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Comparison of Clinical Findings of Positive- and Negative-Patients in Treadmill Test for Adults with Cardiovascular Events

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Abstract

This study was retrospectively designed to investigate differences in physiological variables between positive- and negative-patients in treadmill test for the diagnosis of patients with cardiovascular events and to confirm clinical usefulness of treadmill test. Total leukocyte and neutrophil counts, neutrophils/lymphocytes ratio and mean corpuscular volume were significantly higher, whereas platelet counts were lower in the positive group than in the negative group. Triglyceride and alkaline phosphatase concentrations were significantly higher in the positive group than in the negative group. The patients with abnormal levels of total cholesterol, high density lipoprotein cholesterol were higher in the positive group than in the negative group, but is reverse in hyperglycemia. High sensitivity C-reactive protein, erythrocyte sedimentation rate, lactic dehydrogenase, brain natriuretic peptide, creatinekinase-MB and troponine-I levels in the positive group were significantly greater than those of the negative group. P-, QRS- and T-axis in the EKG were higher whereas PR interval was shorter in the positive group than in the negative group. Most of cardinal symptoms were chest pain (57%) in the both groups. In final diagnosis angina pectoris (53% vs. 37%) and acute myocardial infarction (MI) (10% vs. 3%) were higher in the positive group than in the negative group. Cases of coronary artery angiography (CAG) and medication in the positive group were more than those of the negative group. Cases of normal CAG finding and observation in positive group were more than those of negative group. These data suggest that treadmill test-positive group had higher inflammation, myocardial injury, prevalence of angina pectoris and MI and that treadmill test has clinical usefulness. Also if adults have abnormal range of above mentioned clinical variables, treadmill test is recommended. However, treadmill test is not unique and the best tool for diagnosis of coronary artery disease because the patients with negative finding also had percutaneous coronary intervention and medications.

Keywords: *Coronary artery disease, Treadmill test, Body index, Biochemical marker, Inflammatory marker, Cardiac marker, percutaneous coronary intervention (PCI).*

1. Introduction

Today, we have been exposed to metabolic syndrome and adult disease due to industrialization, introduction of digital society, severe stresses, heavy drinking, smoking and Western diet. These situations and conditions have been risk factors for the increased incidences of diabetes, cancers, cerebrovascular and cardiovascular diseases and thereby

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elevated adult mortality. Especially, for the annulment of stresses attributable to persons and works and harmonious personal relations, the moderns have often active and passive smoking and drinking. However, the incidence of cerebrovascular and cardiovascular diseases in the smokers is higher as 2.87 times than that of the nonsmokers [1]. Tabacco induces platelet activations and agglutinations, leading to thrombogenesis[2,3]. Excessive alcohol consumptions cause hypertension, diabetes, metabolic syndrome and dyslipidemia [4,5]. Finally, Smoking and heavy drinking can contribute to the developments of the atherosclerosis and coronary heart disease (CHD). CHD is major etiology of adult mortality world-wide. In America, 2,300 adults every day die due to CHD, averaging one death every 39 seconds. In 2006, 81 million people in America suffered with $\geq$ one type of cardiovascular disease. One in three Americans has CVD, and 35% of all American deaths are due to the scourge.1 Coronary heart disease (CHD) is the leading cause of death in the US and the UK: one coronary event occurs every 25 seconds in America, with 34% dying within the same year, amounting to one death every minute. On an annual basis, about 785,000 Americans have new coronary attacks, and 470,000 have a recurrence, with an estimated 195,000 first myocardial infarctions (MI) that occur silently [6]. There were about 406,351 deaths in the United States from CHD in 2007, approximately one in six of all deaths [7].

The morbidity and mortality rates of CHD have increasing in Korea. For reducing morbidity and mortality from CAD, earlier diagnosis and treatment are very important.
To diagnose CHD, treadmill test has been universally utilized, which the tool has specificity and sensitivity. Even though the positive criteria of treadmill test are slightly different among hospitals, depression or elevation of ST segment is considered to be positive standard for the diagnosis of CHD. However, the patients with CHD have occasionally negative findings (without depression or elevation of ST segment).

We examined this investigation whether there are any differences in the physiological variables between positive- and negative-patients in treadmill test for the diagnosis of patients with cardiovascular events, and for confirming clinical significance and usefulness of treadmill test.

2. Materials and Methods

2.1 Subjects

The subject of this study was 200 adult patients underwent treadmill test due to cardiovascular events from January 2010 to December 2013 in P hospital. The subjects were classified to two groups. One group was negative in treadmill test (negative group) (n=100) and other was positive in treadmill test (positive group) (n=100). All subjects had chest pain, dyspnea, angina pectoris, myocardial infarction, arrhythmias, syncope, and/or hypertension. The exclusive criteria were patients suffering immunological, hematological, cardiovascular, severe hepatic and/or diseases, recently operation, and cancers. This study was accepted from IRB of the Catholic University of Pusan.

2.2 Analysis and Clinical Data

After the examinations of routine electrocardiography (ECG) with 12 leads, all patients had treadmill test with Bruce protocol such as Table 1. The criteria for the end of this test were significant ST segment depression or elevation, hypertension, chest pain, dyspnea, leg pain, fatigue, and/or dizziness. Body, hematological, biochemical, inflammatory and cardiac indices were analyzed as well as medical treatments and therapy.

2.3 Other Physiological Variables

After fasting for 12 hrs, 10 mL of blood was collected from the study population and 3 mL of the blood was inserted into EDTA tube and was gently mixed for measuring the

hematological variables. Complete blood cell counts (CBC) were analyzed by Cell Dyne 1600 (Abbott Lab., CA., USA). The other blood was separated into serum for detections of biochemical variables and cardiac and inflammatory marker concentrations. The serum total protein, glucose, lipid metabolic markers [total cholesterol (T-ch), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), and triglyceride (TG)], liver markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin], and renal markers [creatinine, uric acid, and blood urea nitrogen (BUN)] were analyzed by THOSHIBA 200FR (Toshiba medical system Co., Japan). The serum troponin-I and myoglobin levels were determined by Immunoassay Analyzer (Bechmen Coulter, America). The serum brain natrieuretic peptide (BNP) levels were measured by Cobas 6000 Analyzer Series (Roch Diagnostics, Switzerland, Switzerland. The serum creatinekinase-MB (CK-MB) and lactic dehydrogenase (LDH) concentrations were analyzed by THOSHIBA 200FR (Toshiba medical system Co., Japan). The serum high sensitivity C-reactive protein (Hs-CRP levels were measured by THOSHIBA 200FR (Toshiba medical system Co., Japan).

2.4 Statistical Analysis

All data were expressed as the mean±standard deviation (SD). For comparison between two groups, unpaired *t*-test was applied with SPSS program (version 12.0). Statistical significance was accepted with $\boldsymbol{P} \leq 0.05$.

3. Results

3.1 Body Indices

As displayed in Table 2. The numbers of female patients were more than those of male patients in the both groups. There were no differences in the body indices between two groups ($\boldsymbol{P}>0.05$).

3.2 Hematological Variables

Total leukocyte and neutrophil counts, neutrophils/lymphocytes ratio, and mean corpuscular volume (MCV) were higher, whereas platelet counts were lower in the positive group than in the negative group ($\boldsymbol{P}<0.05$) (Table 3).

3.3 Biochemical Variables

The serum triglyceride and ALP levels in the positive group were greater than those of the negative group ($\boldsymbol{P}<0.05$) but not different in the other variables ($\boldsymbol{P}>0.05$) (Table 4). The glucose levels in the both groups were higher than the normal ranges but not different between two groups ($\boldsymbol{P}>0.05$) (Table 4).

3.4 The Numbers of Patients having Abnormal Levels in Biochemical Variables

Table 5 shows the numbers of patients having abnormal levels in biochemical variables in the both group. The numbers of patients having high levels of total cholesterol, triglyceride and ALT were more in the positive group than in the negative group ($\boldsymbol{P}<0.05$). In addition, in HDL, the numbers of patients having low levels were more in the positive group than in the negative group ($\boldsymbol{P}<0.05$).

3.5 Inflammatory and Cardiac Markers

The serum high sensitivity C-reactive protein (Hs-CRP) and erythrocyte sedimentation rate (ESR) (inflammatory markers) levels in the positive group were significantly higher than those of the negative group ($\boldsymbol{P}<0.05$) (Table 6).

The serum brain natriuretic peptide (BNP), troponin-I (TNI), creatinekinase-MB (CK-MB) and lactic dehydrogenase (LDH) levels were also greater in the positive group than in the negative group (P<0.05) (Table 6).

3.6 The Results of Routine ECG

PR intervals in the both groups were within normal range, but the values were shorter in the positive group than in the negative group (P<0.05) (Table 7). The values of P-, QRS- and T-axis were higher in the positive group than in the negative group (P<0.05) (Table 7).

3.7 The Final Diagnosis in the Study Population

Angina pectoris and MI cases were more in the positive group than in the negative group (P<0.05) (Table 8).

3.8 The Medical Treatment and Therapy

The cases of coronary artery angiography (CAG) and medications were more, whereas the patients with normal findings of CAG and observation were less in the positive group than in the negative group (P<0.05) (Table 9).

4. Discussion

In this investigation, despite of no difference in body indices between two groups, the study population had abnormal body mass index with over-weights and underwent cardiac interventions and medications for treatment of CHD. These data suggest that over-weight is a risk factor of the development of CHD. Pedersen et al. revealed that 80% of European with CHD had over-weights and obesity [8], indicating importance of the control of the body weights in the middle-aged people.

The data in the present study that total leukocyte and neutrophil counts, neutrophils/lymphocytes ratio, and MCV were higher in the positive group have clinical significances.

Total leukocyte and neutrophil counts were highly associated with the development of acute and chronic heart failures and increased mortality in the Asian and European [9,10,11]. In even healthy adults, total leukocyte and neutrophil counts may have positive relationship with CHD [12,13,14]. Neutrophils are an important of the host defense system, in which they are charged with killing invading pathogens (i.e. bacteria) by a process paradigm of search, recognition and destroy. In the immunological response, the neutrophils respond to intercellular signals that identify the invader as foreign, thereby initiation a complex and effective response to rid the body of such infection or noxious material. However, similar signals of inflammation are generated by endothelial cells and cardiomyocytes, and the neutrophil responses are directed against self in myocardial ischemia-reperfusion [15]. The neutrophils adhered on the vascular endothelium release oxygen free radicals and destructive proteases, leading to the development of CHD [16]. Walker et al. reported that impaired acetylcholine-induced endothelium-dependent dilation in the forearm was related to higher white blood cell count among healthy middle-aged and older adults [17]. Li et al. recently demonstrated that higher total leukocyte counts can be independently related to vascular dysfunction in individuals with low cardiovascular risk [18]. The neutrophils/lymphocytes (N/L) ratio is also a new predictor for CHD risk and mortality. Sönmez et al. recently reported is a strong clinical laboratory value that is associated with presence and complexity of CAD [19]. Previous researches have demonstrated that MCV levels were positively correlated with the development of MI and were predictive risk factor of MI and percutaneous coronary intervention (PCI) [20,21]. In the present, the data concerning leukocyte counts, N/L ratio, and MCV in the positive group indicate clinical usefulness of treadmill test. If platelet

counts in middle-aged adults are raised, platelet-induced aggregations are promoted and finally may cause CHD [22]. Even if we found higher platelet counts in the positive group, the both group had normal levels and thus the significant evaluation between platelet counts and utility of treadmill test should be reserved. Further studies will be needed.

Since biochemical variables are significantly associated with risk factors of CHD [23, 24, 25, 26, 27, 28] we investigated the differences in the numbers of the patients having abnormal levels of them between two groups. The patients having higher levels of total cholesterol, triglyceride and ALT, and lower levels of HDL were more in the positive group than in the negative group, reflecting a utility of treadmill test for diagnosis of CHD.

Moreover, it was observed that Hs-CRP, ESR, LDH, BNP, TNI and CK-MB levels, excepting myoglobin were greater in the positive group than in the negative group. These results represent that the positive group may have the possibility of more severe inflammatory and myocardial injuries, suggesting clinical usefulness of treadmill test.

In the routine ECG, QRS- and T-axis, which are index of right and left ventricular depolarization and repolarization, respectively, in the positive group were higher than those of the negative group. Shirota et al. reported that relative left deviation of QRS-axis reflects the obstructions of ventricular septal coronary artery branch and left anterior descending branch and that relative right axis deviations mean the obstructions of right coronary artery [29].

Nevertheless, other investigations reported that ECG abnormalities, including are not associated with markers of subclinical atherosclerosis and the axis shift response is no more sensitive for the detection of CHD than ST depression [30, 31]. Therefore, more studies must be carried to clarify such discordances.

The changes of ST segment and T-axis have been considered as be predictors of coronary artery calcifications and sclerosis. Rautaharju et al. reported that of 4,173 adults without CHD underwent follow-up study for about 7.4 years, the prevalence of marked T-axis deviation (> or =45 degrees) was 12% and there were 161 CHD deaths, 743 deaths from all causes, and 679 incident CHD events [32]. Also, in adults without CHD, $\geq$45° of T-axis was associated with severity of coronary artery calcification and thus it is a significant indicator for the prognosis of CHD-induced mortality [33, 34].

In the present study, the T-axis in the positive group (52.62°) was significantly higher than that of the negative group (36.64°). These data appear that treadmill test is useful tool for the diagnosis of CHD.

Our observations that the cases of angina pectoris, MI, CAG and medications were more in the positive group than in the negative group also support clinical usefulness of treadmill test.

In summary, these data suggest that treadmill test-positive group had higher inflammation, myocardial injury, prevalence of angina pectoris and MI and that treadmill test has clinical utility. However, treadmill test is not unique and the best tool for diagnosis of coronary artery disease because the patients with negative finding also had percutaneous coronary intervention and medications.

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Table 1. Modified Bruce Protocol for Treadmill Test

Stage	Speed (mph)	Grade (%)	Duration (min)
1	1.7	0	3
2	1.7	5	3
3	1.7	10	3
4	2.5	12	3
5	3.4	14	3
6	4.2	16	3
7	5.0	18	3

Abbreviation: mph, miles per hr.

Table 2. Demographic Characteristics in Two Groups

Variable	Group	
	Negative	Positive
Age (years)	59.66±12.50	60.87±10.35
Gender (male : female)	56 : 44	58 : 42
Height (cm)	164.33±8.29	165.17±8.28
Weight (kg)	65.09±10.79	66.23±11.62
BMI (kg/m^2)	24.03±3.06	24.13±2.75

Data are expressed as mean±standard deviation (SD).
Abbreviation : BMI, body mass index.
Negative group, patients with non-changed ST segment in treadmill test;
Positive group, patients with changed ST segment in treadmill test.

Table 3. Hematological Variables in Two Groups

Variable	Group	
	Negative	Positive
T-leukocyte (10^3/μL)	6.46±2.03	7.03±1.87*
Granulocyte (10^3/μL)	3.77±1.63	4.18±1.59*
Lymphocyte (10^3/μL)	2.03±0.83	2.12±0.74
Monocyte (10^3/μL)	0.49±0.18	0.46±0.21
N/L ratio	1.85±0.97	1.96±0.85*
RBC (10^6/μL)	4.31±0.44	4.38±0.47
MCV (fL)	90.85±14.08	99.50±18.67*
Hemoglobin (g/dL)	13.37±1.42	13.57±1.56
Platelet (10^3/μL)	228.64±70.16	205.38±58.44*

Data are expressed as mean±SD. *, ***P***<0.05 (compared with the negative group).
Abbreviation: N/L ratio, neutrophils/lymphocytes ratio; T-, total; RBC, red blood cells; MCV, mean corpuscular volume.

Table 4. Biochemical Variables in Two Groups

Variable	Group	
	Negative	Positive
T-protein (mg/dL)	6.92±0.55	7.03±0.46
T-cholesterol (mg/dL)	166.72±46.74	171.94±44.62
LDL-ch (mg/dL)	105.82±37.05	108.54±38.88
HDL-ch (mg/dL)	50.99±14.81	47.85±12.85
Triglyceride (mg/dL)	130.30±76.74	153.06±122.29*
Glucose (mg/dL)	128.88±49.96	122.98±41.46
AST (IU/L)	25.32±16.14	24.38±11.15
ALT (IU/L)	23.20±17.70	27.07±19.87
ALP (IU/L)	73.42±15.25	82.34±16.47*
T-bilirubin (mg/dL)	0.75±0.36	0.78±0.35
Creatinine (mg/dL)	0.89±0.24	0.93±0.24
BUN (mg/dL)	15.45±5.691	16.83±4.95

Data are expressed as mean±SD. *, ***P***<0.05 (compared with the negative group).
Abbreviation: T, total; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkine phosphatase; BUN, blood urea nitrogen.

Table 5. The Number of Patients with Abnormal Ranges of Biochemical Markers in Two Groups

Variable	Group	
	Negative	Positive
T-cholesterol (<186.5 mg/dL, no.)	26	38*
HDL (≤35 mg/dL, no.)	5	17*
Triglyceride (>150 mg/dL, no.)	28	34*
Glucose (>90 mg/dL, no.)	67	65
AST (>40 IU/L, no.)	9	8
ALT (>35 IU/L, no.)	12	17*

ALP (>220 IU/L, no.)	3	3

*, ***P***<0.05 (compared with the negative group). Abbreviation: no., number; T, total; HDL, high density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

Table 6. Inflammatory and Cardiac Markers in Two Groups

Variable	Group	
	Negative	Positive
hsCRP (mg/dL)	0.39±0.08	1.08±0.10*
ESR (mm/hr)	9.82±0.86	11.30±2.67*
LDH (U/L)	303.95±29.21	332.94±35.25*
BNP (pg/mL)	69.46±15.82	77.40±13.45*
Myoglobin (ng/mL)	39.69±11.44	37.07±10.87
Troponin-I (ng/mL)	1.23±0.45	1.79±0.69*
CK-MB (U/L)	3.50±1.18	5.74±4.70*

Data are expressed as mean±SD. *, ***P***<0.05 (compared with the negative group). Abbreviation: hsCRP, high sensitivity C-reactive protein; BNP, brain natriuretic peptide; CK-MB, creatinekinase MB, LDH, lactic dehydrogenase.

Table 7. Electrocardiographic Findings in Two Groups

Variable	Group	
	Negative	Positive
P wave (msec)	54.32±18.84	56.74±16.91
QRS complex (mses)	93.67±12.08	94.06±12.23
PR interval (msec)	169.12±27.82	161.89±24.16*
QTc (msec)	93.67±12.08	94.06±12.23
P-axis (degree, °)	45.78±24.84	51.71±20.01*
QRS-axis (degree, °)	36.05±28.98	45.79±24.84*
T-axis (degree, °)	36.64±29.68	52.62±36.69*

Data are expressed as mean±SD. *, ***P***<0.05 (compared with the negative group).

Table 8. Final Diagnosis in Two Groups

Disease	Group	
	Negative	Positive
Angina pectoris (no.)	37	53*
Acute MI (no.)	3	10*
Old MI (no.)	1	2
Acute STEMI (no.)	2	0
STEMI (no.)	5	2
NSTEMI (no.)	2	3
PSVT (no.)	1	0
PSVT with HPN (no.)	1	0
Hypertension (no.)	5	6
Chest pain (no.)	17	12

Arrhythmias (no.)	6	4
Palpitation (no.)	2	1
Syncope (no.)	3	1
The others (no.)	15	6
Total number	100	100

*, ***P***<0.05 (compared with the negative group).
Abbreviation: no., number; STEM, ST-segment elevation myocardial infarction; PSVT, paroxysmal supraventricular tachycardia; HPN, hypertension,

Table 9. Clinical Treatment for Patients in Two Groups

Treatment	Group	
	Negative	Positive
Medication (no.)	25	26
PCI (no.)	33	37
CAG & medication (no.)	16	25*
POBA (no.)	1	1
Normal CAG (no.)	12	2*
Observation (no.)	12	5*
CABG (no.)	0	2
Pacemaker insertion (no.)	1	0
IABP (no.)	0	2
Total number	100	100

*, ***P***<0.05 (compared with the negative group).
Abbreviation: no., number; STEM, ST-segment elevation myocardial infarction; paroxysmal supraventricular tachycardia (PSVT); PCI, percutaneous coronary intervention; CAG, coronary artery angiography; POBA, plain old balloon angioplasty; CABG, coronary artery bypass graft surgery; IABP, intra-aortic ballon pump.