

HAMID REZA KHALIGHI^{1, A, F}, HAMED MORTAZAVI^{1, E}, SAEED ALIPOUR^{2, C},
KAMELIA NADIZADEH^{3, B}

Relationship Between Salivary and Plasma Level of Homocysteine in Coronary Artery Disease

Związek między ślinowym i osoczym stężeniem homocysteiny w chorobie wieńcowej serca

¹ Department of Oral Medicine, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran

² Modares Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³ Private practitioner, Tehran, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. It is believed that an elevated plasma homocysteine concentration conferred an independent risk of cardiovascular disease and plasma homocysteine level can predict the mortality rate of patients suffering from cardiac disease.

Objectives. We designed this study to evaluate the relationship between salivary and plasma level of homocysteine in cardiovascular patients and control group.

Material and Methods. We obtained 5 mL blood and 2.5 mL saliva from 34 patients with coronary artery diseases (CAD) and 32 healthy individuals and detected homocysteine in their blood and saliva by high pressure liquid chromatography (HPLC) method. The difference between homocysteine level of patient and control group was compared with *t*-test. In addition, Pearson-coefficient was also used to determine the relationship between salivary and plasma level of homocysteine.

Results. The average of plasma homocysteine level was 15.43 ± 5.07 mmol/Lit in patients and 9.95 ± 5.88 mmol/Lit in the control group; their difference was statistically significant when compared with the use of Student *t*-test ($p < 0.001$). The average of salivary homocysteine level was 0.24 ± 0.056 mmol/Lit in patients and 0.023 ± 0.013 mmol/Lit in the control group; their difference was statistically significant ($p < 0.001$) with the use of Student *t*-test. There was a relationship between saliva and plasma level of homocysteine in both groups (Person-coefficient = 0.744)

Conclusions. There was a relationship between saliva and plasma level of homocysteine, so it can be used as an alternative media for detecting and measuring homocysteine (**Dent. Med. Probl. 2015, 52, 1, 22–25**).

Key words: coronary heart disease, saliva, homocystein.

Słowa kluczowe: choroba wieńcowa serca, ślina, homocysteina.

Cardiovascular disease (CAD) is one of the most important causes of death and is responsible for 30% of deaths worldwide. The main cardiovascular risk factors such as male sex, family history, smoking, hypertension, dyslipoproteinemia and diabetes account for approximately 60% to 70% of our ability to discriminate CAD patients from healthy subjects [1]. Also, it was discovered that changes in some serum biomarkers such as

homocysteine, creatine kinase, and neopterin occur in this disease [2–4].

Homocysteine is a sulfhydryl-containing amino acid that is formed by methionine demethylation and was discovered for the first time by Duvigneud [acc. 2]. In 1975 Mc-Cully [acc. 2] stated that hyperhomocysteinemia can increase cardiovascular risk. Subsequent investigations, such as the development of atherosclerosis as a result of

hyperhomocysteinemia in animals, have confirmed Mc-Cully's hypothesis. It has recently become clear that hyperhomocysteinemia is an independent risk factor for atherosclerosis and atherothrombosis and there are several studies which show higher homocysteine level in plasma of cardiovascular patients than normal individuals [1–5]. Today it is believed that an elevated plasma homocysteine concentration conferred an independent risk of vascular disease and plasma homocysteine level can predict the mortality rate of CAD patients [2–4].

In the general population, homocysteine level in plasma has a normal distribution. Although this level varies among laboratories, the average value is 7 to 14 mmol/L and in 3–14 years old children was reported 6 mmol/L [2, 5].

Saliva with its composition of organic and inorganic molecules, exfoliated cells and microbes is a rich resource for the assessment of health. Its diagnostic capacity has been documented for nearly 40 years and reviewed in many papers. Advantages of saliva use as a diagnostic test for cardiovascular disease are: it is a noninvasive method vs blood sampling, several biomarkers associated with inflammation, atherosclerosis, plaque stability and myocardial damage can appear and be evaluated in saliva. These advantages have attracted the interest of many investigators in relation to the use of oral fluids to assess aspects of systemic disease, including cardiovascular disease [6, 7]. Some studies have been done in order to detect and measure homocysteine in saliva with same methods that have been used for detection in plasma, including enzymatic method, chromatography and isotropic method [1–8]. Dillion et al. [8] claimed enzymatic method is not efficient in detecting homocysteine in saliva but Boulat-Tolle et al. [9] reported homocysteine can be detected in saliva by isotropic method. However, there is no correlation between saliva and plasma level of it [9]. Balad et al. [10] measured homocysteine in saliva by high-pressure liquid chromatography (HPLC), but he did not compare his results with plasma level in the healthy group and in patients.

So we designed this study to evaluate the relationship between salivary and plasma level of homocysteine in cardiovascular patients and control group.

Material and Methods

Precipitants

Thirty four patients, who were hospitalized in Modares hospital, Tehran, Iran because of cardiovascular disease and their diagnosis was estab-

lished by clinical and echocardiographic criteria, were selected and for control group we matched 34 healthy individuals for their age and sex with patients.

Sampling

We obtained 5 mL blood by a 5 mL syringe (Varid CO, Tehran, Iran) from brachial artery and saved it in a nitrated sterile tube. Saliva sampling was done according to Foely's method [6]. Two aware messenger swabs (Calypte Life Science, Portland, OR) were placed under the tongue simultaneously and adjacent to sublingual salivary duct. After 2 min swabs were placed into sterile tubes containing a protease inhibitor solution (Sigmafast, Sigma, St Louis Mo) tubes were frozen at – 20 and sent to the laboratory.

Homocysteine Measurement

The homocysteine level of saliva and blood was measured by HPLC method in the laboratory. HPLC analysis was performed with the Hewlett-Packard (Waldbronn, Germany) HP 1100 Series System consisting of quaternary pump, an auto sampler, vacuum degasser, diode array detector, and controlled by HP Chem Station software. To 200 μ L of saliva 50 μ L of 0.1M EDTA, 200 μ L of 0.1M pH 7.4 phosphoric buffer and 25 μ L of 10% TBP were added. The mixture was incubated for 30 min at 60°C, followed by adding, after cooling, 50 μ L of 0.1M phosphoric buffer and 50 μ L of 3M PCA and centrifuged (10 min, 10000 \times g). A 20 μ L of the supernatant was transferred into analytical column (ZORBAX SB-C18, 150 \times 4.6 mm, 5 μ m; Waldbronn, Germany) of the HPLC system. The column oven temperature was 25°C, the flow rate 1.2 mL/min, and the detector wavelength 355 nm. Under gradient elution with profile: 0–8 min 10–30% B, 8–10 min 30–10% B (elution solvent A was 0.07 MpH 1.65 TCA buffer prepared from TCA and lithium hydroxide solutions of the same concentration, and B acetonitrile) homocysteine eluted after 6.9 min.

Statistical Analysis

All of data was analyzed with SPSS 20 statistically and the difference between homocysteine level of patient and control group was compared with Student *t*-test. In addition, Pearson coefficient was also used to determine the relationship between salivary and plasma level of homocysteine.

Results

In this study we obtained samples from 68 people, Two saliva samples from healthy individuals were inadequate, so we deleted their blood and saliva samples from our study and reported 66 (29 females and 37 males) remaining cases: 34 CAD patients (14 females and 20 males) and 32 healthy individuals (15 females and 17 males).

The average age in patients was 56.7 ± 25.3 and in the control group was 43.9 ± 34.1 years old. There was no significant difference in terms of age between the two groups. ($p < 0.001$)

The average plasma homocysteine level was 15.43 ± 5.07 mmol/L in patients and 9.95 ± 5.88 mmol/L in the control group. The difference was measured using Student *t*-test and was considered statistically significant ($p < 0.001$) (Table 1).

Table 1. Average of homocysteine level of saliva and plasma in each group

Group	Plasma homocysteine level	Saliva homocysteine level
Patients	15.43 mmol/Lit	0.24 mmol/Lit
Control	9.95 mmol/Lit	0.023 mmol/Lit

The average of salivary homocysteine level was 0.24 ± 0.056 mmol/L in patients and 0.023 ± 0.013 mmol/L in the control group. The difference was considered statistically significant ($p < 0.001$) using the Student *t*-test.

There was a relationship between saliva and plasma level of homocysteine in both groups (Pearson coefficient = 0.744) (Fig. 1).

Discussion

From 1975, when the role of homocysteine in cardiovascular disease was described, many studies have been performed on it and showed that homocysteine level in plasma increases in these patients alone or with other markers such as C-reactive protein, neopterin and pyridoxal-5-phosphate [4, 5, 11, 12]. Similarly, we found an increased level of homocysteine in CAD patients' plasma than in a healthy individual. The homocysteine plasma level was 9.95 ± 5.88 mmol/L in the control group, which is compatible with Humphery's study [2]. The average plasma level of homocysteine in cardiovascular patients was 15.43 ± 5.07 mmol/L, which corresponds with Jarsoz's study [3]. Jarsoz's found that in most cardiovascular patients the homocysteine plasma level is higher than 15 mmol/L.

We detected homocysteine in the saliva by HPLC method in all samples that is in accordance with Balad's et al. study [9], whereas ELISA method did not have good results in detecting it by means of Dillion's study [8].

The salivary homocysteine level was much lower from its plasma level in both groups. Diffusion of substances from plasma to saliva depends mainly on 3 factors: 1) lipophilic substances diffuse more easily than lipophobic molecules do. 2) The saliva to plasma ratio of the ionized compounds varies with salivary PH. 3) The protein binding of substances is an important factor determining the saliva to plasma ratio. So it is not surprising that homocysteine, which is a lipophilic substance and largely bound to plasma protein, was found in saliva in very low concentration [9].

We found a relationship between saliva and plasma level of homocysteine in both groups; this finding suggests that saliva is a suitable alternative for plasma. However, Boulet-Tolle et al. [9] reported the lack of correlation between saliva and plasma level of homocysteine. This difference may have resulted from the difference in the measuring method of homocysteine (HPLC versus isotropic method).

Although we obtained saliva by lingual swabs, but, because of Miller's study [6] which mentions that there is a greater concentration of biomarkers in the whole saliva than in lingual swab, we expect that if the whole saliva is obtained, the same results will be achieved.

There was a relationship between saliva and plasma level of homocysteine, so it can be used as an alternative media for detecting and measurement of homocysteine. However, further investigations should be done to clarify our results.

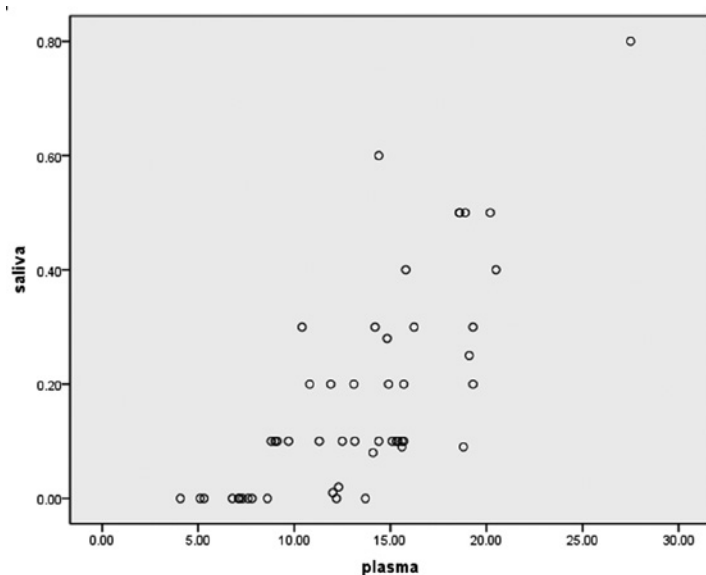


Fig. 1. Correlation between plasma and saliva level of homocysteine

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Address for correspondence

Hamid Reza Khalighi
Shahid Beheshti Dental School
Evin
Tehran
Iran
E-mail: dr.hrkh92@gmail.com

Conflict of interest: None declared

Received: 26.07.2014

Revised: 17.09.2014

Accepted: 30.09.2014