Original Article

ESTIMATION OF ROLE OF HYPERHOMOCYSTEINEMIA, BIOCHEMICAL PARAMETERS, AND CLINICAL MARKERS IN ATHEROSCLEROSIS PATIENTS IN AL-NAJAF AL-ASHRAF GOVERNORATE

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Abstract

Hyperhomocysteinemia is a substantial risk factor for heart disease unrelated to diet. Many clinical and epidemiological research has found a link between elevated plasma homocysteine and the development of atherosclerosis in people. This investigation aims to identify the typical homocysteine levels in the blood. As well as lipids in healthy Iraqi control groups and to compare them with atherosclerotic patients. Blood samples were taken from 62 healthy control subjects (42 male and 20 female), 68 patients suffering from premature signs of Ischemic heart disease admitted to the cardiac care unit (38 male and 30 female), and (15) patients suffering from hyperhomocysteinemia. These participants were divided into five groups based on their age. The blood samples from these groups were analyzed to measure plasma total homocysteine, total serum cholesterol, triglycerides, VLDL, LDL, and HDL. All control subjects have normal fasting blood glucose, serum lipids also blood pressure. According to the findings, patients' plasma total homocysteine levels were considerably higher. Had previous signs of atherosclerosis compared to control groups. The study has also classified plasma homocysteine according to the severity of hyperhomocysteinemia. The findings show that intermediate hyperhomocysteinemia is more frequent (32 patients; 47.05 %) as compared to moderate (25 patients; 36.77%) and severe hyperhomocysteinemia (11 patients; 16.18%). The results also showed that the lipid profile of The patient group was substantially higher than the control group., except for HDL-cholesterol, which has been significantly lower than in the control group.

Keywords: homocysteine, atherosclerosis, dyslipidemia, biochemical parameters, and clinical markers

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1- Introduction:

Atherosclerosis accounts for more deaths and morbidity in developed countries than any other disorder [(1)]. Hypertension, hyperlipidemia, smoking, diabetes, and hyperhomocysteinemia (elevation of homocysteine levels in the blood)are all established risk factors. Hyperhomocysteinemia is a prevalent risk factor for cardiovascular disease unrelated to diet. Hyperhomocysteinemia has been documented in up to 40% of individuals with premature coronary artery, cerebrovascular, or peripheral vascular disease (3). Clinical research on homocystinuria children as early as 1969 demonstrated the significance of severe Hhcy in the early development of atherosclerosis and thrombosis, raising the risk for cardiovascular disease by a factor of two after controlling for traditional risk factors[(4)]. Many clinical and epidemiological research has now demonstrated that total homocysteine in blood or plasma is a powerful predictor of cardiovascular disease risk (5).5 Homocysteine (Hcy), (2-amino-4-mercaptobutanic acid)This amino acid . occurs in free or combined states (but not in proteins) and fulfills essential roles in metabolic processes in mammals[(6)]. Normal Hcy metabolism needs a sufficient amount of folate, vitamin B12, and, to a lesser extent, vitamin B6 and riboflavin. The levels of these vitamins are inversely related to circulating Hcy. (7,8). So folate, along with vitamins B_{12} and $B_{6,}$ are commonly used to treat Hhcy by dietary supplementation of these vitamins[(9,10,11)]. This investigation aims to

identify the typical homocysteine levels in the blood. As well as lipids in healthy Iraqi control groups and to compare them with atherosclerotic patients.

2- Materials and Methods

1-Patients and control groups

The research group consisted of 68 individuals with atherosclerosis, their ages ranging between 29 to 79 years, and 62 age-matched healthy control subjects. The males consisted (55.88%) of the total patients while females (44.12%). The atherosclerotic group consists of patients with angina pectoris, myocardial infarction (MI), and cerebrovascular accident (CVA) admitted to the cardiac care unit (CCU) of Alsader medical city/Najaf. The control group subjects were volunteers. They had no history of ischemic heart disease (IHD), normal resting electrocardiogram (ECG), and no history of hypertension (HT) or diabetes mellitus (DM). Specialist physicians diagnosed the cases. The investigation, which we recorded in the case sheet. The information was collected from the patients by direct interview.

The cases were classified into two categories.:

Group1:

Patients have atherosclerosis or individuals who have been admitted to CCU. The criteria used for the collection of the cases depended on their diagnosis and the information, which was fixed in the case sheet of the patients.

Group2:

Healthy subjects. They had no history of IHD, normal resting ECG, with no history of hypertension, or diabetes mellitus.

2- Blood sample collection

Five milliliters of venous blood were sucked using disposable syringes after an 8–12-hour fast. Samples were taken between 9:00 and 10:30 in the morning. The serum was obtained by centrifuging the blood at 2000 x g for 10 minutes, transferring it into plain plastic tubes, and keeping them frozen at -20 C until analysis in disposable serum tubes. The blood was allowed to clot in plain tubes for 30-45 minutes at room temperature.

3-Instruments :

1. Electronic reading balance.
2. UV-visible spectrophotometer-720 (Shimadzu)
3. centrifuge (Hitachi-Marubeni)
4. cooling centrifuge (Hettich)
5. Cecil spectrophotometer CE 1011(France)
6. Retsch vortex mixer (Germany)
7. Water bath (Memmert- Germany)
8. Micropiprtte (Gilson) Bevin
9. -Highperformance liquid chromatography (Shimadzu)
10. Ultrasonic bath (Karl Kolb)
11. PH meter (Metrohm 632)

4- Chemical analysis:

1. Total cholesterol (TC)

Enzymatic determination of total cholesterol was performed according to Allain's[101]method using (Biomerieax Vitek, Inc. France) kit.

2. Triglyceride (TG)

Enzymatic determination of triglyceride (TG) was performed according to themethod of Fossati and Prencipe[102] by using (Biomerieax Vitek, Inc. France) kit.

3. High-density lipoprotein- cholesterol (HDL)

Enzymatic determination of HDL- cholesterol was performed according to the method of Burstein[103] using (the Biomerieax Vitek, Inc. France) kit. Phosphotungstate and magnesium ions precipitate LDL, VLDL, and chylomicrons, leaving HDL in thesolution. The enzymatic approach is then used to calculate the amount of cholesterol in the supernatant fluid.

4. Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) (LDL)

VLDL and LDL cholesterol were estimated by Fredrickson's method[104]. This procedure can be helpful in the interpretation of lipid profiles, and it is based on the following formula:

$C_{VLDL} = TG/5$

$C_{LDL} = C_{SERUM} - C_{HDL} - C_{VLDL}$

4. **Homocysteine (Hcy)**

Capillary electrophoresis, high-performance liquid chromatography (HPLC), radioenzymology, and gas chromatography connected to mass spectrometry are examples of test techniques with electrochemical or

fluorescent detection. The technique of Araki and Sako was used in the current investigation to measure plasma total homocysteine using HPLC and UV detection at 254 nm (105).

- **statistical analysis**

Minitab, Megastatistical, and SPSS for Excel were used to analyze data on the home computer. The findings were presented as (mean standard deviation). As a level of statistical significance, a p-value of 0.05 was chosen.

3- Results and Discussion:

3.1. The patient's characteristics.

The frequency distributions of subjects regarding their age and sex are shown in table (3-1). A total of 68 patients, 38 males (55.88%) and 30 females (44.12%). For both sexes, it was obvious that previous signs of atherosclerosis were more frequent at age 50 to 59 years. The highest frequency (23.69%) of male patients was in the age group (50-59) years and the lowest frequency (15.78%) in theage groups (30-39). The table also showed that females after 50 years of age became more susceptible than before and more susceptible than males of the same age. The correlation between atherosclerosis and age groups was statistically significant (P values < 0.05).

Table (3-1) The patient's characteristics

Age groups in years	Males		Females		Total
	Control **No. (%)**	**Diseased No. (%)**	***Control*** **No. (%)**	**Diseased No. (%)**	
Group A (≤30-39)	7 (16.67)	6 (15.78)	4 (20)	5 (16.66)	22
Group B (40-49)	10 (23.81)	9 (23.69)	5 (25)	7 (23.33)	31
Group C (50-59)	10 (23.81)	9 (23.69)	5 (25)	8 (26.67)	32
Group D (60-69)	10 (23.81)	7 (18.42)	3 (15)	6 (20)	26
Group E (70-79≥)	5 (11.9)	7 (18.42)	3 (15)	4 (13.34)	19
Total	42	38	20	30	130

Percentage of males in diseased group = 55.88 %

Percentage of females in diseased group = 44.12 %

3.2. Biochemical findings:

3.2.1. Homocysteine:

This study showed a significantly higher plasma total homocysteine concentration in atherosclerotic patients (52.18 ± 31.4 µmol/l) as compared with the control group (10.93 ± 2.2 µmol/l) table (3-2). The above difference was statistically significant (P< 0.05).

The results showed the patient group had considerably higher plasma total homocysteine levels than the control group for different age groups and both sexes. Table (3-3).

In addition, the study indicated that the highest concentration of plasma total homocysteine in Patients ranged in age from 60 to 69 years old, including both sexes. [85.21 ± 34.0µmol/l in male and 80.42 ± 32.9 µmol/l in female]. It was also shown that females had considerably greater plasma total homocysteine concentrations. (58.17±9.3µmol/l) than male (32.33±16.2µmol/l) in the age group (70-79) years. However, Male and female patients' plasma total homocysteine concentrations do not differ significantly., despite plasma homocysteine concentration in males (53.06±32.6 µmol/l) being more significant than plasma homocysteine concentration in females (51.07±30.4 µmol/l).

Table (3-2) Mean of plasma total homocysteine concentration of patients and control groups.

		Homocysteine *µmol/l*
	Number	Mean±SD

Patients	68	52.18 ± 31.47
Control	62	10.93 ± 2.23

Table (3-3) Mean total homocysteine concentration in patients and control according to age and sex groups.

Sex	**Age groups (years)**	**Homocysteine Mean±SD *µmol/l***		**P<0.05**
		Patients	Control	
Males	≤30-39	25.45±5.7	10.89±4.2	Sig.
	40-49	39.4 ± 25.08	10.58 ± 2.8	Sig.
	50-59	76.23 ± 23.3	11.02 ± 0.5	Sig.
	60-69	85.21 ± 34.0	11.68 ±1.06	Sig.
	70-79≥	32.33±16.2	14.18±2.06	Sig.
	Total males	53.06±32.6	11.43±2.5	Sig.
Females	≤30-39	27.17±17.2	9.55±1.6	Sig.
	40-49	23.01±9.8	10.44±0.04	Sig.
	50-59	65.01±21.7	9.36±2.7	Sig.
	60-69	80.42±32.9	9.81±1.05	Sig.
	70-79≥	58.17±9.3	10.41±0.5	Sig.
	Total females	51.07±30.4	9.89±0.79	Sig.

3.2.2. Lipid profile :

As shown in table (3-4), the serum total cholesterol level in the patient's group was (216.53±23.2 mg/dl), which was greater than the level of total cholesterol in the control group (171.44±22.4 mg/dl). The above difference was statistical significantly, with a P less than 0.05.

Also, the table shows that the mean value of serum triglyceride in the patient's group is significantly higher than the control group (146.49±28.3 versus 116.05±14.1mg/dl). A significant difference was also found between the mean of HDL cholesterol in the patient's group (41.77±4.5 mg/dl) and the control group (53.33±14.1 mg/dl). In atherosclerotic patients, the serum LDL- cholesterol mean was (141.12±29.3 mg/dl), which was higher than in the control group (93.87±17.4 mg/dl). Also, VLDL-cholesterol is significantly different in the control and patient groups.

Table (3-4) Lipid profile in Control and Patients groups

	Patients		**Control**		**P<0.05**
	Mean mg/dl	±SD	Mean mg/dl	±SD	
Cholesterol	**216.53**	**23.24**	**171.44**	**22.24**	**Significant**
TG	**146.49**	**28.36**	**116.05**	**18.36**	**Significant**
HDL	**41.77**	**4.52**	**53.33**	**14.18**	**Significant**
VLDL	**29.28**	**6.83**	**22.97**	**7.79**	**Significant**
LDL	**141.12**	**29.36**	**93.87**	**17.42**	**Significant**

Table (3-5) Homocysteine concentration with hypercholesterolemia and hypertriglyceridemia.

		Homocysteine *µmol/l*	
	N	**Mean ± SD**	**%**
Hypercholesterolemia > 220 mg/dl	35	50.48 ± 25.45	51.5
Hypertriglyceridemia> 160 mg/dl	27	47.21 ± 28.36	39.7

Table (3-5) shows that a 35 patients (51.5%) with hypercholesterolemia (cholesterol level > 220 mg/dl) and 27 patients (39.7%) with hypertriglyceridemia (triglyceride level >160 mg/dl). Homocysteine concentration in hypercholesterolemia patients was (50.48±25.45 µmol/l) with an r-value (–0.16).

This result shows a negative correlation between increasing cholesterol levels and homocysteine concentration (r-value >0.05). Also, increasing triglyceride level more than 160 mg/dl, which appears in 27 patients, have a mean plasma homocysteine level of (47.21±28.36 µmol/l) compared to the all patients group (52.18±31.47 µmol/l) with an r-value (0.08) which means that there is a slight correlation between increasing triglyceride level and homocysteine concentration. There has been a rise in interest in increasing the plasma

concentration of Hcy in recent years. Hhcy has been identified as a potential risk factor for atherosclerotic and atherothrombosis. (12).

Interest in Hcy concentrations in healthy and diseased states has been dramatically stimulated since epidemiological research has repeatedly shown that individuals with occlusive vascular disease had higher blood Hcy values than healthy controls [(9)].

Hcy affects anticoagulant effectors such as the Thrombomodulin-protein C system and antithrombin III. Not only does endothelial cell damage impact through direct cytotoxicity, but it may also decrease these cells' capacity to suppress platelet aggregation(109) (13). Numerous investigations on humans and animals have shown that dietary folate or cobalamin deficiencies under baseline conditions result in a slight to intermediate rise in Hcy (4).

The diet of everyday Iraqi individuals does not include adequate amounts of these vitamins, which may influence Hcy concentrations. Therefore the etiological and risk factors, including smoking, diabetes mellitus, alcohol consumption, hypertension, and various serum lipids, are included in this study.

The proportion of patients about age and sex. The results of the present study showed that the highest percentage of atherosclerotic patients were recorded in the age group (50-59) years. However, females after age 50 became more susceptible than before, also more susceptible than males of the same age; this could be due to the decline in females' sex hormones.

4.1. Biochemical findings:

4.2.1 Homocysteine :

Regarding total plasma Hcy level, The results of this investigation showed a significant correlation between Hcy concentration and the risk of vascular disease. The results showed that the concentration of plasma tHcy was significantly higher in different age groups in both sexes in the patient group than in the control group. This relationship was reported in many prospective studies[(3)].

It was also shown that the highest concentration of plasma tHcy in patients was in the age group (60--69) years in both sexes. Durand et al.(4) revealed that Hcy blood concentration steadily rises with age.

Ueland et al. Mentioned that there was no discernible difference in the plasma Hcy levels of men and women, which is consistent with our findings. Men's plasma Hcy levels are 25% greater than women's, according to research by Jacobsen et al. (14).

Regarding the classification of plasma Hcy according to the severity of Hhcy. Our results show that patients with intermediate Hhcy (30- 100 µmol/l) are more frequent compared to moderate (15-30 µmol/l) hyperhomocysteinemic patients and severe Hhcy (>100 µmol/l) patients. Kang and coworkers[(15)]. had classified Hhcy about total plasma Hcy concentrations. However, there is no consensus about the lower limits of Hcy yet; a greater prevalence is shown with concentrations above 16 mol/L. This is due to age, sex, and unhealthy lifestyle factors such as smoking, alcohol misuse, poor eating habits, and lack of physical exercise (16).

Other research indicates that fasting Hcy, defined as reduced levels, is beneficial. (12 µmol/L) is associated with increased odds ratios for atherosclerotic vascular diseases [(17)].

1. Lipid profile:

Based on continuing evidence that Hhcy and hyperlipidemia are strongly related to coronary artery disease risk[(18)], our results showed that serum total cholesterol level in the patient's group was significantly higher than the total cholesterol level in the control group. The same result was reported by Lamarache et al.[(19),] who found in their study that total cholesterol is still a significant predictor of IHD after adjustment of other risk factors.

The present study showed that the values of mean serum triglyceride in the patient's group were significantly higher than in the control group. In atherosclerotic patients, serum LDL- cholesterol mean was also higher than the control group, and VLDL-cholesterol was significantly different in patients and control groups.

A significant difference was also found between the mean of HDL cholesterol in the patients and the control groups.

The higher values of serum total cholesterol and progressive rise in serum lipids with reduced HDL-cholesterol level and the risk for cardiovascular diseases is now clearly established[(20)].

On the other hand, the result demonstrated no relationship between the increase in cholesterol and triglyceride levels and Hcy concentration, which is considered an Atherosclerosis independent risk factor.

These results support several earlier research that showed that Hhcy, which causes vascular dysfunction, may raise the cardiovascular risk without being influenced by other factors [(21).]

4.2.3. Other risk factors:

The distribution of family history of IHD in our study showed that the patient group of both sexes has a higher frequency of positive family history of IHD.

These findings were consistent with the findings of numerous other epidemiological studies like that 0f Goldstein et al.[(22),] who found that (29.8%) of patients had a family history of IHD.

Additionally, smoking has been linked to higher plasma Hcy levels. Both have a higher chance of developing cardiovascular disease. Our results showed that the total Hcy in the smoking group is lower than the

mean Hcy in all patients. This result proved that there is no effect of smoking on the level of Hcy and also indicates that those who smoke and have high plasma Hcy are much more likely to develop cardiovascular disease.

The findings of our study agreed with O'Callaghan et al., which discovered that smokers had a higher chance of developing vascular disease. The presence of elevated plasma Hcy significantly exacerbated this risk [(23)].

On the other hand, Hultberg et al.[(24)] demonstrated that alcoholics have higher tHcy concentrations, perhaps because of malnourishment and malabsorption.

The same results have been found in the current study, there were 8 male patients out of total patients drinking alcohol, and the levels of Hcy in that patient were higher than Hcy values in all patient groups.

Conclusions

1. Atherosclerotic vascular disease is at increased risk due to hyperhomocysteinemia.
2. Patients with peripheral, cerebrovascular, and coronary artery disease consistently have more significant homocysteine amounts than people who do not have these conditions.
3. As in previous studies, we found that hyperhomocysteinemia is An independent- risk factor, and hyperhomocysteinemia may increase Ischemic Heart disease risk via a proatherogenic effect.
4. Homocysteine levels increase with age progression.
5. Lipid profiles of patients were significantly higher than average persons, except for HDL cholesterol, which was significantly lower than normal individuals.

Recommendations:

1. The role of other diseases like diabetes and renal insufficiency in hyperhomocysteinemia.
2. Deficiency of vitamins B6, B12, folic acid, and increased homocysteine.
3. Identification, purification, and isolation of P. fractal plant chemical compounds (active compounds) of P. fractal plant, which are responsible for lowering plasma homocysteine levels.

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