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Clinical study of serum visceral adipose tissue-derived serine protease inhibitor (Vaspin) concentration and some biochemical parameters in Diabetic mellitus type 2 patients

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Abstract

Background: The adipose tissue-derived serine protease inhibitor (vaspin) and biochemical parameters perform many biological activities throughout the human body. Thus, an increase in their level in plasma and blood is directly associated with an increased risk of developing Diabetic mellitus (T2DM). However, the potential association between vaspin and biochemical parameters in patients with Diabetic mellitus (T2DM) is still not well understood and studies in this area are limited. The present study aimed to assessment the relationship between vaspin and biochemical parameters and its association with Diabetic mellitus (T2DM) in the population of Kirkuk, Iraq.

Methods: The current cross-sectional study included a total of 90 participants (53 with Diabetic mellitus (T2DM) and 37 healthy without. Diabetic mellitus (T2DM) and all of them underwent several important routine health examinations. The patients' ages ranged from 18 to 37 years. The samples were collected for the measurement of vaspin and biochemical parameters by biochemical and clinical measurement methods specified in this study. The association between vaspin and biochemical parameters in Diabetic mellitus (T2DM) was evaluated by statistical analysis methods represented by SPSS version 24, and the associations for these variables were found by Pearson's correlation.

Results: The mean value of glucose and vaspin for participants with Diabetic mellitus (T2DM) was significantly higher ($P=0.001$) and ($P=0.01$) compared to the control group respectively. While the mean value of vitamin B12 with Diabetic mellitus (T2DM) was low significantly ($P=0.01$) compared to the control group. Regarding vaspin values and biochemical parameters in Diabetic mellitus (T2DM), found that vaspin was significant positively correlated with blood glucose levels in Diabetic mellitus (T2DM) patients and negatively correlated with vitamin B12. While there was no significant correlation between vaspin and serum creatinine, Troponin and CK-MB in Diabetic mellitus (T2DM) patients.

Conclusions: Elevated levels of blood glucose and vaspin were observed in patients with (T2DM), and it was found decrease levels Vit B₁₂ in patients, that there is a correlation between vaspin and biochemical parameters of (T2DM). This study recommend testing and screening for vaspin and biochemical parameters on a larger scale as it has been shown to be associated with Diabetic mellitus (T2DM).

Keywords: Vaspin, Biochemical parameters, Diabetic mellitus (T2DM)

Introduction

Vaspin, an intriguing adipocytokine, has been shown to possess insulin sensitizing properties [1]. The available research suggests that vaspin may serve as a compensatory mechanism in the progression of obesity and metabolic diseases [2]. The upregulation of vaspin may potentially have a protective benefit against the development of insulin resistance [3]. The administration of central vaspin has been shown to result in a decrease in food consumption and to have prolonged benefits in decreasing blood glucose levels. The results indicate that vaspin may possess an insulin sensitizing impact on the white adipose tissue [4]. There is a prevailing belief that the elevation of vaspin levels might serve as a possible compensatory mechanism to counteract the unidentified proteases that tend to rise in instances of obesity and insulin resistance [5]. The inhibitory impact of vaspin, a member of the serine protease inhibitor family, on kallikrein 7 has been elucidated by Heiker, *et al.* [6].

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Diabetes mellitus (DM) is a prevalent global health issue that poses significant challenges due to its micro vascular and macro vascular consequences, as well as its rising prevalence. The global prevalence of diabetes is expected to impact around 387 million individuals, accounting for 8.3% of the global population [7]. The enzymatic activity of creatine kinase (CK) plays a significant role in the biochemical process of adenosine triphosphate (ATP) synthesis, which is essential for supporting energy-intensive physiological functions in both human and animal cells, particularly in the context of muscular contractions. Both animal and human research have shown associations between creatine kinase (CK) and insulin resistance. Myocytes and adipocytes play significant roles in insulin-mediated processes such as glucose absorption, insulin action, and the development of insulin resistance and type 2 diabetes mellitus (T2DM) [8]. Multiple research have provided evidence indicating a positive correlation between decreased levels of blood creatinine and an increased susceptibility to diabetes. The findings of a prospective cohort research conducted in a representative sample of the general population in China revealed a significant inverse association between blood creatinine concentration and the development of type 2 diabetes mellitus (T2DM). This relationship was seen consistently across both male and female participants [9]. Vitamin B₁₂ functions as a coenzyme within the one-carbon metabolic pathway, which plays a crucial role in the production of methionine, as well as pyrimidine and purine bases. The presence of vitamin B₁₂ deficiencies has been identified as a contributing factor to the development of vascular illnesses, cancer, certain birth malformations and the occurrence of hyperhomocystinemia. These deficiencies are related with DNA damage and impaired repair mechanisms. Vitamin B₁₂ has been recognized as a risk factor for both hypertension and atherosclerosis, often associated with folic acid deficiency [10]. Until far, the majority of research conducted on the relationship between vitamin B₁₂ and diabetes mellitus (DM) has mostly focused on the occurrence of vitamin B₁₂ deficiency among individuals already diagnosed with diabetes. The relationship between the use of metformin and decreased levels of vitamin B₁₂ has been shown by many degrees of evidence [11]. The reason for the significant decrease in blood vitamin B₁₂ levels seen in patients with type 2 diabetes on metformin treatment is likely due to the calcium-dependent nature of vitamin B₁₂ absorption, which is affected by metformin's impact on calcium-dependent membrane activity. Nevertheless, the impact of vitamin B₁₂ on the potential risk of developing diabetes mellitus (DM) in the future remains uncertain, mostly owing to the lack of consistent findings across past research investigations. The findings of a cross-sectional research conducted in a population residing in South India indicated that elevated levels of vitamin B₁₂ were associated with a reduced risk of developing diabetes mellitus [12]. The findings of a research examining the concentrations of high sensitivity Troponin T in diabetic patients have shown a correlation with age, gender, and renal function, whereas no significant link was seen with the presence of coronary artery disease (CAD) [13]. Serum creatinine is mostly a byproduct of creatine metabolism, which is contingent upon the overall skeletal muscle mass and is regularly excreted at a condition of equilibrium. Serum creatinine, although being subject to influences such as age, sex, ethnicity, and dietary protein

intake, remains remarkably constant and is often used as a readily measurable proxy indicator of skeletal muscle mass under conditions of sufficient protein consumption [14]. There exists an association between serum creatinine levels and the onset of Type 2 Diabetes Mellitus (T2DM). The evaluation of the relationship between serum creatinine levels and the incidence of Type 2 Diabetes Mellitus (T2DM) is of interest and use in the early identification of individuals at high risk for T2DM, since testing serum creatinine levels is a more cost-effective and convenient method compared to assessing skeletal muscle mass [15]. The objective of the current research was to assess the levels of vaspin and biochemical markers in individuals with type 2 diabetes mellitus (T2DM) residing in Kirkuk, Iraq.

Materials and Methods

Study design and Participants

The current study is a prospective, cross-sectional, and observational study. The total participants were 145 eligible individuals all of whom underwent medical examinations and of the 145 participants 55 were excluded because their laboratory data were incomplete and they did not meet the inclusion criteria. The study included 90 randomly selected participants who all underwent several important routine health check-ups. This study included 53 patients with confirmed T2DM (28 males and 25 females) and 37 healthy individuals free of diabetes, which is known as the control group. Where the ages of male ranged from 18 to 37 years, and the ages of female ranged from 18 to 37 years. Also, the control group included participants who met all inclusion criteria. The patients' ages ranged from 18 to 37 years. The current study was conducted during the period starting from February 1, 2022 to July 1, 2022, at Hawija general Hospital.

Inclusion and Exclusion Criteria

Inclusion Criteria

Cases were adults within the aforementioned age groups without Diabetic complications.

Exclusion Criteria

Diabetics complications: Heart disease, myocardial infraction, unstable angina, stable angina, heart failure, Diabetic neuropathy, problems with feet, oral health, Diabetic retinopathy, hearing, and Gestational diabetes.

Biochemical and Clinical measurements

Blood samples were collected and drawn from all participants after 15 minutes of rest and a ten-hour fast. Blood samples were kept at 10 °C until centrifugation. All blood samples were stirred and centrifuged at 4000 g for 10 minutes at 28 °C after collection. Plasma was withdrawn and separated from blood samples immediately for vaspin assessment, then stored at -80 °C until vaspin measurement. Plasma vaspin was measured and determined by an enzyme-linked Immunosorbent assay (Elisa, Elabscience Company, USA) technique kit. Plasma concentrations of vaspin were measured and analyzed using (Bio-Tek Instruments, Inc, USA). Biochemical parameters that included blood glucose, creatinine and CK-MB were measured by biometrics. Blood glucose (BG) levels, or blood glucose were assessed by the Trinder reagent test using the god-pap method (Biolabo company, France). Serum creatinine assessed by enzymatic method (Biolabo Company, France). CK-MB assessed by

Immunoinhibition methods (Biolabo Company, France) while troponin concentration assessed by using the One-Step Immunoassay (Nanogen Point of care, Toronto, Ontario, Canada) and Serum vitamin B₁₂ was determined by an enzyme-linked immunosorbent assay (ELISA) (technique Inc. kit, USA) and this analysis was performed using (biotek Instruments, Inc, USA).

Statistical analysis

SPSS version 24 was used to perform the statistical analyses. The value of $p < 0.05$ was taken and determined to indicate statistical significance. Quantitative data are expressed as means \pm standard error (SE), which is used to used also to refer the standard deviation \pm (SD) of the samples. The use of Pearson's correlation was used to find

the correlations between patients' biochemical parameters and vaspin.

Results

In the current study, a total of 90 participants were enrolled. Among them were 53 (58%) participants with T2DM and 37 (41%) without diabetes (control). The ages of the participants with T2DM were recorded and the minimum and maximum ages were 18 and 37 years. Males constituted 28 (53%) of the T2DM participants and females 25 (47%). The mean value of glucose 229.251 mg/dl for participants with T2DM was significantly higher ($P=0.001$) high compared to participants (control) whose mean glucose value was 93.214 mg/dl as shown in Table 1. Figure 1.

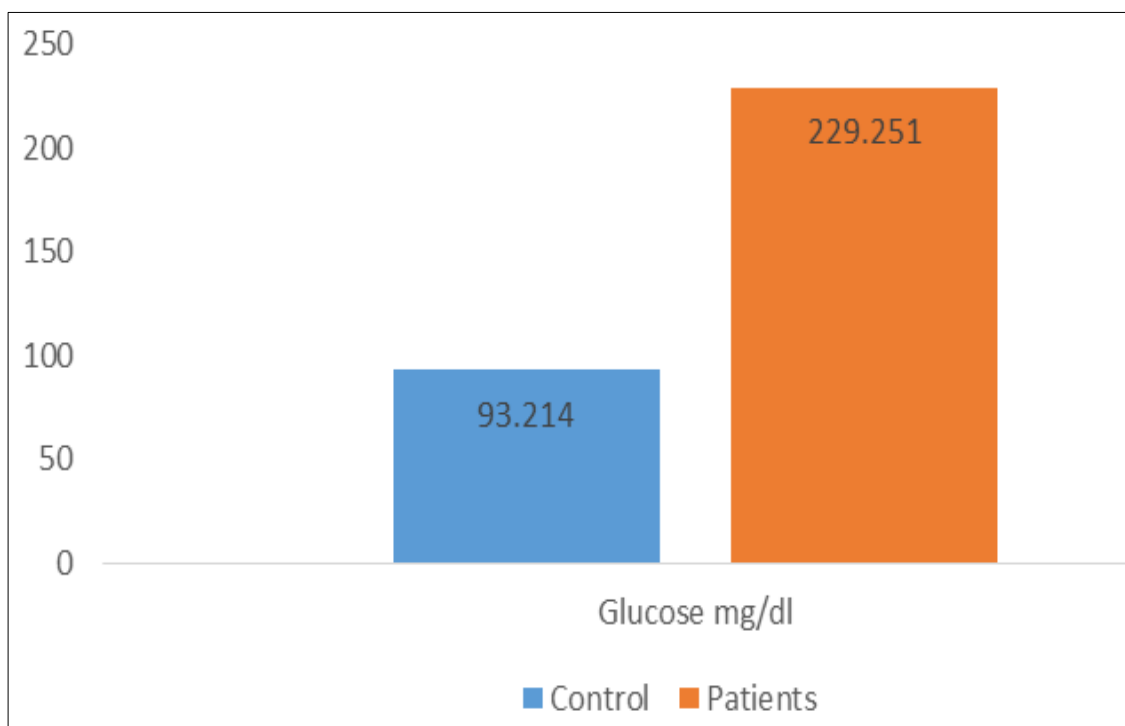


Fig 1: Comparison (means) of Glucose among T2DM patient's participants and healthy participants (control).

Table 1: Comparing between biochemical parameters in T2DM patients and healthy participants (control), (N = 90) in Kirkuk, Iraq, 2022

Parameters	Control Mean \pm SE (N=40)	Patients Mean \pm SE (N=50)	P-Value
Glucose mg/dl	93.214 \pm 1.931	229.251 \pm 0.984**	0.001
Creatinine mg/dl	0.664 \pm 0.071	0.940 \pm 0.180	0.068
Vitamin B ₁₂ pg/ml	697.520 \pm 6.214	173.760 \pm 4.615 **	0.01
Troponin ng/ml	0.0056 \pm 0.014	0.018 \pm 0.021	0.241
CK-MB U/I	19.251 \pm 0.054	26.798 \pm 0.917	0.072

It is also clear from the obtained results that the mean values of creatinine, troponin, and creatine kinase CK-MB were 0.940 mg/dl, 0.088 ng/ml, and 26.798 U/I for participants with T2DM, respectively, while the mean values were 0.664 mg/dl, 0.0056 ng/ml, and 19.251 U/I for the participants

(control), respectively. Moreover, the level of creatinine, troponin, and CK-MB of participants with T2DM and their levels for participants (control) were not significantly correlated ($P=0.068$), ($P=0.241$) and ($P=0.072$), respectively Table 1, Figure 2.

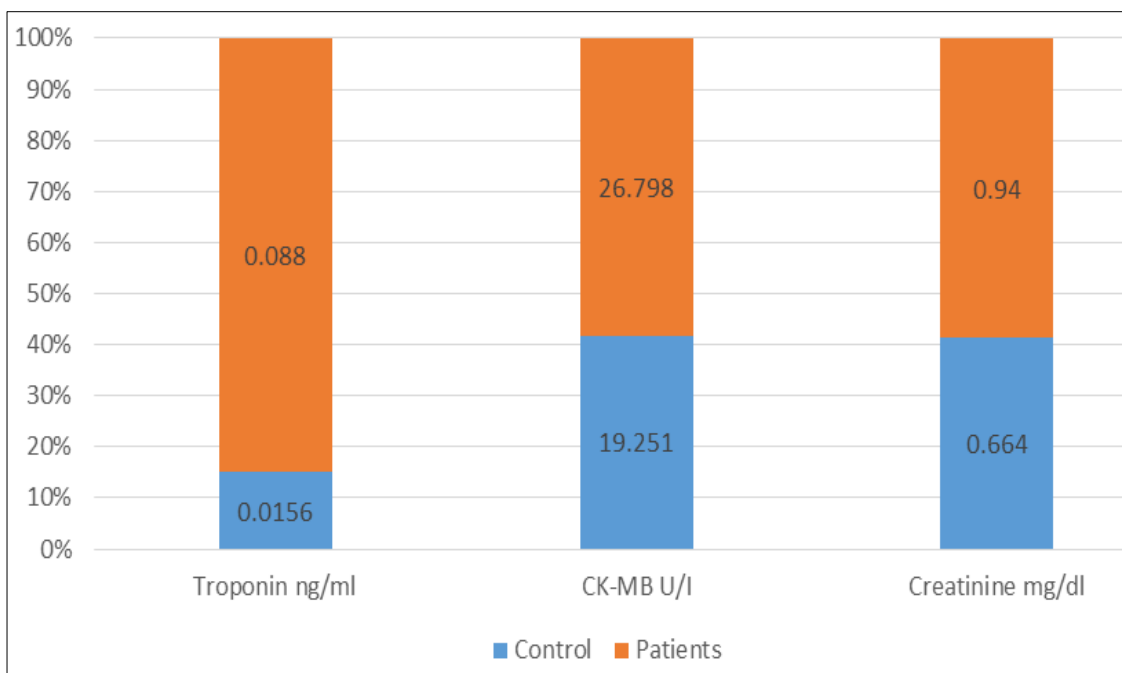


Fig 2: Comparison (means) of Troponin, CK-MB and Creatinine among T2DM patient’s participants and healthy participants (control)

Finally, it was noted through the results that the mean value of vitamin B₁₂ (Vit B₁₂) was 173.760 ng/ml for participants with T2DM and it was 697.520 ng/ml for control group.

Thus the mean values of Vit B₁₂ in participants with T2DM are significantly low compared to the control group (P = 0.01) Table 1, Figure 3.

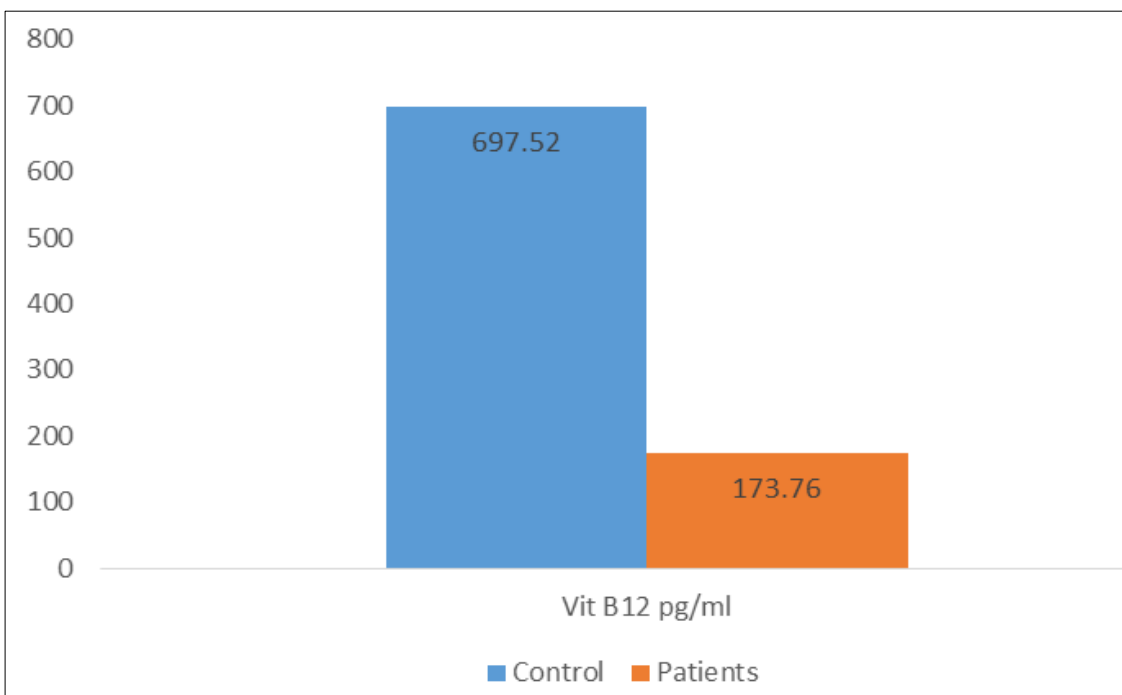


Fig 3: Comparison Vitamin B₁₂ among T2DM patient’s participants and healthy participants (control).

From Table 2 we find that the mean value of vaspin is 773.150 pg/ml for participants with T2DM was significantly high (P = 0.01) compared to participants (control), where the mean value of vaspin was 303.660 pg/ml. Moreover, the results related to vaspin was further illustrated by Figure 2,

which shows the comparison of vaspin between participants with T2DM and healthy participants (control group). It was evident that the vaspin levels of the patients were higher than the control group.

Table 2: Comparing between vaspin levels in T2DM patients and healthy participants (control)

Parameters	Control Mean±SE (N=40)	Patients Mean±SE (N=50)	P-Value
Vaspin pg/ml	303.660±6.842	773.150±7.145**	0.01

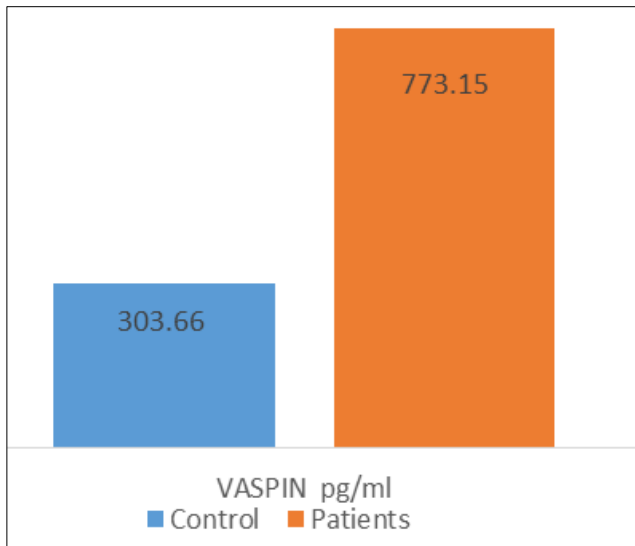


Fig 4: Comparing of vaspin levels between control and patients.

The results showed that the mean values of vaspin were 745.210 pg/ml and 812.280 pg/ml for male and female participants with T2DM, respectively. However, the vaspin level of participants with gender was not significantly associated ($P=0.079$), (Table 3). While Figure 3 shows that the levels of vaspin through the mean values were slightly higher for males with type 2 diabetes than for females with the same disease.

Table 3: Comparing vaspin levels between male and female

Parameters	Male Mean±SE (N=23)	Female Mean±SE (N=27)	P-Value
Vaspin pg/ml	745.210±7.218	812.280±6.175	0.079

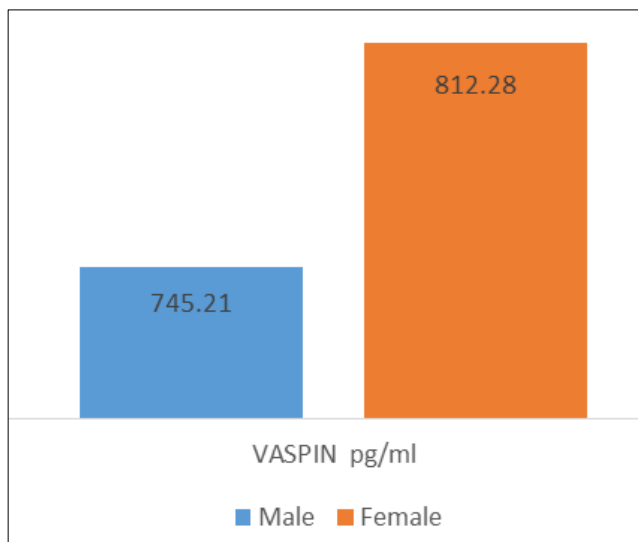


Fig 5: Comparing vaspin levels between male and female.

The results of the correlation between biochemical parameters and vaspin for patients with T2DM are shown in Table 4. There was no significant correlation between vaspin and creatinine, troponin, CK-MB ($p>0.05$) in patients with T2DM, but there was a significant positive correlation between vaspin and glucose ($P=0.05$) in T2DM patients. Also, the results showed that there was a significant negative correlation between vaspin and vitamin B₁₂ ($P=0.05$) in T2DM patients.

Table 4: Correlation between biochemical parameters and vaspin in T2DM patients

Correlation Parameters	Glucose	Creatinine	Vit B12	Troponin	CK-MB
Vaspin	0.937*	0.447	-0.954*	0.214	0.318
P-Value	0.05	0.072	0.05	0.171	0.193

*Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level, Positive number = Direct proportion, and Negative number =inverse proportion

Discussion

In the current study, the blood glucose level was significantly higher in participants with T2DM compared to healthy participants, this because individuals with T2DM had higher levels of plasma glucose concentration [16]. Also, the reason for the higher glucose levels in patients with T2DM than normal is that they have insulin resistance and thus result in the abnormality in plasma glucose balance [17]. Currently, many studies have been conducted that are concerned with the level of creatinine in the blood and its association with T2DM. Through the results of our study, we found that the levels of creatinine in the blood of patients with T2DM were higher compared to healthy participants. This result is consistent with the results of many recent studies that focused on the relationship between creatinine levels in the blood and the risk of developing T2DM, where they found that individuals with T2DM have creatinine levels higher and it was associated with T2DM. The precise mechanism behind the relationship between blood creatinine levels and the likelihood of developing diabetes remains uncertain. Numerous research has shown evidence for a strong correlation between reduced muscle mass and dysglycemia. In a study conducted on Korean individuals aged 65 years or older, it was shown that the incidence rate (IR) was significantly greater in the obese group with lower muscle mass compared to the obese group without low muscle mass [18]. Furthermore, in a cohort research including patients without diabetes, it was shown that hyperinsulinemia, which is a compensatory mechanism aimed at regulating plasma glucose levels within the normal range, was strongly linked to the reduction of skeletal muscle mass throughout the 4.6-year follow-up period [19]. The findings of the study indicated that there was a correlation between an increase in muscle mass and a reduction in insulin resistance, as well as a lower chance of developing diabetes. Additionally, it was seen that the use of nutritional supplements led to an improvement in insulin sensitivity among older individuals with low muscle mass [20]. The involvement of insulin receptors located in the muscle is well recognized as crucial in the control of glucose metabolism. The pathophysiology of type 2 diabetes has been reported to be associated with the deficiency of skeletal muscle insulin resistance (IR), since skeletal muscle is the primary location for insulin-mediated glucose absorption during the postprandial period [21]. The results of the current study revealed that there were significantly elevated levels of the vitamin B₁₂ in participants with T2MD. This occurs in T2MD patients as a result of several reasons that is maybe because documented an increased frequency of vitamin B₁₂ deficiency among type 2 DM (T2DM) patients. Some drug such as Metformin use has been unequivocally demonstrated as the prime factor associated with vitamin B₁₂ deficiency among patients with T2DM [22]. In addition, troponin level Cardiac-specific

troponins (cTn) have received international endorsement as the standard biomarkers for detection of myocardial injury [23]. Moreover, the results in this study indicated that there is a no significant association between elevated levels of troponin and T2DM without chronic coronary artery disease that is agreement with many study showed not associated between the troponin and T2DM without any cardiac disease [24]. Creatine kinase (CK-MB) are among the most important parameter role a biomarker to develop some disease, but at specific levels. In this study appearance no a significant change in level, that is agreement with some study may be because in this study not development the T2DM to another disease [25].

In our study, we aimed to evaluate vaspin and understand the pathophysiological effect of it on individuals with T2DM. An increase in the concentration of vaspin has been associated with several pathological conditions in obese individuals, including insulin resistance, dyslipidemia, oxidative stress, and inflammation [26]. Furthermore, these cases were directly related to patients with T2DM. Thus this association could explain why the concentration of vaspin is increased in patients with T2DM. Our results indicate that the levels of vaspin were significantly higher for those with T2DM than the control group and that there is a close association between high vaspin concentration and T2DM. In view of this, the results we obtained were important and consistent with the results of the study conducted by Pilarski, *et al.* where their results indicated that there is a significantly positive association between vaspin and type 2 diabetes [27]. In addition to that, another study conducted by Yang *et al.* indicated that there is an association between the higher concentration of vaspin and type 2 diabetes, and the association was positive between them, especially in obese patients [28]. Elevated serum vaspin levels in humans are correlated with body mass index (BMI) and insulin resistant, and low serum vaspin concentrations represent a risk factor for the progression of T2DM. A number of studies have confirmed higher serum vaspin concentrations in obese and T2DM patients [29]. Regarding to the gender and its association with vaspin levels in patients with T2DM, the result that showed the relationship of gender (males and females) with type 2 diabetes with different levels of vaspin that there is a difference, but not significantly in the levels of vaspin between males and females, and no significant association between vaspin and gender. There is a agreement with studies that have examined the relationship between these vaspin and gender, but there is one study is found no significant relationship between gender and vaspin [30]. Furthermore, the results of our study regarding the correlation between biochemical parameters and vaspin for patients with T2DM indicated that vaspin was correlated with some parameters and there was no association with the rest. Where we not found correlation between vaspin and creatinine, troponin and CK-MB in patients with T2DM, and we not found previous studies addressing the correlation between vaspin and these biochemical parameters to predict them. Thus, our current study it will be evidence of a lack of association between vaspin levels and serum creatinine, troponin and CK-MB levels of patients with T2DM. Also we found that vaspin levels were significant positively correlated with blood glucose levels in T2DM patients. This is explained by the fact that the high concentration of vaspin in plasma leads to an increase in insulin resistance. Thus this indicates the correlation between vaspin and glucose in

patients with T2DM, in addition to that elevated levels of vaspin also cause variations in glucose metabolism [31]. However, in this study, we evaluated the correlation between vaspin and vitamin B₁₂ in patients with T2DM. These results represent one of the strengths of this study, as when looking at previous and recent studies, this may be due to the fact that the use of metformin is highly associated with vitamin B₁₂ deficiency, and thus the association is inverse between the causes of vitamin B₁₂ deficiency and high vaspin in patients [32].

Conclusion

In conclusion, the results of the current study showed that the levels of blood glucose and vaspin were significantly higher in T2DM patients than in control group participants and significantly associated with T2DM. While the levels of creatinine, troponin and creatine kinase (CK-MB) in T2DM patients were higher but not significant. While vitamin B₁₂ levels were significant lower in T2DM patients compared to control group therefore significantly associated with T2DM. In addition, there was no significant association between gender and vaspin levels in patients with T2DM. Regarding the vaspin and biochemical parameters values in T2DM, we found that vaspin was a significant positively correlated with blood glucose levels and significant negatively correlated with vitamin B₁₂. While there was non-significant positively correlation between vaspin, creatinine, troponin and (CK-MB) in T2DM patients. After the relationship between vaspin and biochemical parameters in T2DM has been evaluated, we recommend that tests and examinations of vaspin and biochemical parameters be conducted more broadly as it has been proven to be associated with type 2 diabetes. This may justify future studies to predict the effect and role of these parameters and vaspin and thus reducing the risk of diabetes.

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