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Homocysteinemia, hypertension, and family history of diabetes in a smoking male population in Saudi Arabia

Research Article

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Abstract: Arabs have a lower incidence of atherosclerosis than other ethnicities, but few studies have examined homocysteine (HCYS) as a risk factor for cardiovascular disease in this population. Here, we investigated the association between serum HYCS levels and risk factors for cardiovascular disease (smoking, hypertension, and family history of diabetes) in Saudi males. A total of 50 smokers and 72 nonsmokers completed a general health questionnaire. In addition, their lipid profiles were measured using routine methods and HCYS levels by high-performance liquid chromatograph with electrochemical detection. Regression analysis showed negative associations between HCYS and glucose ($r = -0.22$; $P < 0.05$) as well as family history of diabetes ($r = -0.21$; $P < 0.05$). HCYS levels were similar between hypertensive and nonhypertensive smokers, but they were significantly elevated in hypertensive nonsmokers ($P = 0.027$) and lower in smokers with family history of diabetes ($P = 0.01$). Levels of HCYS among nonsmokers inversely correlated with history of diabetes and elevated glucose. Nonsmokers' HCYS levels were significantly elevated in the presence of hypertension and correlated with diastolic blood pressure. Thus, HCYS may be a predictor of hypertension among nonsmokers. Until further trials are conducted, we recommend vitamin B6/folic acid supplementation for the Saudi hypertensive population as an adjuvant therapy.

Keywords: *Smoking • Homocysteine • Lipids, Blood pressure • Family history of diabetes • Saudi Arabia*

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1. Introduction

Homocysteine (HCYS) is a sulfur-containing amino acid and is an intermediary product of methionine metabolism. Demethylation of methionine produces HCYS, which can either be remethylated to methionine by a process dependent on folic acid and vitamin B12, or it can be converted to cystathionine in a reaction dependent on vitamin B6. HCYS, like other thiols, readily auto-oxidizes to the disulfide HCYS or to other mixed disulfides, which together account for the major fraction of plasma HCYS. Several studies have identified elevated plasma HCYS as a risk factor for cardiovascular disease (CVD) [1-2]. In particular, hyperhomocysteinemia appears to stimulate oxidative stress and inhibit nitric oxide formation, which could explain the atherogenic effects described in type 2 diabetics [3]. Furthermore, the HCYS level is affected by age, and multiple studies suggest a relationship between HCYS levels and establish risk factors for CVD in elderly subjects [4-5].

In addition, previous studies suggest that HCYS levels are elevated in both smokers and those at risk for hypertension, but the combined effects of smoking and hypertension on the HCYS level remains unclear [6-8]. It is well established that cigarette smoking is the most important risk factor of coronary heart disease (CHD) in countries where the incidence of CHD is high [9-11]. The overall prevalence of smoking in Saudi Arabia has been reported to be as high as 21.1% for males, whereas it is only 0.9% for females. Most smokers (78%) are between 21 and 50 years of age. Within this smoking population prevalence appears higher among married people, uneducated people, and those in certain occupations, namely, manual workers, businessmen, army officers, and office workers [12]. Cigarette smoking is an important public health problem in Saudi Arabia, with the risk of CVD in smokers being two to three times that of

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nonsmokers, and it does not appear that noninhalation or the use of filtered cigarettes offers any protection. In addition, passive smokers are also at risk of developing CHD [13]. Cigarette smoking modifies haemostatic parameters via thrombosis, resulting in a higher rate of cardiovascular events [14] with studies indicating associations between HCYS levels and CVD risk [15].

In the kingdom of Saudi Arabia, the urban population has a higher prevalence of CHD than the rural population (6.2% vs. 4%, respectively) [16]. Additional analysis of diet has shown that smokers have lower levels of the B-vitamins folate, vitamin B6, and vitamin B12 [17-18], all of which affect HCYS levels by acting as cofactors (vitamins B6 and B12) or as a cosubstrate (folate) for enzymes controlling HCYS metabolism [19-21]. Despite these observations, there is no available data on HCYS levels among the Saudi smoking population, and its likely associations with factors linked to the development of CHD.

Here, we carried out a cross-sectional study to investigate whether HCYS levels in smokers are an important biomarker for determining the associated risk of hypertension in the Saudi population. Furthermore, we investigated the influence of factors that can alter HYCS levels in smokers and nonsmokers such as a history of diabetes and other metabolic parameters.

2. Material and Methods

2.1. Subjects

The subject population was drawn from adult, ambulant, non-diabetic Saudi males aged 30-80 years old, attending the blood bank of Riyadh Medical Complex and King Khalid University Hospital in Riyadh, Saudi Arabia. Ethical approval was obtained from the ethics committee of the College of Medicine, King Saud University in Riyadh, Kingdom of Saudi Arabia. Volunteers were asked to give verbal and informed consent prior to inclusion. Subjects were classified as smokers or nonsmokers. For purposes of this cross-sectional study, a smoker is defined as someone who has been actively involved in cigarette smoking for at least one year prior to the study. A nonsmoker was someone who may have previously smoked and with minimum exposure to a smoking environment (e.g., working and living in a nonsmoking environment). All subjects underwent a full physical examination and completed a general questionnaire. Data on socio-demographic characteristics, personal and family medical history including diabetes, and CHD (first-degree relatives who have had diabetes and/or CHD); and health-relevant behaviors including smoking, exercise, and diet were obtained by a standardized interview at the time of presentation. Patients with known illnesses other than hypertension including diabetes or a fasting blood sugar > 7.0 mmol/L and/or creatinine levels > 2000 mg/day were excluded from the study.

2.2. Data Collection

Anthropometric measurements such as height were measured to the nearest 0.5 cm using a standardized stadiometer; weight to the nearest 0.1 kg using a standardized weighing scale; waist and hips to the nearest 0.5 cm using a nonstretchable measuring tape. Blood pressure was measured twice after 30 min rest with a 15-min interval. The mean of the two readings was recorded. The presence of hypertension was defined as a blood pressure ≥ 140/90 mmHg after several readings and/or on hypertensive medications.

Blood samples were collected after a 12-h fast for the determination of total cholesterol, high-density lipoprotein, triglycerides, and HCYS concentrations. Plasma samples were stored at –70°C prior to analysis. Serum glucose, total cholesterol, triglycerides, and 24-h creatinine clearance were measured using standard enzymatic methods and a fully automated analyzer (Kone Instruments, Helsinki, Finland). High-density lipoprotein-cholesterol levels were determined by phosphotungstic acid/magnesium chloride precipitation (Kone Instruments). The low-density lipoprotein level was calculated using the Friedewald equation [22].

HCYS was measured by high-pressure liquid chromatography (HPLC) with electrochemical coulometric detection [23]. Briefly, samples were reduced with dithiothreitol to liberate HCYS, protein was precipitated with sulphosalicylic acid, and the supernatant was analyzed by HPLC.

2.3. Statistical analysis

Data were analyzed using SPSS for Windows, version 10 (SPSS, Chicago, IL, USA). Variables that exhibited a positive skew were log-transformed to normalize the distribution. For these variables, the geometric means are given along with the estimates of the percentage difference in geometric means between never smokers and current smokers. An independent *t*-test was used to compare the levels of the two groups and, if not normally distributed, by a Mann-Whitney *U*-test. The Pearson correlation coefficient was calculated to determine the association between selected parameters and other variables, and stepwise regression analysis was performed to identify significant factors affecting variables of interest.

Table 1. Clinical characteristics and metabolic parameters of Saudi male smokers vs. nonsmokers.

	Nonsmokers	Smokers
n	72	50
Age (years)	47.1 ± 12.6	44.9 ± 11.0
Hypertension (%)	24 (33.3)	11 (22.0)
Family history of type 2 diabetes (%)	22 (30.6)	18 (36.0)
Systolic blood pressure (mmHg)	125.6 ± 19.6	121.1 ± 19.2
Diastolic blood pressure (mmHg)	81.0 ± 9.8	78.3 ± 9.0
Body mass index (kg/m^2)	27.3 ± 6.2	27.2 ± 5.9
Waist circumference (cm)	95.1 ± 12.3	95.0 ± 12.9
Hips circumference (cm)	99.6 ± 13.9	99.3 ± 14.9
Fasting glucose (mmol/l)	5.5 ± 1.3	5.5 ± 1.2
Total cholesterol (mmol/l)	5.5 ± 1.3	5.7 ± 1.6
high-density lipoprotein-cholesterol (mmol/l)	1.0 ± 0.9	1.0 ± 0.6
Triglycerides (mmol/l)	2.0 ± 1.2	2.1 ± 1.1
Low-density lipoprotein-cholesterol (mmol/l)	3.7 ± 1.0	3.9 ± 1.8
24-h urine creatinine (mg/day)	711.1 ± 409.7	765.7 ± 352.6
HCYS (μmol/l)*	10.0 ± 3.6	9.5 ± 2.7

Data are presented as means ± standard deviation and as n (%)
**The Mann-Whitney U-test was used to compare variables.*

Table 2. Pearson correlations between fasting serum HCYS levels and selected metabolic parameters.

Parameters	HCYS			
	Smokers		Nonsmokers	
	Correlation coefficient	P-value	Correlation coefficient	P-value
Age (years)	0.12	0.39	0.20	0.08
BMI (kg/m^2)	−0.11	0.45	0.02	0.83
Glucose (mmol/L)	−0.23	0.11	**−0.250**	**0.03**
Systolic BP (mmHg)	−0.10	0.46	0.19	0.11
Diastolic BP (mmHg)	−0.04	0.76	**0.24**	**0.04**
ApoA1 (mg/dl)	**0.85**	**0.00**	0.20	0.37

Differences were considered significant at $P < 0.05$.

Table 3. HCYS levels (μmol/L) according to presence of a family history of diabetes mellitus among smokers and nonsmokers.

	Without family history of diabetes	With family history of diabetes	*P*-value
Smokers	10.2 ± 2.8	8.2 ± 2.0	**0.01**
Nonsmokers	10.4 ± 4.1	9.2 ± 1.9	NS

Data are presented as means ± standard deviation. Differences were considered significant at $P < 0.05$. NS, not significant.

3. Results

A total of 122 Saudi males were included in this cross-sectional study of whom 50 (41%) were considered active smokers. The mean age of the smokers and nonsmokers was 44.9 ± 11.0 and 47.1 ± 12.6 years, respectively. The clinical and metabolic characteristics of both nonsmokers and smokers are shown in Table 1. For normally distributed variables, independent *t*-tests were performed to determine the significance of differences. For HCYS, significance of differences was assessed using a Mann-Whitney *U*-test. There was no significant difference in baseline characteristics or in HCYS levels between the smokers and nonsmokers.

Pearson correlation analysis was used to determine the significance of associations between HCYS levels and the other clinical and metabolic parameters. Among nonsmokers, the HCYS level significantly correlated with diastolic blood pressure ($r = 0.24$; $P = 0.04$) and inversely correlated with serum glucose ($r = -0.25$; $P = 0.03$). There was a strong positive correlation between ApoA1 and HCYS levels in smokers ($r = 0.85$; $P < 0.001$; Table 2). Regression analysis showed that there was a significant inverse correlation between the presence of a family history of diabetes among smokers and HCYS levels, with 37% of the variance perceived ($P = 0.01$). In all groups, there was a significant inverse correlation between both a family history of diabetes ($r = -0.22$; $P < 0.05$) and glucose levels ($r = -0.21$; $P < 0.05$; Figure 1) and the HCYS levels.

Having confirmed the correlation, we next performed another *t*-test to compare the probable effect of hypertension and a presence of a family history of diabetes among groups. Table 3 reveals significantly elevated HCYS levels among nonsmokers ($P = 0.027$). Table 4, on the other hand, shows significantly lower levels of HCYS in smokers with family history of diabetes than in smokers without a family history of diabetes ($P = 0.01$).

4. Discussion

The current study appears to be the first in the Persian Gulf area to investigate the association between plasma HCYS levels and various metabolic parameters in a smoking male population. Multiple studies have shown that hyperhomocysteinemia is linked to hypertension [24,25]. In addition, even mild hyperhomocysteinemia is a major risk factor for arterial vascular disease, independent of conventional risk factors, such as type 2 diabetes mellitus, cigarette smoking, or hyperlipidemia [26-28]. The mechanism may be related to impairment of vascular endothelial and smooth muscle cell function. It has also been suggested that hyperhomocysteinemia diminishes vasodilation by nitric oxide, increases

Figure 1. Serum HCYS vs. fasting glucose levels.

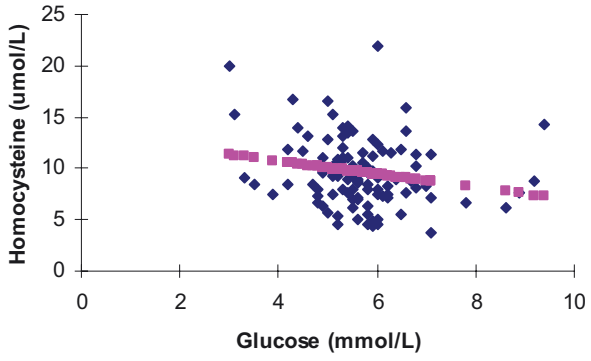

Table 4. HCYS levels (μmol/L) according to presence of hypertension among smokers and non-smokers

	Nonhypertensive	Hypertensive	*P*-value
Smokers	9.6 ± 2.8	9.1 ± 2.4	NS
Nonsmokers	9.3 ± 2.9	11.3 ± 4.3	**0.027**

Data are presented as means ± standard deviation. Differences were considered significant at P < 0.05. NS, not significant.

oxidative stress, alters the elasticity of the vascular wall, and contributes to elevate the blood pressure [28]. In this study, we did not find a significant difference between HCYS levels in smokers and nonsmokers, which confirms previous studies [8,29-30]. This may be due to a connection between HCYS levels and the number of cigarettes smoked and vitamin B6 depletion. Despite the lack of a significant difference, until further prospective studies are done, we recommend the continued use of vitamin B6 vitamin supplements for smokers as previously suggested [10].

Elevated HCYS is considered a strong risk factor for CVD in some parts of the world, but it still remains to be proven in this population, suggesting differences between ethnicities. Even mutations in the MTHFR gene that lead to hyperhomocysteneinemia are not associated with CVD in the Arab population [31], who have a lower prevalence lower of CVD than other ethnicities [32]. Furthermore, previous studies have shown that diabetic Saudi patients have lower HCYS than nondiabetic patients, due to an effect of insulin on the trans-sulfuration of HCYS [33]. In our study, 36% of smokers had a family history of diabetes, which may explain the reduction of HCYS in Saudi smokers. Another probable factor for the fact that hyperhomocysteinemia remains non-threatening in the region is Arab dietary habits; the Mediterranean diet consists mostly of olive oil, wheat bread, yoghurt, and grains rich in vitamin B complexes and other anti-atherogenic elements. This can also explain why the prevalence of CHD as well as certain types of cancer remains lower in these areas than in other industrialized nations.

With respect to blood pressure, Kennedy and coworkers [6] found a significant association between HCYS levels and systolic blood pressure. Blum and colleagues, on the other hand, found no correlation between HCYS and hypertension [33]. Here, we found a weak but significant relationship between HCYS and diastolic but not systolic blood pressure in nonsmokers. Also, apolipoprotein A1 levels strongly correlated with HCYS among smokers in our study, but this needs to be confirmed in retrospective studies using the same cohort because most studies have found instead that the Apo-A1 level inversely correlates with the HCYS level, at least in CHD [34]. In all groups, there was a strong negative association between the HCYS level and a family history of diabetes as well as between HCYS and glucose levels. This reaffirms our previous findings that serum HCYS is lower in Saudi patients with diabetes mellitus because HCYS levels of subjects who are genetically predisposed to develop diabetes also have lower HCYS levels [35].

This study had some limitations. First, the relatively small sample size might have influenced significant factors that are known to affect HCYS levels, such as age and creatinine levels, which did not correlate with HCYS levels in this study. Nevertheless, the subjects were well controlled in terms of gender (males only), renal function (urine creatinine < 2000 mg/day), and age (nonsignificant difference, although there was a wide age range). Second, the staging of hypertension was not considered in this study and may be important in future studies on the influence of HCYS in varying stages of hypertension. Third, the conclusions derived can only be applied to the male population; further studies using Arab females as a cohort will strengthen the findings of this study.

In conclusion, the present findings show that in Saudi male smokers, the circulating HCYS level is negatively associated with a family history of diabetes and glucose levels, while a role of HCYS in hypertension was observed only for nonsmokers. There was no significant difference between smokers and nonsmokers, which may reflect the smokers' family history of diabetes. This suggests that HCYS is a predictor for hypertension in nonsmoking Saudis. Prospective studies and clinical trials need to be performed to confirm these associations in the Saudi population. Finally, we recommend the continued use of vitamin B6 and folic acid supplements for the Saudi hypertensive population in general as an adjuvant therapy until further clinical trials are conducted.

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