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Research Article

Estimation of Chemokine CCL7 Serum Level among Patients with Type 2 Diabetes Mellitus

Kawther Basim Bader Al-Noor[1], Abeer Thaher Naji Al-Hasnawi[2], Hassan Murtadha[3]

[1,2] Department of Microbiology, College of Medicine, University of Kerbala , Karbala, Iraq

[3]Center Imam Hassan for Endocrinology and Diabetes, Karbala Health Directorate, Karbala, Iraq

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Correspondence: Kawther Basim Bader Al-Noor

Email: m11150138@s.uokerbala.edu.iq

ORCID:https://orcid.org/0009-0000-1230-0850

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder with multiple causes that affects adults all over the world. Chemokine ligand (CC motif) 7 (CCL7) is a chemoattractant that attracts immune cells, especially macrophages and monocytes. It is thought to play a role in anti-inflammatory responses and has been associated with several diseases. The study aimed to measure chemokine CCL7 levels in the serum of T2DM patients and compare them with healthy individuals. Also, evaluate the potential diagnostic role of CCL7 as a prognostic biomarker in T2DM patients.

Methods: This is a case-control study that included 100 participants, 50 participants with type 2 diabetes and 50 healthy participants as a control group. Serum was separated from blood samples, and a CCL7-specific ELISA kit was used to calculate its serum level.

Results: Several statistically significant metrics were achieved, including serum CCL7 levels, which were significantly different between patients and healthy controls (p=0.001), with an area under the curve (AUC) of 0.755, which demonstrates strong diagnostic ability. Sensitivity was 0.98, and specificity was 0.5. The cut-off value in the current study was 14.583, with a p-value of 0.000, confirming statistically significant results.

Conclusions: The study revealed that the chemokine CCL7 has a good ability to differentiate T2DM patients, which may make it a potential diagnostic biomarker in the future.

Keywords: Type 2 Diabetic Mellitus; Chemokine CCL7; ELISA test.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease that can also be described as a multifactorial syndrome causing elevated blood sugar levels [1]. It is a concern with an exacerbation due to both aging and significant population growth worldwide, with the majority of those affected being adults between the ages of 40-59 years [2]. In 2017, the global prevalence of T2DM was approximately 462 million people, representing 6.28% [3]. In 2021, the global prevalence rate had risen to 537 million people, with significant concerns about the increasing health burden associated with diabetes [4]. T2DM is a major risk factor for increased mortality, other morbidities, and cardiovascular complications [5]. It is estimated that approximately 20-50% of patients with T2DM develop diabetic kidney disease, which calls for conducting studies to determine the causes and factors influencing the development of T2DM, given its impact on patients' quality of life [6].

Chemokine ligand 7 (CCL7) is a naturally expressed CC chemokine in many immune disorders. It is a chemoattractant that attracts macrophages and other types of white blood cells (neutrophils and monocytes) [7]. Chemokine CCL7, also known as chemokine monocyte-chemotactic protein-3 (MCP-3), is involved in numerous anti-inflammatory responses. This chemokine is associated with a variety of disorders, including cancer, and has multiple receptors, allowing it to exert varying effects on the recruitment and activation of immune cells [8]. Extensive studies have been conducted on numerous inflammatory reactions, including infections, cardiovascular disorders, inflammatory and degenerative neurological diseases, autoimmune diseases, and cancer, concerning the

CCL2 and its co-receptor, CCR2. CCL7 affects a variety of innate and adaptive immune cell types by binding to several receptors, such as CCR1, CCR2, CCR3, CCR5, and CCR10 [8-9].

The chemokine CCL7 is believed to have a primary protective effect by contributing to the migration of immune cells to sites of West Nile virus (WNV) infection and may have future therapeutic potential [10]. In several studies, CCL7 levels have been measured to identify patients with overlap of asthma and chronic obstructive pulmonary disease (COPD), confirming the diagnostic importance of CCL7 in asthma-COPD overlap (ACO) [11]. In men, the CCL7 has been associated with a lower risk of cardiovascular disease at older ages [12]. Serum CCL7 levels were higher in T2DM patients with ischemic stroke compared to those with ischemic stroke alone [13]. The present study aimed to evaluate the potential of CCL7 as an early diagnostic biomarker for T2DM.

Materials and Methods

Patients

The study is a case-control study, which involved 100 participants in two groups: 50 healthy individuals as a control group aged between 25 and 65 years and 50 patients with T2DM aged between 25 and 75 years, who visited the Imam Al-Hassan Center for Endocrinology and Diabetes in Karbala, from November to December 2024.

Inclusion criteria: Patients with T2DM were diagnosed by an endocrinologist and diabetes consultant, based on the clinical and diagnostic criteria approved by the American Diabetes Association (ADA), which include: HbA1c ≥6.5%, fasting blood glucose level ≤126 mg/dL, random blood glucose ≤200 mg/dL, and the presence of clinical symptoms such as thirst, frequent urination, or weight loss [14]. The BMI of participating individuals has been calculated using the BMI formula [15]:

$BMI = \text{weight (kg)} / \text{height}^2 \text{ (m)}^2$

The weight status was classified into five groups according to the value of BMI [16] (Table 1).

Exclusion criteria: Patients with asthma, cancer, autoimmune diseases, pneumonia, and obesity were excluded.

Sample collection

A 3 ml blood samples were drawn from the veins of all participants using a disposable syringe, collected in a gel tube, and the serum was separated by centrifugation. The serum was collected in an Eppendorf tube and stored at -20°C for ELISA to determine the concentration of CCL7 in the samples.

Table 1: Weight values according to the body mass index (BMI)

Weight status	Values of BMI (kg/m^2)
Underweight	<18
Normal	18-24.9
Overweight	25-29.9
Obesity	30-39.9
Morbid obesity	≥ 40

Chemokin CCL7 assay

The CCL-7 serum levels were measured by Sandwich-ELISA type using an ELISA kit (Sunlong Biotech, Chain), the assay range of CCL7 is (3-180 pg/ml).

Ethical Considerations

The relevant committees at the University of Kerbala issued an ethics certificate (No. 24-47, dated September 29, 2024). The Karbala Health Directorate approved our study, No. 3790, on November 4, 2024. All patients provided verbal informed consent before sample collection. The research adhered to the ethical principles outlined in the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Participants' privacy rights were maintained and were not shared within the research.

Statistical analysis

This study was conducted using Microsoft Excel 2010 and IBM Statistical Package for the Social Sciences (SPSS), version 26 (Chicago, Illinois, USA). Means of the studied parameters were compared between the two groups using the t-test. Differences between the two groups were investigated using one-way analysis of variance (ANOVA), and percentages were compared using the Chi-square test. All test results with two-tailed p-values < 0.05 were considered statistically significant. A receiver operating characteristic (ROC) curve has been applied to assess the diagnostic ability of IL-CCL7 as a biomarker to distinguish between T2DM cases and controls, and to determine whether IL-CCL7 can serve as a predictive factor for T2DM. The ROC value was 75%.

Results

The results showed differential values of body mass index (BMI) in T2DM patients and control groups. The results of statistical analysis revealed a significant (p=0.012) increase in BMI values of the

control compared with the patients (Figure 1). Figure 2 shows the distribution of T2DM patients and controls according to BMI categories. The percentage of T2DM patients and controls was 60% and 48%, respectively, within the normal BMI category, while 40% and 52%, respectively, were within the overweight category.

T2DM patients have been divided into four groups according to duration of diabetic disease, including: 1-6, 7-12, 13-18, and <19 years. The percentage of patients was significantly (p=0.0001) higher in the first duration group, 1-6 years, compared with other groups (Figure 3).

Table 2 displayed the levels of CCL7 in both T2DM patients and controls, where the statistical analysis demonstrated a significant (p=0.001) elevation in CCL7 levels in T2DM patients versus control individuals. Also, compares the levels of C-reactive protein (CRP) between T2DM patients and controls, where its levels were increased in T2DM as compared with healthy individuals.

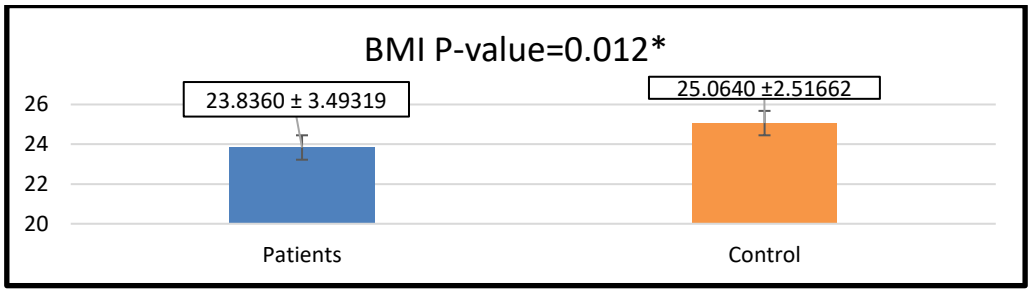

Figure 1: Body mass index value in patients and controls. BMI: Body mass index

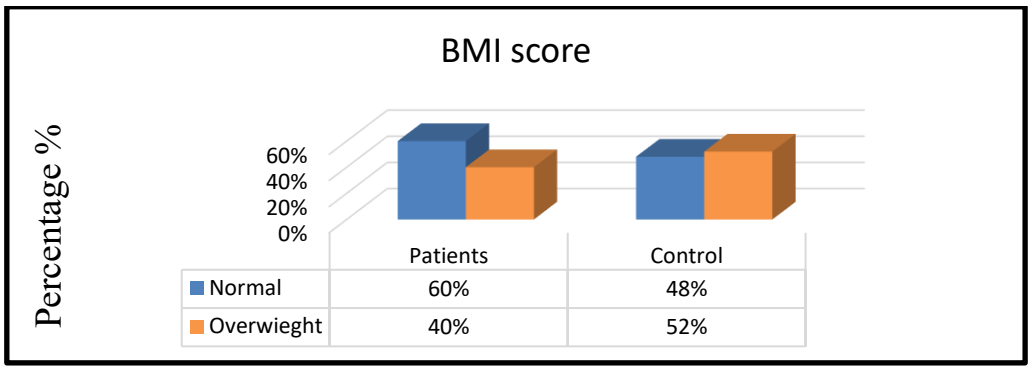

Figure 2: Percentage of body mass index categories in type 2 diabetes patients and controls**.** BMI: Body mass index

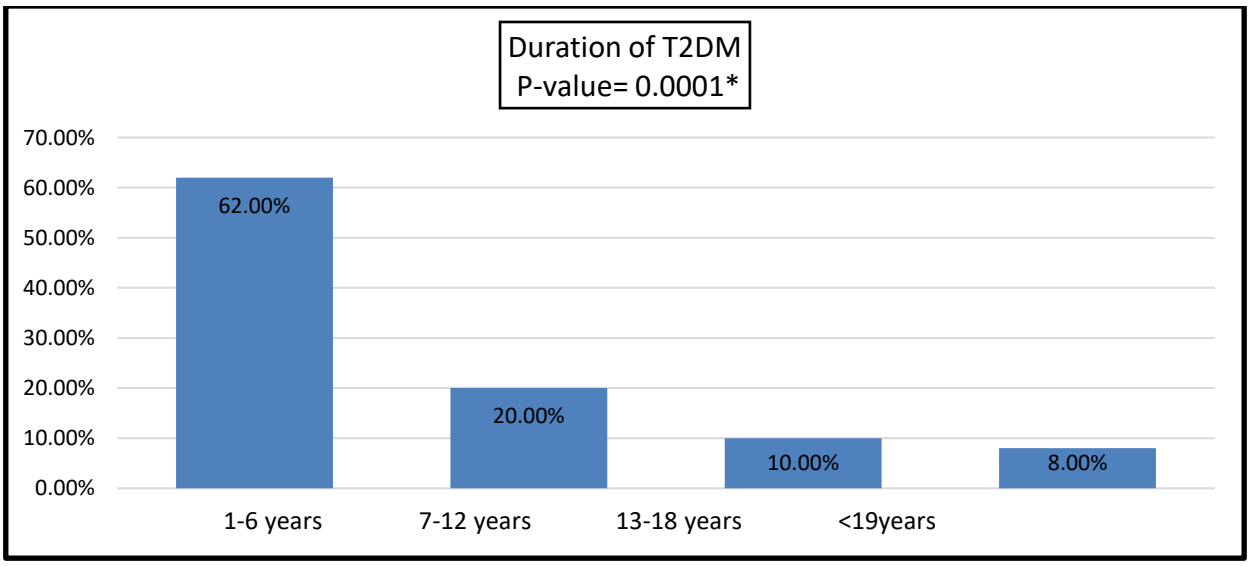

Figure 3: The duration period (years) of type 2 diabetes mellitus patients

Table 2: Serum levels of CCL7 and C-reactive protein (CRP) in type 2 diabetes patients and controls

Parameters	Study population Mean ± SD		P-value
	Patients (n=50)	Control (n=50)	
CCL7 Pg/ml	21.523 ± 4.493	15.705 ±5.959	0.001*
CRP	5.0416 ± 1.4845	2.7020 ±.7625	0.000*

*Significant difference at the 0.05 level by t-test.
SD: Standard deviation

In the Receiver operative characteristic (ROC) curve analysis of CCL7, several significant metrics have been acquired, as shown in Table 3 and Figure 4. Area under the Curve (AUC) was 0.755, which indicated a good diagnostic ability. The sensitivity, which measured CCL7 capacity to accurately detect true positives, was noticeably high at 0.98, which means that 98% of T2DM patients were correctly identified. On the other hand, specificity, which measured the CCL7 ability to accurately detect true negatives, was equal to 50%, indicating that 50% of healthy individuals were properly identified. The cut-off value for the current analysis was set at 14.583, and yielded a p-value of 0.000, confirming a statistically significant outcome.

Table 3: Receiver operative characteristic (ROC) of CCL7 in type 2 diabetes patients

Markers	AUC	Sensitivity	Specificity	Cut-off	P-value
IL-CCL7	0.755 (75%)	0.98 (98%)	0.5 (50%)	14.583	0.000*

AUC: area under curve; *Significant difference at the 0.05 level by ROC analysis

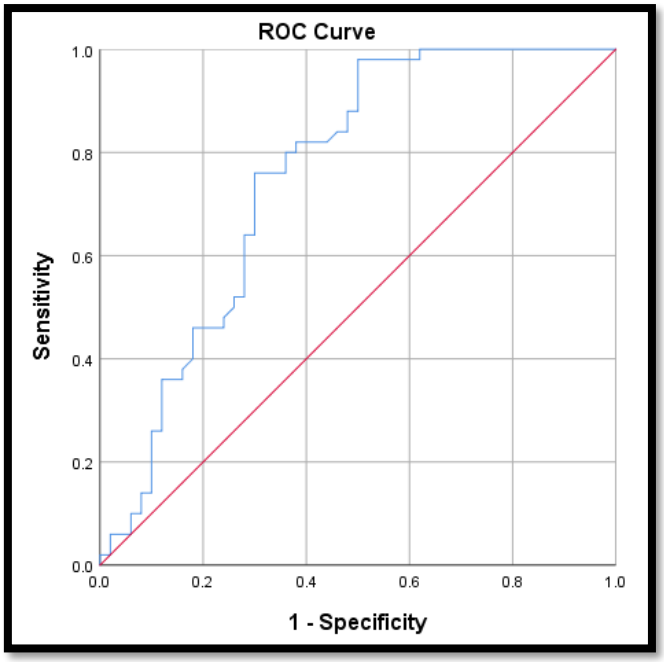

Figure 4: Receiver operative characteristic (ROC) curve for CCL7 in type 2 diabetes mellitus cases

Discussion

In this study, a body mass index (BMI) was assessed in patients with T2DM and compared to that of healthy controls. The results of this study showed a statistically significant difference. The healthy individuals had a higher BMI than the diabetics. This may be due to several reasons, including the weight loss due to disease progression, impaired glucose utilization, and increased fat breakdown, or patients following a healthy diet to control their blood sugar. A study conducted in Iraq on diabetic patients found no significant difference in BMI among patients [17]. Conversely, numerous studies have demonstrated the opposite, indicating that BMI is generally higher in patients with T2DM. Among these studies is a study that they were divided into two groups. The first group was patients with T2DM and were observed to have a higher body mass index, compared to the second group, who were healthy and had a lower body mass index [18]. Also, a study was done in India by Purohit and Tiwari (2014), who also found that the body mass index was high in diabetic patients at 23.94 kg/m², while it was 22.8 kg/m² in people without diabetes. These disparities may also be due to differences in sample size and ethnicity [19].

According to the BMI categories between patients with T2DM and the control group, the present study showed that patients had a normal BMI with low overweight compared to the control group. In a study conducted in Korea on T2DM that showed the HbA1c levels were found in people with a BMI of 18.5–22.9 kg/m² (30%) and with a BMI of ≥30 kg/m² (28.9%). The relationship between BMI and diabetes is complex and influenced by environmental and genetic factors [20]. In a large study conducted in the United Kingdom, which included patients with T2DM, it was noted that South Asians were more likely to have diabetes, even at low BMI less than 30 kg/m², at a rate of 38%, while white Europeans were affected at a rate of 26% and African Caribbeans at a rate of 29%. This disparity may be due to differences in ethnicities [21].

The results related to disease duration and demonstrated statistical significance in the distribution of disease duration showed that the largest proportion was among patients who had suffered from the disease for a short period of time. This reflects several important factors, including the increased incidence of T2DM due to unhealthy lifestyles and low levels of physical activity in recent years, increased health awareness among the population, and improved methods for diagnosing early diabetes [22-23]. A study conducted by Kumar et al. (2024) showed a relationship between gender, duration of diabetes, and the occurrence of complications with increasing duration, with males representing the largest proportion [24]. Yu et al. (2022) classified patients with T2DM into four groups based on the duration of their diabetes: Group 1 (less than one year), Group 2 (1-5 years), Group 3 (5-10 years), and Group 4 (>10 years). The majority of patients in all groups had a family history of diabetes, with lower adherence to proper dietary management and regular physical activity. In group 4, glycated hemoglobin (HbA1c) levels were higher than in the other groups. The results indicated that patients in group 4, who had been living with diabetes for more than 10 years, were more likely to develop vascular complications, including coronary artery disease, peripheral artery disease, and diabetic nephropathy [25]. A large-scale study revealed that complications peaked in individuals with diabetes for more than 15 years, indicating an increased prevalence of stroke, vascular disease, coronary artery disease, and congestive heart failure [26].

In this study, the CCL7 levels in the serum of T2DM patients were significantly higher compared to healthy individuals. The current study is the first study in Iraq to detect the level of this chemokine in T2DM patients. Previous studies have shown the role of this chemokine in other diseases such as a study of Chidimatsu et al. (2023), which found that high concentrations of CCL7 and carcinoembryonic antigen (CEA) combined increase the risk of death in patients with metastatic colorectal cancer compared to patients with a low percentage [27]. Also, the role of CCL7 in psoriasis has been confirmed in previous studies. Increased levels of CCL7 have been observed in areas affected by psoriasis, where it is believed to act as a TNF-α-dependent inflammatory mediator, increasing inflammation in psoriatic skin [28]. In a previous study, an ELISA test was used to determine the level of the chemokine CCL7 and its effect on the development and spread of oral squamous cell carcinoma. It was found that there was an increase in its secretion, and when an anti-CCL7 was given, a decrease in the spread of cancer cells was found [29].

CCL7 is a pro-inflammatory cytokine that plays a major role in the development of T2DM complications. It also plays a role in regulating the response of monocytes and macrophages. Elevated CCL7 levels in T2DM patients may be associated with increased chronic inflammation resulting from insulin resistance [30]. The above studies indicate the role of the chemokine CCL7 in the development of disease conditions, which makes this chemokine a candidate as an effective biomarker for detecting chronic inflammation or a therapeutic target for some conditions in the future.

In the present study, the results of comparing the levels of CRP for T2DM patients and the control group found significant differences, meaning that T2DM patients have high levels of CRP compared with healthy individuals. Another study showed that the majority of T2DM patients (96.7%) had a positive CRP test, with varying degrees of severity [31].

In this study, the area under the curve (AUC) value of 0.755 indicates that CCL7 levels have a moderate to good ability to differentiate between

T2DM patients and healthy individuals. The high sensitivity of 0.98 demonstrates superior ability to detect T2DM patients, making CCL7 a reliable biomarker for early diagnosis and treatment. The cutoff value was set at 14.583, indicating strong statistical significance and demonstrating the reliability of the results. Previous studies have indicated the effective role of CCL7 as a biomarker in several diseases. A study on patients with metastatic colorectal cancer showed that higher serum CCL7 levels had lower survival rates, compared to those with lower CCL7 levels, and the ROC analyses were at significant levels [23]. Another study conducted by Hassan et al. (2022) supported this result. It included COVID-19 patients and conducted ROC analyses of serum CCL7 levels, showing statistically significant results [32]. However, the low specificity highlights the need to improve analytical methods and consider factors that may influence CCL7 levels, such as tissue inflammation and comorbid conditions. Finally, the ROC analysis reveals that CCL7 has potential as a diagnostic biomarker, particularly due to its high sensitivity. However, further improvements are needed to enhance its specificity and overall diagnostic accuracy.

Conclusions

The study revealed that the chemokine CCL7, due to its high serum level and sensitivity, has a good ability to differentiate between patients with T2DM, which may make it necessary as a marker for diagnosis or treatment. However, further studies are needed to improve its identification accuracy and diagnostic precision for T2DM.

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