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C-PEPTIDE AS AN INFLAMMATORY MARKER IN OBESE WOMEN

B. Can[1,*], S. Ozturk[2], K. Gungor[1], M. Sargin[2]

[1]Istanbul Medeniyet University Faculty of Medicine − Department of Internal Medicine, Division of Endocrinology and Metabolism, [2]Department of Family Medicine, Istanbul, Turkey

Abstract

Background. Insulin resistance (IR) is a component of type 2 diabetes and metabolic syndrome and it increases in the presence of chronic inflammation. Lately, "neutrophil-to-lymphocyte ratio" (NLR) has been used as an indicator of inflammation. This study evaluates the association between IR and NLR in obese women.

Material and methods. Obese female patients who were followed up in a university hospital for the last two years were included in the study. Homeostasis model assessment of IR (HOMA-IR), C-peptide, NLR, bioelectrical impedance measurements of 83 patients were analyzed.

Results. The C-peptide levels of our patients showed a highly significant correlation with HOMA-IR ($p<0.001$). A significant positive correlation was found between fasting plasma C-peptide levels and NLR ($r=0.36$ and $p<0.003$) in obese women. The increase in C-peptide levels had a significant effect on the increase in NLR ($r2=0.31$, $p=0.002$), however insulin had no similar effect on NLR ($r2=0.01$, $p=0.544$).

Conclusion. Plasma C-peptide levels are better correlated with NLR compared to other parameters of IR. C-peptide may be used as an efficient laboratory marker with high relevance in IR and chronic inflammatory conditions in obese women.

Keywords: inflammatory marker, neutrophil-to-lymphocyte ratio "NLR", C-peptide, insulin resistance, obesity.

INTRODUCTION

Obesity has been defined as abnormal fat accumulation that impairs health in the body and has been recognized as a disease by the "American Medical Association" (1). Obesity is estimated to shorten life expectancy by 4% (2). According to the 2016 data of the World Health Organization, 52% of the world population is overweight or obese, and it is predicted that this rate will increase to 60% in 2030 (3).

In obesity, there is a low-grade chronic inflammatory process due to proinflammatory cytokines released from increased adipose tissue. Inflammation is also a risk factor for the development of atherosclerosis (4,5). Therefore, an increase in mortality due to atherosclerosis and cardiovascular diseases can be seen in obesity.

Obesity is mostly associated with insulin resistance (IR). IR is a component of type 2 diabetes and metabolic syndrome, and just like these two conditions, there is a chronic, low-grade, inflammatory process in IR. IR is usually associated with increased adipose tissue mass. Substances such as adipose tissue-derived tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), visfatin, and free fatty acids have a negative effect on pancreatic β-cell functions and ultimately cause IR (6,7). Lately, "neutrophil-to-lymphocyte ratio" (NLR) has been used as an indicator of inflammation (8-11). NLR is calculated by dividing the number of neutrophils by the number of lymphocytes. NLR is a reliable and affordable test that is easily accessible (8-11).

The gold standard method for measuring IR is the euglycemic clamp technique. The 'homeostasis model assessment of IR' (HOMA-IR) method is preferred, which gives similar results to this laborious and expensive method. In recent studies, it is emphasized that serum C-peptide level can also be used in IR evaluation (12,13).

In this study, based on the knowledge that obesity progresses with a chronic inflammatory process, the association between insulin resistance and NLR was investigated in obese women.

MATERIALS AND METHODS

This is a case study of obese female patients who have been followed up in the obesity outpatient

*Correspondence to: Bulent Can MD, Istanbul Medeniyet University Faculty of Medicine, Internal Medicine, Division of Endocrinology and Metabolism, Goztepe, Istanbul, 34700, Turkey, E-mail: blntcn34@gmail.com

clinic of a university hospital. All patients included in the study attended their scheduled appointments regularly for at least two years. With the approval of the local ethics committee, the clinical and laboratory data in the patient follow-up forms used in the obesity outpatient clinic of our center were evaluated.

The body mass index (BMI) formula [BMI=weight (kg) / height (m^2)] was used for the diagnosis of obesity. A BMI of 30 or over was evaluated as "obesity" (1,2). Patients were categorized into three groups according to their BMI values; group 1 consisted of patients with BMI= 30-34.9 kg/m^2, group 2 consisted of patients with BMI= 35-39.9 kg/m^2 and group 3 consisted of patients with BMI ≥40 kg/m^2. The body composition analyzer (bioelectrical impedance analysis "BIA" measuring device) was used to evaluate the obesity status (14). Body composition analyses of the patients were performed with TANITA® (MC-780U, Japan) brand body bioelectrical impedance analyzer.

The IR of the patients was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) formula (HOMA-IR= [Fasting insulin (μU/ml) × Fasting glucose (mg/dL)] / 405), simultaneous fasting plasma C-peptide levels were recorded. Neutrophil-to-lymphocyte ratio (NLR), which is a hemogram parameter, was used as an inflammatory marker. Patients with known diabetes, malignancy, pregnancy, acute infection or inflammatory disease and male patients who constituted less than 10% of the study population were excluded from the study. As a result of this screening, 83 women who fulfilled the study criteria were evaluated. The analysis aimed to compare the correlations between insulin and C-peptide, as markers of the inflammatory process.

Statistical analysis

Descriptive analyses were performed, the relationship of independent variables (fasting blood glucose, insulin, C-peptide, HOMA-IR, BMI, body

Table 1. Descriptive data of patients at first admission to the outpatient clinic

	mean ± SD	**(min.-max.)**
Age (year)	49.06±10.54	18-77
BMI (kg/m^2)	37.75±5.64	30-56
Weight (kg)	95.47±15.37	66.9-134.6
Whole body fat mass (**WBFM**) (kg)	38.5±9.73	22.8- 62.3
Central fat mass (CFM) (kg)	15.45±3.72	8.5-26.3
CFM / WBFM (%)	40.7±6.28	20-64
CFM / weight (%)	16.19±2.84	9-24
Body fat percentage (BFP) (%)	39.84±4.2	31-54
Glucose (mg/dL)	98.66±10.14	71-117
Insulin (uU/mL)	13.36±11.91	1.0-89.8
C-peptide (ng/mL)	2.98±1.44	1.14-8.4
HOMA-IR	3.34±3.33	0.18-25.72
HbA1c (%)	5.69±0.39	4.4-6.4
Leucocyte (10^3/μL)	6.967 ± 1.470	3.740-10.500
Neutrophil (10^3/μL)	4.007 ± 1.329	1.400-7.620
Lymphocyte (10^3/μL)	2.312 ± 0.5545	1.210-3.510
NLR	1.84 ± 0.79	0.69-4.12

BMI: Body mass index, WBFM: Whole body fat mass, CFM: Central fat mass, BFP: Body fat percentage (BFP=whole body fat mass /weight), HOMA-IR: Homeostasis model assessment of insulin resistance, HbA1c: glycated hemoglobin, NLR: Neutrophil-to-lymphocyte ratio, SD: standard deviation.

Table 2. Correlation analysis between "neutrophil lymphocyte ratio and C-peptide" and "insulin resistance parameters and body fat components"

	NLR		**C-peptide**	
	r	**p**	**r**	**p**
Fasting blood glucose	0.20	0.07	0.11	0.363
Insulin	0.25	**0.037**	0.72	**<0.001**
C-peptide	0.36	**0.003**	-	-
HOMA-IR	0.26	**0.029**	0.69	**<0.001**
BFP	0.00	0.998	-0.09	0.488
CFM / weight	-0.13	0.26	-0.17	0.16

HOMA-IR: Homeostasis model assessment of insulin resistance, BFP: Body fat percentage (BFP=whole body fat mass / weight), CFM: Central fat mass, NLR: Neutrophil-to-lymphocyte ratio.

fat percentage "BFP", whole body fat mass "WBFM", central fat mass "CFM") with each other and their effects on each other were tested with Pearson correlation analysis and linear regression analysis.

For intragroup BMI analysis, either ANOVA test or Kruskall-Wallis test was used depending on the distribution of data. Receiver operating characteristic (ROC) curves were obtained and the optimal C-peptide value with the greatest total sensitivity and specificity was selected in the prediction of insulin resistance. If the p value was below 0.05, it was considered statistically significant. SPSS Statistics 21.00 (for Windows, Chicago, US) program was used for statistical analysis of our study.

RESULTS

The mean age among the 83 study participants was 49 (±10.54), with a lower limit of 18 and an upper limit of 77 years. The patients were generally in the moderately obese category, with a mean BMI=37.74 (30-56) kg/m^2. It was observed that the mean HbA1c levels of non-diabetic obese patients were at the border of 5.7% (5.69%), which is accepted as prediabetes (Table 1). The mean NLR of the patients was 1.84 ± 0.79.

When the relationship between NLR and C-peptide levels, insulin and HOMA-IR index in obese female patients was examined, it was found that they were significantly correlated with each other (p=0.003, p=0.037 and p=0.029, respectively) (Table 2).

In obese women, linear regression analysis was performed to show which parameters (including BMI, whole body fat mass, central fat mass, HOMA-IR, plasma fasting insulin and C-peptide levels) were more affected by the change in NLR values. We observed that the increase in C-peptide levels had a significant effect on the increase in NLR (r2=0.31, p=0.002), however insulin had no similar effect on NLR (r2=0.01, p=0.544) (Table 3).

Patients were categorized into three groups according to their BMI values; group I (BMI=30.0-34.9) [n=28]), group II (BMI=35.0-39.9, [n=33]) and group III (BMI≥40, [n=22]). We found no statistically significant difference between the three groups in terms of C-peptide (Fig. 1) and NLR values (p=0.187, p=0.071, respectively).

In addition, ROC analysis was performed to determine the C-peptide cut-off value, which predicts insulin resistance, and patients were categorized as those with a C-peptide value below 2.5 and ≥2.5. Then, NLR, CRP and neutrophil levels between the groups were compared. While there was no difference between the groups in terms of NLR and CRP levels, neutrophil levels were statistically significantly higher in the group with C-peptide level ≥ 2.5 (7821±1511 *vs.* 6509±1254, p=0.021).

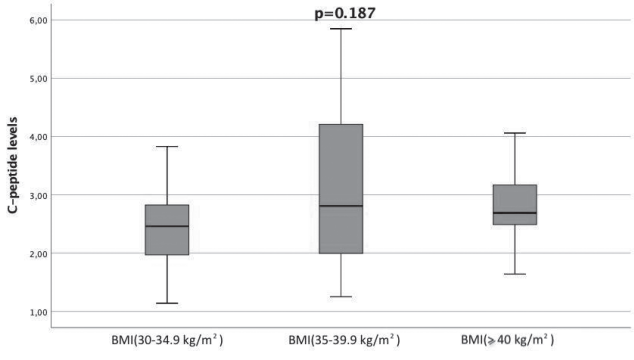

Figure 1. Mean c-peptide levels of BMI groups

Table 3. Linear regression analysis of parameters that may affect the neutrophil-to-lymphocyte ratio (NLR)

	Standardized coefficients Beta *	p
BMI	0.358	0.236
WBFM	-0.017	0.969
CFM	-0.195	0.443
Insulin	-0.103	0.544
C-peptide	0.553	**0.002**

BMI: Body mass index, WBFM: Whole body fat mass, CFM: Central fat mass.
* The beta values on the table are standardized values.

DISCUSSION

Obesity progresses with a chronic, subclinical inflammation. It predisposes the patient to IR, diabetes, and atherosclerotic cardiovascular diseases (15,16). Combating obesity is important in preventing cardiovascular disease and related mortality (17). The level of inflammatory markers in plasma increases in obesity due to chronic inflammation. In this study, neutrophil-to-lymphocyte ratio (NLR), an inflammatory marker that can be used in daily practice, was used. It was shown that NLR was correlated with IR, and that C-peptide levels were significantly associated with NLR.

An indicator of inflammation, NLR, which is a reliable, inexpensive and widely accessible test, has been used frequently in studies conducted in recent years (8-11). Neutrophils are the first immune cells to increase in response to inflammation (18). In diabetic patients, lymphocyte proliferation has decreased due to interleukin-2 receptor expression (19). Tanaka *et al*. showed that T lymphocytes decreased in obese

individuals and lymphopenia was associated with inflammation via TNF-α (20). NLR reflects both lymphocyte and neutrophil count. Therefore, it is a powerful parameter reflecting inflammation (8,21). It has been shown that NLR increases in many conditions which progress with inflammation (21-24). In our study, in accordance with the literature, it was observed that NLR in obese women was correlated with insulin resistance index HOMA-IR and fasting C-peptide levels. The mean NLR of our patients (1.84±0.79) was in the range of 0.78-3.53, which is considered to be the average of normal healthy adults according to the literature (25). The other parameters that may affect the NLR of the patients were examined by linear regression analysis. The increase in C-peptide levels had a significant effect on the increase in NLR. This relationship was not observed with insulin. Therefore, C-peptide may reflect the level of inflammation in obesity as well as IR. Metabolically healthy obesity is defined as obesity that is without metabolic abnormalities or increased cardiometabolic risk factors (26). The absence of inflammatory effects of insulin in non-insulin resistant (NIR) obese individuals supports our findings that C-peptide has possible pro-inflammatory consequences in obese women.

In fact, obesity is mostly associated with IR. The term IR refers to the resistance that develops against the metabolic effects of insulin. There is a chronic, low-grade, inflammatory process in IR. IR is usually associated with increased adipose tissue mass. It is known that an increase in adipose tissue mass has a negative effect on pancreatic β-cell functions. Visceral tissue-derived substances such as TNF-α, IL-6, visfatin, asymmetric dimethyl arginine, and free fatty acids may play a role in the development of IR (6,7). Direct and indirect methods can be used to measure IR. The gold standard method for measuring IR is the euglycemic clamp technique. The 'homeostatic model evaluation' (HOMA) method is favored, which gives similar results to this difficult and expensive method. C-peptide is secreted by the pancreas in equal amounts with insulin, but while insulin undergoes first-pass removal in the liver, C-peptide is degraded in the renal cortex. Therefore, its half-life is much (about 6 times) longer than insulin. The half-life of insulin is 3-5 minutes, while the half-life of C-peptide is 20-30 minutes (12,27). Therefore, it is stated that plasma C-peptide levels and C-peptide indices can be used as a more stable indicator for calculating IR (12,13,27). In our study, there was a highly significant correlation between fasting plasma C-peptide level and HOMA-IR in obese women, supporting this view.

When the patients were categorized according to their BMI, there was no statistically significant difference between the groups regarding their C-peptide levels. This may be due to the relatively small size of our study sample.

C-peptide measurement is used for differential diagnosis of hypoglycemia. Likewise, it has an important role in deciding the treatment regimen of diabetes mellitus. Measuring C-peptide at regular intervals may be beneficial in determining the insulin reserve of diabetic patients as well as predicting the degree of inflammation. It may even be a promising marker for estimating the cardiovascular disease risk.

Body mass index (BMI) is a frequently used scale in the diagnosis of obesity. However, it may not be an accurate and sufficient measurement method in muscle-developing athletes, sarcopenic patients, and in cases of edema such as heart or kidney failure. As it does not directly measure body fat, it may not entirely reflect the inflammation in obesity. However, in the definition of obesity, the abnormally increased amount of body fat is emphasized (1,2). Obesity can be expressed as a body fat percentage exceeding 25% in men and 32% in women (28). Therefore, BMI should be used as a part of clinical evaluation, not as the only diagnosis method of obesity (14). Bioelectrical impedance analysis (BIA) can be used for the diagnosis and follow-up of obesity besides determining body fat mass (14). In our study, it was found that body fat percentages or whole-body fat mass (WBFM) measured by BIA in obese women were not associated with NLR. It can be explained by the exclusion of male patients in our study, and may also be due to the ethnic difference in body fat distribution of women in Turkish population.

Some study limitations are worth mentioning. It was a limitation that only female patients were included in our study. Since the number of male patients meeting the inclusion criteria of our study was less than 10% of the total number of cases, male patients were omitted. Another limitation is that our study had a relatively small sample size. The strength of our study is that the patients who came to the obesity outpatient clinic regularly were followed up for a long time. Additionally, it has been shown that the level of C-peptide in obese women may reflect the level of inflammation besides IR.

In conclusion, when evaluating the association between IR and inflammatory markers in obese women, fasting plasma C-peptide levels were found to be highly correlated with HOMA-IR. In addition,

when examined with linear regression analysis, it was observed that while the increase in C-peptide levels had a significant effect on the increase in NLR, there was no such relationship between insulin and NLR. Therefore, we conclude that plasma C-peptide may be used as an efficient laboratory marker with high relevance in IR and chronic inflammatory conditions including obesity and pre-diabetes. However, prospective clinical studies are required to clarify the possible mechanisms underlying the association between C-peptide and inflammation.

Conflict of interest

The authors declare that they have no conflict of interest.

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