Homocysteine Level and Coronary Artery Disease

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Atherosclerosis, and its most common manifestation, coronary artery disease (CAD), are rather common causes of morbidity and mortality worldwide. Recognition of its various risk factors is important to planning effective preventive measures. After the homocysteine theory was presented in 1969, attention has been directed toward the serum homocysteine level as a coronary artery disease risk factor. The authors aimed to assess the relationship between hyperhomocysteinemia and CAD in an Iranian population. In a case control study, 197 individuals (male: 123 [62.4%]) who were scheduled for coronary angiography were selected. Venous samples were taken from the patients in fasting state before angiography. Data about age, sex, risk factors (eg, hypertension, diabetes, smoking, hyperlipidemia, obesity) were obtained from prepared questionnaires. Homocysteine levels in patients were measured by ELISA method. A homocysteine level above 15 µmol/liter was considered high. Angiography reports and homocysteine levels were analyzed by independent sample t test, one-way ANOVA, multiple linear regression, and stratified analysis. In comparison with the patients with normal angiography reports (32.5%), patients with abnormal angiography reports (67.5%) had increased levels of homocysteine (p=0.001). About 28.1% of patients with normal angiography reports had hyperhomocysteinemia. After further evaluation, linear correlations were detected between the numbers of involved vessels and homocysteine level (p=0.000). Multiple linear regression analysis of data detected that in individuals without any risk factors, the relationship was stronger and more meaningful (p=0.000). These data show that hyperhomocysteinemia is related to CAD as an independent risk factor. In individuals without any risk factors a linear correlation between homocysteine level and numbers of coronary artery involvement was present. If this equation is confirmed prospectively in other studies, the level of plasma homocysteine may he used as a noninvasive way of predicting the number of diseased coronary arteries.

Introduction

Atherosclerosis, and its most common manifestation, coronary artery disease (CAD), are rather common causes of morbidity and mortality worldwide including Iran. Recognition of its various risk factors is important to planning effective preventive measures. Homocysteine (Hcy), a sulfur amino-acid first described by Butz and du Vigneaud in 1932,1 has been linked to atherosclerosis and CAD by McCully in 1969.2-5 He de-
scribed hyperhomocysteinemia as a possible risk factor for CAD. Since then more than 100 papers have been published in the literature probing various clinical and epidemiologic features of this hypothesis, some suggesting hyperhomocysteinemia as a strong risk factor for CAD. The most important parts of this information come from nested case-control studies; all but one found a relation between total homocysteine level and the frequency of vascular disease.

Fasting normal total plasma homocysteine concentrations range from 5 to 15 µmol per liter, with the highest concentrations seen in men and postmenopausal women. Kang and coworkers have classified hyperhomocysteinemia as mild (15–30 µmol per liter), moderate (30–100 µmol per liter), and severe (more than 100 µmol per liter).

We aimed to assess the relationship between hyperhomocysteinemia and CAD in an Iranian population. It could be clinically important not only to the grave consequences of coronary artery disease but also to the easy correction of hyperhomocysteinemia by vitamin supplementation, which may prove to be an effective therapy in future studies.

Methods

Study Population

All adult patients undergoing coronary angiography at Shiraz University of Medical Sciences during October and November 2001 were invited to participate in the study. Consenting patients were enrolled and a questionnaire covering demographic data and known CAD risk factors (hypertension, diabetes mellitus, dyslipidemia, obesity, past or family history of CAD, obesity, current medication use, noncardiovascular medical problems, and some dietary habits) was filled in for each participant. Family history of CAD was considered to be positive whenever there was documented CAD in at least 1 first-degree relative before 55 years of age.

Participants were further subdivided according to their smoking status (current smokers and nonsmokers). Current smokers (including those who had given up smoking less than 30 days before coronary angiography) were subclassified as those smoking less than 10 cigarettes per day and those smoking more than 10 cigarettes per day.

Angiographic Evidence of Coronary Artery Disease

Cardiologists unaware of the subjects’ risk factors status reported the angiograms. Coronary artery disease was defined as any degree of stenosis in major coronary arteries (ie, the left main coronary artery or the left anterior descending coronary artery with its major diagonal branches, the right coronary artery, or the circumflex coronary artery with its major marginal branch). Depending on dominance, the descending or posterior descending coronary artery was included as part of the right coronary artery or the circumflex coronary artery.

CAD was graded from 0 to 3 according to the number of involved vessels: 0 (no disease), 1 (single-vessel disease), 2 (two-vessel disease), or 3 (three-vessel disease).

Left main stem stenosis with a normal right coronary artery was graded as 2.

Biochemical Measurements

After an overnight fast 10 cc venous blood was drawn just before the coronary angiography. Plasma was immediately separated and stored at −20°C until measurement of total homocysteine. Homocysteine was measured with an enzyme-linked immunosorbent (ELISA) method (DRG Instruments GmbH, Marburg, Germany). All the samples were processed by a single technician on 2 consecutive days to minimize the interobserver error. The upper limit of normal provided by the company was 15 µmol/L.

Statistical Analysis

Independent sample t test, one-way ANOVA, multiple linear regression, and stratified analysis were used where appropriate. Two-sided p values below 0.05 were considered to indicate statistical significance.

Results

One hundred and ninety-seven individuals agreed to participate. Of these, 123 (62.4%) were men. One hundred and thirty-three (67.5%) had CAD (Figure 1). Of these, 44 had grade-1, 43 had grade-2, and 46 had grade-3 CAD (Figure 2). The 64 individuals (32.4%) with normal coronary ar-
teries served as controls. The mean homocysteine level in all was 15.84 ± 6.44 µmol/L, 65 of 123 men and 23 of 74 women had homocysteine levels more than 15 µmol/L (52.8% vs 31.1%, respectively, p = 0.003).

The patients were divided into 5 groups by age (21–30, 31–40, 41–50, 51–60, and 61–70), and the mean homocysteine levels in each group were as follows: 13.24, 15.12, 16.61, 16.16, and 18.8 µmol/L, respectively (Figure 3). One-way ANOVA showed no difference in homocysteine level among these groups (p = 0.14). Also, no difference was observed when they were subdivided into 2 groups: below 50 and above 50 years old (p = 0.062). Perhaps, the reason is that all were not from a normal population.

The mean homocysteine level was 16.98 ± 6.49 µmol/L in the 133 patients with CAD compared to 13.47 ± 5.67 µmol/L in the control group (p = 0.000) (Figure 4).
Eighteen of 64 (28.1%) individuals with normal angiography reports had hyperhomocysteinemia, whereas 70 of 133 patients (52.6%) with coronary artery disease had hyperhomocysteinemia ($p = 0.001$).

Regression analysis was used to assess the relationship between plasma homocysteine levels and severity of CAD. There was a linear relationship between increasing plasma homocysteine level and severity of CAD ($p = 0.000$, $r = 0.246$, Figure 5). According to our data the following equation could be calculated to predict the number of diseased vessels, given the plasma homocysteine level:

$$n = 0.655 + (0.045 \times \text{homocysteine level})$$  \hspace{1cm} (1)$$

where $n$ is the number of diseased vessels and 0.056 is the coefficient of determination. To improve the predictive potential of the equation, other major CAD risk factors quantitative variables (ie, age and body mass index) were included in a multiple regression analysis, and the following equation was obtained:

$$n = 0.059 \times \text{homocysteine level}$$  \hspace{1cm} (2)$$

where $n$ is the number of diseased vessels. The coefficient of determination was 0.195. In other words, if the age and body mass index are considered, about 19.5% of involved vessels fluctuation will be stated by homocysteine fluctuation. In the next step, the above-mentioned relation was obtained in the sex subgroups by use of stratified analysis, and it was recognized that the above-mentioned linear relation was significant in men and the following equation was obtained:

$$n = 0.737 + (0.046 \times \text{homocysteine level})$$  \hspace{1cm} (3)$$

where $n$ is the number of diseased vessels. The coefficient of determination in this relation was 0.062, but no significant linear relation was seen in women.

Evaluation of the effect of hyperlipidemia showed that the significant linear relation was as follows only in the individuals with negative history:

$$n = (0.064 \times \text{homocysteine level})$$  \hspace{1cm} (4)$$

where $n$ is the number of diseased vessels. The coefficient of determination in this case was 0.144 and no significant linear relation was seen in the group with a history of hyperlipidemia.

The history of hyperglycemia in the subjects showed no significant relation between the num-
number of involved vessels and homocysteine level. In the subjects with no history of hyperglycemia, the following linear relation was seen:

\[ n = 0.556 + (0.047 \times \text{homocysteine level}) \] (5)

where \( n \) is the number of diseased vessels. The coefficient of determination in this case was 0.062.

Also the following significant linear relation was obtained in nonsmokers:

\[ n = 0.56 + (0.044 \times \text{homocysteine level}) \] (6)

where \( n \) is the number of diseased vessels. The coefficient of determination was 0.053.

In the individuals without a history of hypertension the following linear relation was obtained between homocysteine level and the number of coronary artery involvement:

\[ n = 0.05 \times \text{homocysteine level} \] (7)

where \( n \) is the number of diseased vessels. The coefficient of determination was 0.067 in this case, but the linear relation between homocysteine level and number of coronary artery involvement in these subjects was not significant.

In regard to the obtained equations in confounding variables of subgroups, such as smoking, sex, hyperlipidemia, hypertension, and hyperglycemia, a relation between homocysteine level with the number of involved vessels in the subgroups of nonsmoker individuals without a history of CAD was obtained that led to the following equation \((r = 0.419, p = 0.000)\) (Figure 6).

\[ n = 0.07 \times \text{homocysteine level} \] (8)

where \( n \) is the number of diseased vessels. In this case, the coefficient of determination was 0.163. In other words, 16.3% of variable fluctuation in the number of involved vessels will be stated by homocysteine level fluctuation. This is the strongest relation that could be observed between the number of coronary artery involvement and homocysteine level.

**Discussion**

Hyperhomocysteinemia has been reported in 5–21% of the normal population in different studies from the West. In our study 28.1% of normal people had hyperhomocysteinemia. Although this may be a true difference, it may be related to our sample size and selection bias, i.e., we studied patients referred for coronary angiography and not the general population. If we had looked at the general population with a larger sample size we might have reached different conclusions. This can be sought in further larger scale population-based studies. In the studies by McCully et al, they showed that only 5–7% of normal individuals had hyperhomocysteinemia. In the Framingham heart study conducted by Selhub et al and another study done by Sipahi et al 21% of a normal population had hyperhomocysteinemia, which is lower than that of our study. The higher plasma homocysteine that we found in male subjects is in accord with findings of Uneland et al. Several investigators have found hyperhomocysteinemia significantly more in patients with CAD. Note that the upper limit of normal has not been defined unanimously in these different studies, therefore affecting the comparison. We found that patients with CAD had a significantly higher fasting plasma homocysteine level than those with normal coronary arteries. Our cutoff level was 15 µmol/L, which is higher than that considered as upper limit of normal by several other investigators. Qujeq et al have found that if the upper limit of normal for homocysteine is set at 10 µmol/L, then hyperhomocysteinemia will pose a relative risk of 2.84 (95% CI: 1.01–7.98) for CAD. Their wide confidence interval points to the low sample size on which they worked, and therefore, their data should be interpreted with caution. This is consistent with the findings of Hankey et al, although the border line in their study was 9 µmol/L.

Another source of inconsistency between reports is the way in which homocysteine is measured. Many investigators have used high-power liquid chromatography (HPLC) for this purpose. HPLC, although very accurate, is a cumbersome method that is not widely available, especially at commercial laboratories. For this reason others have used the ELISA method. We used ELISA, according to the method of Frantzen et al for determining the homocysteine level instead of HPLC. Moreover, other studies have confirmed the precision and reliability of ELISA method for measuring homocysteine level, so our results seem to be not much different from those that would be obtained by HPLC.

We calculated a coefficient of determination for plasma homocysteine level’s ability to predict the number of diseased coronary arteries. This was 0.056, which means that hyperhomocysteinemia as an independent variable that counts...
for only about 6% of the diseased coronary arteries, and therefore the relationship, although real, is small. This weak relation is similar to the findings of Nygard et al.6,7 We found that after correction for other known CAD risk factors (ie, sex, smoking, hypertension, hyperlipidemia, and hyperglycemia), plasma homocysteine level is still related to CAD. After correction for all the above-mentioned risk factors, the coefficient of determination for hyperhomocysteinemia is 0.163. Therefore hyperhomocysteinemia alone is responsible for about a 16.3% fluctuation in the number of involved vessels in individuals. This shows a relatively strong relation. In a similar study done in Syria, homocysteine level was associated with the number of vessels involved.23

Finally, our data show that hyperhomocysteinemia is related to CAD as an independent risk factor. In addition, if our equation is confirmed prospectively in other studies, the level of plasma homocysteine may be used as a noninvasive way of predicting the number of diseased coronary arteries.

This relation is stronger in individuals who are not involved with the risk factors such as sex, hypertension, smoking, hyperlipidemia, and diabetes. Since the relation between homocysteine level and the number of involved vessels has been ignored in previous studies, our study may initiate a new approach to prevention of coronary artery disease.

REFERENCES