Serum Total Homocysteine Concentrations in Normal and Cardiovascular Disease Patients

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Introduction

Homocysteine is a sulpher-containing amino acid that is closely related to methionine and cysteine. All homocysteine found in organisms is formed in the metabolism of the essential amino acid, methionine, in the methylation cycle. This is the only known source of homocysteine. Reduced homocysteine is in a highly reactive free thiol group, which is susceptible to auto-oxidation at physiological pH, thereby forming disulphide bonds between two molecules or mixed disulphides with other thiols.

In plasma only about 1% of homocysteine exists in a free reduced form. About 70% is bound to albumin and the rest forms low molecular weight disulphides, predominately with cysteine. The sum of all the forms is termed total homocysteine. The assays generally measure the total homocysteine in plasma or serum, sometimes in cerebrospinal fluid, rarely in urine.

Three enzymes are directly involved in the homocysteine metabolism: methionine synthase, betaine homocysteine methyl transferase, and cystathionine □-synthase.

Vitamins B_6, B_{12}, and folate are cofactors to these enzymes. If the metabolism is disturbed, because of some enzymatic defect or intracellular deficiency of some cofactors to the mentioned enzymes, homocysteine

accumulates in the cell and is then transported to the circulation where it's level rises.

Homocysteine is mainly eliminated by renal catabolism. Only about 1% of the homocysteine filtered by the *glomeruli* is normally found in the urine [Guttormsen, et al., 1997]. The rest is reabsorbed and metabolized. Thus, the kidneys are homocysteine metabolizing rather than homocysteine-excreting [Van-guldener, et al., 1998, and Refsum, 1998].

Homocysteine is metabolized through two major pathways: transsulphuration and methylation. Normally about 50% is catabolised in the transsulphuration pathway, where homocysteine and serine for cystathionine, which is cleared into cysteine and α-ketobutyrae. The other 50% enter the methylation cycle, which is part of the one-carbon metabolism. S-adenosylmethionine is an important regulator of the remethylation and transsulphuration of homocysteine.

Cardiovascular diseases (CVD) are one of the most important health problems and remain to be the major cause of death and disability in many countries, all over the world. Atherosclerosis and thrombosis are the two etiological causes of coronary heart disease (CHD). Atherosclerosis is principally a disease of the large arteries in which lipid deposits called atheromatous plaques in the subintimal layer of the arteries. These plaques contain especially large amounts of cholesterol. Obesity, smoking, diabetes, hypertension and abnormal plasma lipid levels are recognized risk factors that contribute to the development of CHD [Rifai, 1986 and Ramirez, et al., 1992]. This theory hold that cholesterol, mainly its oxidized low density lipoprotein

(LDL) variety, collects in plaques that line the insides of large and small arteries.

The aim of these projects is to measure total homocysteine in normal individual and coronary heart disease patients to fired ant if there is any relationship between homocysteine and CHD.

Materials and Method

Randomly selected normal healthy Saudi citizens (both males and females) living in the Jeddah area were selected to take part in this study. These were 111 males and 80 females (overall 191 subjects) with a range of 18-60 years of age. Male and female cardiovascular disease (CVD) patients were also selected to take part in the present study. They were 61 males and 28 females attending King Abdulaziz University Hospital with a range of 40-70 years of age.

Measurement of serum homocysteine concentration

Serum total homocysteine was measured by AXIS homocysteine EIA assay. The assay procedure is based on the method described by Franzen *et al* [6].

Disulfide bonds were first reduced with dithiothreitol (DTT) to yield free homocysteine (Hcy).

$$\text{Prot} - \text{SS} - \text{Hcy} \xrightarrow{\text{DTT}} \text{Hcy}$$

Homocysteine enzymatically converted to S-adenosyle-L-homocystein (SAH) by the use of SAH hydrolase and excess adenosine (Ad) in a separate procedure prior to the immunoassay.

$$Hcy + Ad \xrightarrow{\text{SAH hydrolase}} SAH + H_2O$$

The enzyme is specific for the L-form of homocysteine, which is the only form present in the blood. The calibrators were run in duplicate and each run was performed with a new calibration curve to avoid run-to-run variation using coated microtitre plates. A four-parameter logistic curve is used for the preparation of the calibration curve and for the calculation of unknown samples.

Results and Discussions

The mean values (± SEM) for the measured serum glucose and total homocysteine of normal and disease males and females are shown in table 2. The patients groups have higher serum glucose significantly ($p < 0.001$). The concentration of total homocysteine in normal males (9.49 ± 0.3) was significantly higher ($p<0.001$) than normal females, (7.54 ± 0.38), whereas no significant difference was noted between the male and female patients. Also, the difference between normal males and diseased males was non-significant; whereas, diseased females have higher serum homocysteine concentration (9.79 ± 0.92) than normal females (7.54 ± 0.38), significantly.

The mean values (± SEM) for the measured serum lipids of normal and cardiovascular disease males and females are shown in table 3. The difference of total cholesterol between normal and diseased groups was statistically non-significant, whereas the concentrations of triacylglycerol and LDL-cholesterol were significantly increased ($p< 0.001$) in the cardiovascular disease males and females. In the normal group, the concentration of triacylglycerol was significantly higher ($p<0.001$) in females with no significant

differences in the concentrations of total cholesterol and LDL-cholesterol. The above descriptive statistics on the biochemical variables of normal and cardiovascular males and females are furtherly shown in figures 35-40.

The mean concentration of total homocysteine in normal Saudi males and females are 9.49 ± 0.33 μmol/L and 7.54 ± 0.38, respectively. These values are close to those values reported in Norwegians [Nygard, et al., 1995], in Non-Hispanic white and Black males and females and in Mexicans [Jaques, et al., 1999]. These results of total homocysteine are also consistent in that males have higher concentration of total homocysteine than in females [Nygard, et al., 1995; Jaques, et al., 1999].

Moreover, the concentration of total homocysteine of cardiovascular disease females was significantly higher ($P < 0.01$) than normal females. This result is in agreement with other published results in that hyperhomocyteinemia is associated with an increased risk of cardiovascular disease [Pancharuniti, et al., 1994: Morrison, et al., 1996; Rimm, et al., 1998].

Conclusion

Serum concentrations of total homocysteine increase with age and are greater in males than in females. Results of the present study show that serum concentrations of total homocysteine are positively correlated with triacylglycerol and LDL-cholesterol and inversely correlated with HDL-cholesterol concentration, which is consistent with other reports [Refsum, et al., 1998]. Despite this correlation of serum total homocysteine with other cardiovascular risk factors, the associations of homocysteine with vascular risk

appears to be independent of other cardiovascular risk factors [Danesh, et al., 1998].

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معدلات تركيز الهوموسيستين الكلي في مصل الدم في الأصحاء ومرضى ضغط الدم

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المستخلص : إن ندرة المعلومات المتوفرة عن توزيع معدلات الهوموسيستين الكلي في مصل الدم لدى المواطنين في المملكة العربية السعودية من الأسباب التي دفعت الفريق البحثي للقيام بهذا المشروع .

يوجد هناك دليل واضح على أن التراكيز العالية من الهوموسيستين الكلي عادة ما تكون مصاحبة مع أمراض القلب و تصلب الشرايين .

و الهدف من هذه الدراسة هو قياس تركيز الهوموسيستين الكلي في عينات دم مجمعة من أفراد أصحاء (ذكور و إناث) بالإضافة الى عينات من مرضى القلب و الأوعية الدموية . كذلك أيضا تم تقدير تراكيز دهون مصل الدم الرئيسية (الكوليسترول الكلي ، ثلاثيات الجليسرول ، البروتين الدهني العالي و منخفض الكثافة) إضافة الى عدد من الخصائص البشرية المرتبطة مثل (السن- الوزن-الطول-و معامل كتلة الجسم) .

تم تجميع ١٩١ عينة دم من أفراد أصحاء (١١١ ذكور و ٨٠ إناث) إضافة الى ذلك تم تجميع ٨٩ عينة دم من مرضى القلب و الأوعية الدموية (٦١ ذكور و ٢٨ إناث) . لقد وجد أن معدل تركيز الهوموسيستين الكلي عند الإناث الأصحاء (٧,٥٤ $^{+}$ ٠,٣٨ ميكرومول/لتر) كان أقل معنويا من الذكور الأصحاء (٩,٤٩ $^{+}$ ٠,٣٣ ميكرومول/لتر) .

إضافة الى ذلك فإن معدل تركيز الهوموسيستين في ذكور مرضى القلب لم يكن مختلفا معنويا عن الذكور الأصحاء ، بينما وجد أن معدل التركيز عند إناث مرضى القلب كان أعلى معنويا عن الإناث الأصحاء . لقد تناول البحث مناقشة هذه النتائج في ضوء ما نشر من أبحاث سابقة .