Annals of Clinical and Analytical Medicine
Original Research

Increased insulin secretion suppresses cortisol levels, exacerbates inflammation and beta-cell dysfunction

Increased insulin resistance with cortisol levels and HOMA- β

Evin Kocatürk[1], Ezgi Kar[2], Zeynep Küskü Kiraz[1], İ. Özkan Alataş[1]
[1]Department of Medical Biochemistry, Faculty of Medicine, Eskişehir Osmangazi University, Eskişehir
[2]Department of Medical Biochemistry, Faculty of Medicine, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

Abstract
Aim: The role of inflammatory mechanisms in the formation of insulin resistance (IR), diabetes and metabolic syndrome has been widely discussed in recent years. The aim of this study was to investigate the relationship between IR and pancreatic β-cell function with hematological inflammatory markers and cortisol levels.
Material and Methods: Four hundred fifteen adult patients whose samples were accepted to the laboratory between the hours of 08:00-12:00; leukocyte, neutrophil and lymphocyte count, mean platelet volume (MPV), insulin, glucose, and cortisol levels were examined retrospectively. The neutrophil-lymphocyte ratio (NLR), the homeostasis model assessment (HOMA)-IR and HOMA-β values were calculated according to the collected data. The patients were divided into two groups: with (HOMA-IR≥2.5) and without IR (HOMA-IR<2.5). All data were statistically evaluated using the SPSS package program.
Results: A statistically significant difference was found in cortisol levels (p=0.003), leukocyte (p<0.001), neutrophil (p<0.001), lymphocyte counts (p=0.003) and NLR (p=0.011) between the groups. However, there was no significant difference between the MPV levels. Both HOMA-IR and HOMA-β showed a weak positive correlation with leukocyte, neutrophil and lymphocyte counts and showed a negative correlation with cortisol levels. There was a weak positive correlation between NLR levels and HOMA-IR. Although there was a negative correlation between cortisol with insulin and NLR levels, no significant correlation was found between cortisol and glucose, neutrophil-lymphocyte count.
Discussion: The significant increase in hematological inflammatory cells in patients with IR suggests that inflammatory mechanisms may have produced insulin resistance. The increase in insulin levels and suppression of cortisol levels may play a role in the progression of inflammation.

Keywords
Insulin Resistance; Neutrophils; Lymphocytes; Mean Platelet Volume; Hydrocortisone

DOI: 10.4328/ACAM.20591 Received: 2021-03-15 Accepted: 2021-05-18 Published Online: 2021-06-17 Printed: 2021-09-15 Ann Clin Anal Med 2021;12(Suppl 4): S365-369
Corresponding Author: Ezgi Kar, Department of Medical Biochemistry, Faculty of Medicine, Çanakkale Onsekiz Mart University, 17020, Terzioğlu, Çanakkale, Turkey.
E-mail: ezgikar@comu.edu.tr P: +90 5546189450
Corresponding Author ORCID ID: https://orcid.org/0000-0003-2134-4067

Introduction

Diabetes mellitus (DM) is a chronic, broad-spectrum metabolic disorder, which requires constant medical care, and the organism cannot benefit enough carbohydrate, fat and proteins [1]. The global prevalence of DM was 9% in 2014, and in 2015 almost 1.6 million deaths were caused directly by DM in the world (available at: https://apps.who.int/iris/bitstream/handle/10665/112736/9789240692763_eng.pdf;jsessionid=A0D24CBF0DD2997742383515E2739EC6?sequence=1).

It is now accepted that diabetes develops through two mechanisms: insufficient insulin release and insulin resistance (IR) due to dysfunction of pancreatic β cells. The homeostasis model assessment (HOMA) model is a widely accepted method for assessing β-cell function (with HOMA-β) and IR (with HOMA-IR) from basal glucose and insulin concentrations [2-4]. Higher HOMA- β level is associated with beta-cell response or insulin secretion [5], and increased HOMA-IR and decreased HOMA-β have been shown to significantly predict type 2 diabetes [6-8].

Inflammation can affect insulin signaling, indirectly increasing the risk of DM [9], and the increase of cytokines disrupts beta cell function [10]. Many studies confirm that increased hematological inflammatory markers (especially WBC levels) are an independent risk factor for insulin resistance, diabetes, metabolic syndrome, and coronary artery disease [11-13].

The neutrophil- lymphocyte ratio (N/L ratio-NLR) is known to be related to inflammation. Both the types of malignancy and chronic diseases have been shown to be related to subclinical inflammation in various literature studies [14, 15]. However, although the relationship between IR and inflammation has been described in the literature, there is not enough information about NLR levels' relationship.

Cortisol plays very important regulatory roles in glucose metabolism and plays an important role in the insulin signaling pathway. It disrupts insulin sensitivity in various tissues, reduces glucose uptake by inhibiting the interaction with the insulin receptor in cells, especially GLUT 4 in which adipose and muscle tissues, and contributes to IR [16, 17]. There is insufficient information about the effect of increased cortisol on pancreatic cell function and insulin secretion [17].

Cortisol is associated with inflammation and increases the number of neutrophils in the peripheral circulation by stimulating the production of neutrophils from the bone marrow in the case of inflammation. Also, it induces the production of anti-inflammatory cytokines while suppressing the proinflammatory cytokines.

In this study, we aimed to investigate the relationship between IR and pancreatic β cell function with hematological inflammatory markers (leukocyte, neutrophil and lymphocyte counts, neutrophil/lymphocyte ratio, mean platelet volume) and cortisol levels.

Material and Methods

Subjects, inclusion and exclusion criteria

For this cohort study, the necessary permission was obtained from the local ethics committee (Decision number: 30.04.2019/34). Four hundred fifteen adult patients (18-60 years) who applied to the Biochemistry Laboratory of Eskişehir Osmangazi University Hospital for checkup between the hours of 08:00-12:00 from January 2017 to 2019 were included the study. Individuals with defined any chronic, (rheumatoid arthritis, thyroid dysfunction, etc.) or malignancy disease, Diabetes Mellitus or obesity disease, pituitary gland diseases (including Cushing syndrome), irregular menstrual cycle disease, post-surgical patients and pregnant women were excluded from the study.

Data collection and calculations

In the early hours of the morning, fasting blood was collected from the participants into one red-capped hemogram tube (for serum samples) and two purple-capped EDTA-containing tube (for plasma and whole blood samples). Fasting insulin, and glucose levels were observed in serum samples, leukocyte (WBC), mean platelet volume (MPV), neutrophil and lymphocyte counts were observed in whole blood samples, and cortisol levels were observed in plasma samples. According to the electrochemiluminescence immunoassay (ECLIA, Elecsys system), sandwich method, insulin levels were measured with appropriate commercial reagents on Roche Cobas e 801 auto-analyzer device (Roche Diagnostic GmbH, Mannheim, Germany). Participants' glucose values were measured on a Roche Cobas c 702 auto-analyzer device using commercial kits, including the reference spectrophotometric hexokinase method. According to the ECLIA competitive method, cortisol levels were measured with appropriate commercial reagents on a Roche Cobas e 601 auto-analyzer device. Sysmex XN-9000 (Sysmex Co., Kobe, Japan) automatic analyzer was used for hematological inflammatory markers. HOMA-IR and HOMA-β values of the participants were calculated by the following equations in the literature, respectively:

HOMA-IR = [fasting plasma glucose (mg/dL) × fasting insulin level (mIU/L)] / 405,

HOMA-β = [360 ×fasting insulin level (mIU/L)] / [fasting plasma glucose (mg/dL) – 63] [2, 4].

IR was defined as a HOMA-IR value equal or greater than 2.5 [18], and patients were divided into two groups according to the absence (HOMA-IR <2.5 – Group I) or presence (HOMA-IR ≥2.5 – Group II) of IR. In addition, the neutrophil/lymphocyte ratio (NLR) levels of patients were calculated by proportioning the obtained neutrophil and lymphocyte count.

Statistical Analysis

All parameters were analyzed using the Kolmogorov-Smirnov and Shapiro-Wilk normality tests. The Mann-Whitney U statistic test was used for the parameters that did not show normal distribution. Correlations of parameters were examined with the Spearman correlation test. All statistical evaluations were performed with the SPSS package programs. Significance value (p) < 0.05 was considered statistically significant.

Results

The baseline characteristics of the participant are summarized in Table 1. According to the HOMA-IR values, 148 of the participants were included in Group I (absence of IR) and 267 in Group II (presence of IR). The average age of the groups was 37.5 (27.0-48.75) and 42.0 (28.0-50.0) years, respectively ((Median (25-75%), Table 1). Females were dominant than males in both groups. As expected, there was a significant difference between insulin and glucose concentrations between

Groups I and II (Figure 1).

When Group I and Group II were compared, a statistically significant difference was found between cortisol levels (p=0.003), leukocyte (p<0.001), neutrophil (p<0.001), lymphocyte count (p=0.003) and NLR (p=0.011). In Group II, cortisol levels were significantly lower and other parameters were significantly higher than in Group I. MPV levels were higher in Group II, but there was no significant difference between Group I (Table 2).

Both HOMA-IR and HOMA-β showed a positive correlation with hematological inflammatory markers and showed a negative correlation with cortisol levels (Table 3). There was a weak positive correlation between NLR levels and HOMA-IR (p =0.007, r =0.132). Although there was a negative correlation between cortisol with insulin and NLR levels (p <0.001, r = -0.182; p =0.008, r = -0.129, respectively), no significant correlation was found between cortisol and glucose, neutrophil and lymphocyte count (Table 3).

Table 1. Age and gender characteristics of patients

Parameters	Group I (n=148)	Group II (n=267)	p- value
Age (years) (Median % (25-75)	37.5 (27.0-48.75)	42.0 (28.0-50.0)	0.249*
Gender (F/M)	110/37	204/64	0.916#

*Mann-Whitney U Test, F: female; M: male, #chi -square test.

Table 2. Comparison of cortisol and hematological inflammation markers between the groups

Parameters	Groups	Median (25-75%)	p*
Cortisol (µg/dL)	Group I	16.1 (13.0 – 19.3)	0.003
	Group II	14.5 (11.6 – 18.1)	
Leukocyte (x10³/HPF)	Group I	6.9 (5.8 – 8.2)	<0.001
	Group II	7.9 (6.8 – 9.4)	
Neutrophil (x10³/HPF)	Group I	3.6 (2.8 – 4.6)	<0.001
	Group II	4.4 (3.5 – 5.4)	
Lymphocyte (x10³/HPF)	Group I	2.4 (2 – 3.0)	0.003
	Group II	2.7 (2.1 – 3.3)	
NLR	Group I	1.6 (1.1 – 2.0)	0.011
	Group II	1.7 (1.3 – 2.2)	
MPV (fL)	Group I	8.9 (8.1 – 10)	0.372
	Group II	9.1 (8.3 – 9.9)	

*Mann-Whitney U Test

Table 3. Correlation of hematological inflammation markers and cortisol levels with insulin resistance parameters

		Cortisol (µg/dL)	Leukocyte (x10³/HPF)	Neutrophil (x10³/HPF)	Lymphocyte (x10³/HPF)	NLR	MPV (fL)
Insulin (mIU/ L)	r	-0.182	0.342	0.289	0.241	0.098	0.049
	p	<0.001	<0.001	<0.001	<0.001	0.046	0.319
Glucose (mg/dl)	r	-0.017	0.086	0.148	-0.052	0.180	-0.013
	p	0.726	0.082	0.002	0.290	<0.001	0.793
HOMAβ	r	-0.157	0.327	0.227	0.301	-0.018	0.060
	p	0.001	<0.001	<0.001	<0.001	0.716	0.224
HOMAIR	r	-0.170	0.320	0.290	0.199	0.132	0.029
	p	0.001	<0.001	<0.001	<0.001	0.007	0.559
Cortisol (µg/dL)	r	1.00	-0.019	-0.053	0.057	-0.129	-0.027
	p	-	0.701	0.283	0.247	0.008	0.578

Spearman correlation test, r= Correlation Coefficient, p= Significance (2-tailed)

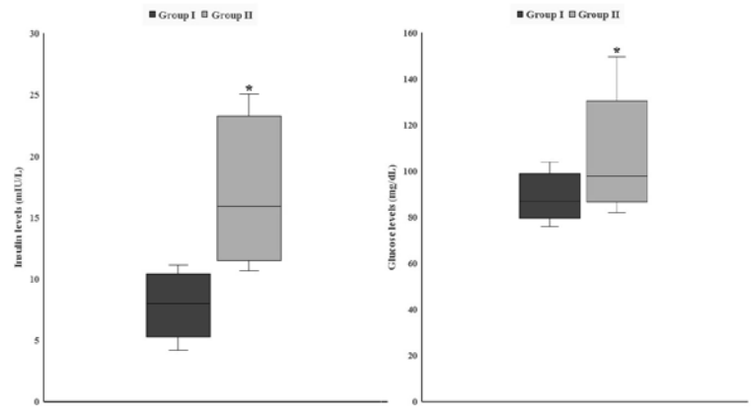

Figure 1. Insulin concentrations, Group I: 8.0 (6.36–9.66), Group II: 17.9 (13.9–25.0) µUI/mL

*p<0.001. Glucose concentrations, Group I: 87 (83–94), Group II: 98 (91–111) mg/dl

*p<0.001. Concentrations are presented as median (25–75%)

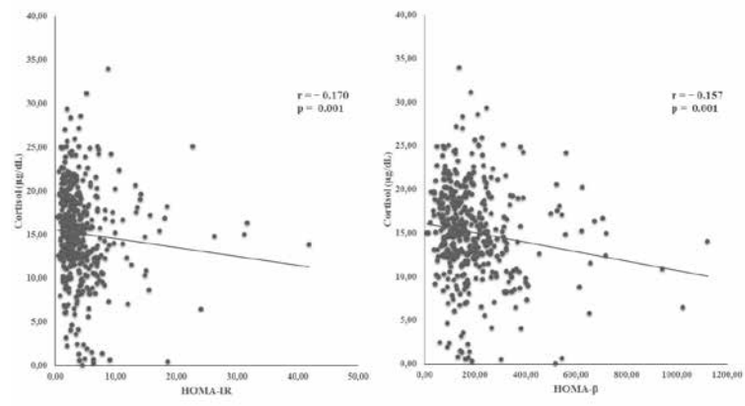

Figure 2. Correlation relationships between cortisol levels with HOMA-IR and HOMA-β (Scatter plot correlation analysis)

Discussion

Many studies in the literature have shown that diabetes is associated with inflammation [19, 20]. Although the molecular mechanisms leading to IR formation are still unclear, epidemiological studies have demonstrated that systemic inflammation and IR coexistence play a role in the pathogenesis of diabetes [20]. Increased neutrophil and decreased lymphocyte count indicate the immune system's response to different physiological diseases, and the neutrophil/lymphocyte ratio (NLR) is now considered an important inflammatory marker for evaluating the degree of disease [19, 21, 22]. In our study, hematological inflammatory markers were found to be high in patients with IR. Although both neutrophil and lymphocyte count was higher, the NLR was also found to be significantly higher in patients with IR, as the increase in neutrophil counts was higher (p = 0.011). The positive correlation of these hematological inflammatory markers with both HOMA-IR and HOMA-β suggested that inflammation was associated with IR and β cell dysfunction.

Glucocorticoids (GC) are a main group of endocrine regulating hormones that are released in our body in cases of stress. GCs and especially cortisol increase serum glucose levels by

increasing gluconeogenic gene expression and gluconeogenesis in the liver, as well as inhibiting the entry of glucose into muscle and adipose tissue by inhibition of GLUT 4 receptor translocation [23, 24]. These mechanisms may lead to impaired glucose tolerance, development of IR and excessive cortisol release leading to diabetes. However, there is no precise information about the development of IR or whether it causes pancreatic β cell dysfunction. In our study, cortisol levels were lower in patients with IR and cortisol levels were negatively correlated with HOMA-IR, HOMA-β and insulin levels (r = -0.17 p =0.001, r = -0.157 p =0.001, r = -0.182 p <0.001, respectively). This shows us that cortisol causes β cell dysfunction rather than IR. Our results suggest that cortisol may directly suppress β-cell function, and in this case, higher cortisol levels may increase the risk of impaired glucose metabolism independent of induction of insulin resistance. At the same time, the negative correlation of cortisol with NLR (r = -0.129, p=0.008) indicates that inflammation may be exacerbated by decreasing cortisol levels. In our study, fasting glucose and insulin levels of the participants were studied in order to determine insulin resistance in a healthy way. In addition, the hours when the cortisol level is the highest in healthy people between 8:00 and 12:00 hours were preferred [24].

MPV is one of the hemogram parameters measured as a marker of blood platelets. MPV values are the subject of recent studies that may be evaluated as early markers of inflammatory diseases such as Type I Diabetes Mellitus and atherosclerosis [25]. Hyperglycemia induces non-enzymatic glycosylation of some proteins present on the platelet surface. Therefore, platelet reactivity is reduced because the glycation of the membrane proteins changes membrane fluidity [25]. Our study aimed to investigate insulin resistance and cortisol levels with MPV levels as potential hematological inflammatory markers. However, we did not observe significant difference or correlation with MPV levels.

The limitations of our study can be summarized as follows: due to the retrospective nature of the study, all the data of the participants were obtained through our laboratory data system, and although it was examined and excluded from the study whether they had chronic diseases, their regular drug use that may affect the parameters could not be determined.

Conclusion

In conclusion, a significant increase in hematological inflammatory markers in patients with IR suggests that inflammatory mechanisms may have produced IR. The increase in insulin levels and suppressing cortisol levels may have played a role in making inflammation more pronounced.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *None*

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Satman İ, İmamoğlu Ş, Yılmaz C. TEMD Diabetes mellitus ve komplikasyonlarının tanı, tedavi ve izlem kılavuzu (TEMD Diabetes mellitus and its complications diagnosis, treatment and follow-up guide). 10th. Ed. Ankara: Bayt Bilimsel Araştırmalar Basın Yayın Tanıtım Ltd. Şti; 2018.

2. Wallace TM, Levy JC, Matthews DR. Use and Abuse of HOMA Modeling. Diabetes Care. 2004; 27(6):1487-95

3. Matthews D, Hosker JP, Rudenski AS. Homeostasis model assessment: IR and β- cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412–9.

4. Baek K, Lee N, Chung I. Association of arsenobetaine with beta-cell function assessed by homeostasis model assessment (HOMA) in nondiabetic Koreans: data from the fourth Korea National Health and Nutrition Examination Survey (KNHANES) 2008-2009. Ann Occup Environ Med. 2017; 29(1):31.

5. Yamauchi K, Sato Y, Nakasone Y, Aizawa T. Comparison of HOMA-IR, HOMA-β% and disposition index between US white men and Japanese men in Japan in the ERA JUMP study: was the calculation of disposition index legitimate? Diabetologia. 2015; 58(7):1679-80.

6. Haffner SM, Kennedy E, Gonzalez C, Kennedy E, Stern MP. A prospective analysis of the HOMA model: the Mexico City Diabetes Study. Diabetes Care. 1996; 19(10):1138–41.

7. Li CL, Tsai ST, Chou P. Relative role of insulin resistance and beta-cell dysfunction in the progression to type 2 diabetes: the Kinmen Study. Diabetes Res Clin Pract. 2003; 59(3):225–32.

8. Osei K, Rhinesmith S, Gaillard T, Schuster D. Impaired insulin sensitivity, insulin secretion, and glucose effectiveness predict future development of impaired glucose tolerance and type 2 diabetes in pre-diabetic African Americans: implications for primary diabetes prevention. Diabetes Care. 2004; 27(6):1439-46.

9. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006; 444(7121): 860-7.

10. Yilmaz H, Celik HT, Namuslu M, Inan O, Onaran Y, Karakurt F, et al. Benefits of the neutrophil-to-lymphocyte ratio for the prediction of gestational diabetes mellitus in pregnant women. Exp Clin Endocrinol Diabetes. 2014; 122(1):39-43.

11. Twig G, Afek A, Shamiss A, Derazne E, Tzur D, Gordon B, et al. White blood cell count and the risk for coronary artery disease in young adults. PloS One. 2012; 7(10):e47183. DOI: 10.1371/journal.pone.0047183.

12. Babi N, Ibarrola-Jurado N, Bulló M, Martínez-González MÁ, Wärnberg J, Salaverría I. White blood cell counts as risk markers of developing metabolic syndrome and its components in the PREDIMED study. PloS One. 2013; 8(3):e58354. DOI: 10.1371/journal.pone.0058354.

13. Park JS, Kim HM, Jeung HC, Kang SA. Association between early nutritional risk and overall survival in patients with advanced pancreatic cancer: A single-center retrospective study. Clin Nutr ESPEN. 2019; 30:94-9.

14. Kar F, Kiraz ZK, Kocatürk E, Uslu S. The Level of Serum C-Reactive Protein and Neutrophil Lymphocyte Ratio According to Thyroid Function Status. Clinical and Experimental Health Sciences. 2020; 10(2):142-7.

15. Geer EB, Islam J, Buettner C. Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism. Endocrinol Metab Clin. 2014; 43(1):75-102.

16. Kamba A, Daimon M, Murakami H, Otaka H, Matsuki K, Sato E, et al. Association between higher serum cortisol levels and decreased insulin secretion in a general population. PloS One. 2016; 11(11):e0166077. DOI: 10.1371/journal.pone.0166077.

17. Gutch M, Kumar S, Razi SM, Gupta KK, Gupta A. Assessment of insulin sensitivity/resistance. Indian J Endocrinol Metab. 2015; 19(1):160-4

18. Lou M, Luo P, Tang R, Peng Y, Yu S, Huang W, et al. Relationship between neutrophil-lymphocyte ratio and insulin resistance in newly diagnosed type 2 diabetes mellitus patients. BMC Endocr Disord. 2015; 15(1):9.

19. Fujita T, Hemmi S, Kajiwara M, Yabuki M, Fuke Y, Satomura A, et al. Complement-mediated chronic inflammation is associated with diabetic microvascular complication. Diabetes Metab Res Rev. 2013; 29(3):220–6.

20. Shoelson S, Lee J, Goldfine A. Inflammation and insulin resistance. J Clin Invest. 2006; 116(7):1793–801.

21. Karakaya S, Altay M, Kaplan Efe F, Karadağ I, Ünsal O, Bulur O, et al. The neutrophil-lymphocyte ratio and its relationship with insulin resistance in obesity. Turk J Med Sci. 2019; 49(1):245-8.

22. Buyukkaya E, Karakas MF, Karakas E, Karadağ İ, Ünsal O, Bulur O, et al. Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. Clinical and applied thrombosis/hemostasis. 2014; 20(2):159-63.

23. Bazotte R.B, de Castro Ruiz Marques A, Krupek T, Eik Filho W. Blood levels of pro-inflammatory and anti-inflammatory cytokines in a patient with a flat glucose curve. Acta Diabetol. 2016; 53(6):1057–9.

24. Rafacho A, Ortsäter H, Nadal A, Quesada I. Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. J Endocrinol. 2014; 223(3):49-62.

25. Erdogan S, Dursun F, Kirmizibekmez H, Guven S, Yildirim UM. Evaluation of Erythrocyte and Thrombocyte Parameters in Pediatric Patients with Diabetes Mellitus. Journal of Clinical and Analytical Medicine. 2017; 8(2):98-101.

How to cite this article:
Evin Kocatürk, Ezgi Kar, Zeynep Küskü Kiraz, İ. Özkan Alataş. Increased insulin secretion suppresses cortisol levels, exacerbates inflammation and beta-cell dysfunction. Ann Clin Anal Med 2021;12(Suppl 4): S365-369