The particular interactions of the traditional cardiovascular risk factors with different circulating specific leukocyte subtype counts in blood: an observational study

Geleneksel kardiyovasküler risk faktörlerinin dolaşımdaki farklı özgül lökosit alt tip sayımları ile belirli etkileşimleri: Gözlemsel bir çalışma

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ABSTRACT

Objective: The pathogenesis of atherosclerosis is multifactorial, however the impact of inflammatory cells in this process is well known. Different traditional cardiovascular risk factors (CVRFs) may have specifically different effects on leukocyte subtype. Thus, these special interactions may induce different vascular involvement forms due to the altered endothelial damage and vascular repair mechanisms. The aim of the present study was to investigate whether there is any specific relationship between the leukocyte subtypes and the traditional CVRFs and to evaluate the independency of possible relationships.

Methods: The study had a cross-sectional observational design. The study population consisted of the patients who underwent coronary angiography with a suspicion of coronary artery disease (CAD) at our institution in an outpatient manner. We enrolled 677 consecutive eligible patients with CAD or normal coronary arteries (NCA) and investigated the associations of traditional CVRFs, demographic properties and biochemical parameters including fasting plasma glucose (FPG), creatinine, serum uric acid level (SUA) and lipids with total circulating inflammatory cell (WBC, leukocytes) and subtype counts including neutrophils (N), lymphocytes (L) and monocytes (M). As a dependent variable, total leukocyte count and subtypes, and neutrophil/lymphocyte ratio (N/L ratio) which has been found to being related with increased vascular risk and events were investigated in the groups determined by the presence or absence of CVRFs and CAD by the univariate analyses and then multiple linear regression analyses.

Results: When we performed multiple linear regression analyses to determine the independent associations of inflammatory cell subtypes, we have found that FPG had an independent incremental association with WBC (β±SE:4.2±1.4, p=0.004) and N (β±SE:4.2±1.2, p=0.001). Current smoking had an independent incremental association with WBC and all cell subtypes (for WBC, N, L, and M: β±SE: 748±161, p<0.001; β±SE: 556±136, p<0.001; β±SE: 185±69, p=0.007; β±SE: 38±20, p=0.061, respectively) and SUA had an independent incremental association with WBC (β±SE: 115±43, p=0.008), N (β±SE: 107±38, p=0.005) and M (β±SE: 26±6, p<0.001). Hypertension had an independent incremental association with WBC (β±SE: 431±140, p=0.002) and N (β±SE: 315±118, p=0.008). Male gender had an independent incremental association with only M (β±SE: 52±20, p=0.010). Family history of CAD had an independent decremental association with WBC (β±SE: -327±139, p=0.019) and N (β±SE: -326±121, p=0.007). Finally, age had an independent decremental association with WBC (β±SE: -32±7, p<0.001) and L (β±SE: -16±3, p<0.001). The N/L ratio was independently related with increased age (p<0.001), FPG (p=0.003) and SUA (p=0.012).

Conclusion: Our study results demonstrate that leukocyte subtypes have different specific associations with traditional CVRFs. We found that FPG affects specifically N while SUA affects specifically N and M, and current smoking affects nonspecifically on all cell subtypes. While hypertension with N and male gender with M were specifically related, age and family history of CAD were only related to L. These different interactions may lead to different endothelial damage and vascular repair mechanisms. *(Anadolu Kardiyol Derg 2011; 11: 573-81)*

Key words: Coronary artery disease, inflammatory cells, neutrophil, monocyte, lymphocyte, cardiovascular risk factors, neutrophil-to-lymphocyte ratio, regression analysis

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ÖZET

Amaç: Aterosklerozun patogenezi çok faktörlüdür, bununla birlikte enflamasyon hücrelerinin bu süreçteki etkin rolleri iyi bilinmektedir. Farklı geleneksel kardiyovasküler risk faktörleri (KVRF) lökosit alt tipleri üzerine özgül olarak farklı etkilere sahip olabilirler ve bu özel etkileşimler değişen endotel hasarı ve vasküler onarım mekanizmalarıyla farklı vasküler tutulum şekillerine sebep olabilir. Bu çalışmanın amacı lökosit alt tipleri ve geleneksel KVRF arasında özgül bir ilişkinin var olup olmadığını incelemek ve olası ilişkilerin bağımsızlığını değerlendirmekti.

Yöntemler: Çalışma gözlemsel ve enine-kesitli olarak planlandı. Çalışma popülasyonu hastanemize ayaktan başvuran ve koroner arter hastalığı (KAH) şüphesi ile koroner anjiyografileri yapılmış hastalardan oluşturuldu. Koroner arter hastalığı ya da normal koroner arterleri (NKA) olan 677 ardışık, çalışma kriterlerine uygun hasta dâhil edildi. Geleneksel KVRF'leri, demografik özellikler ve açlık kan şekeri (AKŞ), kreatinin, serum ürik asit (SUA) düzeyi ve lipit düzeylerinin periferik kan toplam enflamasyon hücre sayısı (WBC, lökositler) ve nötrofil (N), lenfosit (L) ve monositleri (M) içeren alt tiplerinin sayıları ile olan ilişkileri araştırıldı. Bağımlı değişken olarak total lökosit sayısı, alt tipleri ve artmış vasküler risk ve olaylar ile ilişkili olduğu ortaya konmuş olan nötrofil/lenfosit oranı (N/L oranı), KVRF'lerinin ve KAH'nın varlığı ya da yokluğu ile belirlenen gruplarda tek değişkenli ve daha sonra çok değişkenli analizler ile araştırıldı.

Bulgular: Lökosit alt tiplerinin bağımsız öngörücülerini belirlemek için çoklu regresyon analizleri gerçekleştirdiğimizde, AKŞ'nin WBC (β±SE:4.2±1.4, p=0.004) ve N (β±SE:4.2±1.2, p=0.001) ile bağımsız arttırıcı bir ilişkiye sahip olduğunu bulduk. Aktif sigara içimi WBC ve tüm hücre alt tipleri ile bağımsız bir arttırıcı ilişkiye sahipti (WBC, N, L ve M için: 748±161, p<0.001; β±SE: 556±136, p<0.001; β±SE: 185±69, p=0.007 ve β±SE: 38±20, p=0.061, sırasıyla) ve SUA düzeyi WBC (β±SE: 115±43, p=0.008), N (β±SE: 107±38, p=0.005) ve M (β±SE: 26±6, p<0.001) ile bağımsız artırıcı bir ilişkiye sahipti. Hipertansiyon WBC (β±SE: 431±140, p=0.002) ve N (β±SE: 315±118, p=0.008) ile artışımsal bir ilişkiye sahipti. Erkek cinsiyet yalnız monosit sayısı ile artışımsal bir ilişkiye sahipti β±SE: 52±20, p=0.010). Koroner arter hastalığı için aile öyküsü WBC (β±SE: -327±139, p=0.019) ve N (β±SE: -326±121, p=0.007) sayısı ile ters ilişkiye sahipti. Son olarak, yaş WBC (β±SE: -32±7, p<0.001) ve L (β±SE: -16±3, p<0.001) ile bağımsız azaltıcı bir ilişkiye sahipti. Artmış yaş, AKŞ ve ürik asit bağımsız olarak N/L oranı ile ilişkiliydi (p<0.001; p=0.003; p=0.012, sırasıyla).

Sonuç: Bizim çalışma sonuçlarımız lökosit alt tiplerinin geleneksel KVRF'leri ile farklı özgül ilişkilere sahip olduğunu ortaya koymaktadır. Biz SUA'nın özgül olarak N ve M'leri etkilediği ve aktif sigara içiminin özgül olmayan bir şekilde tüm hücre alt tiplerini etkilerken, AKŞ'nin özgül olarak N'leri etkilediğini bulduk. Hipertansiyon N ile ve erkek cinsiyet M ile özgül olarak ilişkiliyken, yaş ve KAH için aile öyküsü varlığı sadece L ile ilişkiliydi. Bu farklı ilişkiler farklı endotel hasarı ve vasküler onarım mekanizmalarına yol açabilir. *(Anadolu Kardiyol Derg 2011; 11: 573-81)*

Anahtar kelimeler: Koroner arter hastalığı, enflamasyon hücreleri, nötrofil, monosit, lenfosit, kardiyovasküler risk faktörleri, nötrofil-lenfosit oranı, regresyon analizi

Introduction

In recent years, it has been recognized that atherosclerosis is an active, chronic inflammatory process. Inflammation is a critical feature of atherosclerosis and its clinical manifestations. The pathogenesis of atherosclerosis is multifactorial; however, the effector roles of inflammatory cells in this process are well known (1).

Although leukocyte subtypes play a crucial role in atherosclerosis (2-4), little attention has been paid to the different relationships of leukocyte subtypes with traditional cardiovascular risk factors (CVRFs), demographic properties and biochemical parameters. Different CVRFs may have specifically different peripheral effects on leukocyte subtype, through which these particular interactions may induce different vascular involvement forms due to the altered endothelial damage and vascular repair mechanisms. These effects may be a prerequisite for initiation and progression of the coronary artery disease (CAD) and may determine the type of vascular lesion with a different inflammatory milieu.

For a long time it has been believed that in vascular system, the damaged endothelial cells can only be repaired or replaced by the proliferation and migration of neighboring endothelial cells (5). However, this concept has changed together with determination of endothelial progenitor cells (EPC) having both of stem cell and endothelial cell markers and being able to transform into the endothelial phenotype (6-9). After first time defined by Asaraha et al. (10), we have more knowledge about their source, roles, levels and functionality. The peripheral effects of CVRFs on progenitors were provided by many studies (11-13).

The relationship between leukocytes and increased cardiovascular risk is well known. Horne et al. (14) have aimed to determine the predictive ability of total white blood cell (WBC) count and its subtypes for risk of death or myocardial infarction (MI) and found that high neutrophil (N), monocyte (M) and low lymphocyte (L) counts as well as high N/L ratio are independently related to increased cardiovascular events. These findings were supported by other studies (15-17). However, there are no studies on specific relationships between the inflammatory cells of atherosclerosis and traditional CVRFs focusing particularly on N, L and M as well as total circulating inflammatory cell count (WBC, white blood cell count, leukocyte).

The aim of the present study was to investigate whether there is any specific relationship between the leukocyte subtype counts and the traditional CVRF, biochemical parameters and to evaluate the independency of possible relationships.

Methods

Study design and patients

This study has a cross-sectional and observational design. The Local Ethics review board approved the study protocol. All the patients had given informed consent before the study. The study population consisted of the patients who underwent coronary angiography with a suspicion of CAD at Gazi University School of Medicine in an outpatient manner between October 2005 and June 2006. The study population consisted of 677 eligible consecutive patients. Five hundred and eight (75%) of 677 patients had CAD (men 76%, mean age: 60±10 years). One hundred sixty nine patients (25%) had normal coronary arteries

(NCA) without any stenotic lesion with visual assessment (men 53%, mean age: 53±11 years).

As a dependent variable, total leukocyte count and subtypes, and neutrophil/lymphocyte were investigated in the groups determined by the presence or absence of CVRFs and CAD.

Total and differential leukocyte counts and biochemical markers, which were obtained at most one week before coronary angiography, were used for analyses. Patients with symptomatic peripheral vascular disease (transient ischemic attack, stroke, intermittent claudication, peripheral revascularization, or amputation), evidence of ongoing infection or inflammation, recent acute coronary syndrome either with or without ST-segment elevation (one month before enrollment), hematological disorders, known malignancy and drug history included anti-gout agent were excluded from the study.

Clinical variables and cardiovascular risk factors

Baseline characteristics were recorded during the direct interview with the patient. Hypertension was defined as the active use of antihypertensive drugs or documentation of blood pressure more than 140/90 mmHg. Diabetes mellitus was defined as fasting plasma glucose (FPG) levels over 126 mg/dl or glucose level over 200 mg/dl at any measurement or active use antidiabetic treatment. Smoking was defined as current smoking. The family history for CAD was defined as a history of CAD or sudden death in a first-degree relative before the age of 55 years for men and 65 years for women.

Traditional CVRFs were defined as presence of hypertension, diabetes mellitus, smoking status, and family history for CAD. Demographic properties included age and gender, and biochemical parameters included fasting plasma glucose (FPG), creatinine, serum uric acid level (SUA) and lipids. Baseline characteristics, predictor variables - presence of CAD and CVRFs, outcome variables - leukocyte count and subtypes were included the analyses.

Laboratory analyses

Fasting blood glucose, serum creatinine, total cholesterol, HDL- cholesterol, LDL- cholesterol, and triglyceride levels were recorded. Blood samples were drawn by venipuncture to perform routine blood chemistry. Serum uric acid (SUA) levels were determined with enzymatic colorimetric method by clinical chemistry auto-analyzer (Aeroset, Abbott Laboratory, Abbott Park, IL, USA). Total and differential leukocyte counts were measured by an automated hematology analyzer (Coulter Gen-S, COULTER Corp, Miami, USA). Absolute cell counts were used in the analyses.

Statistical analysis

The SPSS statistical software (SPSS 15.0 for windows, Inc., Chicago, IL, USA) was used for all statistical calculations. Continuous variables are given as mean±SD; categorical variables are defined as percentage. Data were tested for normal distribution using the Kolmogorov-Smirnov test. The Student's t-test was used for the univariate analysis of the continuous variables and the Chi-square test for the categorical variables. Firstly, the inflammatory status of study population was assessed by Student's t-test as an univariate analysis between NCA and CAD groups. After then, to compare the effect of each risk factor on each leukocyte subtype, we performed univariate analyses including Student's t-test and ANOVA, in which different groups were determined by FPG and the quartiles for SUA levels (for categories of FPG: <100mg/dl, 100-126mg/dl and >126mg/dl and for quartiles of SUA: Q1, 1.5-4.1 mg/dl; Q2, 4.2-5.0 mg/dl; Q3, 5.1-6.2 mg/dl; Q4, 6.3-12.9 mg/dl). Lastly, we performed multiple linear regression models to assess multivariate relations between total and differential leukocyte counts and the CVRFs in all patients and also separately in NCA and CAD groups.

All tests of significance were two-tailed. Statistical significance was defined as $p<0.05$.

Results

Inflammatory cells and presence of CAD (Table 1)

When the inflammatory status of study population was assessed according to circulating inflammatory cells, WBC ($p<0.001$), N ($p<0.001$) and M ($p<0.001$) were higher in patients with CAD than those with NCA. Lymphocytes were not significantly different between two groups.

The univariate relationships of leukocyte subtypes with CVRFs (Table 2)

When the effects of each risk factor on each leukocyte subtype were compared in univariate analyses the following relationships were observed: age with L ($p=0.001$) negatively, male gender with M ($p<0.001$) positively, hypertension with N ($p=0.021$) positively, current smoking with N, L and M positively ($p<0.001$; $p<0.001$; $p=0.005$, respectively), family history of CAD with N ($p=0.017$) negatively, the categories for fasting plasma glucose with N positively (<100mg/dl, 100-126mg/dl and >126mg/dl, $p=0.003$), and the quartiles for SUA with M positively (Q1 to Q4, $p<0.001$). While diabetes mellitus was positively related with N, ($p=0.019$) and especially in patients with NCA was negatively related with M. This reverse relation, which was confined to NCA group was also verified by multivariate analysis. Lipids, creatinine and other biochemical parameters except SUA and FPG were not significantly related to leukocyte subtypes.

The multivariate relationships of leukocyte subtypes with CVRFs (Tables 3-7)

When we performed multiple linear regression analyses to determine the independent associations of inflammatory cell subtypes, we have found that FPG had an independent incremental association with WBC (β±SE:4.2±1.4, $p=0.004$) and N (β±SE:4.2±1.2, $p=0.001$). Current smoking had an independent

Table 1. Characteristics of the study population

Variables	NCA (n=169)	CAD (n=508)	p*
Age, years	53±11	60±10	<0.001
Gender, male, %	53	76	<0.001
Hypertension, %	53	59	0.08
Diabetes mellitus, %	17	29	<0.001
Family history, %	28	32	NS
Smoking, current, %	46	57	0.002
Total cholesterol, mg/dl	202±40	188±41	<0.001
LDL, mg/dl	123±32	116±35	0.001
HDL, mg/dl	46±12	43±10	<0.001
Triglycerides, mg/dl	164±89	147±76	0.002
Fasting plasma glucose, mg/dl	103±25	127±56	<0.001
Serum creatinine, mg/dl	1.0±0.5	1.2±1.1	<0.001
Serum uric acid, mg/dl	4.69±1.35	5.59±1.66	<0.001
Hemoglobin, mg/dl	14.0±1.7	13.9±1.8	NS
Platelets, $10^3/mm^{-3}$	243±72	247±77	NS
Leukocytes, mm^{-3}	6959±1669	8117±2530	<0.001
Neutrophils, mm^{-3}	4084±1327	5100±2331	<0.001
Lymphocytes, mm^{-3}	2170±683	2178±795	NS
Monocytes, mm^{-3}	506±150	594±234	<0.001
Medications			
Aspirin, %	60	70	NS
ACEi/ARB, %	44	52	NS
Calcium channel blockers, %	30	40	NS
β-blockers, %	38	36	NS
Statin, %	30	36	NS

Continuous variables are given as mean ± SD and categorical variables are presented as percentage values

*unpaired Student's t-test and Chi-square test

ACEi - angiotensin-converting enzyme inhibitors, ARB - angiotensin II receptor blockers, CAD - coronary artery disease, HDL - high density lipoprotein, LDL - low density lipoprotein, NCA - normal coronary arteries

incremental association with WBC and all cell subtypes (for WBC, N, L, and M: β±SE: 748±161, p<0.001; β±SE: 556±136, p<0.001; β±SE: 185±69, p=0.007; β±SE: 38±20, p=0.061, respectively). SUA had an independent incremental association with WBC (β±SE: 115±43, p=0.008), N (β±SE: 107±38, p=0.005) and M (β±SE: 26±6, p<0.001). Hypertension had an independent incremental association with WBC (β±SE: 431±140, p=0.002) and N (β±SE: 315±118, p=0.008). Male gender had an independent incremental association with only M (β±SE: 52±20, p=0.010). Family history of CAD had an independent decremental association with WBC (β±SE: -327±139, p=0.019) and N (β±SE: -326±121, p=0.007). Finally, age had an independent decremental association with WBC (β±SE: -32±7, p<0.001) and L (β±SE: -16±3, p<0.001). Similar results were also obtained separately in both NCA and CAD subgroups. The N/L ratio was independently related with increased age (p<0.001), FPG (p=0.003) and SUA (p=0.012).

Discussion

Our study results demonstrate that leukocyte subtypes have different specific associations with traditional CVRFs.

We found that FPG affects specifically N while SUA affects specifically N and M, and current smoking affects nonspecifically on all cell subtypes. While hypertension with N and male gender with M were specifically related, age and family history of CAD were only related to L. Furthermore, these relationships were independent of all confounding factors and CAD.

In an additional analysis, we also as a dependent variable searched the N/L ratio which has been found to being related with increased vascular risk and events in previous studies (14-17) and found that it was independently related with increased age, FPG and SUA.

Atherosclerosis is a multifactorial disease, with hypertension, dyslipidemia, dysglycemia, smoking and other CVRFs. These factors cause endothelial injury and contribute to pathogenesis.

Atherosclerosis develops as a process occurring in vessel wall, which begins with an active cellular and passive infiltrative response to endothelial injury. Endothelial dysfunction is characterized with dysfunction and loss of monolayer cells covering the inside of the vessels, which is endothelium. Endothelial dysfunction is the first stage in atherosclerosis. The regenerative capacity of endothelium provides protection against atherosclerosis. Failure of the endothelial repair initiates atherosclerotic inflammation and lesion formation, so-called plaque, especially in non-laminar flow stress points in vascular bed (18).

In recent years, it has been recognized that atherogenesis represents an active, inflammatory process rather than simply passive injury with infiltration of lipids and other substances in blood (19-22). Inflammation plays a critical key role in CAD and other manifestations of atherosclerosis, in which immune mechanisms interact with metabolic risk factors and hemodynamics to initiate, propagate, and activate lesions in the arterial tree (23). Leukocytes play a major role in these inflammatory processes (24), which may be reparative or pathogenic in nature. The microenvironments produced by CVRFs may determine these roles. Inflammatory cells dominate in early atherosclerotic lesions. Their functional molecules accelerate progression of the lesions, and can elicit acute coronary syndromes. Monocytes (macrophages in tissue) and T-lymphocytes are prevalent and pathogenic within unstable plaques. The pathogenesis of atherosclerosis is multifactorial; however, the effector roles of inflammatory cells for plaque formation is clear (1).

Blood-borne inflammatory and immune cells constitute an important part of an atheroma. Chapman et al. (25) found that an inflammatory cell subtype count, monocyte count, in blood was a better cross-sectional marker for the presence of atherosclerotic plaque than interleukine-6 (IL-6), high-sensitive C-reactive

Table 2. The changes in leukocyte subtypes according to traditional cardiovascular risk factors

Risk factors		WBC, mm^{-3}		p/F*	Neutrophils, mm^{-3}		p/F*	Lymphocytes, mm^{-3}		p/F*	Monocytes, mm^{-3}		p/F*
Age	Overall	7316±1686	7336±1577	0.916	4217±1397	4436±1376	0.164	2367±673	2099±700	0.001	538±161	553±193	0.492
<45 years	NCA	7029±1753	6847±1538	0.477	4138±1446	4011±1243	0.554	2219±603	2122±702	0.366	500±141	503±147	0.885
≥45 years	CAD	7872±1416	7454±1557	0.152	4371±1308	4535±1372	0.525	2653±717	2096±699	<0.001	613±175	565±203	0.213
Gender	Overall	7215±1614	7381±1578	0.206	4431±1453	4397±1348	0.765	2093±721	2149±693	0.335	497±163	574±196	<0.001
Female	NCA	6788±1670	7007±1539	0.356	4024±1423	4071±1189	0.807	2114±705	2188±637	0.451	459±134	544±141	<0.001
Male	CAD	7537±1498	7460±1568	0.633	4740±1405	4462±1351	0.052	2078±736	2143±704	0.379	526±176	581±207	0.010
Hypertension	Overall	7211±1563	7395±1610	0.162	4242±1259	4499±1426	0.021	2196±666	2102±743	0.114	542±189	551±193	0.572
Absent	NCA	6758±1576	7038±1575	0.249	3918±1189	4140±1341	0.255	2138±587	2185±750	0.648	510±141	501±147	0.675
Present	CAD	7429±1514	7507±1594	0.617	4399±1265	4605±1406	0.124	2224±701	2078±737	0.043	558±207	569±205	0.618
Diabetes Mellitus	Overall	7244±1665	7534±1520	0.053	4315±1355	4616±1387	0.019	2146±706	2124±733	0.744	548±189	543±198	0.771
Absent	NCA	6963±1630	6577±1222	0.261	4071±1323	3811±895	0.345	2169±701	2140±522	0.844	519±145	424±104	0.002
Present	CAD	7383±1592	7695±1483	0.060	4436±1356	4736±1355	0.038	2135±709	2132±763	0.971	563±207	566±205	0.901
Smoking, current	Overall	7100±1551	8019±1490	<0.001	4260±1357	4813±1282	<0.001	2070±694	2375±720	<0.001	535±191	587±186	0.005
Absent	NCA	6545±1440	8039±1391	<0.001	3820±1229	4731±1137	<0.001	2050±646	2504±641	<0.001	488±142	557±135	0.006
Present	CAD	7308±1529	8011±1536	<0.001	4418±1341	4846±1340	0.005	2081±710	2321±747	0.004	553±204	599±207	0.051
Family history of CAD	Overall	7398±1566	7145±1629	0.068	4480±1373	4198±1318	0.017	2149±730	2130±674	0.758	544±187	552±200	0.653
Absent	NCA	6976±1631	6733±1446	0.335	4130±1340	3809±1074	0.129	2136±683	2224±655	0.435	512±143	488±143	0.318
Present	CAD	7566±1494	7294±1669	0.090	4612±1334	4338±1372	0.048	2160±747	2095±680	0.391	558±202	575±212	0.421
Fasting plasma glucose	Overall	7185±1620	7341±1562	7646±1548	0.015 F=4.2	4259±1393	4402±1305	4734±1408	0.003 F=6.0	2164±695	2115±696	2125±772	0.721 F=0.3	542±189	559±178	554±213	0.596 F=0.5
T1 (<100 mg/dl)	NCA	7018±	6785±	6873±	0.698	4111±	3981±	4030±	0.844	2209±	2077±	2163±	0.533	498±	519±	418±	0.561
T2 (100-126 mg/dl)		1738	1385	1417	F=0.4	1454	1079	948	F=0.2	718	637	524	F=0.6	143	145	134	F=0.6
T3 (>126 mg/dl)	CAD	7275±	7521±	7715±	0.038	4339±	4539±	4784±	0.016	2140±	2127±	2132±	0.986	566±	572±	562±	0.922
		1549	1578	1486	F=3.3	1357	1346	1378	F=4.2	683	715	792	F=0.01	207	186	220	F=0.08
Serum uric acid	Overall	7025±	7254±	7505±	7450±	0.037	4247±	4293±	4471±	4573±	0.145	2094±	2151±	2249±	2007±	0.029	479±	554±	569±	607±	<0.001
Q1 (1.5-4.1 mg/dl)		1673	1431	1589	1526	F=2.9	1498	1187	1347	1396	F=1.8	733	695	741	623	F=3.0	163	176	195	222	F=11.4
Q2 (4.2-5.0 mg/dl)	NCA	6780±	6721±	7454±	7289±	0.143	4031±	3977±	4265±	4150±	0.800	2117±	2015±	2418±	2305±	0.051	443±	494±	556±	616±	<0.001
Q3 (5.1-6.2 mg/dl)		1742	1585	1398	1272	F=1.8	1567	1145	1041	1175	F=0.3	663	630	671	662	F=2.7	127	124	129	147	F=10.5
Q4 (6.3-12.9 mg/dl)	CAD	7212±	7455±	7519±	7471±	0.535	4411±	4413±	4529±	4628±	0.558	2076±	2202±	2203±	2968±	0.033	507±	553±	597±	605±	0.003
		1604	1322	1644	1559	F=0.7	1431	1187	1420	1417	F=0.6	786	713	755	609	F=3.0	183	188	209	230	F=4.7

Continuous variables are given as mean±SD (in mm^{-3})
*unpaired Student's t-test and ANOVA test
CAD - coronary artery disease, NCA - normal coronary arteries, WBC - white blood cell (leukocyte) counts, Q1 to Q4 are quartiles for serum uric acid and T1 to T3 are tertiles for fasting plasma glucose

protein (hsCRP), fibrinogen, and white blood cells. Furthermore, Johnsen et al. (26) showed that monocyte count was an independent predictor of future atherosclerotic plaque formation. Except the patients with chronic kidney disease, cross-sectional studies reported increased numbers of circulating monocytes in individuals with prevalent atherosclerotic disease (27, 28). Additionally, prospective studies suggested that monocyte count can predict cardiovascular events independently (29, 30).

Recent research has focused on the use of inflammatory biomarkers and cells (31) in the prediction of cardiovascular risk. However, information is scant regarding the association between particularly these inflammatory cells and CVRFs. To our knowledge, our study is the first report focusing on the specific relationships between CVRFs and leukocyte subtypes.

A recent research has shown that WBC count has significant correlation with fasting (32) and postprandial glucose (33). In another study, SUA was significantly and independently associated with neutrophil count (34). In a recently published study, hyperlipidemia-triggered neutrophilia was shown to promote early atherosclerosis (35).

Table 3. Effects of demographical and traditional cardiovascular risk factors, and laboratory parameters on leukocyte (white blood cell) count in blood

Variables	Overall (n=677)			CAD (n=541)			NCA (n=136)		
	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*
Age, years	-32±7	-0.214	<0.001	-31±8	-0.199	<0.001	-29±13	-0.194	0.022
Hypertension, +	431±140	0.136	0.002	321±168	0.102	0.056	763±254	0.244	0.003
Current smoking, +	748±161	0.202	<0.001	554±192	0.151	0.004	1122±292	0.311	<0.001
Family history of CAD, +	-327±139	-0.097	0.019	-311±164	-0.095	0.058	-463±272	-0.135	0.091
Fasting plasma glucose, mg/dl	4.2±1.4	0.125	0.004	4.7±1.5	0.159	0.002	-8.7±6	-0.109	NS
SUA, mg/dl	115±43	0.114	0.008	94±49	0.098	0.054	234±100	0.180	0.021
CAD, +	574±162	0.164	<0.001	-	-	-	-	-	-
Constant	7260±455	-	<0.001	7985±607	-	<0.001	7675±962	-	<0.001
R^2	0.145			0.106			0.231		

*Linear regression with stepwise method: dependent variable - leukocyte count, independent variables- age, gender, HT, DM, current smoking, family history of CAD, total cholesterol, LDL, HDL, triglyceride, fasting plasma glucose, creatinine, SUA and presence of CAD
After exclusion of gender, DM, total cholesterol, LDL, HDL, triglyceride and creatinine variables from model, linear regression with enter method was performed
β±SE- Beta±standard error, CAD - coronary artery disease, DM - diabetes mellitus, HDL - high density lipoprotein, HT - hypertension, LDL - low density lipoprotein, NCA - normal coronary arteries, SUA - serum uric acid level

Table 4. Effects of demographical and traditional cardiovascular risk factors, and laboratory parameters on neutrophil count in blood

Variables	Overall (n=677)			CAD (n=541)			NCA (n=136)		
	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*
Hypertension, +	315±118	0.116	0.008	277±143	0.101	0.054	447±221	0.176	0.036
Current smoking, +	556±136	0.176	<0.001	438±164	0.137	0.008	797±243	0.271	0.001
Family history of CAD, +	-326±121	-0.114	0.007	-323±144	-0.113	0.026	-441±230	-0.159	0.058
Fasting plasma glucose, mg/dl	4.2±1.2	0.153	0.001	4.7±1.3	0.190	<0.001	-5.9±5	-0.091	NS
SUA, mg/dl	107±38	0.124	0.005	109±43	0.129	0.012	115±86	0.110	NS
CAD, +	268±134	0.089	0.047	-	-	-	-	-	-
Constant	2905±256	-	<0.001	3152±318	-	<0.001	3799±669	-	<0.001
R^2	0.107			0.081			0.127		

*Linear regression with stepwise method: dependent variable - neutrophil count, independent variables- age, gender, HT, DM, total cholesterol, LDL, HDL, triglyceride, fasting plasma glucose, creatinine, SUA and presence of CAD
After exclusion of age, gender, DM, total cholesterol, LDL, HDL, triglyceride and creatinine variables from model, linear regression with enter method was performed
β±SE- Beta±standard error, CAD - coronary artery disease, DM - diabetes mellitus, HDL - high density lipoprotein, HT - hypertension, LDL - low density lipoprotein, NCA - normal coronary arteries, SUA - serum uric acid level

In another recent study, Tian et al. (36) searched the association between circulating specific leukocyte types and blood pressure and found that blood pressure is specifically related to N and L. Kawada et al. (37) showed an independent relationship between N and hypertension. In the study by Yen et al., the investigators searched the relation between hsCRP, standard CVRFs and WBC, neutrophils and monocytes. Although SUA and L were not included analyses, several overlapping results were obtained in multivariate analyses. While hsCRP was related to nonspecifically all CVRFs, glucose was only related with N. Smoking was, in a manner consistent with our findings, related with WBC, N and M nonspecifically. Other than these, diastolic blood pressure, body mass index, lipids were related WBC, N and M (38). In our opinion, SUA could change the specificity of these relationships and this is a limitation for their multivariate analyses. Lavi et al. (39) searched the effect of smoking status on leukocyte subtypes and found that smoking was related with WBC, N, L and M. Although, in these studies, the investigators had not focused specifically on the relationship between leukocyte subtypes and CVRFs, their findings were consentient with our results.

The specific detrimental peripheral effects of CVRFs on circulating leukocyte subtypes and also on vascular progenitors (11, 12) are important to be recognized in the pathogenesis of atherosclerosis. Since these different effects may lead to different kinds of defects in endothelial function and vascular repair mechanisms, solutions that

Table 5. Effects of demographical and traditional cardiovascular risk factors, and laboratory parameters on lymphocyte count in blood

Variables	Overall (n=677)			CAD (n=541)			NCA (n=136)		
	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*
Age, years	-16±3	-0.251	<0.001	-20±4	-0.274	<0.001	-8.3±5	-0.132	0.093
Current smoking, +	185±69	0.112	0.007	108±83	0.063	NS	384±122	0.247	0.002
CAD, +	114±67	0.072	0.089	-	-	-	-	-	-
Constant	2970±164	-	<0.001	3313±223	-	<0.001	2497±270	-	<0.001
R^2	0.084			0.089			0.100		

*Linear regression with stepwise method: dependent variable -lymphocyte count, independent variables- age, gender, HT, DM, current smoking, family history of CAD, total cholesterol, LDL, HDL, triglyceride, fasting plasma glucose, creatinine, SUA and presence of CAD
After exclusion of gender, DM, HT, family history of CAD, total cholesterol, LDL, HDL, triglyceride, fasting plasma glucose, creatinine and SUA variables from model, linear regression with enter method was performed
β±SE- Beta±standard error, CAD - coronary artery disease, DM - diabetes mellitus, HDL - high density lipoprotein, HT - hypertension, LDL - low density lipoprotein, NCA - normal coronary arteries, SUA - serum uric acid level

Table 6. Effects of demographical and traditional cardiovascular risk factors, and laboratory parameters on monocyte count in blood

Variables	Overall (n=677)			CAD (n=541)			NCA (n=136)		
	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*
Gender, male	52±20	0.121	0.010	52±28	0.102	0.061	50±23	0.175	0.032
Diabetes mellitus, +	15±19	0.033	NS	37±24	0.081	NS	-77±29	-0.198	0.008
Current smoking, +	38±20	0.082	0.061	37±26	0.074	NS	34±25	0.104	NS
SUA, mg/dl	26±6	0.209	<0.001	24±7	0.185	<0.001	34±9	0.305	<0.001
CAD, +	29±20	0.068	NS	-	-	-	-	-	-
Constant	340±31	-	<0.001	373±44	-	<0.001	322±40	-	<0.001
R^2	0.097			0.057			0.264		

*Linear regression with stepwise method: dependent variable - monocyte count, independent variables - age, gender, HT, DM, current smoking, family history of CAD, total cholesterol, LDL, HDL, triglyceride, fasting plasma glucose, creatinine, SUA and presence of CAD
After exclusion of age, HT, family history of CAD, total cholesterol, LDL, HDL, triglyceride, fasting plasma glucose, and creatinine variables from model, linear regression with enter method was performed
β±SE- Beta±standard error, CAD - coronary artery disease, DM - diabetes mellitus, HDL - high density lipoprotein, HT - hypertension, LDL - low density lipoprotein, NCA - normal coronary arteries, SUA - serum uric acid level

Table 7. Effects of demographical and traditional cardiovascular risk factors, and laboratory parameters on neutrophil / lymphocyte ratio in blood

Variables	Overall (n=677)			CAD (n=541)			NCA (n=136)		
	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*	β±SE	Standardized coefficients	p*
Age, years	0.2±0.006	0.156	<0.001	0.02±0.01	0.163	0.001	0.02±0.01	0.121	NS
Family history of CAD, +	-0.3±0.1	-0.088	0.041	-0.3±0.2	-0.088	NS	-0.4±0.3	-0.134	NS
Fasting plasma glucose, mg/dl	0.01±0.001	0.129	0.003	0.01±0.001	0.169	0.001	-0.003±0.01	-0.041	NS
SUA, mg/dl	0.1±0.04	0.109	0.012	0.1±0.04	0.159	0.002	-0.05±0.1	-0.044	NS
Constant	0.3±0.4	-	<0.001	-0.2±0.5	-	NS	1.9±1.0	-	0.048
R^2	0.068			0.081			0.036		

*Linear regression with stepwise method: dependent variable - neutrophil / lymphocyte ratio, independent variables - age, gender, HT, DM, current smoking, family history of CAD, total cholesterol, LDL, HDL, triglyceride, fasting plasma glucose, creatinine, SUA and presence of CAD
After exclusion of gender, HT, DM, family history of CAD, presence of CAD, total cholesterol, LDL, HDL, triglyceride, and creatinine variables from model, linear regression with enter method was performed
β±SE - Beta±standard error, CAD - coronary artery disease, DM - diabetes mellitus, HDL - high density lipoprotein, HT - hypertension, LDL - low density lipoprotein, NCA - normal coronary arteries, SUA - serum uric acid level

are more specific may be provided by understanding these relationships to protect against the peripheral effects of CVRFs.

The CVRFs have detrimental effects on circulating progenitors, which partially may be related to their peripheral effect on inflammatory cells and the inflammatory pathological microenvironment as well as direct detrimental effects on progenitor cells and blocking effects on the mobilization of progenitors from bone marrow.

In our opinion, increase in circulating inflammatory cells, which is induced by some uncontrolled CVRFs may lead to more complicated diffuse and severe vascular involvement due to the accelerated progression of atherosclerotic process. The plaque stabilization providing a specific decrease in leukocyte subtypes may be achieved by the controlling blood sugar, lowering uric acid levels and cessation of smoking. In long term, these methods may provide a decrease in acute and chronic manifestations of atherosclerosis, such as, triggering of acute coronary syndromes and recurrent adverse events.

In a recent intracoronary ultrasound study, atherosclerotic plaque regression and simultaneous inflammatory cell count suppression were achieved with statin therapy. In this study, authors concluded that especially monocyte count may serve as a simple marker of plaque regression with statins. Since atherosclerosis is characterized by recruitment of monocytes to the arterial wall, monocyte count may be a more specific measurement of inflammatory activity in the arterial wall than total leukocyte count (40). In addition, these relationships between risk factors and inflammatory cell subtypes may also provide a simple and risk factor-specific biomarker for follow up in clinical settings. Therefore, the easily measurable neutrophil, monocyte counts by automatic counter can be used to determine the effectiveness of risk factor modification and anti-atherogenic treatment in clinical practice. In our opinion, if we know which risk factor is associated with which specific cell subtype, we can trace a specific cell subtype for related risk factor in individual course of patient.

Study limitations

Our study had some limitations. The main limitation of our study may be the absence of the other inflammatory markers in the analyses. But, we focused more specifically on the relationship between the circulating inflammatory cell subtypes and the traditional CVRFs rather than the relationship between leukocyte subtypes and coronary artery disease, therefore hsCRP is not necessary to search the main goal in current study.

We assessed leukocyte and subtypes by an automatic cell counter and more specific cell determinations could be performed by a flow cytometer with cluster of differentiation (CDs) antigens. Another limitation in current study, it is to have not been done a work for in vitro cell functions, which may be in reparative or pathological nature for physiologic and pathological micro-circumstances. This kind of analysis would probably provide additional information on atherosclerosis.

Lastly, in our study, the patients included in the control group were not completely normal. Although they have angiographically normal coronary arteries, they still have cardiac risk factors or may have cardiac syndrome-X. Therefore, the statistical differences would be difficult to determine between normal and pathologic group. Otherwise, our study population proved many significant relations among study groups.

Conclusion

Our findings show special influences of age, FPG, SUA levels and male gender, current smoking and presence of hypertension on specific subtypes of circulating inflammatory cells. Further studies with larger study populations would be warranted to demonstrate whether the risk factor modification and anti-atherogenic treatment could provide simultaneous decrease in specific cell subtype count and number of adverse clinical events.

Conflict of interest: None declared.

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