Circulating levels of endothelin-1 in a homogenous Gulf Arab population with untreated essential hypertension

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BACKGROUND: Racial variations are reported in the natural history of hypertension. For example, hypertension is significantly more prevalent in blacks than whites. Endothelial cells are important regulators of vascular tone and homeostasis, in part through secretions of vasoactive substances including endothelin-1 (ET-1), a small peptide with potent vasopressor actions. In black hypertensives, ET-1 levels are higher than in normotensive blacks and in both hypertensive and normotensive whites. Since ET-1 might play a significant role in the development and severity of hypertension in the indigenous Arab population of the United Arab Emirates, we investigated the circulating levels of ET-1 in this homogenous population.

PATIENTS AND METHODS: ET-1 levels were measured in plasma samples from 60 untreated hypertensive Arabs and compared with 60 age- and sex-matched normotensive controls.

RESULTS: ET-1 levels were significantly higher in hypertensives (mean 10.1 ± 1 pmol/L) than normotensives (mean 2.2 ± 0.5 pmol/L). Body mass index (BMI) was slightly higher among the hypertensives. For all subjects these levels significantly ($P<0.001$) correlated with systolic blood pressure and less significantly ($P<0.05$) with diastolic blood pressure and body weight. The correlation between ET-1 and both systolic and diastolic blood pressure was persistently significant after adjusting for BMI.

CONCLUSION: Plasma concentrations of ET-1 are significantly higher in hypertensive Gulf Arabs as compared with reported levels in white hypertensives and ET-1 could be a risk factor for cardiovascular diseases in this population. The endothelial system might be particularly important with respect to hypertension in this racial group and merits further study.

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The dysfunction of endothelium, a monolayer group of cells within blood vessels, is widely recognized to play a significant role in most types of vascular diseases and may contribute to the pathophysiology of cardiovascular diseases (CVD) and obesity-associated hypertension.[1,2] The endothelium itself is responsible for the production of vasoconstrictors, known as endothelium-derived constricting factors (EDCF), and vasodilators named endothelium-derived relaxing factors (EDRF). Among others, the endothelin (ET) family of peptides, identified by Yanagisawa et al in 1988,[3] includes powerful endogenous vasoconstrictors and pressor agents. They consist of three distinct isopeptides, ET-1, ET-2, and ET-3, which are encoded by three different genes. Of the isopeptides, ET-1, the focus of this study,

is the most abundant in the cardiovascular system. It originates not just from endothelial cells, but also from vascular smooth muscle cells and cardiomyocytes. Various growth factors, cytokines, angiotensin II, bradykinin as well as other vasoactive substances stimulate ET-1 production and release.[4]

In recent years, emerging evidence has suggested that ET-1 plays an important role in the pathophysiology of cardiovascular diseases since it apparently regulates vascular tone and blood pressure.[5,6] When administered exogenously in vivo (either by intravenous or intracoronary routes) or in vitro to isolated heart preparations, it triggers potent coronary constriction that may result in myocardial ischaemia.[7,8] In addition, its positive chronotropic and inotropic effects increase oxygen consumption and may predispose to signs and symptoms of ischaemic heart disease (IHD).[6] Moreover, the infusion of ET-1 increases blood pressure and decreases cardiac index and renal blood flow, whilst both calculated systemic and renal vascular resistance are increased.[9]

At the cellular level, ET-1 exerts its diverse effects via two distinct G protein-coupled receptor subtypes.[1-3] Endogenous over-expression of preproET-1 (the precursor protein for ET-1) is accompanied by a six-fold elevation of plasma ET-1 concentration and by a mean increase in blood pressure of 28 mm Hg. This systemic hypertension results from the activation of the ETA receptor by ET-1. In contrast, however, intravenous infusion of an ETA receptor antagonist in this model reduces blood pressure to physiologically normal levels.[10] Other risk factors may also contribute to making essential hypertensive subjects more sensitive to the vasoconstrictive effects of ET-1; circulating levels of ET-1 have been shown to be elevated in hypertensive patients with a high-risk profile for developing vascular damage,[11] in patients with pre-eclampsia,[12] atherosclerosis,[13] angina pectoris,[14] and coronary artery disease (CAD) compared with healthy normal controls.[15] Likewise, the production of ET-1 is increased by a high-salt diet in hypertensive rats.[16] It has also been shown that plasma levels of ET-1 are increased in several cardiovascular disorders such as myocardial infarction, congestive heart failure, atherosclerosis, hypertension, and septic shock.[17] However, conflicting reports have clouded these findings, showing no cardioprotective effects when ET receptor antagonists were administered.[18]

The involvement of racial differences with regard to the alterations of plasma levels of ET-1 in both hypertensives and normotensives is yet to be under-stood. In the first report on racial differences, plasma levels of ET-1 were significantly higher in hypertensive blacks compared with whites.[19] Other authors, however, have reported that only some patients with moderate to severe hypertension exhibit enhanced endothelial expression of ET-1, which increases an individual's susceptibility to hypertension as well as its severity.[20]

Thus, a critical area of research continues to be the elucidation of the diverse effects of ET-1 and its potential role in the pathophysiology of CVDs and hypertension,[21] especially among different ethnic groups. Racial differences in the prevalence and the severity of CVD and hypertension have been well documented, but remain unexplained. A possible determinant is perhaps the circulating levels of ET-1. The aim of the current study was to investigate the levels of immunoreactive ET-1 in a genetically homogenous Gulf Arab Bedouin population, an ethnic group so far unstudied in this context.

Patients and Methods

The United Arab Emirates (UAE), located in the northeast of the Arabian Peninsula, is a federation made up of seven Sheikhdoms (the largest being the Emirate of Abu Dhabi). The inhabitants of the UAE include many expatriate groups, but the indigenous population of Emiratis is largely Gulf Arabs of Bedouin descent.

A total of 60 unrelated, well-characterized but untreated hypertensive Emirati Gulf Arabs were recruited from the inpatient and outpatient units of two tertiary referral facilities at the Al Mafraq Hospital in Abu Dhabi and the Tawam Hospital at Al Ain, UAE. These were matched to 60 volunteer normotensive Gulf Arabs of the same sex and similar age. Most hypertensive and normotensive subjects did not undertake any regular exercise regimen, but admitted to moderate physical activity including walking. In fitting with their cultural background, only a very small minority of individuals admitted to cigarette smoking, alcohol consumption or excessive coffee consumption. Furthermore, none of the subjects from either group was on any form of regular medication. To ensure accuracy, a staff nurse was assigned throughout the project to measure blood pressures by means of a mercury sphygmomanometer after the individual had rested for 15 minutes in the supine position. Height and weight were also measured to calculate body mass index (BMI) as kg/m². The history was taken and a physical examination made for each hypertensive individual by a consul-

tant physician to exclude any cases of secondary hypertension and other cardiovascular events. Prior to blood sample collection, patients and controls both signed a consent proforma and were requested to fill out a well-explained self-reporting questionnaire with the help of a trained nurse to obtain information regarding lifestyle. The protocol for this study was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences (UAE University, Al Ain, UAE).

The hypertensive group of patients comprised 35 men and 25 women with a mean age of 46±1 years. Individuals were considered as hypertensive if they had untreated systolic blood pressure (SBP) (140 mm Hg and or diastolic blood pressure (DBP) (90 mm Hg on at least three separate occasions according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7).[22] No known risk factors for CVD were observed. The normotensive group also comprised 35 men and 25 women with a mean age of 42±2 years. These were healthy individuals with no family history of hypertension with normal blood pressure (SBP <120 mm Hg and DBP <80 mm Hg) on at least three different occasions. As this was considered to be a cross-sectional representation of the Emirati Gulf Arab population, obesity as such was not an exclusion factor for this study, but none of these normotensive individuals had any previous history of CVD, lipid disorders or other risk factors for CVD.

After overnight fasting, a venous blood sample (5 mL) was taken and anticoagulated with disodium EDTA. Samples were mixed by gentle inversion at room temperature and were transported within 3 hours in an ice container at 4ºC to our research laboratories by our courier service. Following the collection, plasma was separated by centrifugation in a refrigerated centrifuge (2000xg, 15 min, 4ºC) and aliquotted into plastic tubes. Three aliquots from each plasma sample were immediately frozen at -80ºC and thawed once only before analysis.

Immunoreactive ET-1 levels in plasma were measured by ELISA using a commercially available kit obtained from Assay Designs, Inc., Ann Arbor, USA. Insulin levels were also determined by ELISA using reagents supplied by Pako Diagnostics Ltd., Ely, UK. Glucose was measured colorimetrically using a kit obtained from Randox Laboratories Ltd, Crumlin, UK.

Data were processed using the Statistical Package for the Social Sciences (SPSS) version 10.0 for Windows (SPSS, Chicago, IL, USA). Unless otherwise stated, the results are presented in mean values ± SE. The significance of differences between mean values for hypertensives and normotensives were assessed using the Student t-test and P values <0.05 were considered to be statistically significant. Correlations between different variables for the hypertensives and normotensives, treated as a single group, were calculated using the Pearson correlation coefficient. Further, we used stepwise logistic regression (backward selection) to regress hypertension on ET-1, BMI, age and sex. Values for insulin, which show a skewed distribution, were approximately normalized by converting to log values. Once again, P values <0.05 were considered to be statistically significant.

Results

Demographic and blood pressure data for normotensive and hypertensive Gulf Arabs are shown in Table 1 with plasma concentrations of insulin and glucose shown in Table 2. The mean BMI for the

Table 1. Age, body mass index and haemodynamic parameters for normotensive and hypertensive Gulf Arabs.
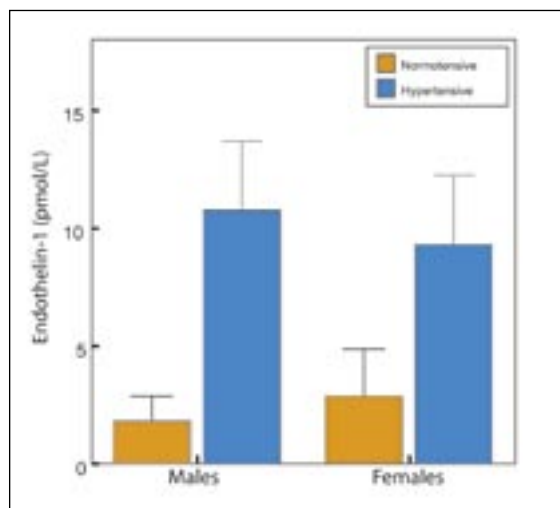
	Normotensives					Hypertensives				
	Age (years)	BMI (kg.m-2)	Pulse (sec-1)	SBP (mm Hg)	DBP (mm Hg)	Age (years)	BMI (kg.m-2)	Pulse (sec-1)	SBP (mm Hg)	DBP (mm Hg)
All subjects (n=60)	42±2	27.3±0.9	79±2	123±2	80±1	46±1	30.3±0.9*	87±2**	156±2***	98±2***
Males (n=35)	43±2	25.0±0.9	76±2	123±3	81±2	46±1	31.0±1.3***	87±3**	158±3***	98±3***
Females (n=25)	41±3	30.1±1.4	83±3	122±3	79±3	45±2	29.5±1.0	87±2	153±3***	98±1***

BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure. Significant differences normotensive and hypertensives: *P< 0.05; **P<0.01, ***P<0.001.

Table 2. Plasma levels of glucose and insulin for normotensive and hypertensive Gulf Arabs.

	Normotensives		Hypertensives	
	Glucose (mmol/L)	Log insulin (µIu/ml)	Glucose (mmol/L)	Log insulin (µIu/ml)
All subjects (n=60)	4.8 ± 0.2	1.09 ± 0.04	4.8 ± 0.4	1.10 ± 0.04
Male (n=35)	5.1 ± 0.3	1.13 ± 0.06	4.3 ± 0.4	1.16 ± 0.05
Female (n=25)	4.5 ± 0.3	1.04 ± 0.05	5.2 ± 0.6	1.00 ± 0.07

normotensive Gulf Arabs (27.3±0.9) was significantly less than for the hypertensives (30.3±0.9), but was nevertheless high for a normal population at more than 27 kg/m². This high mean BMI for normotensives was due to the female members of the group and in fact, for females, the hypertensive group had a slightly lower BMI than for the normals, but not significantly so. By contrast, the mean BMI for male normotensives was more modest and significantly less than for the male hypertensives. Plasma insulin levels were not statistically different between hypertensives and normotensives. Glucose levels were also similar in both groups. By contrast, the mean plasma ET-1 levels in the hypertensive group were over four times greater than in the normotensives and there was no significant difference in the sexes between hypertensives and normotensives (Figure 1). For the normotensive group, plasma ET-1 levels were higher in females compared to males, but not significantly so.

Of particular interest in the multivariate correlation analysis for all parameters measured in both controls and patients of this Gulf Arab population is the fact that ET-1 levels were significantly correlated with systolic blood pressure and to a lesser significance with diastolic blood pressure (Table 3). Stepwise logistic regression to explore the effect of ET-1 on hypertension after adjusting for other variables (BMI, age, sex, and weight), selected only ET-1 (adjusted OR=1.27, 95% CI=1.12-1.44) and weight (adjusted OR=1.06, 95% CI=1.01-1.12) with a Nagelkerke R square = 0.47.

Discussion

In this study, every effort was made to match each hypertensive subject with a normotensive subject of the same sex and similar age. For cultural reasons it was not always possible to recruit healthy volunteers in the older age range and for this reason the mean age of the normotensive group is slightly but not significantly lower than for the hypertensives. However, as far as is known, this marks the first study of ET-1 plasma levels in a Middle East population. Clearly the results are of interest in relation to other studies in which racial differences in ET-1 levels have been shown.

The Gulf Arab population studied is of Bedouin origin and is particularly homogenous. This study clearly shows that not only are ET-1 levels significantly raised in hypertensive Arabs, but that the absolute levels are on the average more than four times those of the normotensive subjects and similar to those reported for black Americans.[16] The data also address differences in ET-1 levels between the two sexes. In normotensive Gulf Arab women, the levels were higher than for men, but the difference was not significant. In the hypertensives, the levels of ET-1 in females were lower than for men, but not significantly so, showing that in this population, marked increases in ET-1 in hypertension occur in both sexes just as for black Americans.

P<0.0001 normotensives vs hypertensives

Figure 1. Comparison of the plasma concentrations of immunoreactive endothelin-1 (ET-1) for hypertensive and normotensive Gulf Arab males (n=35) and females (n=25).

Table 3. Correlati⊠
group (n=120).

	Age	Height	Weight	BMI	Pulse	SBP	DBP	Glucose	Log Insulin	ET-1
Age	1.000									
Height	0.017	1.000								
Weight	0.128	0.332***	1.000							
BMI	0.135	-0.300**	0.792***	1.000						
Pulse	0.092	-0.011	0.062	0.028	1.000					
SBP	0.350***	0.050	0.289**	0.249*	0.190	1.000				
DBP	0.132	-0.040	0.248*	0.264*	-0.077	0.706***	1.000			
Glucose	-0.046	0.125	-0.005	-0.077	-0.040	0.066	-0.08	1.000		
Log insulin	0.105	0.226*	0.136	0.125	0.047	0.180	0.053	0.109	1.000	
ET-1	0.170	0.173	0.227*	0.026	0.185	0.430***	0.246*	0.010	0.021	1.000

BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, ET-1; endotheline-1. Significant differences: *P< 0.05; ** P<0.01, *** P<0.001.

Whether ET-1 is involved in the aetiology of hypertension is still unclear. Several groups have reported that plasma ET-1 levels are not elevated in patients with mild to moderate hypertension.[23,24] However, in some studies,[18,25,26] patients with essential hypertension were found to have higher plasma ET-1 levels than control subjects as shown in this study. Ergul et al[19] demonstrated for the first time that black hypertensive patients have significantly higher levels of ET-1 than white hypertensives as well as white or black normotensives, suggesting that ET-1 may be one of the factors behind known racial differences in the development and severity of hypertension and its complications. In this context, the highly significant direct correlation between ET-1 levels and systolic blood pressure found in the present study clearly supports a role, direct or indirect, for ET-1. The corresponding correlation between ET-1 and diastolic blood pressure was weaker, but was nonetheless, significant. Clearly, the increased BMI in the hypertensive group might have adverse effects on the circulating levels of ET-1, but since the data was analyzed as continuous variables for all subjects, such an effect of BMI may not be apparent.

Only a few studies have assessed racial variations in ET-1 levels. Evans et al,[27] reported that ET-1 levels were significantly increased in healthy black men compared with white men, but not in black versus white women. In an age-matched control study of a Japanese population, Miyauchi and co-workers found no increase in ET-1 concentrations in hypertensive subjects, but did conclude that the levels were higher in men than in women and also that they increased with age.[28] In the present study, no significant sex differences in ET-1 levels were observed, nor was there any significant correlation between ET-1 levels and the age of the subjects.

Racial differences in renal physiology as well as socio-economic factors have been suggested as possible causes for the higher prevalence of essential hypertension in the black population in comparison with whites.[23,29,30] In particular, it has been reported that the T235 allele of the angiotensinogen gene is linked to human hypertension and that this allele is predominant in blacks.[31] Furthermore, it has also been demonstrated that plasma angiotensinogen concentrations are higher in hypertensive blacks than in comparable whites.[32] Interestingly, ET-1 has been shown to enhance the production of angiotensin II from angiotensin I in cultured endothelial cells via an angiotensin converting enzyme-sensitive mechanism.[33] It is also known that angiotensin II stimulates ET-1 synthesis,[33] suggesting that the hormones may mutually reinforce each other in a synergistic fashion to induce vasoconstriction.[34,35]

The present finding that plasma ET-1 levels are elevated in the hypertensive Gulf Arab population compared with their normotensive counterparts suggests that the interaction of angiotensin II and ET-1 could also be an important factor in blood pressure regulation in Gulf Arabs. Also, since ET-1 has various biological effects on the cardiovascular and renal systems,[7] elevated levels of ET-1 in hypertensive Bedouin Arabs may contribute significantly to the

occurrence of complications in this population.

In conclusion, plasma concentrations of ET-1 in Gulf Arabs were measured and the levels were significantly higher in hypertensive subjects than in the age-and sex-matched normotensive population. Interestingly, direct correlation between ET-1 levels and blood pressure, both systolic and diastolic, has also been demonstrated, apparently for the first time. Further studies of this population will be of interest to determine whether elevated levels of plasma ET-1 in hypertensives might be involved in the pathophysiology of complications in hypertension as well as to investigate any possible interaction between ET-1 and angiotensin II.

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