ORIGINAL ARTICLE

Effect of obesity and serum leptin level on clopidogrel resistance

Obezite ve serum leptin seviyelerinin klopidogrel direnci üzerine etkisi

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ABSTRACT

Objective: Clopidogrel inhibits platelet aggregation by blockade of platelet adenosine diphosphate (ADP) P2Y12 receptor. Leptin is the obesity gene product, and its serum level increases with obesity. Platelets have leptin receptors on their surfaces. Hyperleptinemia may induce ADP-mediated platelet aggregation. It has been proposed that clopidogrel effect could be diminished with high serum leptin levels. The aim of the present trial was to further investigate the relationship between serum leptin level and clopidogrel resistance.

Methods: A total of 100 subjects who underwent percutaneous coronary intervention were enrolled. Two groups were organized according to presence of clopidogrel resistance, and serum leptin levels were compared. Threshold for clopidogrel resistance and hyperleptinemia were accepted as ≥P2Y12 reaction unit (PRU) 240 and ≥15 ng/mL leptin, respectively. Body mass index (BMI) of 30 kg/m^2 or greater was considered obese.

Results: A total of 37% of patients were considered clopidogrel-resistant. Comparison of groups revealed significantly higher clopidogrel resistance (p=0.017) and PRU levels (p=0.001) in hyperleptinemic patients. No significant difference in serum leptin levels (p=0.116) was found. Increased clopidogrel resistance was observed in patients with BMI >30 kg/m^2 (p=0.015).

Conclusion: Clopidogrel resistance is more common in obese and hyperleptinemic patients. Dosage should be individualized in these populations.

ÖZET

Amaç: Klopidogrel trombosit adenozin difosfat (ADP) P2Y12 reseptörlerini bloke ederek trombosit agregasyonunu inhibe eder. Leptin obezite geni ürünü olup, serum seviyesi obezite ile artar. Trombositler, yüzeylerinde leptin reseptörleri barındırırlar. Hiperleptinemi ADP aracılı trombosit agregasyonunu uyarabilir. Klopidogrel etkisinin yüksek serum leptin seviyeleri ile azalabileceği düşünülmektedir. Çalışmamızda serum leptin seviyeleri ile klopidogrel direnci arasındaki ilişkiyi araştırmayı amaçladık.

Yöntemler: Çalışmaya perkütan koroner girişim uygulanmış 100 hasta alındı. Klopidogrel direnci bulunuşuna göre iki grup oluşturuldu ve serum leptin seviyeleri iki grup arasında karşılaştırıldı. Klopidogrel direnci ve hiperleptinemi için eşik değerler sırasıyla, ≥ PRU (P2Y12 reaction units) 240 ve ≥15 ng/ml (leptin) olarak kabul edildi. Vücut kitle indeksinin (VKİ) 30 kg/m^2 ve üstü oluşu obezite olarak değerlendirildi.

Bulgular: Hastaların %37'sinde klopidogrel direnci olduğu bulundu. İki grubun karşılaştırılması klopidogrel direnci (p=0.017) ve PRU seviyelerinin (p=0.001) hiperleptinemik hastalarda anlamlı olarak daha yüksek olduğunu ortaya koydu. İki grup arasında serum leptin seviyelerine göre anlamlı fark yoktu (p=0.116). VKİ >30 olan hastalarda, daha fazla sayıda klopidogrel direnci gözlendi (p=0.015).

Sonuç: Klopidogrel direnci obez ve hiperleptinemik hastalarda daha fazla görülür. Klopidogrel dozu bu hasta grubunda bireyselleştirilmelidir.

Platelet activation and aggregation play crucial roles in the pathogenesis of atherothrombosis, which can lead to acute coronary syndrome (ACS) and is also of import following percutaneous coronary intervention (PCI).[1] Clopidogrel is a thienopyridine derivative that inhibits platelet aggregation by blockade of platelet adenosine diphosphate (ADP) P2Y12 receptor.[2,3] It is an alternative to aspirin and, in combination, provides additional antiplatelet effect.[4] This dual therapy is the standard combination for the prevention of subacute stent thrombosis.[5] Inhibition of platelet aggregation with clopidogrel may vary between patients.[6,7] Inadequate platelet inhibition is associated with increased cardiovascular event risk.[8,9]

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Clopidogrel resistance can be described as the persistent activity of clopidogrel receptor in spite of adequate antiplatelet therapy.[10] Clopidogrel resistance varies from 4–44% in different populations.[11] Response to clopidogrel can be determined by various methods. Light transmittance aggregometry is considered the gold standard of platelet function tests. However, certain limitations are present, including the time consumed, and the required provision of technical information from specialized laboratories.[12] Results of various platelet function tests have indicated that light transmittance aggregometry has the strongest correlation with results of the VerifyNow P2Y12 test (Accumetrics, Inc., San Diego, CA, USA).[13]

Leptin, a product of the obesity gene, is a protein consisting of 167 amino acids, and it regulates the storage of energy in mammals. Obesity and hyperphagia are caused by either absence or deterioration of leptin, and reversible with leptin replacement therapy.[14] Leptin is primarily found in adipocytes, and has a positive correlation with obesity,[15] a response to reduced leptin sensitivity in obese patients. Obesity is related to hyperleptinemia, due to leptin resistance in spite of high-circulating levels of leptin.[16] In 1999, the long form of the leptin receptor was found on the platelet surface. It has been suggested that activation of the leptin receptor could induce platelet aggregation.[17] Several studies have shown that ADP-mediated platelet aggregation was more common in obese patients, partially due to the increase in leptin levels. Platelet stimulation with leptin causes platelet aggregation in healthy subjects. Furthermore, platelets in obese patients are also susceptible to leptin-induced platelet aggregation, in contrast to the results of previous studies.[18] Therefore, the antithrombotic effect of clopidogrel could be diminished by hyperleptinemia-induced, ADP-mediated platelet aggregation.

The present aim was to investigate the correlation of serum leptin levels and hyperleptinemia with clopidogrel resistance.

METHODS

Study population

The present population consisted of 100 patients admitted between January and April 2012, who underwent PCI following stable angina pectoris, unstable angina pectoris, or ACS without ST-segment elevation myocardial infarction. The study was approved by the local ethics committee, and participants provided prior written consent. Patients were administered 300 mg clopidogrel loading dose followed by 75 mg/day maintenance or at least 5 consecutive clopidogrel 75 mg/day doses prior to procedure. Patients with a history of malignant disease, active infection or inflammatory disease, advanced liver failure, end-stage renal failure, cerebrovascular disease, hypothyroidism, hyperthyroidism, or who were taking corticosteroids were excluded. Body mass index (BMI) was calculated, and subjects with BMI of 30 kg/m^2 or higher were considered obese.

Abbreviations:

ACS	*Acute coronary syndrome*
ADP	*Adenosine diphosphate*
BMI	*Body mass index*
PCI	*Percutaneous coronary intervention*
PRUs	*P2Y12 reaction units*

Blood samples and laboratory methods

Prior to PCI procedure, 10 mL of blood was collected into anticoagulant-free containers to determine leptin level and other parameters. Serum was immediately separated after the draw and stored at -80°C with special labelling. Quantitative serum leptin levels were measured by leptin enzyme-linked immunosorbent assay (DIAsource ImmunoAssays SA, Louvain-la-Neuve, Belgium), and other parameters were measured by standard laboratory methods. Threshold for hyperleptinemia was ≥15 ng/mL.

Clopidogrel inhibition level was measured from venous blood samples collected 12–24 hours after procedure. Inhibitory effect of clopidogrel was measured by VerifyNow P2Y12 test, which primarily measures the effect of the drug on the P2Y12 receptor, and was developed to serve as a quick, cartridge-based platelet function test. Increase in light transmittance is measured as aggregation, and an algorithm expresses this in P2Y12 reaction units (PRUs). High number of PRUs shows high ADP-mediated platelet reactivity. Patients with ≥240 PRU were considered resistant, while those with lower levels were considered responsive to clopidogrel.

Statistical analysis

Statistical analyses were performed using SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean±SD or median (min–max). Number of cases and percentages were used for categorical data. Kolmogorov-

Smirnov test was used to determine normalcy of distribution. Student's t-test was used to compare mean variables among groups for parametric assumptions. Mann-Whitney U test was used for non-parametric assumptions. Intergroup comparisons of categorical data were performed using continuity-corrected chi-square or Fisher's exact test. Correlation between serum leptin and clopidogrel PRU level was performed using Spearman's correlation analysis. A p value of <0.05 was considered statistically significant.

RESULTS

Overall, 37% of patients were found to be clopidogrel-resistant. Average age and BMI was compared between resistant and responsive groups (age: 65.94±9.37 years *vs.* 62.73±8.99 years, p=0.092; BMI: 28.38±3.45 *vs.* 27.42±2.91, p=0.139).

No statistically significant difference was found between groups regarding diabetes mellitus, hypertension, disease status (stable coronary artery disease

Table 1. Comparison of clinical features between clopidogrel-responsive and -resistant groups

n=100	Clopidogrel resistance						*p*
	Responsive (n=63)			Resistant (n=37)			
	n	%	Mean±SD	n	%	Mean±SD	
Age[a]			62.73±8.99			65.94±9.37	0.092
Sex[b]							
Female	10	15.9		8	21.6		0.651
Male	53	84.1		29	78.4		
Disease status[b]							
Stable coronary artery disesase	49	77.8		31	83.8		0.641
Acute coronary syndrome	14	22.2		6	16.2		
Diabetes mellitus[b]							
–	41	65.1		23	62.2		0.938
+	22	34.9		14	37.8		
Hypertension[b]							
–	18	28.6		5	13.5		0.138
+	45	71.4		32	86.5		
Smoking[b]							
–	36	57.1		19	51.4		0.723
+	27	42.9		18	48.6		
Stent type[b]							
Bare metal stent	26	41.3		20	54.1		0.303
Drug-eluting stent	37	58.7		17	45.9		
Statin usage[c]							
–	6	9.5		2	5.4		0.707
+	57	90.5		35	94.6		
Body mass index[a]			27.42±2.91			28.38±3.45	0.139
Body mass index[b]							0.015*
Body mass index <30	50	79.4		20	54.1		
Body mass index >30	13	20.6		17	45.9		

SD: Standard deviation; +: Indicates presence; –: Indicates absence. [a]Indicates performance of Student's t-test; [b]Indicates performance of continuity-corrected chi-square test; [c]Indicates performance of Fisher's exact test.

vs. ACS), type of stent (drug-eluting vs. bare-metal), use of statins, or smoking (p>0.05). However, increased clopidogrel resistance was found in patients with BMI >30 kg/m^2 (p=0.015, Table 1).

Median serum leptin levels and minimum–maximum leptin levels were 4.72 (0.46–25.33) and 3.72 (0.11–38.88) in clopidogrel-resistant and -responsive groups, respectively. However, the difference was not statistically significant (p=0.116), though it was supported by correlation analysis between serum leptin and clopidogrel PRU levels (r: 0.259; p=0.009).

At a leptin threshold of 15 ng/mL, clopidogrel PRU was significantly higher in hyperleptinemic patients than in those with leptin levels of <15 ng/mL (p=0.001). Clopidogrel resistance was also significantly higher in hyperleptinemic patients (p=0.017). Data distribution is shown in Table 2.

DISCUSSION

Concomitant use of aspirin and clopidogrel in patients undergoing PCI is particularly vital in the reduction of short- or long-term risk of major cardiac events, particularly in cases of stent thrombosis.[19] However, it has been demonstrated that efficacy of clopidogrel treatment varies individually, and causes high residual platelet activity.[20] Patients with high post-clopidogrel platelet activity experience a higher number of ischemic events than those with normal clopidogrel response.[21] Platelet response to clopidogrel may vary according to clinical, cellular, and genetic occasions.[7]

It has been shown that patients with high BMI had increased platelet reactivity. In these patients, platelets respond weakly to the inhibitory effect of insulin. Angiolillo et al. demonstrated suboptimal platelet response in patients with high BMI after 300 mg of clopidogrel loading dose.[22] Although BMI was not statistically different between the present groups, clopidogrel resistance was more common in patients with BMI of or over 30 kg/m^2.

Obesity is an independent risk factor for atherosclerosis, thrombosis, stroke, and myocardial infarction— a serious health problem, particularly in developed countries.[23] Recent studies have shown that increased leptin level increases risk for cardiovascular disease, metabolic irregularity and changes in the coagulation system.[24] Angiolillo et al. indicated that obesity was also associated with lower sensitivity to clopidogrel.[25] The impetus of investigating the relationship between clopidogrel and leptin was the presence of leptin receptors on the platelet surface.[17] Most obese patients have higher leptin levels, a condition primarily caused by leptin resistance. The key question to be asked at this point should be: Do platelets have leptin resistance? Dellas et al. showed that platelets are not resistant to leptin-induced, ADP-mediated platelet aggregation in obese patients,[18] a result contradictory to findings of Corsonello et al., which suggested that the platelet could be a site of leptin resistance.[26]

Although serum leptin levels in patients with clopidogrel resistance seem to have been higher in

Table 2. Comparison of PRU level according to cut-off leptin level of 15 ng/mL between clopidogrel-responsive and -resistant groups

n=100	Leptin level				*p*
	<15 (n=89)		≥15 (n=11)		
	Median	Minimum–Maximum	Median	Minimum–Maximum	
Clopidogrel PRU[a]	204.00	47–401	302.00	146–366	0.001*
	Responsive (n=63)		Resistant (n=37)		*p*
	n	%	n	%	
Leptin Level[b]					
Leptin <15	60	95.2	29	78.4	0.017*
Leptin ≥15	3	4.8	8	21.6	

[a]Indicates the performance of Mann-Whitney U test; [b]Indicates the performance of continuity-corrected chi-square test.

the present series, the difference was not statistically significant. When 15 ng/mL of leptin is assumed as a cutoff for hyperleptinemia, patients with leptin levels above this cutoff have increased clopidogrel resistance. This difference becomes more prominent when comparing clopidogrel PRU levels of groups.

Gatto et al. also investigated the relationship between clopidogrel resistance and leptin level, and found that leptin level was significantly higher in the clopidogrel-resistant group, compared to the responsive group.[27] Similarly, clopidogrel PRU values were significantly different, corresponding to a cutoff leptin level of 15 ng/mL. Unlike the results of the Gatto et al. study, clopidogrel resistance was not presently found to be related to serum leptin level. However, incidence of clopidogrel resistance was higher in the hyperleptinemia group, in accordance with the results of the previous study. These findings support hyperleptinemia as a potential cause of clopidogrel resistance in obesity.

The present limitations included relatively small sample size, which may have contributed to the inability to detect clopidogrel response and resistance by serum leptin level. Only 11 patients had leptin level above 15 ng/mL, a statistical disadvantage. In addition, in spite of good correlation with gold-standard method, platelet function testing was performed by a single test (VerifyNow).

Conclusions

The effect of high leptin levels and obesity on clopidogrel resistance was confirmed by the present results. These factors must be taken into consideration during clopidogrel treatment. No correlation was presently determined between serum leptin level and clopidogrel resistance. However, clopidogrel resistance was more prominent in patients with leptin levels above 15 ng/dL. Studies with larger sample sizes are warranted, to determine dosage of clopidogrel or choice of alternative drug in obese people.

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REFERENCES

1. Steinhubl SR, Moliterno DJ. The role of the platelet in the pathogenesis of atherothrombosis. Am J Cardiovasc Drugs 2005;5:399–408. Crossref
2. Savi P, Beauverger P, Labouret C, Delfaud M, Salel V, Kaghad M, et al. Role of P2Y1 purinoceptor in ADP-induced platelet activation. FEBS Lett 1998;422:291–5. Crossref
3. Herbert JM, Savi P. P2Y12, a new platelet ADP receptor, target of clopidogrel. Semin Vasc Med 2003;3:113–22. Crossref
4. Ma TK, Lam YY, Tan VP, Kiernan TJ, Yan BP. Impact of genetic and acquired alteration in cytochrome P450 system on pharmacologic and clinical response to clopidogrel. Pharmacol Ther 2010;125:249-59. Crossref
5. Bhatt DL, Bertrand ME, Berger PB, L'Allier PL, Moussa I, Moses JW, et al. Meta-analysis of randomized and registry comparisons of ticlopidine with clopidogrel after stenting. J Am Coll Cardiol 2002;39:9–14. Crossref
6. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908–13. Crossref
7. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. J Am Coll Cardiol 2007;49:1505–16.
8. Matetzky S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004;109:3171–5. Crossref
9. Hochholzer W, Trenk D, Bestehorn HP, Fischer B, Valina CM, Ferenc M, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol 2006;48:1742–50. Crossref
10. Ben-Dor I, Kleiman NS, Lev E. Assessment, mechanisms, and clinical implication of variability in platelet response to aspirin and clopidogrel therapy. Am J Cardiol 2009;104:227–33. Crossref
11. Vlachojannis GJ, Dimitropoulos G, Alexopoulos D. Clopidogrel resistance: current aspects and future directions. Hellenic J Cardiol 2011;52:236-45.
12. Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. Comparison of methods to evaluate clopidogrel-mediated platelet inhibition after percutaneous intervention with stent implantation. Thromb Haemost 2009;101:333–9.
13. van Werkum JW, van der Stelt CA, Seesing TH, Hackeng CM, ten Berg JM. A head-to-head comparison between the VerifyNow P2Y12 assay and light transmittance aggregometry for monitoring the individual platelet response to clopidogrel in patients undergoing elective percutaneous coronary intervention. J Thromb Haemost 2006;4:2516–8. Crossref

14. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70. Crossref
15. Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q, et al. The metabolic significance of leptin in humans: gender-based differences in relationship to adiposity, insulin sensitivity, and energy expenditure. J Clin Endocrinol Metab 1997;82:1293–300. Crossref
16. Bjørbaek C, El-Haschimi K, Frantz JD, Flier JS. The role of SOCS-3 in leptin signaling and leptin resistance. J Biol Chem 1999;274:30059–65. Crossref
17. Nakata M, Yada T, Soejima N, Maruyama I. Leptin promotes aggregation of human platelets via the long form of its receptor. Diabetes 1999;48:426–9. Crossref
18. Dellas C, Schäfer K, Rohm I, Lankeit M, Ellrott T, Faustin V, et al. Absence of leptin resistance in platelets from morbidly obese individuals may contribute to the increased thrombosis risk in obesity. Thromb Haemost 2008;100:1123–9. Crossref
19. Smith SC Jr, Feldman TE, Hirshfeld JW Jr, Jacobs AK, Kern MJ, King SB 3rd, et al. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/SCAI Writing Committee to Update 2001 Guidelines for Percutaneous Coronary Intervention). Circulation 2006;113:166–286. Crossref
20. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. J Am Coll Cardiol 2005;45:246–51. Crossref
21. Geisler T, Langer H, Wydymus M, Göhring K, Zürn C, Bigalke B, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. Eur Heart J 2006;27:2420–5. Crossref
22. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Sabaté M, Hernández-Antolín R, et al. Is a 300 mg clopidogrel loading dose sufficient to inhibit platelet function early after coronary stenting? A platelet function profile study. J Invasive Cardiol 2004;16:325–9.
23. Eckel RH, Krauss RM. American Heart Association call to action: obesity as a major risk factor for coronary heart disease. AHA Nutrition Committee. Circulation 1998;97:2099–100.
24. Coleman DL. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 1973;9:294–8. Crossref
25. Angiolillo DJ, Bernardo E, Ramírez C, Costa MA, Sabaté M, Jimenez-Quevedo P, et al. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. J Am Coll Cardiol 2006;48:298–304. Crossref
26. Corsonello A, Perticone F, Malara A, De Domenico D, Loddo S, Buemi M, et al. Leptin-dependent platelet aggregation in healthy, overweight and obese subjects. Int J Obes Relat Metab Disord 2003;27:566–73. Crossref
27. Gatto L, Nusca A, Contuzzi R, Di Giovanni G, Ricottini E, Patti G, et al. Leptin levels and clopidogrel non-responsiveness in patients undergoing PCI: a dangerous relationship not yet described. European Heart Journal 2010;31(Supplement):157.

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