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Circulating CCN6/WISP3 in type 2 diabetes mellitus patients and its correlation with insulin resistance and inflammation: statistical and machine learning analyses

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Abstract

Introduction Cellular Communication Network Factor 6 (CCN6) is an adipokine whose production undergoes significant alterations in metabolic disorders. Given the well-established link between obesity-induced adipokine dysfunction and the development of insulin resistance and type 2 diabetes mellitus (T2DM), this study investigates the potential role of CCN6 as a biomarker for T2DM. The present study aimed to investigate the association between serum CCN6 levels and T2DM, as well as its risk factors, for the first time.

Methods In this case-control study, a total of 80 individuals diagnosed with T2DM and 80 healthy control individuals, who referred to Shariati hospital (Tehran, Iran), were included in the study. Biochemical parameters including fasting blood glucose (FBG), aspartate transaminase (AST), alanine transaminase (ALT), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined using the AutoAnalyzer instrument. The circulating levels of CCN6, adiponectin, Tumor necrosis factor-α (TNF)-α, Interleukin 6 (IL-6), and insulin were quantified using ELISA. The Student t-test was applied to data that presented as mean ± standard deviations (SD). Moreover, the Gini Index was utilized to determine the weight of each factor in T2DM classification. Additionally, various machine learning models were employed to develop classifiers for predicting T2DM.

Results T2DM patients demonstrated significantly lower levels of CCN6 (1259.76 ± 395.02 pg/ml) compared to controls (1979.17 ± 471.99 pg/ml, $P < 0.001$), as well as lower levels of adiponectin ($P < 0.001$) and higher levels of TNF-α and IL-6 ($P < 0.001$) compared to non-T2DM individuals. In the T2DM group, CCN6 exhibited negative correlations with insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), body mass index (BMI), IL-6, and TNF-α. Logistic regression analysis indicated an increased risk of T2DM, with a CCN6 cutoff value of 1527.95 pg/mL distinguishing T2DM patients with 86.3% sensitivity and 73.8% specificity. The Gini Index highlighted that HOMA-IR, IL6, and CCN6 had the highest weighting on T2DM.

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Conclusion Our research identified a significant and negative association between serum CCN6 levels and the likelihood of T2DM, as well as inflammation biomarkers (IL-6 and TNF-α). CCN6 shows promise as a potential biomarker for T2DM; however, further investigations are necessary to validate this finding and assess its clinical utility.

Keywords WISP3/CCN6, Type 2 diabetes mellitus, Inflammatory cytokines, Insulin resistance, Adiponectin

Introduction

Diabetes Mellitus is a medical condition characterized by challenges in the body's efficient storage and utilization of glucose [1]. The most prevalent form of this condition is type 2 diabetes mellitus (T2DM), characterized by persistent elevation of blood glucose and insulin levels. This elevation is primarily triggered by insulin resistance and impaired insulin function in vital tissues such as the liver, adipose tissue, and skeletal muscle [1, 2]. In our current era, obesity represents a substantial threat to human well-being [1] this has resulted in a surge of obesity-related diseases, including insulin resistance and T2DM. Diet stands out as one of the significant factors influencing the onset and progression of these conditions [1, 3].

Obesity carries significant health implications, triggering inflammation and insulin resistance, ultimately culminating in diabetes. Furthermore, insulin resistance can be associated with various factors such as oxidative stress, endoplasmic reticulum stress, hypoxia, aging, and lipodystrophy, all contributing to the development of T2DM [4, 5]. In cases of obesity, M1 macrophages in adipose tissue release numerous pro-inflammatory cytokines, disrupting insulin signaling and promoting insulin resistance [1]. Moreover, other immune cells in adipose tissue influence inflammation and insulin resistance, including mast cells, dendritic cells, neutrophils, and B and T lymphocytes. Beyond fat storage, adipose tissue also plays a crucial role in releasing adipokines, a group of secretory cytokines [6]. These adipokines play a crucial role in the pathogenesis, prognosis, diagnosis, and treatment of metabolic disorders triggered by obesity [3, 7, 8]. Due to the association of obesity with T2DM, the expression of a group of adipokines is increased and reduced in the induction of insulin resistance associated with obesity and T2DM [9].

Adipokines are crucial in regulating carbohydrate and lipid metabolism, maintaining body energy balance, promoting homeostasis, and modulating inflammation. Notably, specific adipokines and cytokines, such as vaspin, resistin, apelin, ghrelin, omentin, TNF-α, and interleukin-6 (IL-6), are implicated in the development of insulin resistance associated with obesity and T2DM [9]. Another significant adipocytokine in this context is cellular communication network factor 6 (CCN6/WISP3), which belongs to the CCN matrix protein family and is synthesized and secreted from adipose tissue [10].

WISP3 is one of the six members of the CCN protein family, and it holds significant importance in the mitochondrial electron transport system and tumor suppression, particularly in certain metastatic cancers [10, 11]. Significantly, earlier research has shown that CCN6 acts as a tumor suppressor and inhibits the action of insulin-like growth factor 1 (IGF-1) when breast cancer advances. Epithelial cells could become invasive and migrate if the function of this adipokine is reduced because it may alter the IGF-1 growth factor receptor [12, 13]. Additionally, a 2022 study found that in NASH (Non-Alcoholic Steatohepatitis) mice, elevated CCN6 expression decreased fibrosis, hepatic steatosis, and the inflammatory response [14]. However, there is currently little knowledge of the function of CCN6 in T2DM patients. Further investigation is required to comprehend its potential significance in the context of T2DM patients.

Furthermore, machine learning techniques have demonstrated promising outcomes in identifying biomarkers for various diseases, including coronary artery disease, breast cancer [15], and Alzheimer's disease [16]. In metabolic diseases such as T2DM, machine learning has emerged as a powerful method for complex data analysis and pattern recognition regarding disease progression, insulin resistance, and obesity-related complications. Machine learning algorithms, for example, have been applied to predict T2DM risk from clinical, biochemical, and genetic variables with the prospect of personalized medicine approaches [17–19]. These techniques permit the integration of diverse variables, such as adipokine levels, inflammatory markers, and anthropometric parameters, to improve diagnostic sensitivity and identify novel biomarkers. Machine learning techniques are employed in the current research to assess the utility of CCN6 as a biomarker in diagnosing patients with T2DM.

Through the application of sophisticated algorithms, we aim to contrast the diagnostic potential of CCN6 and its relationship with other conditions such as inflammation, insulin resistance, and obesity. This type of analysis not only gives greater insight into CCN6 in T2DM but also offers an understanding of how machine learning assists in the speeding up of biomarker discovery for metabolic disorders. Considering the association between CCN6/WISP3 serum levels and obesity, inflammation, insulin resistance, and other physiological processes that could influence T2DM development, there is potential for CCN6 to be used as a biomarker for the early diagnosis and treatment of T2DM. As far as we know, the relationship between CCN6 serum levels and type 2 diabetes has not been addressed in any previous research.

Early detection of T2DM is crucial in order to intervene early and prevent complications such as cardiovascular disease, neuropathy, and nephropathy. Current tests for diagnosis, including HbA1c and fasting plasma glucose, typically are not successful at identifying those individuals who are pre-diabetic or at high risk of T2DM. New biomarkers, including CCN6, can enhance early diagnosis and enable tailored therapeutic strategies to ultimately benefit patient outcomes.

Hence, the current study will be the first to investigate the serum levels of CCN6 in T2DM patients and explore its potential correlation with TNF-α, IL-6, and other biochemical parameters.

Methods

Participants

The current study comprised 80 patients in the T2DM group (52 males and 28 females) and 80 individuals in the control group (53 males and 27 females), aged between 45 and 75 years, who referred to Shariati hospital (Tehran, Iran) [20]. The T2DM group was diagnosed according to the American Diabetes Association (ADA) compliant clinical tests [21]. The medical histories of all individuals were checked for the absence of renal diseases, autoimmune disorders, chronic inflammations, cancer, steroid medication, a family history of thyrotoxicosis, immune system suppression, and anti-inflammatory drugs in the past six months, as well as individuals who had smoked or used tobacco products during the previous three months, were not allowed to participate in the study. Comprehensive medical records of both participant groups were recorded. The current study was conducted with adherence to ethical principles and received approval (IR.TUMS.SPH.REC.1401.280) from the Ethics Committee of the University of Medical Sciences, with written consent obtained from all participants. Ethical guidelines in accordance with the Helsinki Declaration were followed.

Assessment of anthropometric parameters

The Body Mass Index (BMI) of the participants was calculated using the standard formula of weight (kg)/height (m^2). Furthermore, a standard aneroid sphygmomanometer was used to monitor their resting systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Assessment of biochemical parameters

A volume of 7 ml of fasting venous blood was collected from each participant in both groups. Subsequently, the serum levels of fasting blood glucose (FBG), aspartate transaminase (AST), alanine transaminase (ALT), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were examined using an autoanalyzer and commercially available test kits. The serum insulin concentration in both groups was assessed using the enzyme-linked immunosorbent assay (ELISA) method and the commercial Monobind kit. Additionally, the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index in this study was determined using the standard formula: [FBG (mg/dl)] × [fasting blood insulin (μU/mL)/405].

Assessment of cytokines and adipokines

The serum concentrations of CCN6, TNF-α, IL-6, and adiponectin were measured using the ELISA method. The CCN6 kit (KEH04333, Aviva system biology; USA) had intra-assay and inter-assay coefficients of variation (CV) of 8% and 10%, respectively, and a minimum detectable concentration of less than 50 pg/mL. The levels of adiponectin were determined using the Adipogen kit (South Korea) with intra-assay and inter-assay CV values of 3.4% and 4.3%, respectively, and a minimum detectable range of 0.1 nanograms per milliliter.

The R&D Systems kit, which has a minimum detectable range of 5.5 pg/mL for TNF-α and 0.11 pg/mL for IL-6, was used to measure the serum levels of these two chemicals. The intra-assay and inter-assay CV values for TNF-α were 7.4% and 5.2%, respectively, while for IL-6, they were 9.6% and 6.9%, respectively.

Statistical analysis

All statistical analyses were performed using SPSS version 27, and a significance level of $p < 0.05$ was considered statistically significant. Categorized data were tested using the chi-square test and presented in terms of frequency and percentage. The evaluation of data normality was carried out by employing the Kolmogorov-Smirnov test. Following this assessment, the Student t-test was applied to data that demonstrated a normal distribution, and the resulting results were presented as mean values alongside their corresponding standard deviations (SD). In cases where the data did not exhibit a normal distribution, the U Mann-Whitney test was employed, and the results were reported as median values along with their respective interquartile ranges (IQR). Pearson correlation analysis was utilized to evaluate the relationship between CCN6 and continuous variables, while binary logistic regression was employed to investigate the connection between CCN6 and the probability of developing T2DM. To assess the diagnostic performance of the CCN6 ELISA test in distinguishing between the patient and control groups, the ROC curve was utilized. Moreover, the cutoff value, sensitivity, and specificity of the CCN6 ELISA test were determined based on the ROC curve.

Machine learning modeling and evaluation

RapidMiner 10.1 was used for the modeling and assessment procedure [22]. To determine the significance of

Table 1 Basic anthropometric, biochemical and immunological characteristics of 80 control people and 80 T2DM patients. The student t-test was applied to data that presented as mean ± standard deviations (SD)

variables	Non-T2DM		T2DM		*P*-value
	Mean ± SD		Mean ± SD		
Age (Year)	55.71	7.06	57.48	7.85	0.13
BMI (kg/m^2)	25.73	3.87	25.91	4.08	0.77
SBP (mmHg)	128.56	15.90	129.40	17.49	0.75
DBP (mmHg)	79.80	13.42	80.85	13.10	0.62
FBG (mg/dl)	90.91	10.95	155.41	22.92	< 0.001
Insulin (μU/ml)	4.77	3.13	10.52	4.77	< 0.001
Adiponectin (μg/ml)	13.56	3.52	9.12	2.74	< 0.001
TNF-alpha (pg/ml)	19.34	7.14	28.69	7.62	< 0.001
IL-6 (pg/ml)	4.76	1.95	9.78	3.32	< 0.001
HOMA-IR	1.08	0.72	4.11	2.13	< 0.001
Creatinine (mg/dl)	1.13	0.16	1.14	0.12	0.51
AST (U/l)	18.47	5.66	17.63	5.54	0.34
ALT (U/l)	19.18	8.41	18.14	7.39	0.40
TG (mg/dl)	125.30	50.18	162.10	54.83	< 0.001
TC (mg/dl)	169.42	45.93	191.49	42.91	0.002
LDL-C (mg/dl)	101.14	33.20	117.66	34.03	0.002
HDL-C (mg/dl)	45.28	8.17	42.12	5.50	0.005
CCN6 (pg/ml)	1979.17	471.99	1259.76	395.02	< 0.001

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, IL-6: Interleukin-6, TNF-alpha: Tumor necrosis factor α, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, AST: Aspartate transaminase, ALT: Alanine transaminase, TG: Triglycerides, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, CCN6: Cellular communication network factor 6

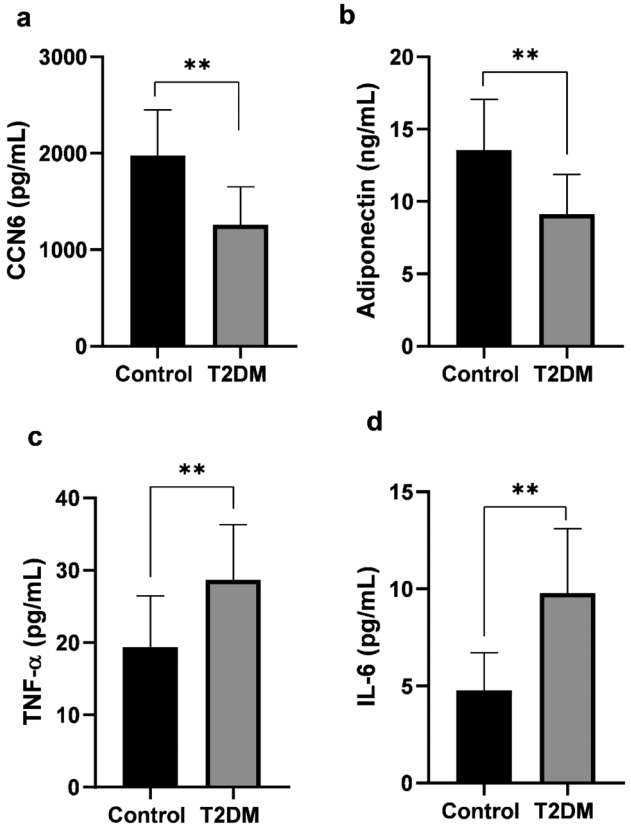

Fig. 1 **a**, **b** Serum levels of CCN6 and adiponectin were lower in the T2DM group than the control group, respectively. **c**, **d** Serum levels of TNF-α and IL-6 were higher in T2DM group than the control group, respectively. Note: *<0.05, **<0.01.

each factor about T2DM, we employed the Gini Index [23]. Following that, we utilized five different machine learning algorithms: Naïve Bayes [24], Decision Tree [25], Gradient Boosted Trees [26], K-Nearest Neighbor (KNN) [27], Random Forest [26], and, to develop predictive models for T2DM. The performance of these models was assessed using various metrics such as accuracy, area under the curve (AUC), f-measure, kappa, sensitivity, and specificity [28]. To ensure the reliability of our results, we implemented a 10-fold cross-validation technique [29].

Results

Anthropometric parameters

The data presented in Table 1 reveals that there were no statistically noteworthy differences observed in BMI ($P = 0.77$), SBP ($P = 0.75$), or DBP ($P = 0.62$) when comparing the control group with the T2DM group. However, it's worth noting that the mean age of the T2DM group shows a marginal elevation compared to the control group ($P = 0.13$).

Biochemical parameters

In contrast to the control group, the T2DM group showed considerably higher levels of FBG, HOMA-IR, and insulin ($P < 0.001$), as shown in Table 1. Additionally, TG, TC, and LDL-C values were higher in the T2DM group than in the control group ($P < 0.001$, $P = 0.002$, and $P = 0.002$, respectively). On the other hand, it was discovered that the patient group's serum HDL-C concentration was lower than the control group's ($P = 0.005$).

Cytokines and adipokines

There was a considerable reduction in the average serum levels of CCN6 among individuals with T2DM, declining from 1979.17 ± 471.99 pg/ml to 1259.76 ± 395.02 pg/ml compared to the control group ($P < 0.001$). As well as, the serum concentration of adiponectin decreased from 13.56 ± 3.52 μg/ml to 9.12 ± 2.74 μg/ml ($P < 0.001$) (Fig. 1 (a, b) and Table 1). Moreover, the serum levels of inflammatory cytokines IL-6 and TNF-α were notably elevated in the T2DM group compared to the control group ($P < 0.001$ for both). These findings are graphically represented in Fig. 1 (c, d) and detailed in Table 1.

Correlation of CCN6 serum level with clinical parameters

Within both the non-T2DM and T2DM groups, a negative notable correlation was identified between serum

CCN6 levels and BMI [(r = -0.298, P = 0.007) and (r = -0.251, P = 0.025), respectively], as showed in Tables 2 and 3.

As demonstrated in Tables 2 and 3, a clear negative and inverse relationship was evident between CCN6 levels and insulin within both the non-T2DM and T2DM groups, as indicated by the correlation coefficients [(r = -0.231, P = 0.040) and (r = -0.334, P = 0.002), respectively]. Additionally, in control and patient groups a similar negative and significant correlation was observed between CCN6 and HOMA-IR [(r = -0.240, P = 0.032) and (r = -0.349, P = 0.002), respectively].

In the T2DM group, a negative and robust correlation was identified between CCN6 and TNF-α (r = -0.257, P = 0.022) and IL-6 (r = -0.403, P < 0.001), as detailed in Table 3. These findings highlight the potential role of CCN6 in the onset of type 2 diabetes by suggesting that the relationships between CCN6 and several variables varied between the control and T2DM groups.

Association of CCN6 serum level with the risk of T2DM

The effect of a 100-unit change in serum CCN6 concentration on the risk of acquiring type 2 diabetes was assessed using a binary logistic regression analysis. The findings consistently indicated a significant relationship between both the unadjusted model (OR [95% CI] = 0.67 [0.60–0.76]) and the adjusted models, which accounted for factors like age, gender, and BMI (OR [95% CI] = 0.62 [0.54–0.72]) as demonstrated in Table 4.

Figure 2 displays the ROC curve analysis, revealing a CCN6 threshold value of 1527.95 pg/mL for discriminating T2DM patients from the control group. The sensitivity (86.3%) and specificity (73.8%) of the study were excellent. At a 95% confidence level, the computed area under the curve was 0.88 (0.83–0.93, p < 0.001). These results suggest that CCN6 shows potential as a prospective diagnostic indicator for T2DM, demonstrating favorable precision in distinguishing between T2DM patients and individuals without the condition.

Machine learning modeling and evaluation

Figure 3 illustrates the relative weight of factors associated with T2DM. Among these factors, HOMAIR, IL6, and CCN6 exhibit the highest weighting on T2DM. Also, Fig. 4 shows the Decision Tree Model.

The performance comparison of the models is depicted in Table 5. The Random Forest model demonstrates the highest performance across multiple metrics, making it the best model for T2DM classification in this comparison. The Random Forest model has the highest overall correctness in predicting cases of type 2 diabetes, with an accuracy of 92.50% +/- 3.95%. It also demonstrates a high degree of agreement between its predicted classifications and the actual T2DM labels, with a kappa value

Table 2 The correlation analysis of various variables with CCN6 in the control group. Pearson correlation analysis was utilized to evaluate the relationship between CCN6 and continuous variables

Variables	Pearson correlation (r)	P-value
Age	-0.05	0.62
BMI	-0.29**	0.007
SBP	-0.20	0.07
DBP	-0.12	0.26
Creatinine	-0.06	0.56
AST	-0.19	0.08
ALT	-0.09	0.40
TG	-0.01	0.86
TC	-0.06	0.55
LDL-C	0.01	0.86
HDL-C	0.05	0.65
FBG	-0.12	0.26
Insulin	-0.23*	0.04
HOMA-IR	-0.24*	0.03
Adiponectin	0.10	0.37
TNF-alpha	-0.17	0.12
IL-6	-0.21	0.06

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, IL-6: Interleukin-6, TNF-alpha: Tumor necrosis factor α, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, AST: Aspartate transaminase, ALT: Alanine transaminase, TG: Triglycerides, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol. Note: *<0.05, **<0.01.

Table 3 The correlation analysis of various variables with CCN6 in the T2DM group. Pearson correlation analysis was utilized to evaluate the relationship between CCN6 and continuous variables

Variables	Pearson correlation (r)	P-value
Age	0.12	0.26
BMI	-0.25*	0.02
SBP	0.09	0.41
DBP	0.001	0.99
Creatinine	-0.10	0.34
AST	0.02	0.79
ALT	-0.01	0.91
TG	-0.15	0.17
TC	-0.12	0.27
LDL-C	-0.16	0.14
HDL-C	0.01	0.91
FBG	-0.17	0.11
Insulin	-0.33**	0.002
HOMA-IR	-0.34**	0.002
Adiponectin	0.18	0.10
TNF-alpha	-0.25*	0.02
IL-6	-0.40**	< 0.001

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, AST: Aspartate transaminase, ALT: Alanine transaminase, TG: Triglycerides, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, IL-6: Interleukin-6, TNF-alpha: Tumor necrosis factor α. Note: *<0.05, **<0.01.

Table 4 Binary logistic regression for odd ratio of T2DM status according to 100-unit change in CCN6

Model	B	S.E.	Wald	df	*P.*	Odd ratio (B)	95% CI.for odd ratio (B)	
							Lower	Upper
Crude model	-0.39	0.06	41.59	1	< 0.001	0.67	0.60	0.76
Adjusted*	-0.46	0.07	40.38	1	< 0.001	0.62	0.54	0.72

* Adjusted for age, sex and BMI

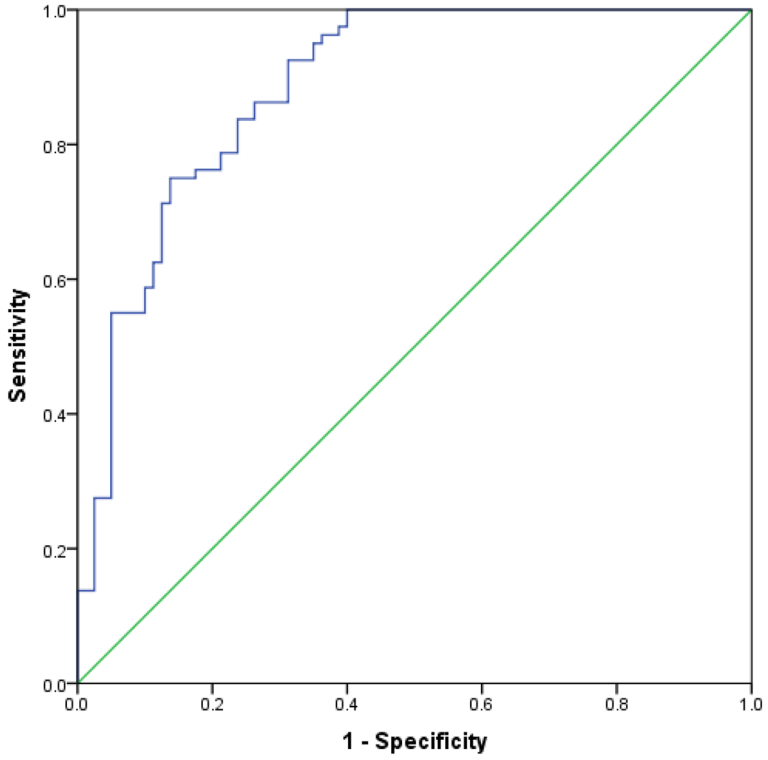

Fig. 2 ROC curve for diagnosis of T2DM according to CCN6 serum levels. A cut-off value of CCN6 (1527.95 pg/mL) was identified to distinguish between T2DM patients and the control group, demonstrating good sensitivity and specificity (86.3% and 73.8%, respectively). The area under the curve was calculated as 0.88 [0.83–0.93 (95% CI), $p < 0.001$]

of 0.850 +/- 0.079 when accounting for the likelihood of agreement by chance. This implies that it successfully differentiates between positive (T2DM) and negative cases and that its predictions are highly reliable. The Random Forest model also demonstrates excellent performance in correctly identifying positive instances (T2DM) while minimizing false positives and false negatives. Its F-Measure of 92.39% +/- 4.27% shows that precision and recall are well balanced. The Random Forest model exhibits remarkable performance in terms of sensitivity and specificity.It exhibits a sensitivity of 92.50% +/- 8.74%, reflecting its ability to accurately identify T2DM cases. Simultaneously, it achieves a specificity of 92.50% +/- 6.45%, indicating its capability to correctly identify non-T2DM cases.

The Random Forest model performs well on several criteria, such as sensitivity, specificity, accuracy, kappa, AUC, and F-Measure. Its strong performance places it at the top of this comparison of T2DM classifications, indicating that it can be used to forecast T2DM instances with high accuracy and dependability.

Discussion

It has been established that adipokines, like hepatokines and myokines [30–32], play critical roles in the regulation of metabolic parameters [3, 7]. Accordingly, the objective of our study is to investigate, for the first time, the association between serum levels of CCN6 and T2DM disease. Previous studies have explored the relationship of CCN6 with breast cancer, skeletal disorders like rheumatoid arthritis and pulmonary fibrosis [33–35]. Additionally, the association of other members of this family, such as CCN1, CCN2, and CCN3, has been studied in conditions like obesity, insulin resistance, and diabetic wound healing [36–40]. Nevertheless, the relationship between serum CCN6 levels and type 2 diabetes has not yet been investigated. Consequently, our research offers a fresh look at serum CCN6 levels in T2DM patients and

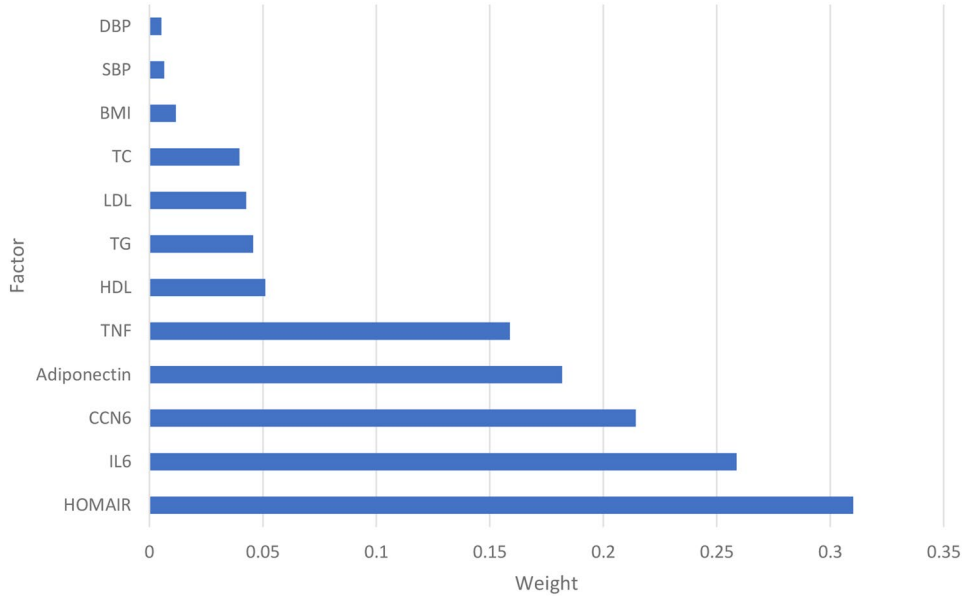

Fig. 3 Factors weight on T2DM calculated by gini index

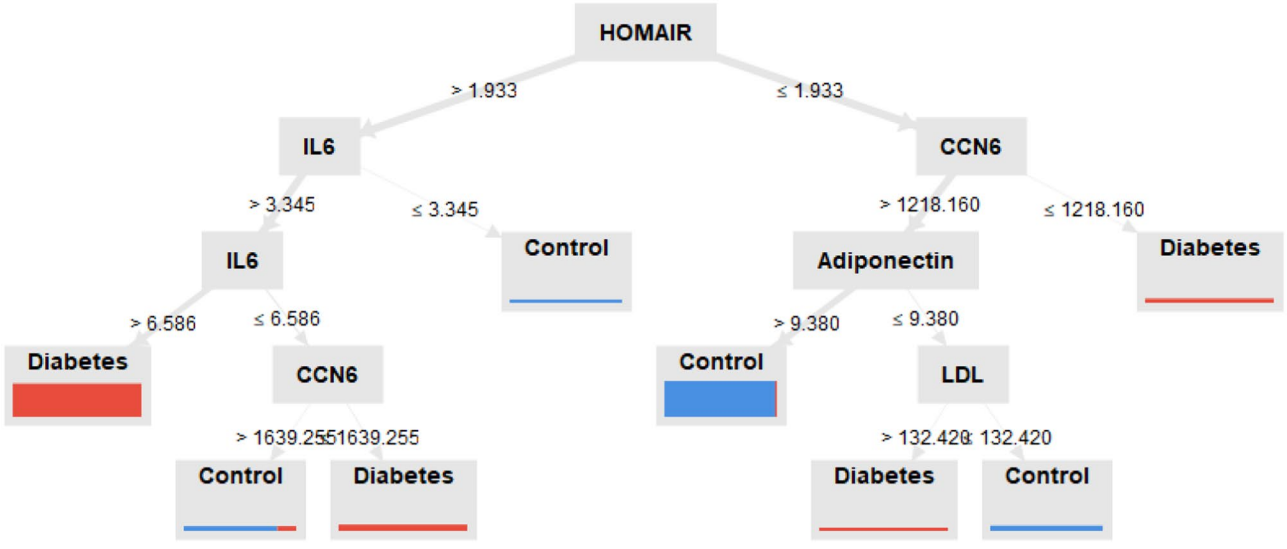

Fig. 4 Decision tree model for classifying T2DM cases

Table 5 The T2DM classification models performance comparison

Model	Accuracy	Kappa	AUC	F-Measure	Sensitivity	Specificity
Decision Tree	89.38% +/- 5.93%	0.787 +/- 0.119	0.852 +/- 0.155	88.51% +/- 6.83%	83.75% +/- 10.29%	95.00% +/- 6.45%
Gradient Boosted Trees	88.75% +/- 5.74%	0.775 +/- 0.115	0.953 +/- 0.050	88.46% +/- 6.17%	87.50% +/- 10.21%	90.00% +/- 7.91%
Random Forest	**92.50% +/- 3.95%**	**0.850 +/- 0.079**	**0.989 +/- 0.017**	**92.39% +/- 4.27%**	**92.50% +/- 8.74%**	**92.50% +/- 6.45%**
Naïve Bayes	90.00% +/- 6.04%	0.800 +/- 0.121	0.967 +/- 0.032	89.69% +/- 6.44%	88.75% +/- 10.94%	91.25% +/- 8.44%
KNN	81.88% +/- 12.31%	0.637 +/- 0.246	0.874 +/- 0.129	80.89% +/- 12.07%	76.25% +/- 12.43%	87.50% +/- 15.59%

explores how they relate to TNF-α, IL-6, and other biochemical markers.

Our study revealed that, in contrast with the control group, those with type 2 diabetes had significantly lower serum levels of CCN6. This discovery suggests a potential link between lower CCN6 levels and the onset of T2DM. Notably, the established cutoff value has proven effective, with sensitivity and specificity exceeding 70%, in accurately distinguishing T2DM patients from the control group. These results are consistent with previous studies

that have emphasized the role of CCN6 in metabolic disorders.

For instance, research findings have indicated that WISP1/CCN4 may play a role in the development of diabetes and obesity. WISP1 has thus been established as a viable clinical marker for the assessment of metabolic diseases and diabetes as a result of these findings [41]. As well as, in an animal-based investigation, it was demonstrated that the expression levels of CCN1 and CCN2 exhibited a substantial increase within the retinal tissues of diabetic mice as compared to their non-diabetic counterparts [42].

In addition, in a study conducted in 2023 by Song and colleagues, they demonstrated that serum levels of CCN6 have decreased in patients and mice models afflicted with NASH. They also found that an increase in this adipokine leads to reduced inflammation, steatosis, and hepatic fibrosis in NASH-affected mice [14].

Consistent with our study, in 2019, Yang Li and fellow researchers demonstrated that individuals with T2DM exhibited elevated levels of CCN3 in their serum when contrasted with the control group. Furthermore, they established a positive association between the serum levels of this adipokine and inflammatory markers, including CRP, TNF-α, and IL-6 [38].

In our study, we observed a significant negative correlation between CCN6 and inflammatory factors such as IL-6, TNF-α. Also, we noticed a negative and noteworthy correlation between CCN6 and indicators of insulin resistance, specifically insulin levels and HOMA-IR. This discovery implies that CCN6 may have the potential to serve as a therapeutic target for enhancing insulin sensitivity in individuals diagnosed with T2DM. Previous researches also demonstrated a correlation between serum levels of CCN6 and insulin resistance. They indicated that increased CCN6 expression in NAFLD-afflicted mice inhibits the Ask1-p38MAPK/JNK signaling pathway in adipose tissue, leading to reduced inflammation and insulin resistance [14]. Moreover, in animal hepatic cells and human muscle cells, CCN4 acts as an inhibitor of phosphorylation of Akt and its glycogen synthase kinase 3β substrates, insulin receptor, FOXO1, p70S6 kinase, suppression of gluconeogenic gene expression, and inhibition of insulin-stimulated glycogen synthesis. This has led to disruptions in insulin functionality [43, 44].

As evidenced, in 2019, Klimontov and colleagues identified CCN4 as a potential biomarker for obesity in individuals with Type 2 Diabetes. Their research revealed a connection between CCN4 serum levels, central abdominal fat mass, and dysfunction of adipose tissue [43]. In line with these findings, our study also demonstrated a significant negative correlation between CCN6 serum levels and BMI within both the patient and control groups.

Furthermore, inhibition of WISP3 expression was shown to enhance the effects of the IGF-1 factor and neoplastic progression in breast epithelial cells [45]. In a separate study in 2018, the impact of CCN6 inhibition on apoptosis of pulmonary cells under hypoxic conditions was investigated, and their results indicated that CCN6 can inhibit the extrinsic apoptosis pathway by suppressing caspase 8, thereby safeguarding pulmonary cells [46].

It's worth highlighting that our research did not uncover any substantial or statistically significant link between CCN6 serum levels and the serum lipid profile or FBG in individuals diagnosed with T2DM. However, a different research investigation documented a notable and positive correlation between CCN3 levels and TG as well as FBG [38].

On the other hand, the Gini Index indicated that the levels of CCN6 in the serum play a significant role in identifying patients with T2DM. The random forest model demonstrated the highest performance in this study, which is consistent with a previous study that also showed the Random Forest model outperforms the Decision Tree model for T2DM prediction. In a study, a random forest model was proposed with an accuracy of 71.1%, while our study reported a model with 92.50% accuracy. This improvement in accuracy indicates that the current study successfully selected suitable features for the T2DM prediction.

In another recent study [47] the random forest model achieved an accuracy of 94.4% and outperformed both the decision tree and multiple logistic regression models for T2DM classification. The improved performance in this study may be attributed to the implementation of the SMOTE (Synthetic Minority Over-sampling Technique) technique, which aims to balance the number of records in the minor class with those in the majority class [48]. By applying the SMOTE technique and conducting tests on the synthetic records generated by SMOTE, the models achieved higher performance compared to their real performance on the original dataset.

The random forest model could serve as a viable approach for predicting the risk of T2DM by considering various factors such as, IL6, CCN6, Adiponectin, TNF, HDL, TG, LDL, TC, BMI, SBP, and DBP. Moreover, this model might be employed in decision support systems to assist clinical professionals.

Conclusion

Our results reinforce the idea that CCN6 serum concentrations was decreased in individuals with T2DM, and also, exhibited correlations with TNF-α, IL-6, BMI, insulin and HOMA-IR measurements. This implies that CCN6 may serve as a prospective biomarker for

anticipating the onset of T2DM. Nonetheless, additional research is imperative to establish definitive conclusions.

Abbreviations

T2DM	Type 2 diabetes mellitus
CCN6	Cellular Communication Network Factor 6
IGF-1	Insulin-like growth factor 1
NASH	Non-Alcoholic Steatohepatitis
IGT	Impaired glucose tolerance
ELISA	Enzyme-linked immunosorbent assay
BMI	Body mass index
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
FBG	Fasting blood glucose
IL-6	Interleukin-6
TNF-alpha	Tumor necrosis factorα
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
AST	Aspartate transaminase
ALT	Alanine transaminase
TG	Triglycerides
TC	Total cholesterol
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
CCN6	Cellular communication network factor 6
FOXO1	Forkhead box protein O1

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Author contributions
RA and SHA participated in study design. NM, RF and SMA carried out experimental tests and interpreted results. YJ, OKGH and VF wrote the draft of the manuscript. RF and RA edited the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets utilized and/or scrutinized throughout the study are obtainable upon reasonable request from the corresponding author.

Declarations

Ethical approval
The protocols were performed in compliance with the Declaration of Helsinki and approved by the Ethics Committee of Tehran University of Medical Sciences (ID number: IR.TUMS.SPH.REC.1401.280). As well as, informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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