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Research Article

An imbalance in serum concentrations of inflammatory and anti-inflammatory cytokines in hypertension

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Abstract

Hypertension is an important risk factor for cardiovascular disease and there is increasing evidence that inflammation and abnormal immune responses are involved in the pathogenesis of hypertension. However, the data on the association between specific cytokine concentrations and hypertension are inconsistent. We have evaluated the association between 12 cytokines/growth factors and the presence of different degrees of hypertension, comparing these concentrations to values in a healthy group of subjects. The concentrations of interleukin (IL)-1α, -1β, -2, -4, -6, -8, -10, tumor necrosis factor (TNF-α), interferon-γ (IFN-γ), monocyte chemoattractant protein (MCP-1), epidermal growth factor, and vascular endothelial growth factor were measured in 155 hypertensive patients and 148 healthy subjects, using EV-3513 cytokine biochip arrays, a competitive chemiluminescence immunoassay. Univariate and multivariate analyses were used to evaluate the association of specific cytokines and growth factors with systolic blood pressure (SBP) and diastolic blood pressure (DBP). Hypertensive subjects had higher serum concentrations of IL-1α, -2, -8, vascular endothelial growth factor, IFN-γ, TNF-α, MCP-1, and epidermal growth factor; and lower concentrations of anti-inflammatory cytokine, IL-10 ($P < .05$), compared with the healthy individuals. The serum concentrations of IL-4, -6, and -1β did not differ between the hypertensive subjects and control group. Univariate and multivariate analyses revealed that IL-1α and IFN-γ were independent predictors of a high SBP, while IFN-γ, IL-1α, TNF-α, and MCP-1 remained statistically significant for DBP after correction for age, gender, Body mass index, smoking, fasting blood glucose, and triglycerides. There was a significant association between the concentrations of several cytokines and hypertension. These associations may either be related to common underlying factors that cause hypertension and may

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also be proinflammatory or because these inflammatory cytokines might directly be involved in the etiology of hypertension.
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Introduction

Cardiovascular disease is now the most important cause of morbidity and mortality globally, and is usually due to atherosclerosis.[1] The prevalence of atherosclerosis has increased over past decades and is now a major cause of morbidity in developing countries. There is increasing evidence suggesting a key role of inflammation during all stages of atherogenesis.[2] Inflammatory cells, including activated macrophages and T lymphocytes, are present during all stages of atherosclerotic lesion development and contribute to the proinflammatory milieu that modulates the local inflammatory responses within the plaque.[3–7] Serum tumor necrosis factor (TNF)-α concentration has been shown to predict the severity of peripheral arterial disease.[8] Serum interleukin (IL)-2 concentrations are reported to be increased in patients with stable, but not unstable, angina.[9,10] There is also evidence that increased serum concentrations of IL-8 are associated with an increased risk of coronary artery disease in healthy subjects.[11] IL-10 is an anti-inflammatory cytokine with a potential protective role that limits the local inflammatory response. IL-10 can regulate the production of proinflammatory cytokines derived from type 1 T-helper lymphocytes promoting a type 2 T-helper cell immune response that it is necessary in the modulation of the inflammatory process.[12]

Hypertension is strongly associated with cardiovascular mortality.[13] The prevalence of hypertension is also increasing globally[14] and within the Iranian population specifically.[15] Hypertension was defined by the World Health Organization as a blood pressure >140/90 mm Hg, and more recently by the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP-III) guidelines as >130/85 mm Hg.[16,17] Chronic inflammation may be an independent risk factor for the development of hypertension. Serum TNF-α and IL-6 concentrations have been reported to be associated with hypertension in otherwise healthy subjects.[18]

Previous studies have been inconsistent in their reports on the relationship between hypertension and serum cytokine concentrations. This may, in part, be related to other confounding factors including diet. In the present study, the association between serum cytokines concentration

Table 1
Demographic and biochemical characteristics of subjects in each group

Characteristics	Healthy (N = 148)	Hypertension (N = 155)	*P* Value
Age (year)	49.62 ± 12.31	50.29 ± 13.03	.647
Sex [n (%)]			
Male	61 (41.2)	64 (41.3)	.990
Female	87 (58.8)	91 (58.7)	
Smoking [n (%)]			
Yes	25 (19.1)	43 (30.3)	.085
No	93 (71)	84 (59.2)	
Former	13 (9.9)	15 (10.5)	
Height (cm)	1.61 ± 0.07	1.60 ± 0.09	.956
Weight (kg)	70.12 ± 12.10	76.15 ± 14.04	**<.001**
BMI (kg/m^2)	27.16 ± 4.73	29.51 ± 4.74	**<.001**
WC (cm)	91.58 ± 11.45	98.54 ± 11.84	**<.001**
HC (cm)	102.96 ± 8.99	106.68 ± 10.08	**.002**
ArmC (cm)	29.91 ± 5.07	31.75 ± 3.47	**<.001**
FBG (mg/dL)	83.55 ± 12.63	86.34 ± 10.48	**.040**
TC (mg/dL)	193.89 ± 35.80	195.33 ± 39.08	.582
TG (mg/dL)	117.00 (83.00–154.25)	138.00 (100.00–186.50)	**.002**
hsCRP (mg/dL)	2.42 (1.24–6.02)	2.55 (1.30–6.25)	.434
HDL-C (mg/dL)	44.02 ± 8.57	42.70 ± 9.30	.962
LDL-C (mg/dL)	123.55 ± 32.53	118 ± 31.79	.396
SBP (mm Hg)	111.70 ± 11.03	136.57 ± 13.57	**<.001**
DBP (mm Hg)	75.62 ± 6.92	88.95 ± 7.21	**<.001**

ArmC, arm circumference; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

Values are expressed as mean ± SD, median and IQR for normally and nonnormally distributed variables, respectively.

Bold values represent significant *P* value.

and hypertension has been investigated in groups of well-characterized hypertensive and healthy subjects from the north east of Iran.

Methods

This case–control study was undertaken in groups of hypertensive and healthy volunteers who were matched for gender and age. As shown in Table 1, there were no significant differences in age or gender distribution between the groups.

Subjects were recruited by using a population-based cluster sampling from Mashhad city, the second largest city in Iran, and the surrounding regions in north eastern Iran. Control group had no known history of major systemic inflammation or infection diseases and without any family history of stroke, myocardial infarction, and diabetes mellitus. A diagnosis of hypertension was based on the NCEP-ATP-III criteria: systolic blood pressure (SBP) of ≥130 mm Hg and diastolic blood pressure (DBP) of ≥85 mm Hg.[12] The classification of hypertension was in accordance with NCEP-ATP-III guidelines: SBP <120 mm Hg or DBP <80 mm Hg considered as normotensive, prehypertensive subjects had a SBP 120–139 mm Hg or DBP 80–89 mm Hg, and Stage 1 and 2 hypertension were described as the SBP 140–159 mm Hg or DBP 90–99 mm Hg and SBP ≥160 mm Hg or DBP ≥100 mm Hg, respectively.[19] Individuals with other medical conditions including endocrinological abnormalities and chronic liver and/or renal diseases were excluded. Subjects being treated with hypoglycemic or other medications and those consuming alcohol were excluded and therefore, taking any drug was one of our exclusion criteria. Informed written consent was obtained from all subjects using approved protocols by Research Ethics Committee of Mashhad University of Medical Sciences.

Measurements

Anthropometric Measurements

Anthropometric parameters (height, body weight, waist, and hip circumference[C]) were measured using standardized procedures. Body mass index (BMI) was calculated as body weight (kg) divided by squared height in meters (m^2). SBP and DBP were measured in duplicates by sphygmomanometers (a standard mercury sphygmomanometer) on the left arm in the sitting position after a 15 minutes rest using a standardized procedure and the mean recorded. If difference between two measurements was more than diastolic 15 mm Hg or systolic 25 mm Hg, a third reading was performed and the average of the two closest readings was used as the mean blood pressure.

Lipid Profile, Fasting Glucose and High-Sensitivity C-Reactive Protein Measurements

Fasting blood samples were collected after a 12 hour overnight fast to determine serum glucose, lipid profile level (total cholesterol, triglyceride [TG], and high-density lipoprotein cholesterol), and high-sensitivity C-reactive protein (hsCRP) using an auto-analyzer (Eppendorf, Germany). If TG level was <400 mg/dL, low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the Friedewald formula,[20] while for samples with TG concentrations >400 mg/dL, LDL-C was determined.

Serum Cytokines Level

Blood samples were taken from an antecubital vein, and collected into plain tubes. They were centrifuged at 1500×*g* for 20 minutes at room temperature to separate the serum, and samples were then stored at −80°C until

Table 2
Serum cytokines and growth factors in healthy vs. hypertensive subjects

Cytokines/Growth Factors	Healthy (N = 148)	Hypertension (N = 155)	*P* Value	*P** Value
IL-2	2.64 (2.40–3.06)	2.70 (2.42–3.39)	**.047**	**.016**
IL-4	1.89 (1.62–2.46)	1.83 (1.55–2.23)	.399	.738
IL-6	0.97 (0.65–1.82)	0.89 (0.63–1.63)	.598	.400
IL-8	4.73 (3.26–8.23)	5.43 (3.61–11.39)	**.028**	**.002**
IL-10	0.87 (0.71–1.23)	0.81 (0.66–1.11)	**.041**	**.044**
VEGF	75.57 (42.02–126.11)	103.70 (39.55–176.47)	**.026**	**.019**
IFN-γ	0.48 (0.00–0.59)	0.55 (0.00–0.70)	**.019**	.154
TNF-α	1.60 (1.23–1.90)	1.76 (1.21–2.22)	**.025**	**.002**
IL-1α	0.52 (0.45–0.60)	0.54 (0.49–0.63)	**.029**	**.007**
IL-1β	0.58 (0.45–0.78)	0.54 (0.40–0.75)	.149	.629
MCP-1	66.95 (35.32–134.74)	107.15 (33.11–182.36)	**.044**	**.011**
EGF	19.78 (7.04–63.50)	29.72 (11.59–114.99)	**.014**	**.021**

BMI, body mass index; EGF, epidermal growth factor; FBG, fasting blood glucose; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein; TG, triglyceride; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Serum cytokines level expressed as pg/mL; values are expressed as median (interquartile range); comparisons were performed by Mann–Whitney *U* test.

Bold values represent significant *P* value.

* Adjusted for age, sex, BMI, smoking, FBG, and TG.

they were analyzed. Analysis of serum cytokines and growth factors (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, interferon γ [IFN-γ], monocyte chemoattractant protein [MCP-1], epidermal growth factor [EGF], and vascular endothelial growth factor [VEGF]) was performed using an EV 3513 cytokine biochip array (Randox Laboratories, Crumlin, UK) using sandwich and competitive chemiluminescence immunoassays (Randox Laboratories, Crumlin, UK).[21,22]

Statistical Analysis

Data analyses were undertaken using the Statistical Package for Social Sciences (SPSS version 16). The normality of distribution was assessed using the Kolmogorov–Smirnov test. Descriptive statistics including mean, frequency, and standard deviation were determined for all variables. In addition, quantitative data were expressed as the mean ± standard deviation for normally distributed variables (or as the median and interquartile range for nonnormally distributed variables). For normally distributed variables, the student *t* test was used to compare the clinical characteristics and baseline demographics between the groups. The Mann–Whitney *U* test was used for continuous variables if they are not normally distributed. Chi-square or Fisher exact tests were used for categorical variables. To control for the effects of confounding factors including age, gender, and smoking and also the variables that differed between the groups such as BMI, fasting blood glucose (FBG), and TG, regression analysis was used. The correlations between all the cytokines and growth factors were assessed using Spearman correlation analysis. A multivariate analysis model was used to examine associations between SBP and DBP and serum pro- and anti-inflammatory cytokines, and growth factors level with a value of $P < .05$ in univariate analysis. A P value of <.05 was considered as statistically significant.

Results

Basic Characteristics

The groups comprised 155 hypertensive (aged 50.29 ± 13.03 years) and 148 healthy subjects (aged 49.62 ± 12.31 years). The hypertensive subjects comprised 64 males (41.3%) and 91 females (58.7%), and the healthy subjects included 61 males (41.2%) and 87 females (58.8%). There were no significant differences in gender distribution and smoking habit between the groups ($P = .990$ and $P = .085$, respectively; Table 1).

Hypertensive patients were significantly more adipose than healthy subjects with higher weight, BMI, waist C, hip C, and arm C ($P < .05$; Table 1). The mean SBP and DBP in the two groups are shown in Table 1.

Table 3
Comparison of cytokines and growth factors in individuals with different degrees of hypertension

Cytokines/Growth Factors	Healthy (N = 71)	Prehypertension (N = 142)	Stage 1 (N = 70)	Stage 2 (N = 20)	P_0 (n/p)	P_1 (n/s_1)	P_2 (n/s_2)	P_3 (p/s_1)	P_4 (p/s_2)	P_5 (s_1/s_2)
IL-1α	0.51 (0.67–0.572)	0.54 (0.76–0.630)	0.52 (0.72–0.615)	0.615 (0.769–0.702)	**.01**	.108	**.001**	.126	.111	**.015**
IL-1β	0.60 (0.445–0.73)	0.57 (0.445–0.785)	0.52 (0.39–0.79)	0.51 (0.32–0.792)	.910	.228	.186	.205	.175	.525
IL-2	2.53 (2.40–3.00)	2.70 (2.50–3.38)	2.70 (2.42–3.22)	2.46 (2.27–3.00)	**.014**	**.026**	.498	.950	**.027**	**.042**
IL-4	1.86 (1.62–2.13)	1.86 (1.56–2.36)	1.89 (1.53–2.37)	2.10 (1.61–2.47)	.523	.619	.132	.978	.340	.329
IL-6	0.91 (0.63–1.50)	0.95 (0.68–1.81)	0.93 (0.60–1.81)	0.86 (0.62–1.57)	.483	.905	.863	.666	.551	.783
IL-8	4.48 (2.87–7.32)	5.20 (3.42–8.23)	7.15 (4.16–13.87)	5.06 (2.39–9.30)	**.049**	**< .001**	.580	.007	.708	.105
IL-10	0.83 (0.63–1.09)	0.84 (0.71–1.29)	0.78 (0.66–1.02)	0.83 (0.73–1.14)	.174	.363	.878	**.010**	.459	.369
MCP-1	64.65 (27.03–137.66)	80.44 (37.11–150.23)	140.99 (43.11–184.55)	50.94 (20.69–138.41)	.144	**.004**	.701	**.035**	.192	.052
IFN-γ	0.46 (0.00–0.57)	0.53 (0.00–0.67)	0.58 (0.44–0.755)	0.44 (0.00–0.822)	**.050**	**.003**	.554	.090	.832	.263
TNF-α	1.55 (1.22–1.97)	1.741 (1.225–2.01)	1.63 (1.18–2.40)	1.64 (1.01–2.20)	.105	.054	.484	.356	.955	.531
EGF	21.83 (8.90–70.44)	23.04 (8.26–79.81)	49.28 (14.25–140.69)	17.025 (7.55–46.11)	.968	**.021**	.399	**.006**	.377	**.011**
VEGF	77.021 (72.97–109.68)	97.65 (263.64–180.85)	97.72 (179.27–169.63)	65.18 (248.41–104.97)	.052	.189	.316	.683	.052	.090

EGF, epidermal growth factor; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.
Values are expressed as median (interquartile range); serum cytokines level expressed as pg/m; since the distribution of cytokines is nonnormal, comparisons were performed by Mann–Whitney *U* test.
P_0, comparison between groups of healthy and prehypertension; P_1, comparison between groups of healthy and Stage 1; P_2, comparison between groups of healthy and Stage 2; P_3, comparison between groups of prehypertension and Stage 1; P_4, comparison between groups of prehypertension and Stage 2; P_5, comparison between groups Stage 1 and Stage 2.
Bold values represent significant P value.

Lipid Profile and Fasting Glucose

No significant difference in total cholesterol, high-density lipoprotein cholesterol, and LDL-C concentrations were observed between hypertensive and healthy subjects ($P = .582$, $P = .962$, and $P = .396$, respectively). However, serum TG and fasting blood glucose were significantly higher in the hypertensive patients ($P = .002$ and $P = .040$, respectively; Table 1).

Level of hsCRP in Patients with Hypertension and Healthy Group

We also measured the level of hsCRP in all patients with hypertension and healthy groups. No significant difference was detected in hsCRP level between the two groups ($P = .434$).

Alteration in the Levels of Cytokines

As shown in Table 2, hypertensive patients had a significantly higher serum concentration of IL-2, IL-8, VEGF, IFN-γ, TNF-α, IL-1α, MCP-1, and EGF and lower serum IL-10 concentrations than healthy subjects ($P < .05$). Moreover, IL-4, IL-6, and IL-1β levels did not differ significantly between the patients and controls ($P > .05$; Table 2). Adjusted P values are shown in Table 2, controlling for confounder factors including age, sex, and smoking and the variables differing significantly between two groups such as BMI, FBG, and TG. Weight, waist C, hip C, and arm C were also significantly different between two groups, but because weight, waist C, hip C, arm C, and BMI were highly correlated, only BMI was entered as a factor into the model. Serum IFN-γ of hypertensive patients was not statistically different compared with healthy subjects after adjustment of the confounding factors (adjusted $P > .05$; Table 2).

Comparison of cytokines and growth factors in the different sub-categories of hypertension are shown in Table 3. In particular, the serum IL-1α concentration was significantly higher in the prehypertensive and Stage-2 hypertensive patients than for normotensive subjects ($P = .001$, $P = .001$, respectively). Moreover, IL-2 and IL-8 levels were significantly higher in the prehypertensive patients, compared with the normotensive subjects ($P = .014$, $P = .049$, respectively). The mean concentration of IL-1α was significantly lower, whereas IL-2 and EGF concentrations were markedly higher when compared with Stage-1 and Stage-2 hypertensive patients ($P = .015$, $P = .042$ and $P = .011$, respectively). IL-2 level was decreased in Stage-2 of hypertensive patients with respect to the prehypertensive subjects ($P = .027$). In addition, hypertensive patients

Table 4
Correlation matrix between the inflammatory, anti-inflammatory, and growth factors for healthy subjects using Spearman correlation analysis

		IL-2										
IL-4 (pg/mL)	*r*	0.222										
	P	.071	IL-4									
IL-6 (pg/mL)	*r*	−0.87	−0.106									
	P	.293	.211	IL-6								
IL-1β (pg/mL)	*r*	0.083	0.110	0.138								
	P	.317	.186	.094	IL-1β							
EGF (pg/mL)	*r*	−0.119	**−0.259**	0.11	−0.129							
	P	.149	**.002**	.179	.118	EGF						
IL-8 (pg/mL)	*r*	0.93	0.024	0.220	0.017	**0.295**						
	P	.264	.775	.008	.838	**<.001**	IL-8					
VEGF (pg/mL)	*r*	−0.137	**−0.391**	**0.285**	−0.045	**0.422**	0.159					
	P	.097	**<.001**	**<.001**	.587	**<.001**	.055	VEGF				
IL-1α (pg/mL)	*r*	**0.176**	**0.233**	0.061	0.098	**−0.168**	0.060	−0.081				
	P	**.032**	**.005**	.458	.235	**.041**	.470	.329	IL-1α			
MCP-1 (pg/mL)	*r*	**0.163**	**0.173**	0.053	0.058	**0.206**	**0.409**	−0.080	0.156			
	P	**.048**	**.036**	.053	.480	**.012**	**<.001**	.333	.058	MCP-1		
IFN-γ (pg/mL)	*r*	**0.273**	0.142	0.131	0.062	−0.056	0.125	0.103	0.156	0.141		
	P	**.001**	.085	.112	.455	.499	.132	.211	.059	.87	IFN-γ	
IL-10 (pg/mL)	*r*	**0.184**	0.133	0.033	**0.199**	**−0.192**	−0.048	**−0.209**	**0.248**	0.009	0.145	
	P	**.025**	.109	.86	**.015**	**.019**	.564	**.011**	**.002**	.910	.080	IL-10
TNF-α (pg/mL)	*r*	0.029	−0.136	**0.318**	**0.266**	**0.265**	**0.230**	**0.225**	0.024	−0.008	−0.062	0.111
	P	.725	.1	**<.001**	**.001**	**.001**	**.005**	**.006**	.768	.927	.455	.178

EGF, epidermal growth factor; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Values are expressed as *r* (Spearman coefficient) and *P* (*P* value).

Bold values represent significant *P* value.

with Stage-1 had a higher serum IL-8 compared with normotensive ($P < .001$) and prehypertensive ($P = .007$) subjects. The values of MCP-1, IFN-γ, IL-2, and EGF were significantly higher in Stage-1 hypertensive patients than subjects with normal blood pressure ($P = .004$, $P = .003$, $P = .026$, and $P = .021$, respectively). Serum EGF and MCP-1 levels were higher in Stage-1 hypertensive patients than for the prehypertensive subjects ($P = .006$ and $P = .035$, respectively), whereas IL-10 concentration was lower ($P = .010$; Table 3). A correlation matrix between the inflammatory, anti-inflammatory, and growth factors for healthy and hypertension groups are shown in Tables 4 and 5.

Furthermore, to assess the effect of each cytokine and growth factors on SBP and DBP, multivariate analysis was performed for the variables that had a $P < .05$ in the univariate analysis. We performed a multivariate analysis of each cytokine along with confounding and different factors including BMI, smoking, FBG, TG, age, and sex. As illustrated in Table 6, serum IFN-γ and IL-1α remained statistically significant independent predictors of SBP ($P = .011$ and $P < .001$, respectively). Each unit increase in serum IFN-γ and IL-1α level was associated with increased SBP. Moreover, concentrations of IFN-γ, TNF-α, IL-1α, and MCP-1 remained statistically significant, independent predictors of DBP after correction for age, gender, BMI, smoking habit, FBG, and TG ($P < .05$; Table 7).

Discussion

Several previous studies have demonstrated that adiposity, hypercholesterolemia, and high blood glucose are strongly associated with hypertension.[23–25] We also found that weight, BMI, waist C, hip C, arm C, FBG, and TGs were significantly higher in hypertensive patients than healthy subjects. Moreover, there was a significantly higher serum concentration of IL-2, IL-8, VEGF, IFN-γ, TNF-α, IL-1α, MCP-1, and EGF in the hypertensive group, whereas serum IL-10 concentrations were lower, compared with the healthy subjects. There are little reported data on the relationship between serum cytokines in hypertensive subjects, and some previous studies have reported conflicting results.[5,26–31] Most studies have only evaluated differences in serum IL-6,[18,32] and TNF-α.[32]

Some studies have shown a positive association between TNF-α level and elevated BP;[28,33] however, several others studies are failed.[29,34] The activation of the TNF-α system has been shown to be associated with SBP and DBP.[35] TNF-α can decrease the expression of endothelial nitric oxide synthase by shortening its half-life.[36] This may result in decreased bioavailability of nitric oxide and lead to endothelial dysfunction and hypertension. We also found that hypertensive subjects had substantially higher levels of TNF-α, compared with the healthy subjects.

Table 5
Correlation matrix between the inflammatory, anti-inflammatory, and growth factors for hypertensive patients using Spearman correlation analysis

		IL-2										
IL-4 (pg/mL)	*r*	0.09	IL-4									
	P	.266										
IL-6 (pg/mL)	*r*	0.095	−0.018									
	P	.242	.825	IL-6								
IL-1β (pg/mL)	*r*	0.144	0.129	**0.212**								
	P	.073	.110	**.008**	IL-1β							
EGF (pg/mL)	*r*	−0.053	−0.155	**0.267**	0.056							
	P	.516	.054	**.001**	.486	EGF						
IL-8 (pg/mL)	*r*	0.002	−0.057	0.142	−0.014	**0.329**						
	P	.979	.482	.079	.866	**<.001**	IL-8					
VEGF (pg/mL)	*r*	0.031	**−0.422**	**0.195**	−0.060	**0.342**	0.097					
	P	.706	**<.001**	**.015**	.459	**<.001**	.231	VEGF				
IL-1α (pg/mL)	*r*	−0.054	**0.162**	−0.038	0.091	−0.096	0.093	−0.148				
	P	.505	**.044**	.641	.262	.235	.248	.066	IL-1α			
MCP-1 (pg/mL)	*r*	0.137	**−0.275**	0.131	−0.041	**0.264**	**0.287**	**0.214**	−0.015			
	P	.090	**.001**	.106	.616	**.001**	**<.001**	**.007**	.853	MCP-1		
IFN-γ (pg/mL)	*r*	0.099	**0.255**	0.157	−0.009	0.147	0.043	−0.051	−0.023	0.022		
	P	.222	**.001**	.053	.910	.068	.597	.527	.777	.782	IFN-γ	
IL-10 (pg/mL)	*r*	0.138	0.139	**0.224**	0.157	0.085	−0.085	0.014	0.065	−0.055	**0.251**	
	P	.088	.083	**.005**	.051	.292	.293	.861	.421	.50	**.002**	IL-10
TNF-α (pg/mL)	*r*	−0.023	−0.101	**0.308**	**0.174**	**0.251**	**0.096**	**0.220**	−0.059	0.093	0.085	**0.288**
	P	.774	.211	**<.001**	**.030**	**.002**	**.233**	**.013**	.468	.250	.295	**<.001**

EGF, epidermal growth factor; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Values are expressed as *r* (Spearman coefficient) and *P* (*P* value).

Bold values represent significant *P* value.

Table 6
Impact of serum cytokines and growth factor level on systolic blood pressure by using multivariate analysis

Predictors	Univariate			
	β	CI		*P*
IL-2	0.566	−0.170	1.301	.131
IL-4	2.32	−0.279	4.911	.080
IL-6	−0.231	−1.33	0.873	.681
IL-8	0.015	−0.017	0.048	.355
IL-10	−0.791	−2.30	0.714	.302
VEGF	0.017	−0.003	0.037	.102
IFN-γ	1.933	0.451	3.414	**.011**
TNF-α	1.684	−0.035	3.403	.055
IL-1α	20.22	10.21	30.232	**<.001**
IL-1β	−3.20	−8.28	1.863	.214
MCP-1	0.003	−0.019	0.024	.808
EGF	0.004	−0.010	0.019	.566
hsCRP	−0.190	−0.571	0.191	.327
	Multivariate*			
	β	CI		*P*
IFN-γ	1.48	0.70	2.90	**.040**
IL-1α	15.87	5.24	26.49	**.004**

β, regression coefficient; BMI, body mass index; CI, confidence interval; EGF, epidermal growth factor; FBG, fasting blood glucose; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; IFN, interferon; MCP, monocyte chemoattractant protein; TG, triglyceride; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Bold values represent significant *P* value.

* Adjusted for age, sex, BMI, smoking, FBG, and TG.

Table 7
Impact of serum cytokines and growth factor level on diastolic blood pressure by using multivariate analysis

Predictors	Univariate			
	β	CI		*P*
IL-2	0.178	−0.260	0.616	.425
IL-4	0.833	−0.712	2.379	.289
IL-6	−0.175	−0.850	0.499	.610
IL-8	0.010	−0.009	0.030	.300
IL-10	−0.397	−1.292	0.498	.383
VEGF	0.011	−0.002	0.023	.086
IFN-γ	1.162	0.282	2.042	**.010**
TNF-α	1.277	0.251	2.303	**.015**
IL-1α	11.323	5.354	17.293	**<.001**
IL-1β	−3.079	−6.078	−0.080	**.044**
MCP-1	0.021	0.009	0.034	**.001**
EGF	0.005	−0.004	0.014	.253
hsCRP	0.166	−0.060	0.392	.150
	Multivariate*			
	β	CI		*P*
IFN-γ	0.87	0.07	1.67	**.033**
TNF-α	1.45	0.32	2.57	**.011**
IL-1α	9.45	3.42	15.49	**.002**
IL-1β	−2.18	−5.02	0.66	.132
MCP-1	0.02	0.004	0.30	**.010**

β, regression coefficient; BMI, body mass index; CI, confidence interval; EGF, epidermal growth factor; FBG, fasting blood glucose; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; IFN, interferon; MCP, monocyte chemoattractant protein; TG, triglyceride; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Bold values represent significant *P* value.

* Adjusted for age, sex, BMI, smoking, FBG, and TG.

Results from studies on serum IL-6 and hypertension have been inconsistent.[26,27] IL-6 can stimulate the synthesis of many acute-phase reactant proteins including CRP,[37] and also regulates the expression of TNF-α and IL-1β.[38] There is increasing evidence that IL-6 can exacerbate endothelial dysfunction,[39] and it has been suggested that variations in lifestyle and diet, may be responsible for this association.[18,40,41] However, we did not find an association between serum IL-6 and hypertension.

We found that serum concentrations of the anti-inflammatory cytokine and IL-10[12,42] were lower in hypertensive patients than healthy subjects, whereas serum EGF and VEGF were higher. Previous studies have shown that EGF can play a key role in blood pressure regulation and endothelial dysfunction.[43] Moreover, there is accumulating evidence showing that VEGF can increase vascular inflammation and endothelial and vascular smooth muscle cells proliferation.[44–48] VEGF mRNA levels were found to be associated with TNF-α expression in peripheral blood mononuclear cells, and hence, there is a plausible biological connection between VEGF and inflammatory markers in healthy subjects.[49]

Higher level of serum MCP-1 has been shown to be present in hypertensive patients compared with healthy subjects.[50,51] Moreover, in rats, hypertension is associated with an upregulation of MCP-1 expression in arterial tissue.[52] Chen et al[53] showed that the level of urinary MCP-1 was positively correlated with increased SBP and DBP. Similarly, we found that serum MCP-1 levels were higher in hypertensive patients than normotensive controls.

Furthermore, we observed that serum IL-6 concentration was positively correlated with the IL-10. This correlation may be related to immunosuppressive activity of IL-10.[54] We also found a relationship between different degrees of hypertension and cytokines concentrations, which may suggest the role of each cytokine in different stages of evolving hypertension. In particular, IL-1α concentration was significantly higher in the prehypertensive and Stage-2 hypertensive patients than for normotensive subjects, suggesting the role of IL-1α in early stages compared with IL-2, IL-8, MCP-1, and EGF. This may indicate that IL-1α is a part of the initial process and may be derived from smooth muscle cells within the artery wall; MCP-1 attracts further monocyte influx, whereas IL-8 and growth factors are involved at a later stage.

We also demonstrated that IL-8 and MCP-1 levels were higher in Stage 1 than normotensive and prehypertensive subjects. Previous studies have reported IL-8 and MCP-1 are involved in monocyte adhesion and migration into the inflamed vessel wall in atherosclerosis.[55] When the endothelium is activated and chemokines including MCP-1 and IL-8 are expressed, this may lead to monocyte and/or lymphocyte recruitment and infiltration in the sub endothelium.[56] The present findings also showed that IL-1α is the most related factor to SBP and DBP. Moreover, this analysis showed no evidence of a relationship between serum IL-1α and high blood pressure. Previous studies have reported results about some other members of IL-1 family including IL-1β and IL-1 receptor antagonist. It has been reported that IL-1β is implicated in stress-induced hypertension.[57] Voelkel et al[58] showed that recombinant human IL-1ra might inhibit the development of pulmonary hypertension in the animal monocrotaline inflammatory model. Furthermore, our results demonstrated that transition from normal pressure to Stage-1 hypertension was associated with down regulation of Th1 responses (such as TNF-α) and Th2 cytokines (including IL-10).

Conclusions

This study demonstrates a significant association between some circulating cytokines and hypertension. This may be related to the underlying causes of hypertension that may also be related to an inflammatory response or because inflammatory cytokines are involved in the etiology of hypertension.

Limitations

Some demographic data, including the dietary habits of the study population were not recorded, and these may have an important impact on the interpretation of our data.

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