



ISSN: 0067-2904
GIF: 0.851

The Correlation of Lipocalin-2 and Retinol Binding Protein-4 with the Inflammatory State in Iraqi Patients with T2DM

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Abstract

Adipokines are associated with insulin resistance and obesity-related metabolic disorders in many diseases. The levels of Lipocalin-2 and Retinol binding protein-4 were evaluated in sera of patients with Type 2 diabetes (T2DM) to study the association between them and the inflammatory state as established by high levels of C - reactive protein and with atherogenicity. Serum Lipocalin-2 and Retinol binding protein-4 levels measured in 73 subjects: 48 patients with T2DM with the mean level of C- reactive protein 23.989 mg/dL. For comparison, 25 age- and gender-matched control participants enrolled with C- reactive protein CRP level 1.476 mg/dL. The results showed that circulating Lipocalin-2, and Retinol binding protein-4 levels were significantly higher in T2DM patients when compared with that of the control group (78.688vs.38.463ng/mL) and (0.0499vs.0.041μg/mL; P<0.05) respectively. Serum lipocalin-2 levels of patients group were negatively associated with RBP-4 (r= -0.248; P<0.039), and positively correlated with CRP (r=0.512; P<0.005) and with atherogenic index (AIP). Lipocalin-2, but not RBP-4, was independently associated with inflammatory state and atherogenicity that confirm the presence of cardiovascular diseases risk. A study with a large number of patients is needed to determine serum lipocalin-2 value as an early predictor for the development of cardiovascular diseases in type 2 diabetes patients.

Keywords: lipocalin-2, retinol binding protein-4, inflammation, atherogenicity, diabetes mellitus.

العلاقة بين مستويات Lipocalin-2 و Retinol binding protein-4 والحالة الالتهابية في

العراقيين المصابين بمرض السكري من النوع الثاني

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الخلاصة

تعتبر adipokines من البروتينات المرتبطة مع مقاومة الانسولين و اضطرابات السمنة- ذات العلاقة بالايض في العديد من الامراض. تم قياس مستويات Lipocalin-2 و Retinol binding protein-4 في المرضى المصابين بمرض السكري من النوع الثاني وتقدير علاقتها مع الحالة الالتهابية والمثبتة من خلال تركيز البروتين التفاعلي - C (CRP) ودالة تصلب الشرايين atherogenicity . تم قياس مستويات Lipocalin-2 و Retinol binding protein-4 في 73 موصول شخص: 48 مريض بالسكري من النوع الثاني والذين لديهم معدل مستوى CRP (23.989 mg/dL) . ولغرض المقارنه، تم استخدام مجموعة

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سيطره مكونه من 25 شخص اصحاء مطابقين بالعمر والجنس لمجموعة المرضى. أظهرت نتائج هذه الدراسه أن مستويات Lipocalin-2 و Retinol binding protein-4 في المرضى كانت اعلى معنويا من تلك لمجموعة السيطره (78.688 vs. 38.463 ng/ mL) و (0.0499 vs. 0.041µg/mL; P<0.05) على التوالي.

يرتبط Lipocalin-2 بعلاقه سالبه مع Retinol binding protein-4 ($r = -0.248$; $P < 0.039$) بينما يرتبط بعلاقه موجبه مع CRP ($r = 0.512$; $P < 0.005$) وكذلك مع دالة تصلب الشرايين (AIP) ($P < 0.05$). ان وجود ارتباط معنوي بين Lipocalin-2 و Retinol binding protein-4 (وليس CRP) مع حاله الالتهابيه (CRP) وكذلك دالة تصلب الشرايين يؤكد وجود خطر الاصابه بامراض القلب الوعائيه. أن الحاجه لدراسه مكثفه وبعده اكبر من المرضى ضروري لتعيين قيمة Lipocalin-2 المتوقعه كداله للتنبأ الاولي لتطور الاصابه بأمراض القلب الوعائيه في مرضى السكري من النوع الثاني.

Introduction:

The worldwide epidemic of obesity and T2DM has focused attention on adipocytes biology and the role of adipose tissue in the integration of systemic metabolism [1]. Adipose tissue is an endocrine tissue secreting some proteins as adipokines mostly affecting lipid and glucose metabolism, as well as insulin resistance (IR); whereas some of them as adiponectin have a protective effect in insulin resistance state, and many of these molecules exert positive or negative actions on inflammation [2].

Lipocalin-2 and retinol binding protein -4(RBP-4) are lipocalin proteins they have a common tertiary structure known as the 'lipocalin fold' in which small molecules such as lipids are attached [3].

Lipocalin-2, also known as neutrophil gelatinase-associated lipocalin, siderocalin, and 24p3, was reported to be associated with obesity and IR in both mice and humans [4, 5]. Also, lipocalin modulates inflammation in adipocytes and microphages [6]. Since different inflammatory processes can induce Lipocalin-2 expression, it was considered as an acute phase protein [7]. In obesity, its association with CRP may be an independent predictor of inflammation and could be the link between obesity and cardiovascular risk [4]. Lipocalin-2 suppresses lipopolysaccharide-induced cytokine production that could be an anti-inflammatory process [8].

The RBP- 4, a fat- derived adipokine, is expressed in adipose tissue and was linked to inflammation through its association with adiposity, T2DM, IR, and several components of the metabolic syndrome [9-12], but still the causes of its expression changes and its relationship with these states are unknown.

Furthermore, in human studies, inconsistent results were found [13, 14]. So, further studies are needed. The lipid-binding properties of lipocalins [15], may act as lipid sensors for hyperlipidemia, inflammation and atherosclerosis [16]. The pro-inflammatory and pro-atherogenesis activities of lipocalins are still unknown if it is due to its lipid-binding properties. Lipocalin-2 and RBP4 might be the links between lipid metabolism and inflammation in atherogenesis and may help to design more efficient strategies for early intervention of this disease.

In this study, we determined the levels of lipocalin-2 and retinol binding protein-4 in the serum of T2DM patients to find out their association with the inflammatory state (CRP) and atherogenicity (atherogenic index AIP).

Methods

Baseline clinical evaluation

Detailed medical, as well as, family history of obesity and cardio-metabolic risk obtained from all subjects. Selected forty-eight patients with T2DM (28 male and 20 females) from National Diabetes Center for Treatment and Research, based on their medical history according to American Diabetes Association ADA [17]. The criteria are plasma glucose (FPG) ≥ 7.0 mmol/L (126 mg/dL) or with an oral glucose tolerance test (OGTT), two hours after the oral dose a plasma glucose ≥ 11.1 mmol/L (200 mg/dL). All T2DM patients were enrolled from October 2012 to March 2013 with the ages ranging from 20 to 65 years. The control group was twenty-five healthy volunteers (11 males and 14 females) with the same age range. T2DM patients and volunteers excluded if they had a history or even manifestation of cardiovascular disease, peripheral vascular disease, coagulation disorders, neuropathy, nephropathy, insulin therapy, psychiatric illness, smoking and any acute or chronic diseases. Written informed consent obtained from all subjects.

The blood drawn from all subjects enrolled in this study in the morning after 12 -14 hours of fasting. The serum obtained after at least 10 minutes of clotting by centrifugation at 2500 rpm for 10 minutes. Then stored at (-70°C) until assayed.

Laboratory measurements

Serum Lipocalin-2, Retinol binding protein-4, and CRP measured by ELISA kits from (RayBio com., USA). The levels of CRP of patients group were (23.989 mg/dL) and (1.476 mg/dL) for the control group.

The atherogenic index of plasma (AIP) was calculated using the formula: $\log(\text{TG}/\text{HDL-C})$.

Statistical analysis

The statistical software package (SPSS) (Version 21.0, Chicago, IL) was used to analyze the data that represented as the means \pm SD. Differences between parameters were tested using ANOVA or Student's t-test, and the correlation between variables of interest was performed using Pearson's correlation. The P value less than 0.05 were considered statistically significant.

Results:

Table-1 shows that serum Lipocalin-2, and RBP-4 levels were found to be elevated in patients with T2DM (78.688vs. 38.463ng/mL, and 0.0499 vs.0.041 $\mu\text{g}/\text{mL}$ respectively; $P<0.05$).

Table 1- Mean levels (\pm SD) of Lipocalin-2, and RBP-4 of T2DM patients and control groups.

Parameters	Mean \pm SD		P value ≤ 0.05
	Patients (n=48)	Controls (n=25)	
Lipocalin-2(ng/mL)	78.688 \pm 17.736	38.463 \pm 5.589	0.000
RBP-4($\mu\text{g}/\text{mL}$)	0.0499 \pm 0.015	0.041 \pm 0.013	0.014

Significant if P values < 0.05.

Pearson correlation coefficient was calculated to elucidate further the association between serum Lipocalin-2, RBP-4, and CRP levels. Sera Lipocalin- 2 levels were negatively associated with RBP-4 ($r = -0.248$; $P < 0.013$), and positively correlated with CRP ($r = 0.512$; $P < 0.03$) as shown in Table-2.

Table 2- The Pearson correlation coefficient values of sera lipocalin-2, RBP-4 and CRP levels of T2DM patients group.

Parameters	CRP		Lipocalin-2	
	r	p	r	P
Lipocalin-2	0.512*	0.03	1	-
RBP-4	- 0.249	0.087	- 0.248*	0.013

Significant correlations if P values < 0.05, r: Pearson correlation coefficient

Lipocalin-2, but not RBP-4, showed significant correlation with atherogenic index, as shown in Table 3. A 45% of T2DM patients were at low risk while 37% were at high risk and only 16% were at intermediate risk.

Table 3- The association between Lipocalin-2 and RBP-4 levels with atherogenicity in T2DM patients

	Atherogenic Index of Plasma			P
	Low risk (<0.11)	Intermediate risk (0.11-0.2)	High risk (0.21+)	
RBP-4 ($\mu\text{g}/\text{mL}$)				0.29[NS]
Range	(0.024 to 0.127)	(0.026 to 0.067)	(0.027 to 0.135)	
Median	0.045	0.052	0.055	
Inter-quartile range	(0.033 to 0.06)	(0.041 to 0.056)	(0.043 to 0.062)	
N	22	8	18	
Mean rank	22	24.5	29.06	
Lipocalin-2 (ng/mL)				0.048*
Range	(28.8 to 118.7)	(25.5 to 108.4)	(27.7 to 93.3)	
Mean	82	76.5	68.2	
SD	19.5	24.6	22.5	
SE	4.07	8.69	5.3	
N	22	8	18	

Significant if P values < 0.05.

Discussion:

Inflammation is now established to be a key mediator of coronary artery disease development [18]. Consistent independent association found between CRP elevations and coronary risk in many epidemiologic studies. Also other studies had recognized CRP as an independent risk factor for T2DM and cardiovascular diseases [19- 21].

A positive association between lipocalin-2 concentrations and hs-CRP was reported by Wang et al. [4], suggesting that lipocalin-2 may be considered a marker of obesity-related to low-grade inflammation. Hemdahl and his colleagues have shown that increased lipocalin-2 expression in coronary atherosclerotic plaques developing myocardial infarction in mice [22].

Adipose tissue produces acute phase reactants as its contribution to the immune response that is related to cardiovascular diseases [23]. Lipocalin-2 suggested as an acute-phase protein that can be induced by a variety of in vitro inflammatory stimuli [7, 8]. Consistent with the previous data, a high positive correlation between lipocalin-2 and CRP was observed in this study, that establish the suggestion that lipocalin-2 is associated independently with inflammation.

While the results of other study had suggested that lipocalin-2 is a novel and potent molecular target for diagnosis and treatment of diabetic vascular complications [24, 25]. Other study suggested that mild inflammation may have some roles in up-regulating lipocalin -2 levels [26].

The mathematical relationship between TG and HDL-C, which represents the atherogenic index, was used to predict cardiovascular risk. An AIP values above 0.24 are associated with high cardiovascular diseases while values from 0.1 to 0.24 with medium risk and values from -0.3 to 0.1 with little risk [27, 28].

In this study, lipocalin-2 showed a significant association with AIP, leading us to suggest that lipocalin-2 may be used to predict the risk of cardiovascular diseases. In obesity states, the probable sources that contribute to the increased lipocalin-2 concentrations are adipose tissue and liver and might be useful for evaluating the outcomes of various obesity-related metabolic and cardiovascular diseases [29].

Entirely inconsistent results in human studies, RBP-4 concentrations were increased in obese individuals, and higher levels correlated with lower insulin sensitivity and other components of the metabolic syndrome [10, 30]. Also, the lack of association with T2DM, obesity, and fasting insulin levels have also been reported [31, 32]. Results concerning the relationship between RBP-4 and glucose transport protein (GLUT4) [30, 32] were different.

The results of this study agree with two studies that demonstrated elevated RBP-4 levels in impaired glucose tolerance and T2DM patients. However, no significant relationship was found between RBP4 levels with impaired insulin secretion and with insulin resistance [33]. It is possible that the correlation between RBP4 and glucose concentrations represents a secondary, rather than a primary, phenomenon as suggested by Franco et.al (2009) [34]. A question needed to clarify whether increased RBP-4 is a causative factor or merely an irrelevant bystander in T2DM pathogenesis.

The proposition that in the progression from T2DM to atherosclerosis, lipocalin might serve as a relay between inflammation and lipid metabolism. However, lipocalin-2 and RBP4 showed different correlations with various metabolic parameters, suggesting that they should have different roles in regulation [35].

In conclusion, our study suggests that serum lipocalin-2 levels, but not RBP-4, reflect the progression of atherosclerosis in T2DM, and provide a possible perspective biomarker for early detection of high- risk cardiovascular diseases and potential therapeutic targets for atherosclerosis.

Acknowledgment:

The authors are very grateful to the workers in National Diabetes Center for Treatment and Research who kindly provided us the blood samples of the patients and their information.

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