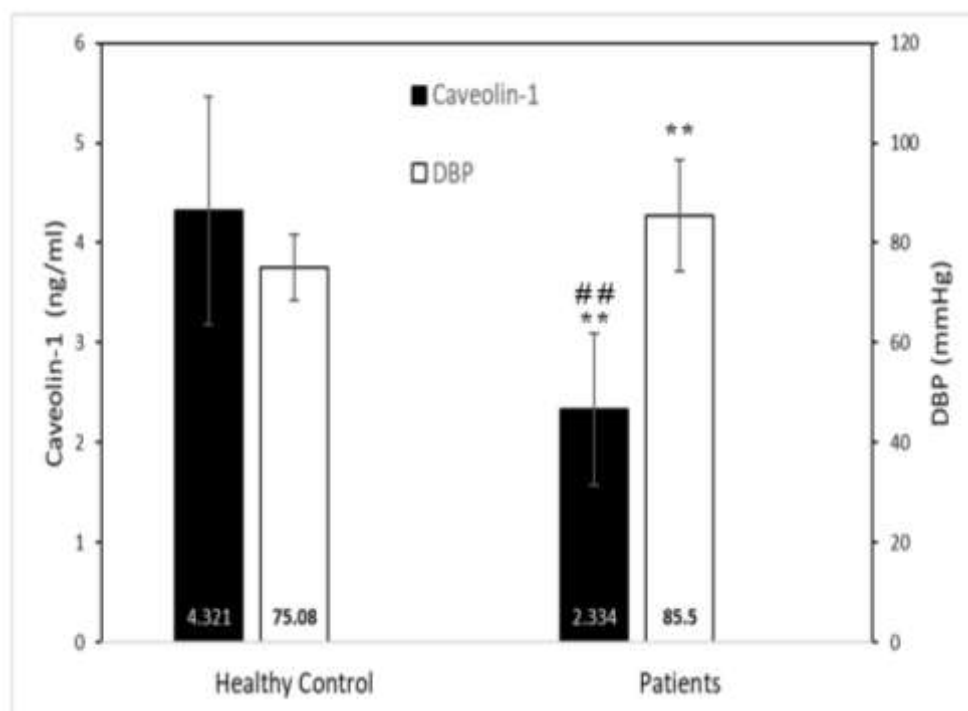th controls. The significant decrease in serum Cav-1 inversely correlated with HbA1c level (p< 0.001) (Fig. 4). Finally, SBP and DBP were both increased significantly in individuals with T2DM compared with controls (p< 0.001). An inverse significant association was detected between serum Cav-1 concentrations and SBP and DBP in patients with T2DM compared with controls (p< 0.001) (Figs. 5 and 6).

Fig. 4. The relationship between serum Caveolin-1 levels and glycohaemoglobin (HbA1c). Compared with controls, a significant decrease in serum Caveolin-1 levels (**p< 0.001) was correlated with a significant increase in HbA1c (**p< 0.001). Significant differences in HbA1c levels have been detected between cases and controls ($^{##}$p< 0.001). Each bar is a mean ±SEM.

Fig. 5. The relationship between serum Caveolin-1 levels and systolic blood pressure (SBP). Compared with controls, a significant decrease in serum Caveolin-1 levels (**p< 0.001) was correlated with a significant increase in SBP (**p< 0.001). Significant differences in SBP have been detected between cases and controls (##p< 0.001). Each bar is a mean ±SEM.

Fig. 6. The relationship between serum Caveolin-1 levels and dystolic blood pressure (DBP). Compared with controls, a significant decrease in serum Caveolin-1 levels (**p< 0.001) was correlated with a significant increase in DBP (**p< 0.001). Significant differences in DBP have been detected between cases and controls (##p< 0.001). Each bar is a mean ±SEM.

Discussion

The sharp increase in the prevalence of obesity and associated health complications including T2DM during the last few decades has represented a major public health challenge worldwide (1). Due to associated health risks, individuals with diabetes have shorter life expectancy compared with general population (2, 20). T2DM represents most diabetes cases and because of the harmless symptoms most patients with T2DM are only diagnosed incidentally (3). Thus, searching for a potential marker to predict T2DM is highly important, because any breakthrough within this area may help in decreasing the incidence rate or delaying disease development. In line with the former, the current study was carried out to evaluate the relationship between serum Cav-1 and serum adipokines vaspin, visfatin, HbA1c and hypertension. Compared with the nondiabetic controls, serum Cav-1 level was significantly decreased among men with T2DM. Furthermore, the significant decrease in serum Cav-1 concentration was associated with significant elevation of serum of vaspin

and visfatin levels. In addition, the significant decrease in serum Cav-1 was inversely associated with FSG and HbA1c values. Regrouping of the patients with T2DM according to their HbA1c levels reveals that individuals with the highest HbA1c values have had the lowest Cav-1 concentrations suggesting possible roles of Cav-1 in the pathogenesis of T2DM.

In newly diagnosed patients with T2DM, blood serum exhibits extremely higher level of insulin which mirrored by higher level of C-peptides compared with patients who already living with T2DM for a longer period (21). This is a compensatory mechanism, where pancreatic β-cells expanded, increases their secretory activities to cover tissues needs of insulin to overcome the vicious condition. Based on these details, we hypothysed that, the significant decrease of serum Cav-1 in patients' with T2DM of the current study is a part of the compensatory mechanism to maintain pancreatic β-cells survival to promote more insulin secretion to overcome insulin resistance.

Furthermore, this compensatory action most probably started before an individual entered the stage of prediabetes or impaired glucose tolerance stage. In another word, T2DM will never develop until pancreatic β-cells fail to cover insulin needs of tissues. A previous study has shown that impairment of serum Cav-1 is associated with a significant decrease in β-cell death in mice (22). In addition, silencing Cav-1 gene promotes the proliferation of pancreatic β-cell, decreases apoptosis, and encourages insulin secretion (23). Thus, decreasing Cav-1 concentrations is an essential action aiming to maintain pancreatic β-cells and expand their activity in order to overcome insulin demand. The current study has provided strong evidence that the significant prolonged decrease of serum Cav-1 concentrations in patients with T2DM is associated with a poor glycaemic control. Although, serum Cav-1 was the focus of many previous studies (14, 16, 18, 21, 22, 23, 24), however, this is the first study targeted Arabs, and the first to link low serum Cav-1 concentrations with the poorer prognosis in patients with T2DM. In addition, the results of the current study suggest that Cav-1 has the potential to be a therapeutic target in patients with T2DM and can be used as effective prognostic marker in the management of T2DM.

In another study on mice, it has shown that cav-1 gene expression was associated with a significant reduction in non-alcoholic-fatty liver disease (NAFLD), while knockdown of Cav-1 gene in these mice has led to aggravation of steatosis. At cellular level, down regulation of Cav-1 increases fat accumulation in L02 and AML12 cells and over expression inhibit cellular fat accumulation in these cells (24). In another word, the compensatory decrease in Cav-1 concentration is associated with alteration in serum lipid profile in parallel with increases insulin resistance. Severe insulin resistance together with poor lipid profile is a direct reflection of metabolic disturbances including non-alcoholic fatty liver and cellular fat accumulation especially as visceral fat (25). The accumulative side effects of such metabolic disturbances may complicate the management of T2DM. Thus, the stability of serum Cav-1 concentrations is crucial, and the prolonged significant reduction of Cav-1 is closely associated with wide-range of chronic diseases such as cardiovascular disease, liver disease, pulmonary disease, and different types of cancers (16, 17, 18).

Although Cav-1 has been established as a marker for some types of cancers and as a target for molecular therapy for some others, however no such progress was reported in T2DM. Over a longer period of poor glycaemic control in patients with T2DM, a further reduction of serum Cav-1 can cause up-regulation of transforming growth factor-β (TGF- β). The up-regulation of TGF-β is associated with a significant decline in β-cell function, while the inhibition of inhibition of TGF- β signaling promotes β-cell replication in human (27). Although the exact mechanism linking TGF-β with β-cell dysfunction is not yet known, however it was suggested that Cav-1 and TGF-β working in two different directions. Thus, knocking down Cav-1 gene and /or inhibit its expression resulting in a significant decrease in β-cell death (22), promotes pancreatic β-cells proliferation and increases insulin secretion under normal physiological glucose levels (23) and may trigger the up-regulation of metabolic diseases related genes (25). Insulin resistance and a defective of insulin receptor were observed in adipose tissue obtained from Cav-1 deficient mice (28). Furthermore, homozygous mutation in human's Cav-1 gene results CGL3, where patients with such mutation have severe insulin resistance, postprandial hyperinsulinemia, hypertriglyceridemia, and lipodystrophy (29).

The association between serum vaspin level and biochemical parameters of obesity and glucose metabolism in adults suggests an effect similar to insulin sensitizing agents (30). It is believed that visceral adipose tissue induces vaspin as a compensatory response in order to attenuate the side effect of severe insulin resistance, obesity and T2DM. Vaspin inhibits cytokines production through the downregulation of Nuclear Factor-Kappa B (NF-kB), thereby increases insulin sensitivity

and act as anti-inflammatory and anti-atherogenic adipokine (31, 32). Thus, in an individual with obesity and/or T2DM, vaspin and visfatin are acting in two different directions, while high visfatin level is associated with poor prognosis (8, 11, 12), high level of vaspin on the other hand is preassembly associated with better prognosis (13, 14, 31, 32), although not all studies agree (33).

Compared with controls, the significant decreased in Cav-1 concentrations in patients with T2DM was associated with significant increase in FSG and HbA1c and this association was in parallel with elevation of serum vaspin and visfatin. In patients with T2DM serum visfatin has been reported to be strongly correlated with HbA1c, although this association may be mediated partially by factors related to hyperglycemia and/or oxidative stress (12). In patients living with T2DM, higher HbA1c and /or FSG values may reflect poor glycaemic control and/or severe deterioration in β-cell function due to longer duration living with diabetes (34, 35). Pancreatic β-cells dysfunction commonly observed among older patients with prolonged T2DM. In older individuals with prolonged T2DM, it becomes difficult to preserve beta-cell functioning, because β-cells dysfunction is increasingly worsening over longer duration of the disease. In addition, other health risks including those associated with T2DM also increase with age progression. As a result, most patients with T2DM ended relying on insulin therapy (36).

HbA1c value does not give an idea about daily glucose spike which is an important in patients with diabetes to keep it low as possible to minimize the risk of organs' damage such as retinopathy, neuropathy and nephropathy. Sustaining HbA1c value of less than 7.6 was found to be enough to avoid all these complications (37). At time of diagnosis, patients with T2DM are usually presented with higher FSG and HbA1c values than those observed in patients with T1DM. This could be due to differences in the pathophysiology of the disease and related symptoms (2). While genetic predisposition and family history play critical roles in the development of T1DM, the development of T2DM is more affected by environmental, lifestyle and socioeconomic related factors with possible interaction with genetics (2, 3).

We have further observed a significant increase in SBP and DBP in patients with diabetes compared with controls. Hypertension is frequently reported in patients with diabetes and is usually associated with history of poor glycaemic control (35). A previous epidemiological study has reported that blood pressure≥ 115/75 mmHg is associated with increased health risks such as CVD, retinopathy, and chronic kidney disease (38). Among patients with T1DM hypertension may reflect the onset of diabetic nephropathy (39), whilst, among patients with T2DM, hypertension is mostly reflecting metabolic syndrome and related contributors such as abdominal obesity, insulin resistance and hyperlipidemia (39). In the current study all the patients were either overweight or obese. A poor glycaemic control is associated with a poorer lipid profile including high total cholesterol, high LDL-cholesterol, decreased HDL/LDL ratio and high triglycerides all of which considered as risk factors for atherosclerosis and CVD (35). The former suggests that the higher prevalence of hypertension in patients with T2DM is most probably occurred due to the diabetes complications rather than due to obesity, although obesity may confound this relationship. Hypertension in the current study was associated with a significant reduction in serum Cav-1. If not treated, hypertension may cause serious health consequences such as stroke, CVD, nephropathy, retinopathy, and neuropathy (35). An adequate glycaemic control and promoting satisfactory lipid profile target are necessary to decrease co-morbidities and increase life expectancy in patients with diabetes. Furthermore, lifestyle modification, physical activity, regular tracking of blood pressure and enhancing antihypertensive medications all may help to reverse the unfavorable effect and improve quality of life (35).

Being the first study that targeted Arabs to evaluate the role of serum Cav-1 in T2DM and its association with vaspin and visfatin levels is amongst the strength of the present study. In addition, for the first time we were able to provide evidence that the decreased level of Cav-1 is associated with poor prognosis in patients with T2DM, suggesting that Cav-1 is a potential as therapeutic target. This study, however, has some drawbacks such as small sample size which was probably a direct impact of the Covid-19 pandemic. In addition, the current study may be prone to selection bias, since we have targeted men only. In Middle Eastern societies, and due to cultural/religious and other unidentified reasons, it is difficult to recruit women in a study, irrespective of the aim of such study (40). The current Covid-19 pandemic made this situation even worse. The crisis is among the major challenges that we and other researchers within the medical field around the world have ever faced. Consequently, encountering some difficulties in recruiting participants or implementing a larger study was expected. Iraq and most countries around the world have been disrupted by the pandemic which is associated with shutting down most of public and private sectors, spreading fear around the globe and promoting population-wide social isolation.

In conclusion, serum Cav-1 level in newly diagnosed men with T2DM was found to be significantly lower than non-diabetic controls. Furthermore, serum Cav-1 has an inverse significant relationship with serum concentrations vaspin and visfatin. The significant reduction of serum Cav-1 was also associated with a significant elevation of plasma FSG and HbA1c. Increased SBP and DBP in diabetics with low concentrations of Cav-1 provided a novel perception into the role of Cav-1 in the development of hypertension related diseases such as atherosclerosis, CVD and CKD. The current study for the first time has provided strong evidence that the significant reduction of serum Cav-1 in patients with T2DM is associated with poor prognosis. As a result, serum Cav-1 concentrations may have the potential to be used as a target therapy in patients T2DM.

Acknowledgements

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