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C-REACTIVE PROTEIN/ALBUMIN RATIO IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract

Type 2 diabetes mellitus (T2DM) is an inflammatory condition. C-reactive protein (CRP) is a well-known inflammatory marker for demonstrating systemic inflammation in T2DM. We aimed to compare the diagnostic value of CRP and C-reactive protein to albumin ratio (CAR) in discriminating T2DM patients and healthy controls and to investigate the association of CAR and glycemic control markers. This cross-sectional retrospective study included 173 T2DM patients and 104 healthy control subjects. Serum albumin and CRP levels were measured with spectrophotometric and nephelometric methods, respectively. CAR was calculated by dividing CRP level by albumin level. The median (interquartile range, IQR) CAR in the T2DM patients was significantly higher than those in the controls (0.15 (0.26) vs. 0.06 (0.20), $p = 0.002$, respectively). In receiver operating characteristics curve analysis (ROC), CAR > 0.0653 had 77.21% sensitivity and 52.44% specificity in discriminating T2DM. The area under ROC curve for CAR was 0.623 (95% CI =0.544–0.701, p=0.002), which was similar to CRP. A positive correlation was found between CAR and body mass index, fasting blood glucose and HbA1c levels. As a result, CAR demonstrated poor sensitivity and specificity for discriminating T2DM patients from healthy subjects and it has little diagnostic utility in diabetes.

Key words: albumin, C-reactive protein, inflammation, type 2 diabetes

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Introduction. Type 2 diabetes is a major global public health concern and its worldwide prevalence is increasing rapidly. Type 2 diabetes can lead to both long-term microvascular and macrovascular complications associated with high morbidity and mortality rates [1]. Hyperglycemia develops due to either inadequate insulin secretion from the pancreas or insulin resistance in target cells. In the early stage of T2DM, also called prediabetes, the pancreas secretes more insulin to compensate for hyperglycemia. Over time, this leads to pancreatic insufficiency linked with decreased insulin production and ultimately the development of T2DM. Inflammation participates in the pathogenesis of T2DM. Many commonly coexisting conditions related to diabetes are thought to originate via inflammatory mechanisms [2,3]. CRP and interleukin-6 (IL-6), sensitive markers of subclinical systemic inflammation, are the most investigated inflammatory markers in diabetes to date [4]. IL-1, IL-8, transforming growth factor-beta 1 (TGF-β1) and tumour necrosis factor-alpha (TNF-α) have also been investigated in T2DM [5]. However, the measurement of these inflammatory markers is not considered practical for routine laboratory analysis.

CRP is the acute-phase plasma protein synthesized by the liver. It is produced in response to proinflammatory cytokines released from activated immune system cells and adipocytes and is a sensitive marker of systemic inflammation [6]. A meta-analysis of 18 prospective studies concluded that elevated levels of IL-6 and CRP are significantly associated with an increased risk of T2DM [7]. Albumin is a negative acute phase reactant produced by the liver. Serum albumin levels are decreased in individuals with chronic inflammation [8]. Albumin provides the majority of the total antioxidant capacity of plasma [8] and it has been reported that lower albumin levels may be associated with an increased risk of coronary heart disease, cardiovascular mortality, and carotid atherosclerosis [9].

The CAR, first introduced by Fairclough et al. [10], has been proposed to be a marker of systemic inflammation. CAR has been explored in a variety of cancer types as a prognostic predictor and it has been indicated that high pretreatment CAR can be used in the evaluation of cancer prognosis [11]. The limited number of studies investigating the CAR were conducted in patients with polycystic ovary syndrome (PCOS), a disease closely associated with insulin resistance and type 2 diabetes [12]. The authors concluded that CAR, irrespectively of body mass index (BMI), was found to have a stronger association with PCOS than either androgen excess or insulin resistance. When compared with CRP, they found that the CAR has an improved ROC curve for predicting PCOS [12]. Since diabetes is an inflammatory condition, there are many inflammatory markers related to the disease, including CRP, IL-6 and TNF-α. Despite their clinical utility, these markers, except for CRP, are not widely used in the majority of clinical laboratories. Therefore, we aimed to investigate whether we can identify new and simpler inflammatory indicator with a notable discriminative power for T2DM. To the best of our knowledge, there is a limited number of studies examining CAR

in T2DM. In the present study, we compared serum CRP levels and CAR in the patients with T2DM as a possible discriminative indicator for disease. Also, we investigated the correlation between CAR and glycemic control markers such as fasting blood glucose (FBG), fasting insulin, hemoglobin A1c (HbA1c), C-peptide levels.

Materials and methods. ***Patients.*** We retrospectively reviewed a database of 294 randomly selected patients with T2DM who attended the outpatient service of Internal Diseases at the Istanbul Education Research Hospital between 2015 and 2018. We recruited the patient group regarding only exclusion criteria to prevent bias. Blood pressure, weight, and height were measured during the visit. Body mass index (BMI) was calculated with the Quetelet formula (kg/m^2) and patients were classified as obese with a BMI $\geq$ 30 kg/m^2. All participants were questioned for the history of cardiovascular diseases. The presence of dyslipidemia was defined according to the current use of statins (a total of 43 patients were taking 20 mg statin/day), and/or abnormal lipid findings on blood chemistry. T2DM was diagnosed based on the American Diabetes Association consulting criteria [13] according to the patient's clinical anamnesis, physical examination and laboratory findings. T2DM patients population consisted of 173 subjects (78 men, 95 women). The control group included 104 age-, sex-, and BMI-matched healthy subjects (43 men, 61 women) without T2DM who visited the hospital for routine physical examinations during the same period. We included participants aged $\geq$ 30 with normal liver, thyroid and kidney function. Patients who had acute and/or chronic inflammatory diseases (defined as liver disease, kidney disease, rheumatoid arthritis, inflammatory bowel disease, sepsis or multiple sclerosis), malignancy, and cases who were taking vitamin supplements or herbal drugs for the last 3 months were excluded from the study.

The study protocol complied with the standards of the Declaration of Helsinki and the current ethical guidelines and was approved by the Institutional Ethics Board of Istanbul Education and Research Hospital (date: April 26, 2019, number: 1808). Written informed consent was obtained from each patient at the time of enrollment.

Clinical assessment and laboratory data. Demographic characteristics and laboratory data of the study cases are given in Table 1. On admission, peripheral venous blood samples were drawn from the antecubital vein of patients after overnight fasting (10–12 h) from 8 to 10 am for the routine biochemical tests. FBG and C-peptide were measured by an automatic analyzer (Beckman Coulter AU5800, Beckman Coulter, USA). Insulin was measured with an automatic analyzer (Beckman Coulter DX1800, Beckman Coulter, USA) and HbA1c was analyzed with high-performance liquid chromatography (HPLC) by Adams HA-8180V (BIODPC, Japan). Measurement of CRP and albumin levels were performed by nephelometric and spectrophotometric methods, respectively. All the measurements were completed within 2 h after collecting the samples collection.

Table 1

Demographic characteristics and laboratory data of the patient and control groups

		T2DM patients ($n = 173$)	Controls ($n = 104$)	p
Sex	Women, n (%)	95 (54.90)	61 (58.65)	0.516
	Men, n (%)	78 (45.10)	43 (41.35)	
Age (years)		55 (14)	54 (15)	0.066
BMI (kg/m^2)		30.86 ± 5.09	31.73 ± 5.45	0.193
Family history of T2DM, n (%)		96 (57.80)	8 (7.70)	**0.001**
Comorbidities, n (%)				
Obesity, n (%)		101 (58.38)	57 (54.80)	0.617
Dyslipidemia, n (%)		137 (79.19)	11 (10.58)	**0.001**
Cardiovascular diseases, n (%)		17 (9.83)	9 (8.66)	0.731
FBG (mg/dl)		136 (95)	96.5 (13)	**0.001**
Insulin (mU/L)		10.56 (8.03)	8.15 (5.80)	**0.012**
HOMA-IR		3.49 (4.09)	1.93 (1.51)	**0.001**
HbA1c (%)		7.20 (2.80)	5.60 (0.40)	**0.001**
C-peptide (ng/ml)		1.91 (1.07)	1.81 (1.16)	0.830
Total cholesterol (mg/dl)		216 (60.5)	221 (55.2)	0.113
LDL cholesterol (mg/dl)		131 (46.7)	141 (49.2)	**0.011**
HDL cholesterol (mg/dl)		46.0 (13.0)	48.0 (15.5)	**0.024**
Triglyceride (mg/dl)		148 (113.2)	129.5 (66.6)	**0.003**
CRP (mg/dl)		0.63 (1.04)	0.28 (0.83)	**0.003**
Total protein (g/dl)		7.40 (0.50)	7.40 (0.60)	0.257
Albumin (g/dl)		4.30 (0.40)	4.30 (0.30)	0.287
CAR		0.15 (0.26)	0.06 (0.20)	**0.002**

Values represent mean ± SD, median (IQR) or absolute numbers (percentages) according to the type of variables and normality of data distributions.
Legend: BMI – Body mass index; FBG – Fasting blood glucose; HOMA-IR – Homeostatic model assessment for insulin resistance; CRP – C-reactive protein; CAR – C-reactive protein to albumin ratio

CAR was calculated by dividing CRP by albumin values. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR), defined as fasting serum insulin (IU/mL) × fasting plasma glucose (mg/dl)/405 [14].

Statistical analysis. For continuous variables with normal distributions data were presented as mean ± standard deviation (SD), whereas continuous variables with non-normal distribution were presented as median (IQR). Categorical variables were presented as percentages. The Kolmogorov–Smirnov test was used to evaluate the distribution of continuous variables. Continuous variables with normal distribution were analyzed by Student's t-test (independent-sample t-test), whereas continuous variables without normal distribution were analyzed by the Mann–Whitney U test. The categorical data were analyzed by Pearson's Chi-square test. Spearman Rho correlation analyses were used to assess the relationships between variables. The optimal cut-off level for the CRP and CAR were

determined by a ROC curve analysis. The areas under the curve (AUC) were calculated to assess the power of a model to identify patients for CRP and CAR. Cut-off values showing the greatest accuracy were determined using a sensitivity/specificity versus criterion value plot. p-values of < 0.05 were considered to indicate statistical significance. The data analysis was performed by IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, 2013).

Results. The patients included 95 women (54.90%) and 78 (45.10%) men with median age of 55 years (IQR, 14 years), while the control group consisted of 61 women (58.65%) and 43 (41.35%) men with median age of 54 years (IQR, 15 years). No significant differences were observed in age ($p = 0.066$), BMI ($p = 0.193$) and sex ($p = 0.516$) between the two groups. The presence of a positive family history of T2DM was found to be significantly higher in the patient group relative to the control group ($p = 0.001$). The rate of dyslipidemia was found to be statistically higher in the patient group than in the control group ($p = 0.001$). HDL cholesterol and LDL cholesterol levels were lower, triglyceride level was higher in the T2DM group than those in the control group (p-values 0.024, 0.011, 0.003, respectively). The patient group showed significantly higher FBG values, fasting insulin values, HbA1c values, HOMA-IR, CRP values and CAR than the control group (p-values 0.001, 0.012, 0.001, 0.001, 0.003, and 0.002, respectively). No significant differences in C-peptide, total protein and albumin levels were detected between the patient and control groups (p-values 0.830, 0.257, and 0.287, respectively). Discrimination of patients and controls was assessed using the AUC. The AUC for CAR and CRP were statistically significant to discriminate between patients and controls (p-values 0.002 and 0.003, respectively). ROC curve analysis showed that CAR had almost similar discriminatory power to differentiate between T2DM and controls (AUC: 0.623, 95% Cl 0.544 to 0.701) compared with CRP (AUC: 0.621, 95% Cl 0.542 to 0.699) (Fig. 1). We determined the optimal cut-off value for CAR as 0.0653 which yielded a sensitivity of 77.21% and a specificity of 52.44%, the optimal cut-off value for CRP as 0.28 mg/dl which yielded a sensitivity of 76.47% and a specificity of 51.22% (Fig. 1). The comparison of laboratory data of T2DM group regarding the cut-off values for CRP (0.28) and CAR (0.0653) were given in Table 2 and Table 3, respectively.

CRP was found to be correlated with BMI, FBG and HbA1c (r: 0.195 $p = 0.004$, r: 0.251 $p = 0.001$, r: 0.259 $p = 0.001$, respectively). CAR was correlated with BMI, FBG, HbA1c, CRP and albumin (r: 0.204 $p = 0.003$, r: 0.251 $p = 0.001$, r: 0.262 $p = 0.001$, r: 0.998 $p = 0.001$, r: -0.214 $p = 0.001$, respectively).

Laboratory data of the T2DM group were compared regarding the cut-off values of CRP and CAR. FBG, HbA1c levels were higher, albumin level was lower under the cut-off values for CRP and CAR (Table 2 and Table 3).

Discussion. Our study is one of the limited numbers of studies in the literature assessing the diagnostic utility of CAR in diabetes. In the present study, we identified a significantly increased CAR in T2DM patients compared with healthy

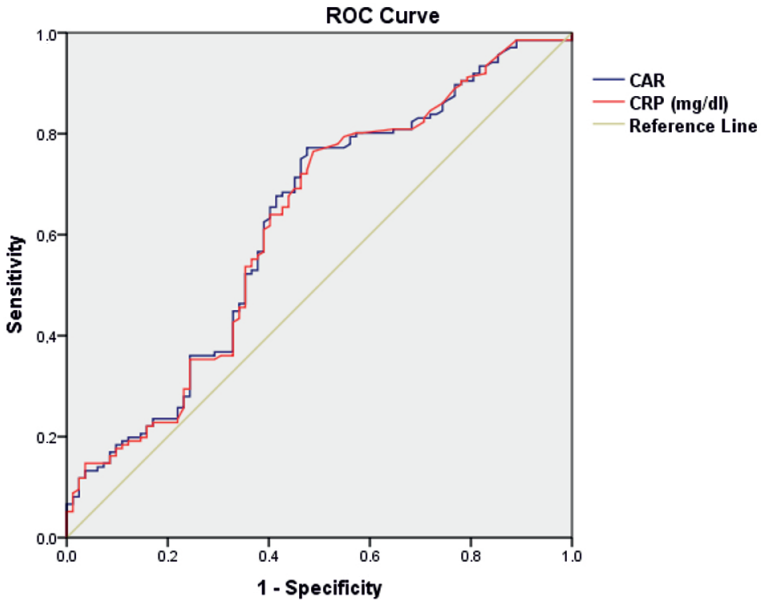

Fig. 1. Receiver operating characteristic curve plotting the true positive rate against the false positive rate for CAR (blue line) and CRP (red line) in differentiating patients and controls. The area under the curve for CAR 0.623, 95% Cl 0.544 to 0.701; for CRP 0.621, 95% Cl 0.542 to 0.699

T a b l e 2

Comparison of laboratory data of T2DM group regarding the cut-off values for CRP

	CRP $\leq$ 0.28	CRP $>$ 0.28	p
FBG (mg/dl)	101.5 (30)	123 (79)	**0.022**
Insulin (mU/L)	9.12 (5.67)	9.42 (8.30)	0.466
HOMA-IR	2.53 (1.63)	3.12 (3.71)	0.117
HbA1c (%)	5.80 (0.95)	6.60 (2.5)	**0.032**
C-peptide (ng/ml)	1.83 (1.09)	1.91 (1.24)	0.342
Total protein (g/dl)	7.4 (0.60)	7.4 (0.58)	0.381
Albumin (g/dl)	4.4 (0.40)	4.3 (0.40)	**0.028**
CAR	0.039 (0.03)	0.206 (0.46)	**0.001**

For Legend see Table 1.

subjects. However, despite this statistically significant difference, there was not a reliable relationship between CAR and diabetes as illustrated by the ROC analysis.

It is well known that systemic inflammation is an underlying contributor to T2DM and chronic subclinical inflammation is recognized as being common in the context of insulin resistance. Several studies have demonstrated that the

Table 3

Comparison of laboratory data of T2DM group regarding the cut-off values for CAR

	CAR $\leq$ 0.0653	CAR $>$ 0.0653	p
FBG (mg/dl)	101 (26)	123 (79)	**0.025**
Insulin (mU/L)	9.00 (5.87)	9.49 (8.13)	0.468
HOMA-IR	2.39 (1.64)	3.09 (3.68)	0.131
HbA1c (%)	5.80 (1.00)	6.60 (2.50)	**0.045**
C-peptide (ng/ml)	1.85 (0.98)	1.88 (1.26)	0.439
Total protein (g/dl)	7.40 (0.60)	7.40 (0.60)	0.277
Albumin (g/dl)	4.40 (0.40)	4.30 (0.40)	**0.020**
CRP (mg/dl)	0.17 (0.137)	0.91 (2.08)	**0.001**

For Legend see Table 1.

presence of inflammation predicts the development of T2DM. Duncan et al. [15] regarding the data from the Atherosclerosis Risk in Communities study, reported that low-grade systemic inflammation markers including sialic acid, orosomucoid, IL-6, and CRP are related to the development of diabetes in middle-aged adults. However, after additional adjustment for BMI, waist-to-hip ratio, fasting glucose and insulin, only the IL-6 predicts the development of T2DM [15]. The ATTICA study, performed by Pitsavos et al. [16] in Greece from 2001 to 2002, reported that the prevalence of T2DM was strongly associated with high CRP, IL-6 and TNF-α levels. In a systematic review and meta-analysis conducted by Wang et al. [7], including 10 prospective studies with a total of 19 709 participants and 4480 cases, it was concluded that elevated CRP levels were significantly associated with increased risk of type 2 diabetes. Recently, CAR has been defined as a novel potential prognostic factor and systemic inflammation marker in cancer and a predictive marker for PCOS. In a recent study, Bayrak [17] reported serum CAR levels were significantly higher in complicated diabetic patients compared to controls. Besides, any validated CAR value for predicting diabetic complications including neuropathy, nephropathy, coronary artery disease, and retinopathy was not observed [17]. Calculating CAR is simpler and cheaper than measuring other inflammatory cytokines, such as IL-6 and TNF-α. In the present study, CRP and CAR values of the T2DM patients were found to be significantly higher than those of the healthy control cases ($p = 0.003$ and 0.002, respectively). However, ROC curve analysis showed that the CAR had no greater discriminatory power to differentiate between T2DM patients and controls in comparison to CRP (Fig. 1). Increased serum albumin levels were reported to be associated with metabolic syndrome and insulin resistance [18, 19]. In contradiction with earlier findings, we found no association between HOMA-IR and albumin level and there was no significant difference between patient and control groups for plasma albumin levels. Mean albumin level was lower in the T2DM group, however, it did not reach

statistical significance, thus the increase in the CAR was mainly derived from the increase in the CRP. The magnitude of the increases in the concentrations of acute phase proteins varies from one protein to another. There is evidence that the response to chronic inflammation varies from one protein to another. It is about 50% in the case of CRP [20] but might be less for albumin. Perhaps this may be the answer to the question why the discriminatory power of CAR to differentiate between T2DM patients and controls was no greater than that of CRP alone.

As for the potential limitations of the current study, we used HOMA-IR to measure IR and not the hyperinsulinemic-euglycemic clamp which is the gold standard. Also, pre-existing comorbid conditions with inflammatory processes such as dyslipidemia and prescribed medications that we cannot manage may have affected the level of CAR and CRP. As a matter of fact, LDL cholesterol levels were found to be lower in the T2DM group because of statin usage.

All these findings of our research support the link between systemic inflammation and T2DM. Although the performance of CAR is nearly equal to that of CRP to discriminate between T2DM patients and healthy subjects, our findings suggest that CAR can possibly serve as an inflammatory biomarker for patients with T2DM in clinical practice. CAR may be particulary relevant in evaluating the efficacy of new treatments targeting inflammation in T2DM. Further prospective research is necessary to assess the diagnostic utility of CRP and CAR in diabetes. For example, prospective follow-up cohorts may determine optimal cut-off points for different age groups. Our findings may be improved by large-scale prospective population studies to compare CAR and CRP in predicting the incident of T2DM and monitoring the disease. Also, we suggest a multi-centre study to clarify the diagnostic role of CAR in diabetes.

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