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Age-Associated Increasing of MCP-1 in Adults

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Monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) play a key role in development of atherosclerosis. To take into account the atherogenic properties of MCP-1 and IL-8 and its influence on insulin resistance, we examined circulating levels of MCP-1 and IL-8 in adults. We recruited 292 subjects (84 males and 208 females) aged between 29 and 79 years. MCP-1 and IL-8 levels were measured by enzyme-linked immunosorbent assay. Age, total cholesterol, HDL-cholesterol, and LDL-cholesterol levels were significantly higher in female subjects (P<0.01, respectively), but diastolic blood pressure (BP) was significantly lower in female subjects compared to male subjects. MCP-1 and IL-8 levels were tended to increase with age, the highest in their seventies. MCP-1 (P=0.05) and IL-8 (P<0.01) levels were higher in males than in females. MCP-1 was positively correlated with age (r=0.17, P<0.05), IL-8 (r=0.26, P<0.01), fasting insulin (r=0.30, P<0.01), and HOMA-IR (r=0.29, P<0.01). In linear regression analysis, age was found to be independent factor associated with MCP-1 adjusted by age, BMI, fasting glucose, triglyceride, and systolic BP. In conclusion, age was found to be independent factor associated with MCP-1. It is possible that an increase of MCP-1 in adults with age may be risk to atherosclerosis and diabetic properties.

Key Words: MCP-1, IL-8, Age, Gender, Atherosclerosis

INTRODUCTION

Numerous reports demonstrated that chronic low-grade inflammation is involved in the pathogenesis of type 2 diabetes and cardiovascular disease (CVD). The chemokine, monocyte chemoattractant protein-1 (MCP-1) attracts monocytes and interleukin-8 (IL-8) attracts neutrophils (Rollins, 1997; Krishnaswamy et al., 1999). These chemokines play a key role in development of atherosclerosis by enhancing adhesion molecules on leukocytes and endothelial cells, as well as by inducing leukocyte infiltration into the vascular subendothelial area (Reape et al., 1999; Yu et al., 2004). Elevated MCP-1 levels have been found to be associated with older age (Inadera et al., 1999), hypertension (Parissis et al., 2000), hypertriglycemia (Inadera et al., 1999), and diabetes (de Lemos et al., 2003). IL-8 levels were significantly increased in patients with type 1 or 2 diabetes (Zozulińska et al., 1999), and obese (Bruun et al., 2003). These indicate the involvement of MCP-1 and IL-8 in insulin resistance, diabetes and CVD.

Normal ageing in humans is associated with several hormone, inflammation and metabolic alterations. Moreover, advanced age is one of the major risk factor of atherosclerosis. But, the consequence of ageing on inflammation about chemokines has not fully examined.

Therefore, we examined circulating levels of MCP-1 and IL-8 in adults, and to evaluate the relationship between aging and both MCP-1 and IL-8.

MATERIALS AND METHODS

We recruited 292 subjects (84 males and 208 females) aged between 29 and 79 years. The participants visited the hospital for a periodic health check-up. We excluded patients with bleeding tendencies and thrombotic events, such as stroke and ischemic heart disease. Prior to testing, the part-

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icipants independently completed a questionnaire regarding their medical disease and medications.

Body weight was measured to the nearest 0.1 kg using an electronic scale. Patients were weighed in light clothing without shoes. Height was measured to the nearest 0.1 cm using a wall mounted stadiometer. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

Biochemical tests were performed on blood samples collected after fasting for more than 12 hours. Serum levels of glucose, total cholesterol, HDL-cholesterol, triglyceride, and high-sensitivity C-reactive protein (hs-CRP) were assayed using an ADVIA 1650 Chemistry system (Bayer, Tarrytown, NY, USA). LDL-cholesterol was calculated by Friedewald's formula, if serum triglyceride levels were below 400 mg/dL (Friedewald et al., 1972). Fasting insulin levels were measured by a competitive immunoassay using an Immulite 2000 (DPC, Pacific Concourse, LA, USA). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) index [(Insulin (μIU/ml) × Fasting blood glucose (mg/dl)/18) / 22.5]. MCP-1 and IL-8 levels were measured by enzyme-linked immunosorbent assay (R&D system, Minneapolis, USA).

Data are expressed as mean ± SD. Variables such as fasting insulin, HOMA-IR, hs-CRP, triglyceride, IL-8 levels were logarithmically transformed prior to statistical analysis to approximate a normal distribution. Baseline characteristics and the difference of chemokines such as MCP-1 and IL-8 between genders were compared using a *t*-test for continuous variables. An analysis of variance (ANOVA) was performed to assess the differences in MCP-1 and IL-8 levels between age groups in both genders. Pearson correlation coefficients were calculated to evaluate a relationship between MCP-1 or IL-8 and metabolic variables such as age, BMI, blood pressure (BP), lipid profiles, fasting glucose, insulin, and HOMA-IR index. Multple linear regression analysis was used to determine the interactions between MCP-1, adjusted by gender, BMI, fasting glucose, triglyceride, and systolic BP. Significance was defined at the 0.05 level of confidence. All calculations were performed using the Statistical Package for Social Sciences software, version 15.0 (SPSS, Chicago, IL, USA).

Table 1. Clinical and metabolic characteristics of study subjects

Characteristics	Male (N=84)	Female (N=208)	*P*-value
Age (years)	51.4±9.8	55.6±8.7	<0.01
BMI[a] (Kg/m^2)	24.9±3.2	24.8±3.2	0.69
Blood pressure (mmHg)			
Systolic	129.5±15.8	131.4±20.8	0.46
Diastolic	82.7±12.1	78.7±12.4	<0.05
Glucose tolerance index			
Fasting glucose (mg/dl)	102.6±36.5	101.8±49.0	0.89
Fasting insulin[b] (μIU/mL)	4.84±3.3	4.91±3.1	0.88
HOMA-IR[b,c]	1.2±1.0	1.2±0.8	0.96
Lipid profile			
Total cholesterol (mg/dl)	194.3±33.8	210.3±38.9	<0.01
Triglyceride[b] (mg/dl)	148.4±90.5	134.9±156.2	0.46
HDL-cholesterol (mg/dl)	44.4±12.0	49.9±12.1	<0.01
LDL-cholesterol (mg/dl)	119.8±30.1	134.2±35.5	<0.01
Chemokines			
MCP-1[d] (pg/ml)	387.2±109.0	360.1±108.8	0.05
IL-8[e] (pg/ml)	54.3±61	36.7±30.0	<0.01

Data are shown as means ± the standard deviation.
P-values are calculated by t-test and χ^2-test.
[a] Body mass index.
[b] Values were analysed after log-transformation
[c] Homeostasis model assessment insulin resistance.
[d] Monocyte chemoattractant protein-1
[e] Interleukine-8

RESULTS

The clinical characteristics of the subjects are shown in Table 1. Age, total cholesterol, HDL-cholesterol, and LDL-cholesterol levels were significantly higher in female subjects (*P*<0.01, respectively), but diastolic BP was significantly lower in female subjects compared with male subjects. MCP-1 (*P*=0.05) and IL-8 (*P*<0.01) levels were higher in males than in females.

MCP-1 and IL-8 levels according to age and gender group are shown in Table 2. MCP-1 and IL-8 levels were tended to increase with age, the highest in their seventies.

MCP-1 was positively correlated with age (*r*=0.17, *P*<0.05), whereas IL-8 levels were not significantly correlated with age (*r*=0.04, *P*=0.50) as shown in Fig. 1. In addition, MCP-1 was positively correlated with fasting insulin (*r*=0.30, *P*<0.01), HOMA-IR (*r*=0.29, *P*<0.01), and IL-8 (*r*=0.26, *P*<0.01) data was not shown.

In linear regression analysis, age was found to be in-

Table 2. Serum MCP-1[a] and IL-8[b] values according to age and gender

Gender	Age	Chemokines				
		N	MCP-1 (pg/ml)	*P*	IL-8 (pg/ml)	*P*
Male	30~39	10	370.5±145.1		45.8±49.5	
	40~49	23	333.1±69.7		53.5±49.4	
	50~59	33	410.8±122.1		51.9±58.2	
	60~69	16	416.2±79.7		48.5±44.4	
	70~	2	471.5±46.0	0.123	191.4±236.1	0.569
Female	30~39	8	326.6±93.7		49.2±54.2	
	40~49	31	308.7±92.0		30.8±9.7	
	50~59	97	367.4±109.9		35.4±25.4	
	60~69	62	372.3±110.4		36.9±29.9	
	70~	10	399.7±110.8	0.077	56.6±67.2	0.711

Data are shown as means ± the standard deviation.
[a]monocyte chemoattractant protein-1, [b]Interleukine-8

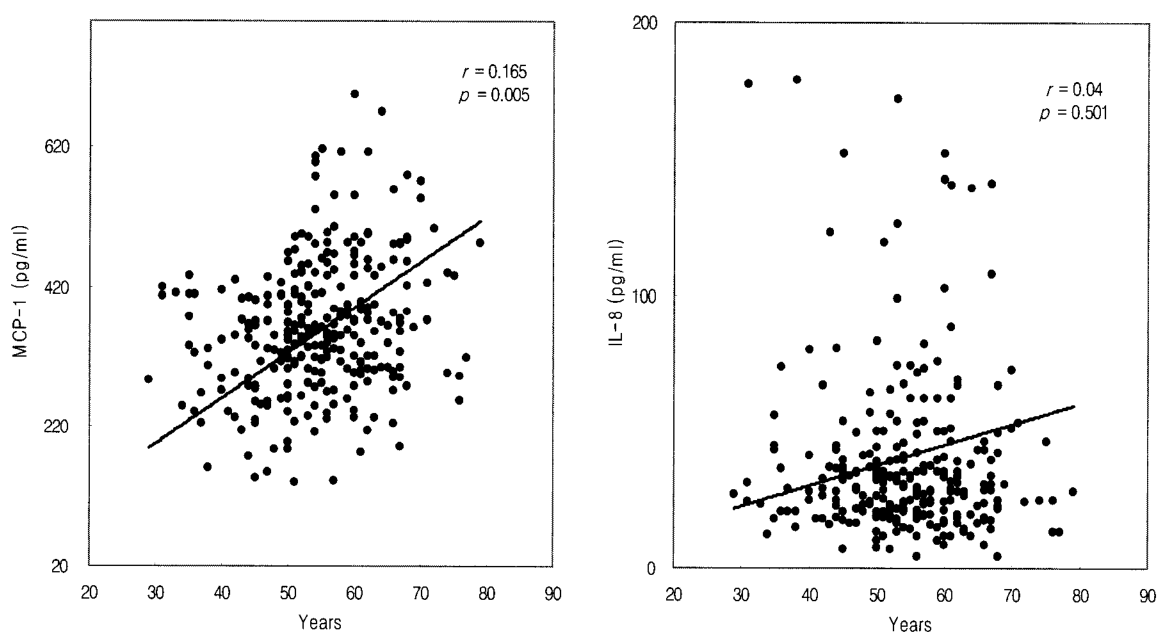

Fig. 1. Relationship between chemokines and age. MCP-1 levels were positively correlated with age (r=0.17, P=0.005), whereas IL-8 levels were not significantly correlated with age (r=0.04, P=0.50).

dependent factor associated with MCP-1 adjusted by age, BMI, fasting glucose, triglyceride, and systolic BP (Table 3).

Table 3. Association between several factors and MCP-1

Variables	β	S.E	*P*-value
Age	2.601	0.625	<0.01
Body mass index	3.067	2.023	0.13
Fasting glucose	0.042	0.125	0.74
Triglyceride	0.029	0.041	0.47
Systolic blood pressure	-0.034	0.331	0.92

DISCUSSION

In this study, we examined the circulating levels of MCP-1 and IL-8, and the impact of age and gender. We found that MCP-1 and IL-8 levels tended to increase with age, the highest in seventies. However, MCP-1 was decreased in fourties of males and females, whereas IL-8 was decreased in fourties of females only. Low levels of both MCP-1 and IL-8 in fourties might be explained by increase of healthy behavior such as exercise, and ingestion of vitamins.

In present study, MCP-1 and IL-8 levels were higher in males than in females although statistically not significant in MCP-1. Gender-dependent difference in MCP-1 levels was found previous studies (Inadera et al., 1999; Deo et al., 2004; Berrahmoune et al., 2006). In another study, Tabara et al. (2003) failed to find such association; elderly persons did not show a significant difference between men and women. A recent *in vivo* study revealed that physiologic concentrations of estradiol suppress MCP-1 expression in rabbit (Pervin et al., 1998). Thus, the circulating MCP-1 levels may be influenced by sex hormones.

In multiple regression analysis, age was independently and significantly associated with MCP-1. This result is in accordance with other reports. Several studies reported that the levels of MCP-1 and IL-8 were significant positive association with age (Tabara et al., 2003; Mukaida et al., 2003; Juarranz et al., 2004). Age dependent increasing of the level of MCP-1 may indicate possible atherosclerotic lesions (Inadera et al., 1999). Epidemiologic studies have established that aging is a risk factor for atherosclerosis (Vinereanu, 2006). Gerli et al. (2000) postulated an age-dependent shift in the cytokine network. Apart from athero-sclerosis, many studies have reported that oxidative stress increase with age. This oxidative stress-induced activity promotes the production of a number of proinflammatory cytokines, which can contribute to the pathology of many diseases states associated with aging (Rajindar, 2002; Junqueira et al., 2004).

In this study, serum levels of MCP-1 showed significantly positive associations with insulin and HOMA-IR. Recent studies found that relationship between MCP-1 level and insulin resistance, as well as type 2 diabetes (Piemonti et al., 2003; Herder et al., 2006). MCP-1 or IL-8 level was positively related to the HOMA-IR in obese subjects (Kim et al., 2006). This result supports the idea that these chemo-kines contribute to insulin resistance. Circulating levels of IL-8 have also been shown to be high in atherosclerosis patients (Gerszten et al., 1999), and are associated with insulin sensitivity (Bruun et al., 2003). However, our data failed to show any significant correlation between IL-8 and metabolic related parameter. The lack of the relation is in concordance with others, IL-8 is expressed in adipose tissue (Bruun et al., 2003), but IL-8 levels did not correlated with BMI and obesity related parameter (Herder et al., 2005).

The limitation of this investigation is that it is only an observational study. Therefore, we are not able to follow circulating level of MCP-1 and IL-8 in individuals with ageing.

In conclusion, age was found to be independent factor associated with MCP-1. It is possible that an increase of MCP-1 in adults with age may be risk to atherosclerosis and diabetic properties.

REFERENCES

Berrahmoune H, Lamont JV, Herbeth B, FitzGerald PS, Visvikis-Siest S. Biological determinants of and reference values for plasma interleukin-8, monocyte chemoattractant protein-1, epidermal growth factor, and vascular endothelial growth factor: Results from the STANISLAS cohort. Clin Chem. 2006. 52: 504-510.

Bruun JM, Verdich C, Toubro S, Astrup A, Richelsen B. Association between measures of insulin sensitivity and circulating levels of interleukin-8, interleukin-6 and tumor necrosis factor-alpha. Effect of weight loss in obese men. Eur J Endocrinol. 2003. 148: 535-542.

de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, McCabe CH, Cannon CP, Braunwald E. Association between plasma levels of monocyte chemoattrac-tant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003. 107: 690-695.

Deo R, Khera A, McGuire DK, Murphy SA, Meo Neto Jde P, Morrow DA, de Lemos JA. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. J Am Coll Cardiol. 2004. 44: 1812-1818.

Friedewald WT, Levy RI, Fridrikson DS: Estimation of concen-trations of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. Clin Chem. 1972. 18: 499-502.

Gerli R, Monti D, Bistoni O, Mazzone AM, Peri G, Cossarizza A, Di Gioacchino M, Cesarotti ME, Doni A, Mantovani A, Franceschi C, Paganelli R. Chemokines, sTNF-Rs and sCD30 serum levels in healthy aged people and centenarians. Mech Ageing Dev. 2000. 121: 37-46.

Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone MA, Luster AD, Luscinskas FW, Rosenzweig A. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 1999. 398: 718-723.

Herder C, Baumert J, Thorand B, Koenig W, de Jager W, Meisinger C, Illig T, Martin S, Kolb H. Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. Diabetologia 2006. 49: 921-929.

Herder C, Haastert B, Müller-Scholze S, Koenig W, Thorand B, Holle R, Wichmann HE, Scherbaum WA, Martin S, Kolb H. Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Diabetes 2005. 54: S11-S17.

Inadera H, Egashira K, Takemoto M, Ouchi Y, Matsushima K. Increase in circulating levels of monocyte chemoattractant protein-1 with aging. J Interferon Cytokine Res. 1999. 19: 1179-1182.

Juarranz MG, Santiago B, Torroba M, Gutierrez-Cañas I, Palao G, Galindo M, Abad C, Martinez C, Leceta J, Pablos JL, Gomariz RP. Vasoactive intestinal peptide modulates proinflammatory mediator synthesis in osteoarthritic and rheumatoid synovial cells. Rheumatology (Oxford) 2004. 43: 416-422.

Kim CS, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, Kwon BS, Erickson KL, Yu R. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes (Lond) 2006. 30: 1347-1355.

Krishnaswamy G, Kelley J, Yerra L, Smith JK, Chi DS. Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease.? J Interferon Cytokine Res. 1999. 19: 91-104.

Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. Am J Physiol Lung Cell Mol Physiol. 2003. 284: L566-L577.

Parissis JT, Venetsanou KF, Kalantzi MV, Mentzikof DD, Karas SM. Serum profiles of granulocyte-macrophage colony-stimulating factor and C-C chemokines in hypertensive patients with or without significant hyperlipidemia. Am J Cardiol. 2000. 85: 777-779.

Pervin S, Singh R, Rosenfeld ME, Navab M, Chaudhuri G, Nathan L. Estradiol suppresses MCP-1 expression In vivo: implications for atherosclerosis. Arterioscler Thromb Vasc Biol. 1998. 18:1575-1582.

Piemonti L, Calori G, Mercalli A, Lattuada G, Monti P, Garancini MP, Costantino F, Ruotolo G, Luzi L, Perseghin G. Fasting plasma leptin, tumor necrosis factor-alpha receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. Diabetes Care 2003. 26: 2883-2889.

Rajindar S. Sohal. Oxidative stress hypothesis of aging. Free Radic Biol Med. 2002. 33: 573-574.

Reape TJ, Groot PH. Chemokines and atherosclerosis. Atherosclerosis 1999. 147: 213-225.

Rollins BJ. Chemokines. Blood 1997. 90: 909-928.

Tabara Y, Kohara K, Yamamoto Y, Igase M, Nakura J, Kondo I, Miki T. Polymorphism of the monocyte chemoattractant protein (MCP-1) gene is associated with the plasma level of MCP-1 but not with carotid intima-media thickness. Hypertens Res. 2003. 26: 677-683.

Vinereanu D. Risk factors for atherosclerotic disease: present and future. Herz. 2006. 31 Suppl 3: 5-24.

Junqueira VB, Barros SB, Chan SS, Rodrigues L, Giavarotti L, Abud RL, Deucher GP. Aging and oxidative stress. Mol Aspects Med. 2004. 25: 5-16.

Virginia B. C. Junqueira, Silvia B. M. Barros, Sandra S. Chan, Luciano Rodrigues, Leandro Giavarotti, Ronaldo L. Abud and Guilherme P. Deucher. Aging and oxidative stress. Mol Aspects Med. 2004. 25: 5-16.

Yu R, Kim CS, Kawada T, Kwon TW, Lim TH, Kim YW, Kwon BS. Involvement of leukotactin-1, a novel CC chemokine, in human atherosclerosis. Atherosclerosis 2004. 174: 35-42.

Zozulińska D, Majchrzak A, Sobieska M, Wiktorowicz K, Wierusz-Wysocka B. Serum interleukin-8 level is increased in diabetic patients. Diabetologia 1999. 42: 117-118.