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Correlation between Albuminuria Levels and Chitinase 3 like 1 Protein in Iraqi Patients with Type 2 Diabetes Mellitus

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Abstract

Diabetes mellitus (DM) is a metabolic diseases attributed to lack of insulin secretion, insulin activity, or both. The most serious medical problems in hyperglycemia is diabetic nephropathy (DN), originating from the aggregation of inflammatory cells in high numbers. Chitinase 3 like 1 protein (CH3L1P) is a new biomarker for chronic and severe inflammatory conditions. It has been suggested to have a role in the progress of diabetes-associated micro and macro-vascular complications. This paper aims to measure CH3L1P levels and examine their correlation with albuminuria levels in Iraqi patients with type 2 diabetes mellitus (T2DM). Our study involved 66 T2DM patients (41males and 25 females with age ranging from 30 to 70 years, who were distributed into three groups; (A1) normoalbuminuria, (A2) microalbuminuria and (A3) macroalbuminuria, each group consisting of twenty-two patients. The control (C) group involved 22 healthy individuals (11 males and 11 females). Whole blood was used to estimate Glycated hemoglobin (HbA1c), while serum was used to measure other biochemical parameters, and the urine to measure urine creatinin (U.CR), urine albumin (U.A) & albumin to creatinine ratio (ACR). A quantitative sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine serum CH3L1P level. Results revealed that the levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c and fasting blood glucose (FBG) were significantly higher in the patients (A1, A2 and A3 groups) compared to C group ($P < 0.001$). Moreover, mean CH3L1P level significantly increased in the three T2DM patient group (A1, A2 and A3) (1327.20 ± 294.44 , 1434.82 ± 305.26 , 1602.90 ± 409.50 pg/ml, respectively) as compared to the C group (626.81 ± 103.15 pg/ml). CH3L1P levels in the blood were substantially higher in T2DM patients, with a clear correlation with the increase in albuminuria levels. As a result, it can be concluded that serum CH3L1P level may be used as an early detection marker of DN in T2DM patients.

Keywords: Diabetic nephropathy (DN), chitinase 3 like 1 protein (CH3L1P), YKL-40, Type 2 diabetes mellitus (T2DM).

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العلاقة بين مستويات albuminuria و chitinase 3-like 1 protein لدى المرضى العراقيين المصابين بداء السكري من النوع الثاني

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الخلاصة

مرض السكري (DM) هو من أمراض التمثيل الغذائي الناجمة عن نقص في إفراز الأنسولين، ونشاط الأنسولين، أو كليهما. واحدة من أخطر المشاكل الطبية في مرضى السكري هو اعتلال الكلية السكري (DN)، وتجميع الخلايا الالتهابية في مستويات عالية يؤدي إلى اعتلال الكلية السكري Chitinase 3 like 1 protein (CH3L1P). هو علامة حيوية جديدة للحالات الالتهابية المزمنة والحادة، وقد ثبت أن لها دورا مزعوما في التقدم المحرز في مضاعفات الأوعية الدموية الناجمة عن مرض السكري. الهدف من الدراسة: تهدف هذه الدراسة إلى قياس مستويات chitinase 3 like 1 protein، ودراسة العلاقة بين مستويات CH3L1P مع مستويات الزلال في المرضى العراقيين الذين يعانون من مرض السكري النوع الثاني.

المواد والأسلوب: شملت دراستنا ستة وستين مريضا بالسكري من النوع الثاني (41 من الرجال، 25 من النساء) تتراوح أعمارهم بين 30 إلى 70 عاما، مقسمين إلى ثلاث مجموعات (normoalbuminuria، microalbuminuria & macroalbuminuria)، تتكون كل مجموعة من اثنين وعشرين مريضا. مجموعة الكونترول (C) تتكون من اثنين وعشرين من الأفراد الأصحاء (11 الرجال و 11 النساء)، النماذج المستخدمة الدم لقياس (HbA1c)، السيرم لقياس المعلمات الحيوية، و الادرار لقياس مستوى الكرياتينين في الادرار (U.CR)، الالبومين (U.albumine) و نسبة الالبومين الى الكرياتينين (ACR)، تم قياس تركيز مستوى chitinase 3 like 1 protein باستخدام تقنية الاليزا.

النتيجة: نلاحظ من النتائج ان مستويات سكر الد الصائم، خضاب الدم، ضغط الدم الانبساطي و ضغط الدم الانقباضي أعلى بكثير في مجموعات المرضى بالمقارنة مع مجموعة الكونترول ($P < 0.001$). كما ان مستوى CH3L1P يعاني زيادة كبيرة في مرضى السكري من النوع الثاني بالمقارنة مع مجموعة الكونترول بقيم (103.15±626.81، 409.50±1602.90، 305.26±1434.82، 294.44±1327.20) pg/ml على التوالي.

الخلاصة: تم زيادة مستويات CH3L1P بشكل كبير في مجموعة مرضى السكري من النوع الثاني مقارنة مع مجموعة الكونترول، وترتفع مستويات CH3L1P مع زيادة مستويات الزلال. ونتيجة لذلك، يمكننا القول أن CH3L1P يمكن أن تكون علامة مفيدة للكشف المبكر عن اعتلال الكلية السكري.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease described by the existence of hyperglycemia caused by deficiency in insulin secretion, activity, or both [1]. In modern societies, DM is considered a common disease [2]. Around 90-95% of persons with DM have T2DM. It is more frequently found in adults, particularly in individuals who are obese. The main cause of T2DM is the relative insulin deficiency and resistance [3]. T2DM is the outcome of insulin resistance (IR) and, consequently, insulin levels in the blood are higher in the patients [4]. In DM, uncontrolled chronic hyperglycemia is related to several macro and micro-vascular complications, including hypertension, retinopathy, nephropathy, neuropathy, and ischemic heart disease (IHD) [5, 6]. Diabetic nephropathy (DN) is a serious medical problem in

diabetic patients [7]. It is a micro-vascular complication of diabetes that is a leading cause of end-stage renal disease (ESRD), which has high morbidity and mortality rates [8].

Diabetic nephropathy-associated pathological factors include mesangial expansion, extracellular matrix protein accumulation, glomerular hypertrophy, basement membrane thickening, increased albumin secretion in the urine, and glomerular hyper-filtration [9]. The main factor in the growth of DN is inflammation, demonstrating the significance of hyperglycemia in the development of inflammatory damage in diabetics [10], which is accompanied by fibrosis and oxidative stress [11]. Adhesion molecules, chemokines, and proinflammatory cytokines are among the immune system components involved in the onset and progression of DN. Renal tissues from patients with DN show elevated levels of inflammation molecules and high intensity of inflammatory cells aggregation [12]. As nephropathy progresses, the content of inflammatory components rises, all being linked to higher urinary albumin excretion (UAE) and clinical markers of glomerular and tubule-interstitial damage [7]. This inflammatory path supports the suggestion that DN is caused not only by unregulated hemodynamics and hyperglycemia, but also by a chronically stimulated innate immune response and a low-grade inflammatory condition in diabetic patients [13]. Although microalbuminuria may be used as an indicator of DN, it is not sufficient alone because non-diabetic patients who are suffering from chronic renal disease can also show progressive microalbuminuria. Also, microalbuminuria sometimes does not develop to ESRD. Therefore, specific and sensitive biomarkers are required to predict diabetic nephropathy [14]. CH3L1P is a glycoprotein that causes inflammation [15]. It has been known as a biomarker for chronic and acute inflammatory conditions and proved to have a role in the progress of diabetes micro- and macro-vascular complications [16]. CH3L1P can promote cell migration, adhesion, and chemotaxis, beside its role in endothelial dysfunction [8]. Several types of cells, such as macrophages, neutrophils, fibroblast-like cells, hepatic stellate cells, endothelial cells, and cancer cells, can secrete CH3L1P [17]. Overexpression of CH3L1P has been observed in a variety of infection conditions including cirrhosis, sepsis, diabetes, asthma, rheumatoid arthritis, preeclampsia, and coronary artery disease [18]. Some studies have recently found a correlation between circulating CH3L1P levels and albuminuria in patients with type 2 diabetes mellitus, suggesting that CH3L1P may be a potential early marker of micro-vascular complications and diabetes nephropathy [19].

The aim of the present study is to determine CH3L1P levels and examine their correlation with albuminuria levels in Iraqi patients with T2DM.

1.2 Materials and Methods

1.2.1 Subjects

This study was performed at the National Center for Diabetes Treatment and Research at Al-Mustansiriya University in Baghdad, Republic of Iraq, during the period from December 2019 to February 2020. The study involved 66 T2DM patients (41 males and 25 females) with age ranging from 30 to 70 years. The patients were categorized into 3 groups based on the stage of albuminuria, i.e. albumin to creatinine ratio (ACR), each consisting of 22 patients As follows; Normoalbuminuria (A1) with $ACR < 3.4$ mg/mmol, microalbuminuria (A2) with $ACR 3.4- 34$ mg/mmol, and macroalbuminuria (A3) with $ACR \geq 34$ mg/mmol.

The control (C) involved 22 healthy individuals (11 male and 11 female), with age ranging from 30-70 year, who had no hypertension, high cholesterol, family history of DM, or renal disease. The World Health Organization's (WHO) guidelines were used to diagnose diabetes [20].

Exclusion criteria included T1DM patients, T2DM patients who were using insulin as a hypoglycemic treatment, pregnancy, heart diseases, chronic hepatic disease, fever, and urinary tract infection or other renal disorders. People who have been standing for a long time, people who have walked a long way before having their urine sampled, and patients with acute

infections on the day of sampling were also excluded. The study was approved by the center's ethics committee and written consent was obtained from all patients.

1.2.2 Sample collection

About 10 ml of venous blood was obtained from each participant after overnight fasting. The blood sample was separated into two parts; 2 ml of blood was placed in K-ethylene diamine tetra acetic acid (K-EDTA) tube and used for the measurement of HbA1c. In addition, 8 ml of the sample was placed in a gel tube and allowed to clot at room temperature for serum separation. The following information were collected from all participants: age, sex, waist circumference (WC), body mass index (BMI), waist-to-hip ratio (WHR), SBP, and DBP. Laboratory investigation included FBG, HbA1c, blood urea (B.UA), serum creatinine (S.CR), serum uric acid (S.UA), serum albumin (S.A), lipid profile (total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C)), C-reactive protein (CRP), and CH3L1P.

1.2.3 Sample Measurements

Urine albumin and creatinine were measured by using a Combolyzer13 device. A modified Diet in Renal Disease (MDRD) formula was used to calculate the estimated glomerular filtration rate (eGFR), as follows [21]:

$$\text{eGFR} = 186 * (\text{serum creatinine [mg/dl]})^{-1.154} * (\text{age in years})^{-0.203} * (0.742 \text{ for women})$$

The following equation was used to estimate creatinine clearance (CCR) [21]:

$$\text{CCR} = (140 - \text{age}) \times \text{weight (kg)} / 72 \times \text{serum creatinine (Scr) (mg/dL)} \quad (\times 0.85 \text{ for females})$$

The homeostasis model assessment of insulin resistance (HOMA-IR) index was used to express insulin resistance from levels of fasting insulin and glucose.

The HOMA-IR cutoff point for determining insulin resistance is 3.8 [22]. HbA1c and CRP were measured by using the sandwich immune detection method iCHROMA kit (Boditech, Korea). A commercially available enzyme-linked immunosorbent assay (ELISA) kit was used to test fasting serum insulin (FINS) (Monobind Inc., U.S.A). Human CH3L1P level was determined using a commercial ELISA kit (MyBioSource system, USA). The tests were carried out in accordance with their respective company's protocols.

1.3 Statistical Analysis

The IBM SPSS software package (version 18.0) was used to analyze the data statistically. Data are presented as mean and standard deviation. One way analysis of variance (ANOVA) was used to compare the groups by using the least significant difference as a post hoc test to make individual comparisons. The association between serum CH3L1P and other parameters was determined using Pearson correlation analysis. Statistically significant differences were determined by a P value of 0.05.

1.4 Results

The anthropometric and biochemical characteristics of the study population are demonstrated in Table 1. The BMI and WHR values showed highly significant increases in A1, A2 and, A3 groups compared with the control group ($P < 0.01$). Moreover, waist and hip measurements revealed highly significant differences between A2 and A3, but not A1, groups vs. the control group ($P < 0.01$). However, SBP levels were found to vary significantly between patient groups and the control group, as well as between A1 vs. A3 and A2 vs. A3 groups ($P < 0.01$). A1 group displayed a significant increase in DBP ($P < 0.05$), whereas A2 and A3 groups showed highly significant elevations ($P < 0.01$). The results also demonstrated highly significant increases in FBG and HbA1c levels in the three groups of patients in comparison with the healthy one ($P < 0.001$). A2 and A3 groups showed highly a significant increase of FINS and HOMA-IR as compared with the control group. Furthermore, A3 with A1 and A2 groups showed a highly significant increase in FINS and HOMA-IR ($P < 0.001$). The study did not find substantial differences between the patient and control groups in the serum levels of

TC, HDL, and albumin. A1 group showed a highly significant increase in TG level as compared to the control group at $P<0.01$, whereas similar significant differences were found in A2 and A3 groups, but at $P<0.05$. All the three patient groups showed significant increases in the serum levels of LDL-C as compared with the control group ($P<0.05$). A3 showed in comparison to the C group, A1 and A2 groups, a substantial difference in blood urea ($P<0.01$). Serum creatinine revealed a highly significant increase ($P<0.001$) in A3 compared the control group, while A1 and A2 groups did not appear any significant difference in comparison to control group. We also observed that A2 and A3, but not A1 groups, have highly significant differences in microalbumine levels as compared with the control group ($P<0.001$). Moreover, A3 group showed highly significant differences with A1 and A2 groups. A3 group showed a highly significant increase in urine creatinine (U.CR), ACR and CCR as compared with A1, A2, and C groups. The results showed a significant increase in the levels of eGFR in A1 and A2 groups compared with the control group ($P<0.05$). However, A3 group showed highly significant differences when compared with A1, A2, and C groups ($P<0.001$). CRP level did not show any significant differences when T2DM groups were compared with the control group. CH3L1P levels showed a highly significant elevation ($P<0.001$) in the T2DM groups in comparison with the control group. Also, significant differences appeared between A3 and A1 groups ($P<0.05$). Table 2 shows the correlations between serum CH3L1P and the other tested parameters. Serum CH3L1P showed a significant positive correlation with height ($r=0.481$, $P=0.023$), S.UA ($r=0.482$, $P=0.023$) and S.CR ($r=0.446$, $P=0.037$) in A1 group. CH3L1P showed a significant positive correlation with HIP ($r=0.551$, $P=0.008$) and a significant negative correlation with WHR ($r=-0.554$, $P=0.007$) in A2 group. However, CH3L1P had a significant positive correlation with insulin ($r=0.465$, $P=0.029$) and duration of T2DM ($r=0.665$, $P=0.001$) in A3 group, as shown in Figure (1- A, B, C).

Table 1-Anthropometric and biochemical characteristics of patients and control groups

Parameter	Type 2 DM				P Value
	Control N=22	Normoalbuminuria N=22	Microalbuminuria N=22	Macroalbuminuria N=22	
Age (year)	45.27±11.06	52.77±9.97	59.05±8.66 ^{**b}	64.64±9.19 ^{**c,e}	<0.001
Weight (kg)	71.91±18.12	83.05±12.26	90.09±16.44 ^{**b}	86.23±17.42 ^{*c}	0.003
Height (cm)	166.18±13.39	165.00±8.58	170.59±9.91	168.50±9.93	0.312
BMI (kg/m ²)	25.68±4.04	30.58±4.24 ^{**a}	30.93±5.43 ^{**b}	30.32±5.05 ^{**c}	0.001
Waist (cm)	90.36±14.57	101.09±21.88	108.09±11.64 ^{**b}	107.77±12.20 ^{**c}	0.001
HIP (cm)	102.27±8.76	109.36±7.23	112.64±12.61 ^{**b}	111.91±9.88 ^{**c}	0.003
WHR	0.87±0.09	0.96±0.06 ^{**a}	0.95±0.06 ^{**b}	0.96±0.05 ^{**c}	<0.001
SBP (mmHg)	117.30±8.8	135.00±10.47 ^{**a}	141.8±10.59 ^{**b}	155.90±10.71 ^{**c,e,f}	<0.001
DBP (mmHg)	72.23±7.5	81.18±10.14 ^{*a}	85.59±10.22 ^{**b}	85.55±10.14 ^{**c}	<0.001
FBG (mg/dl)	92.14±6.55	152.64±46.56 ^{**a}	162.09±40.38 ^{**b}	178.95±67.63 ^{**c}	<0.001
HbA1c (%)	4.4±0.40	6.32±1.24 ^{**a}	6.82±1.65 ^{**b}	7.50±1.39 ^{**c,*c}	<0.001
FINS (μIU/ml)	13.65±2.98	16.55±4.77	20.04±6.65 ^{**b}	25.22±3.36 ^{**c,e,f}	<0.001

HOMAIR (%)	1.76±0.36	2.36±0.73	2.90±0.89 ^{**b}	3.88±0.99 ^{**c,e,f}	<0.001
TC (mg/dl)	171.18±26.77	180.45±42.53	190.45±40.10	175.50±51.66	0.451
TG (mg/dl)	90.95±32.63	162.77±64.21 ^{**a}	150.45±55.56 ^{*b}	148.95±41.77 ^{*c}	0.003
HDL (mg/dl)	37.91±12.48	46.45±10.45	45.45±14.17	41.44±15.22	0.129
LDL (mg/dl)	47.82±18.56	93.41±28.65 ^{*a}	91.11±32.06 ^{*b}	86.55±25.75 ^{*c}	0.007
S.UA (mg/dl)	3.73±0.85	4.57±1.06	4.52±1.24	5.14±1.38 ^{**c}	0.002
S.CR (mg/dl)	0.62±0.14	0.71±0.22	0.74±0.16	1.07±0.38 ^{**c,e,f}	<0.001
BUA (mg/dl)	26.06±8.35	27.25±9.76	28.10±6.29	40.42±15.73 ^{**c,e,f}	<0.001
s.albumine (g/dl)	3.90±0.45	4.00±0.43	4.07±0.53	3.98±0.41	0.688
Microalbumine (mg/l)	11.82±3.89	15.45±5.12 ^{**d}	110.91±38.71 ^{** b}	150±00 ^{**c,e,f}	<0.001
U.CR (mmol/l)	7.41±2.96	9.20±3.18	10.24±3.06	3.60±1.50 ^{*c,**e,f}	<0.001
ACR (mg/mmol)	1.70±0.33	1.79±0.46	11.19±3.33	63.09±20.28 ^{**c,e,f}	<0.001
CCR (ml/min)	167.44±43.91	165.21±52.9	150.77±42.08	100.07±38.33 ^{**c,e,f}	<0.001
Egfr (ml/min/1.73m ²)	134.29±29.54	110.55±25.42 ^{*a}	110.45±27.70 ^{*b}	78.22±29.54 ^{**c,e,f}	<0.001
CRP (mg/l)	3.27±0.9	5.89± 1.9	6.67±2.35	7.31±2.39	0.093
Ch311p (pg/ml)	626.81±103.1 5	1327.20±294.44 ^{**a}	1434.82±305.2 6 ^{**b}	1602.90±409.50 ^{**c,* e}	<0.001

* $P < 0.05$ is significant, ** $P < 0.01$ is highly significant.

a: significant difference between control and normoalbuminuria groups, b: significant difference between control and microalbuminuria, c: significant difference between control and macroalbuminuria, d: significant difference between normoalbuminuria and microalbuminuria, e: significant difference between normoalbuminuria and macroalbuminuria, f: significant difference between microalbuminuria and macroalbuminuria. FBG=fasting blood glucose; BMI = body mass index; WC = waist circumference; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; HbA1c = glycated hemoglobin; FINS=fasting insulin; HOMA-IR = homeostasis model assessment evaluation of insulin resistance; TC= total cholesterol; TG= triglyceride; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; BUA=blood urea; S.CR=serum creatinine; S.UA=serum uric acid; U.CR= urine creatinine; ACR = albumin-to-creatinine ratio; CCR= creatinine clearance; egfr estimated glomerular filtration rate; CRP C-reactive protein; CH3L1P chitinase 3 like 1 protein.

Table 2-Correlation results of serum CH3L1P level with anthropometric and biochemical parameters of T2DM patients.

Parameter	T2DM					
	Normoalbuminuria N=22		Microalbuminuria N=22		Macroalbuminuria N=22	
	R	P	R	P	R	P
Age (year)	-0.023	0.918	0.026	0.909	0.082	0.718
Weight(kg)	0.232	0.299	-0.003	0.991	-0.234	0.294
Height(cm)	0.481	0.023*	-0.335	0.128	-0.008	0.973
BMI (kg/m ²)	-0.210	0.349	0.234	0.294	-0.278	0.211
Waist (cm)	0.091	0.688	0.230	0.303	-0.143	0.527
HIP (cm)	-0.034	0.879	0.551	0.008**	-0.148	0.510
WHR	0.346	0.115	-0.554	0.007**	-0.040	0.510
Smoker	000	1.00	-0.137	0.542	-0.171	0.447
SBP (mmHg)	0.228	0.307	-0.287	0.195	0.074	0.745
DBP (mmHg)	0.174	0.440	-0.125	0.580	0.127	0.574
Duration (year)	0.257	0.248	-0.075	0.741	0.665	0.001**
FBG (mg/dl)	-0.023	0.920	0.235	0.293	0.114	0.613
HBA1C (%)	-0.031	0.891	0.111	0.624	0.065	0.775
FINS (μ U/ml)	-0.219	0.327	-0.157	0.486	0.465	0.029*
HOMA-IR(%)	-0.208	0.352	-0.116	0.608	0.291	0.189
TC(mg/dl)	0.378	0.083	0.250	0.261	-0.107	0.635
TG (mg/dl)	0.094	0.678	0.068	0.763	-0.025	0.912
HDL (mg/dl)	-0.129	0.567	0.034	0.880	0.285	0.198
LDL (mg/dl)	0.316	0.152	0.032	0.889	-0.106	0.639
S.UA (mg/dl)	0.482	0.023*	0.216	0.333	-0.224	0.316
CRP (mg/l)	0.159	0.481	0.157	0.484	0.081	0.721
S.CR (mg/dl)	0.446	0.037*	0.035	0.878	0.273	0.220
BUA (mg/dl)	-0.119	0.598	-0.271	0.222	0.280	0.206
s.albumine (g/dl)	0.002	0.994	-0.137	0.544	-0.259	0.244
Microalbumine(mg/l)	-0.114	0.613	0.242	0.278	-	-
U.CR (mmol/l)	-0.015	0.948	0.115	0.611	-0.404	0.062
ACR (mg/mmol)	0.020	0.929	0.211	0.346	0.411	0.058
Ccr (ml/min)	-0.306	0.166	0.037	0.870	-0.234	0.294
Egfr (ml/min/1.73m ²)	-0.362	0.098	-0.216	0.335	-0.008	0.973

FBG=fasting blood glucose; BMI = body mass index; WC = waist circumference; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure;; HbA1c = glycated hemoglobin; FINS=fasting insulin; HOMA-IR = homeostasis model assessment evaluation of insulin resistance; TC= total cholesterol; TG= triglyceride; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; BUA=blood urea; S.CR=serum creatinine; S.UA=serum uric acid; U.CR= urine creatinine; ACR = albumin-to-creatinine ratio; CCR= creatinine clearance; egfr estimated glomerular filtration rate; CRP C-reactive protein; CH3L1P chitinase 3 like 1 protein.

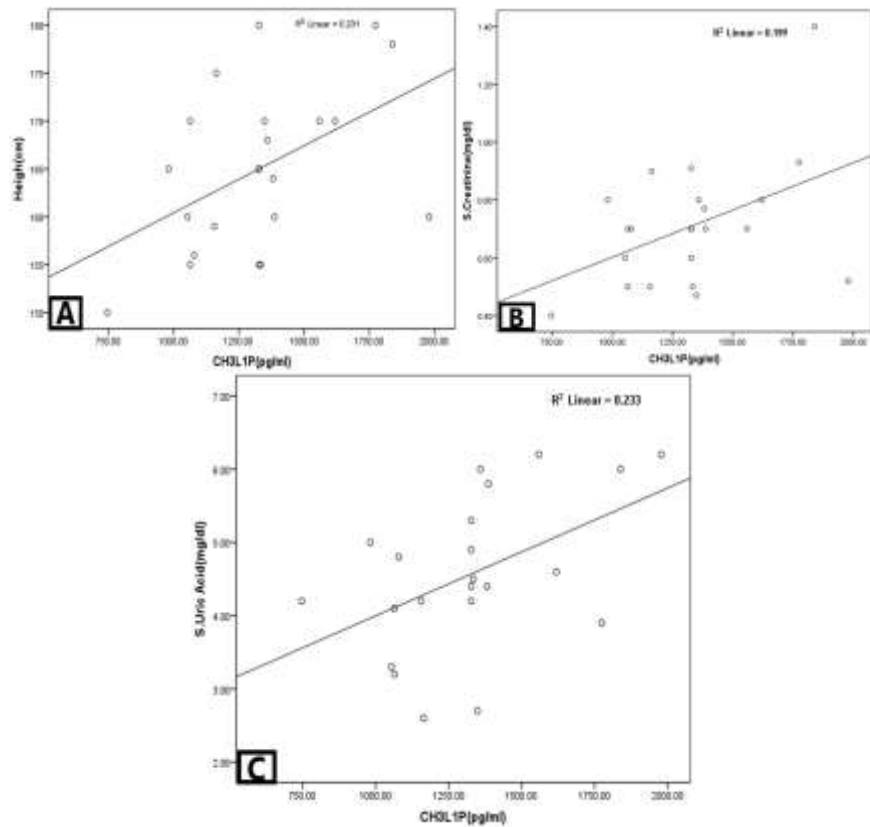
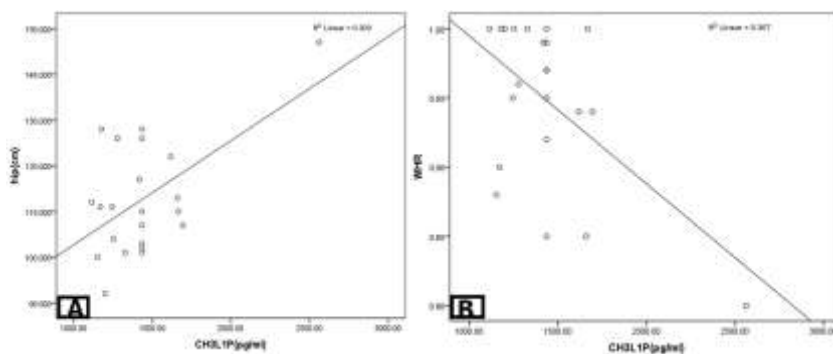


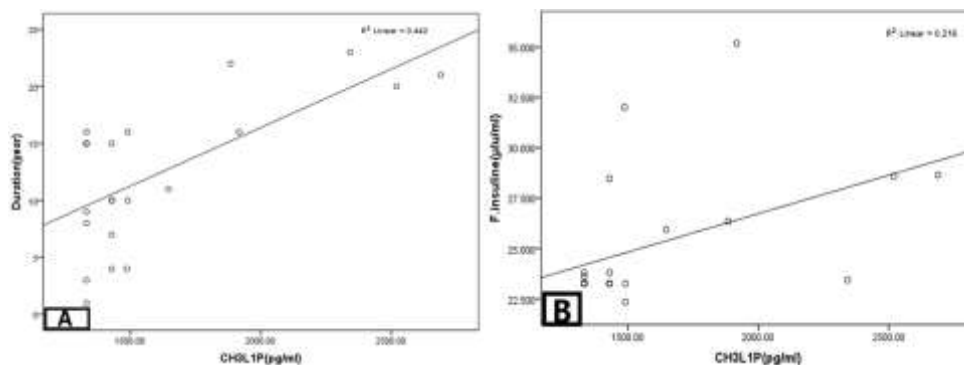
Figure1-1(A): Correlation between CH3L1P with (A) height, (B) creatinine, and (C) uric acid in A1 GROUP



Figures 1-1(B): Correlation between CH3L1P with (A) hip, (B) WHR and in A2 group

1.5 Discussion

The present study explains the relationship between chitinase 3 like 1 protein and albuminuria levels in type 2 diabetes mellitus. This study presented increased levels of serum CH3L1P in T2DM patients with the development of albuminuria status. We observed an elevation of serum CH3L1P in all T2DM patient groups (A1, A2 and A3) compared with the control group. Our results are in agreement with those of Luo *et al.*, 2021, who found



Figures 1-1(C): Correlation between CH3L1P with (A) duration and (B) insulin in A3 group.

significantly higher level of CH3L1P in DM patients compared with healthy individuals [23]. Furthermore, the level of CH3L1P was reported to be higher in DM patients with various stages of albuminuria than in healthy subjects, and it rises as the severity of albuminuria worsens [23]. In addition, our findings are in line with those of Rathcke *et al.*, 2009, who found that patients with T1DM and T2DM have higher serum CH3L1P levels [24]. Another study was conducted by Paarivalavan *et al.*, 2015 to display the role of urinary and plasma CH3L1P in early detection of nephropathy in T2DM. They found that plasma and urinary CH3L1P levels were significantly raised in T2DM patients compared to healthful subject, which is in consistence with our results [25]. In A1 group, plasma concentrations of CH3L1P were significantly higher than in healthy participants, which can be described as an early indication of diabetic nephropathy (according to their albuminuria status in early stages of nephropathy of T2DM patients ($eGFR \geq 60$ ml/min/1.73 m²).

Some researchers reported that serum CH3L1P levels were elevated in patients with T1DM and T2DM, with an independently positive relationship between CH3L1P and albuminuria [19]. In this study, we observed a significant association of gender, height, HIP, WHR, duration of T2DM, FINS, S.UA and S.CR with CH3L1P in different albuminuria states, while no correlation between CH3L1P and CRP as an inflammatory marker appeared. These findings agree with previous findings by Lee *et al.*, 2012, who found no association between the albuminuria and CRP levels in T2DM patients [19]. They also demonstrated that both urine and plasma CH3L1P did not have a significant correlation with CRP. Moreover, our data are consistent with those of Brix *et al.*, 2011, who found that CH3L1P levels did not have any correlation with CRP level [26]. This finding could suggest the use of CH3L1P as a specific marker for the early detection of diabetic nephropathy instead of the general marker of CRP. Diabetic nephropathy is one of the most severe medical problems in the diabetic community. One-third of all diabetic patients are influenced by this pathology in the Western world [27]. In the expansion of DN, Chronic kidney disease (CKD). Diabetes-related kidney disease is a strong risk factor of the end-stage renal disease (ESRD) [28]. Glomerular hypertrophy, glomerular hyperfiltration, elevated urinary albumin excretion, basement

membrane thickness, mesangial extension, and extracellular matrix protein aggregation have all been identified as pathological signs of DN [9].

A significant predictor of DN occurrence and development is microalbuminuria. DN has been linked in recent years to pro-inflammatory cytokines and cell signaling pathways [29]. However, microalbuminuria is not an ideal DN predictor, due to pathological changes and progressive renal functions during impaired glucose tolerance. Therefore, a suitable indicator is required that can detect DN at an early stage [8]. CH3L1P is a new inflammatory marker that is linked to acute and chronic inflammations. Patients with purulent meningitis, rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, and inflammatory bowel disease have higher levels of CH3L1P, according to some reports [30]. Obesity is associated with increased macrophage infiltration to adipose tissue and represents a significant characteristic in patients with advanced insulin resistance [31]. CH3L1P may be linked to insulin resistance based on the macrophages infiltration and adipose tissue. All the studies mentioned above indicated that CH3L1P may have a link to DM [23]. Additionally, a previous research by Luo *et al.*, 2021, is in agreement with our study which showed that the level of CH3L1P is significantly higher in T2DM patients compared with healthy individuals. The concentration of CH3L1P is increased with the increase in albuminuria levels. In diabetic patients, microalbuminuria is considered as a risk factor for cardiovascular disease. CH3L1P was linked with type 1 diabetic nephropathy [24], also having elevated levels in type 2 diabetics [32]. It was also indicated that CH3L1P could play a role in the development of micro-vascular difficulties during renal vascular injury. Therefore, it is thought that CH3L1P could be beneficial as an autonomous marker [24].

Conclusion

Our study explains the correlation between serum CH3L1P level and albuminuria in patients with T2DM. The level of CH3L1P increases significantly in T2DM patients and with the development of albuminuria state; therefore, it is possible to use CH3L1P as an early indicator to detect diabetic nephropathy.

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