Evaluation of Homocysteine and Some Biochemical Parameters in Patients with Coronary Atherosclerosis in Erbil Governorate


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ABSTRACT

An elevated homocysteine (Hcy) level in blood causes endothelial dysfunction and damage. Individuals with higher Hcy concentration have increased risks of cardiovascular disease. Ninety individuals were taken at Hawler Cardiac Surgery Center and diagnosed by coronary angiography. Blood sample collections started in October 2014 and ended in January 2015. The present study includes 60 patients who had stenosis and 30 individuals who had no stenosis as healthy group, patient and healthy groups aged >40 years old. The results of this study show that, the mean ± S.E. levels of tHcy in patient group was 17.170 ± 0.748 μmol/L, which was located above normal range for tHcy and in healthy group was 13.780 ± 0.679 μmol/L, which was located within normal range for Hcy and the concentration of tHcy was significantly higher in patient group compared to healthy group. Also, the mean values of malodialdehyde (MDA) and blood glucose concentrations were significantly higher in patient group than healthy group. Meanwhile, vitamin B12, folic acid levels were significantly higher in healthy group but TAC level was higher and not significant in healthy group when compared to patient group. On the other hand, Tch, TG, LDL and VLDL levels were significantly higher in patient group compared to healthy group. While, HDL level was significantly higher in healthy group rather than patient group. In correlation analysis, tHcy was positively and significantly correlated with LDL (r = 0.338). As well as tHcy was positively (not significantly) correlated with MDA, CH, TG, VLDL and urea, glucose (r = 0.3072; r = 0.07717; r = 0.1063; r = 0.1063; r = 0.2612; r = 0.003459) respectively. While, tHcy was negatively correlated with vitamin B12, folic acid, TAC and HDL (r = -0.02752; r = -0.2076; r = -0.021; r = -0.1554), respectively.

1. INTRODUCTION

Cardiovascular diseases (CVD) are the class of diseases that involve the heart or blood vessels (Kamal, 2012). Atherosclerosis is an inflammatory disease affecting the arterial blood vessels, the prevalence of atherosclerosis leading to CVD (VanderLaan, Reardon and Getz, 2004).

Homocysteine is sulfur containing intermediary amino acid which is derived by the demethylation of methionine (Met) (Ridker et al., 1999), is an amino acid with the chemical formula (HSCH2CH2CH(NH2)CO2H) (Champe, Harvey and Ferrier, 2008). The primary source of Met is animal protein (Hankey and Eikelboom, 1999). Hyperhomocysteinemia may be due to genetic insufficiencies of the
enzymes needed for its metabolism, or to nutritional deficits in vitamin cofactors, or to other circumstances such as drugs and medical conditions (Kang, Wong and Malinow, 1992). Hyperhomocysteinemia is defined as tHcy concentrations elevated above 15 μmol/L. Hyperhomocysteinemia has been strongly associated with the pathogenesis of CVD, and correspondingly has been identified as a contributing factor in four main disease mechanisms including thrombosis, oxidative stress, apoptosis and cellular proliferation (Agoston-Coldea et al., 2011).

Vitamin B12 often occurs with protein bound as methylcobalamin, hydroxycobalamin and deoxyadenosycobalamin in nutrients (Warad et al., 2014). Folate is the term used to describe a group of compounds derived from tetrahydrofolate (THF) which is a B-vitamin mainly present in green leafy vegetables such as asparagus, spinach and broccoli, in legumes, whole grains and citrus fruits (Wu et al., 2012). B-vitamins, specifically B12 and folate, are important coenzymes of several enzymes involved in Hcy metabolism; thus dietary intake of these vitamins greatly affects plasma Hcy levels (Schneeberg, 2007).

Oxidative stress plays a major role in the development of CVDs, and hyperhomocysteinemia, also it is an independent risk factor for these diseases, and it may contribute by inducing production of oxygen free radicals (Cavalca, 2001).

2. MATERIALS AND METHODS
2.1. Study population

The present study was carried out on 90 individuals, 60 of which patients (30 males and 30 females) who had stenosis (diameter stenosis >50%) (Coronary atherosclerosis disease) and admitted in the Hawler Cardiac Surgery Center. As a healthy group blood samples of 30 individuals (15 males and 15 females) were taken who had no stenosis in the Hawler Cardiac Surgery Center. The patient and healthy groups were aged >40 years old.

2.2. Blood sample collection

Blood sample collections started in October 2014 and ended in January 2015. The collected blood samples were left for a while without anticoagulant to allow bloods to clot. Sera were obtained by centrifugation at 900 g for 15 minutes and kept at -70°C until analysis. Serum tHcy and folate were determined by using fully automated immunoassay analyzer (Cobas e 411), The ELx508 absorbance microplate reader (BioTek, USA) was used to measure the absorbance of vitamin B12 and TAC in serum by (ELISA kit), Tch, TG, HDL, LDL and serum glucose were measured with the Cobas c 311 diagnostic kits (Roche/Hitachi Cobas).

2.3. Statistical analysis

The data were compared by student’s t-test, results were express as Mean ± S.E and Pearson correlation was used to determine relationships, all analyses were performed with Graph pad-prism (version 6.0). P<0.05 considered statistical significant.

3. RESULTS AND DISCUSSION

3.1. Serum total homocysteine (tHcy)

The mean ± S.E. level of tHcy in coronary atherosclerotic patient group was 17.170 ± 0.748 μmol/L, which was located above normal range for Hcy (5-15 μmol/L) and in healthy group was 13.780 ± 0.679 μmol/L, which was located within normal range for Hcy (5-15 μmol/L) as shown in (Table 1). The results show that the concentrations of tHcy were significantly higher in patient group when compared to healthy group. Hcy causes an increase in the production of free radicals and an increase in lipid peroxidation. The
production of peroxide anions and H2O2, however, leads to reduced production and increased deactivation of NO. An increase in Hcy is thus related with acute endothelial dysfunction, and oxidative stress is implicated in this process (Chambers et al., 2000). Toboreck et al., (1995) reported tHcy which induce atherosclerosis by damaging endothelial cells through decreasing the plasma concentrations of antioxidants.

3.2. Serum homocysteine and vitamin status

The mean ± S.E. values of vitamin B12 and folic acid were 538.000 ± 25.850 pg/ml, 5.729 ± 0.218 ng/ml, respectively in coronary atherosclerotic patient group, while in healthy group were 673.900 ± 35.700 pg/ml, 6.638 ± 0.425 ng/ml, respectively (Table 2). The results show that the mean concentration of vitamin B12 and folic acids were significantly higher in healthy group rather than patient group. Vitamin B12 and folic acid are major determinants of Hcy level, and a nutritional deficiency in either of these two vitamins results in hyperhomocysteinemia (Sabry, Sabry and Hashim, 2014). Deficiency in vitamin B12 leads to an increase in serum methylmalonyl-CoA, and its metabolic product, methyl malonic acid. The second reaction uses cobalamin as a coenzyme in the synthesis of Met from Hcy. Hcy levels increase in vitamin B12 deficiency, also increased in folic acid deficiency (Al-Jumaily, Khaleel and Al-Rawi, 2009). In correlation analysis, tHcy was inversely correlated with vitamin B12 and folic acid (r = -0.02752, r = -0.2076) respectively (Figure 1 and 2).

3.3. Serum homocysteine and malondialdehyde (MDA)

Malondialdehyde is used as a marker of oxidative stress. These data show that the sera concentration of MDA was 5.762 ± 0.255 μmol/L in coronary atherosclerotic patient group, while in healthy group was 4.529 ± 0.252 μmol/L (Table 3). The result shows higher value of MDA in patient group compared to healthy group and there was a significant difference between patient and healthy groups.

Malondialdehyde is a decomposition product of auto-oxidation of polyunsaturated fatty acid, which is used as an index of oxidative damage, rise in MDA concentration indicates increased membrane lipid peroxidation, characterized by hyperlipidemia,
specifically hypercholesterolemia, in these patients. Rise in MDA could be due to increased generation of reactive oxygen species, due to the excessive oxidative damage generated in these patients. These oxygen species in turn, can oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported in patients with coronary artery disease (CAD) (Kaur et al., 2008).

In the correlation analysis, it was found that the tHcy level was no correlated with MDA ($r = 0.3072$) (Figure 3).

![Figure (3): Correlation coefficient between tHcy and MDA.](image)

### 3.4. Serum homocysteine and total antioxidant capacity (TAC)

Mean ± S.E. serum TAC level was $1.250 ± 0.063$ mmol/L in coronary atherosclerotic patient group, meanwhile in healthy group was $1.331 ± 0.100$ mmol/L (Table 4), this result shows TAC level was slightly higher in patient group than the healthy group.

Demirbag, Yilmaz and Kocyigit (2005) showed that an inverse association between TAC and the number of damaged vessels, in logistic regression analysis only low levels of HDL were significantly associated with CAD risk. Moat, Bonham and Powers (2001) conducted a study on role of amino thiols as a component of the plasma antioxidant system and relevance to Hcy-mediated vascular disease and their study showed plasma tCys and TAC were significantly lower in the subjects with severe hyperhomocysteinemia compared healthy control subjects, also tHcy was inversely correlated with TAC, whereas tCys was positively associated with TAC.

In the correlation analysis, tHcy levels were negatively and not significantly correlated with TAC ($r = -0.021$) (Figure 4).

![Figure (4): Correlation coefficient between tHcy and TAC.](image)

### 3.5. Serum homocysteine and lipid profile

The Mean ± S.E values of total cholesterol (Tch), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) in coronary atherosclerotic patient group were $213.900 ± 5.194$, $235.100 ± 10.980$, $155.500 ± 4.389$, $41.280 ± 1.212$, and $21.97 ± 1.172$ mg/dL, respectively, meanwhile in healthy group were $155.800 ± 4.897$, $109.800 ± 5.858$, $102.100 ± 4.378$, $47.930 ± 1.339$, and $47.30 ± 2.153$ mg/dL, respectively (Table 5). These data show significant higher levels of CH, TG, LDL and VLDL in patient group compared with healthy group, whereas HDL level was significantly higher in healthy group rather than patient group.

Total cholesterol has been singled out as the primary factor in development of atherosclerosis. HDL is regarded as one of the
most important protective factors against arteriosclerosis. HDL’s protective function has been attributed to its active participation in the reverse transport of Tch (Tomas et al., 2004). The concentration of LDL correlates positively whereas HDL correlates inversely to the development of CVD (Thakur et al., 2011). TG, Tch and lipoproteins are implicated in the pathogenesis of CAD, especially atherosclerosis. Reduced concentrations of HDL and increased TG have been shown to be responsible for the genesis of atherosclerotic lesions (Navab et al., 2000). Oxidative modified LDL contributes to the pathogenesis of atherosclerosis, increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to atherosclerotic lesions (Mohammed et al., 2011).

In the correlation analysis, the result of the present study found tHcy levels were positively correlated with Tch, TG, LDL, and VLDL, whereas negatively correlated with HDL (r = 0.07717, r = 0.1063, r = 0.338, r = -0.1554 and r = 0.1063) respectively (Figure 5, 6, 7, 8, 9), there were no significant correlations between plasma tHcy with Tch, TG, HDL and VLDL, while significantly correlated with LDL.
3.6. Serum homocysteine and hyperglycemia

Mean serum concentration of glucose was 250.800 ± 12.810 mg/dL in coronary atherosclerotic patient group, while in healthy group was 111.900 ± 4.459 mg/dL. The study shows that serum concentration of glucose was significantly higher in patient group than in healthy group (Table 6).

Diabetes is a significant risk factor for atherosclerosis; the diabetic state promotes oxidative stress mediated by reactive oxygen species, these consume NO and lead to endothelial dysfunction (Libby, Ridker and Maseri, 2002). Hyperglycemia in diabetics may induce dysfunctional endothelium which is involved in the genesis of atherosclerosis (McNair, 2006). Atherosclerosis is considered to be an inflammatory process triggered by response to injury and oxidative stress. Increasing in the plasma inflammation markers were shown to be related with the risk of vascular disease in type II diabetes patients (Emoto et al., 2001). Both type I and type II diabetes are powerful and independent risk factors for CAD, stroke, and peripheral arterial disease (Grundy et al., 1999).

In correlation analysis, there was a positive correlation between serum thcy and concentration of glucose fasting blood sugar (FBS) (r = 0.003459), which was statistically not significant (Figure 10).
Table 1: Mean ± S.E. of tHcy in patient and healthy groups

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Patient group Mean ± S.E.</th>
<th>Healthy group Mean ± S.E.</th>
<th>Statistical evaluation P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (μmol/L)</td>
<td>17.170 ± 0.748</td>
<td>13.780 ± 0.679</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

Table 2: Mean ± S.E. of vitamin B12 and folic acid in patient and healthy groups

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Patient group Mean ± S.E.</th>
<th>Healthy group Mean ± S.E.</th>
<th>Statistical evaluation P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>538 ±25.850</td>
<td>673.900 ± 35.700</td>
<td>0.003</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>5.729 ±0.218</td>
<td>6.638 ± 0.425</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Table 3: Mean ± S.E. of MDA in patient and healthy groups.

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Patient group Mean ± S.E.</th>
<th>Healthy group Mean ± S.E.</th>
<th>Statistical evaluation P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μmol/L)</td>
<td>5.762 ± 0.255</td>
<td>4.529 ± 0.252</td>
<td>0.0043</td>
</tr>
</tbody>
</table>

Table 4: Mean± S.E. of TAC in patient and healthy groups.

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Patient group Mean ± S.E.</th>
<th>Healthy group Mean ± S.E.</th>
<th>Statistical evaluation P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (U/ml)</td>
<td>1.250 ± 0.063</td>
<td>1.331 ± 0.100</td>
<td>N.S*</td>
</tr>
</tbody>
</table>

*N.S: non-significant
Table 5: Mean ± S.E. of Tch, TG, LDL, HDL and VLDL in patient and healthy groups.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Patient group Mean ± S.E.</th>
<th>Healthy group Mean ± S.E.</th>
<th>Statistical evaluation P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tch (mg/dL)</td>
<td>213.900 ± 5.194</td>
<td>155.8 ± 4.897</td>
<td>0.001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>235.100 ± 10.980</td>
<td>109.8 ± 5.858</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>155.500 ± 4.389</td>
<td>102.1 ± 4.378</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>41.280 ± 1.212</td>
<td>47.93 ± 1.339</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>47.30 ± 2.153</td>
<td>21.97 ± 1.172</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 6: Mean ± S.E. of glucose inpatient and healthy groups.

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Patient group Mean ± S.E.</th>
<th>Healthy group Mean ± S.E.</th>
<th>Statistical evaluation P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Glucose (mg/dL)</td>
<td>250.800 ± 12.810</td>
<td>111.900 ± 4.459</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

REFERENCES


