The Role of Some Biochemical Parameters in The Formation of Gallstones

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الخلاصـــة

تناولت الدراسة (52) مريضاً مشخصاً بحصوات كيس المرارة غير العرضية. تم قياس مستويات معدلات الدهون الكلية في مصل الدم (الكولسترول الكلي، ثلاثي الكليسيرايد، كولسترول البروتين الدهني عالي الكثافة، كولسترول البروتين الدهني واطئ الكثافة وكولسترول البروتين الدهني واطئ الكثافة جداً) بالإضافة إلى معدلات فيتامينات مانعة الأكسدة وتركيز المالونداياليديهايد في مصل الدم (فيتامين A، فيتامين C وفيتامين E) وقورنت مع مجموعة سيطرة من (20) شخصاً سليماً.

وضحت النتائج وجود ارتفاع معنوي في مستويات الكولسترول الكلي، ثلاثي الكليسيرايد، كولسترول البروتين الدهني واطئ الكثافة وكولسترول البروتين الدهني واطئ الكثافة جداً بالإضافة إلى تركيز المالونداياليديهايد لدى المرضى المصابين بحصوات كيس المرارة عند مقارنتهم بمجموعة السيطرة ولكلا الجنسين. كما لوحظ وجود انخفاض في مستوى كولسترول البروتين الدهني العالي الكثافة بالإضافة إلى انخفاض في مستويات فيتامينات مانعة الأكسدة (فيتامين A، فيتامين C وفيتامين E) في مصل دم المرضى بالمقارنة مع مجموعة السيطرة ولكلا الجنسين. توصلت الدراسة إلى استنتاج بان الفيتامينات مانعة الأكسدة تقوم بدورها في منع تكوين حصوات المرارة بطريقتين، الأولى مباشرة عن طريق منع الأكسدة الإجهادية في الكبد والتي تؤدي إلى تغيرات غير مرغوبة في مكونات عصارة المرارة، والثاني غير مباشر عن طريق تأثير هذه الفيتامينات على معدلات الدهون الكلية.

ABSTRACT

The study dealt with (52) patients diagnosed with asymptomatic gallstones. Serum lipid profile (total cholesterol TC, triglycerides TG, high density lipoprotein cholesterol HDL-C, Low density lipoprotein cholesterol LDL-C and very low density lipoprotein cholesterol VLDL-C) as well as antioxidant vitamins (vitamin A, vitamin C and vitamin E) and malondialdehyde were measured for them and were compared with a control group of (20) healthy individuals.

Results revealed a significant increase in TC, TG, LDL-C and VLDL-C as well as malondialdehyde concentration in the sera of the patients with gallstones when compared to control group for both sexes. A significant decrease in HDL-C as well as the antioxidant vitamins (vitamin A, vitamin C and vitamin E) were detected in sera of the patients compared to control group for both sexes. The study reached to a conclusion that antioxidant vitamins play their role in the prevention of the formation of gallstones in two ways, the first is direct, by preventing the oxidation stress within the liver leading to unfavorable changes in the bile composition and the second way is indirect through their influence on lipid profile.

Introduction

Gallstones are crystalline structures formed by concretion or accretion of normal or abnormal bile constituents. These stones are divided into three major types, cholesterol and mixed stones account for 80 percent of the total, with pigment stones comprising the remaining 20 percent. Mixed and cholesterol gallstones usually contain more than 70 percent cholesterol monohydrate plus an admixture of calcium salts, bile acids and bile pigments, proteins, fatty acids, and phospholipids. Pigment stones are composed primarily of calcium bilirubinate; they contain less than 10 percent cholesterol [(1)].

Bile plays a vital role in the metabolism of lipids in mammalians, whereas it is used as a shuttle to excrete cholesterol, bilirubin and a number of hormones and drugs. The liver excretes bile in the form of a diluted solution which contains (95-98 %) water, it is then converted to a concentrated solution of sodium and bile salts in the gallbladder [(2)].

Gallstones can block the normal flow of bile if they lodge in any of the ducts that carry bile from the liver to the small intestine. That includes the hepatic ducts, which carry bile out of the liver; the cystic duct, which takes bile to and from the gallbladder; and the common bile duct, which takes bile from the cystic and hepatic ducts to the small intestine. Bile trapped in these ducts can cause inflammation in the gallbladder, the

ducts, or, rarely, the liver. Other ducts open into the common bile duct, including the pancreatic duct, which carries digestive enzymes out of the pancreas. If a gallstone blocks the opening to that duct, digestive enzymes can become trapped in the pancreas and cause an extremely painful inflammation called gallstone pancreatitis [3].

Cholesterol is excreted from the human body via bile with changing and as a cholesterol or after be modified to bile acids [4,5]. This approach is done by the integration between the hepatocytes and lipoproteins, whereas cholesterol is transferred from the body to the liver to be excreted to the bile by high density lipoprotein (HDL-C) [6,7].

The aim of this study is to evaluate the role of lipids in the formation of gallstones and to determine whether antioxidant vitamins take part in the formation of such stones or not. by the measuring serum lipid profile and antioxidant vitamins in patients with gallstones and comparing the results with a control group of healthy individuals.

Experimental

Blood samples of (52) patients (23 male and 29 female) with asymptomatic gallstones were collected as well as a control group of (20) healthy individuals (10 male and 10 female) after a period of fasting ranged between (9-12) hours. The age of the patients ranged between (50-60) years as in the control group too.

Measured parameters

1. Serum Lipid Profile

A. Total cholesterol (TC): was measured enzymatically[8] using manufactured kit from (BioMerieux).

B. Triglycerides (TG): was measured enzymatically[9] using a manufactured kit from (Labkit).

C. High density lipoprotein cholesterol (HDL-C): was measured enzymatically [10] using a kit manufactured by (BioMerieux).

D. Low density lipoprotein cholesterol (LDL-C): it was calculated using the equation below [11]:

$$LDL-C = (Total\ cholesterol) - (HDL-C) - (Tg/5)$$

E. Very low density lipoprotein (VLDL-C): it was calculated using the equation below [11].

$$VLDL-C = Tg/5$$

2. Serum Antioxidant vitamins

A. Vitamin A (Vit. A): vitamin A was determined by UV. degradation of the vitamin which is measured at wavelength (327 nm) [12].

B. Vitamin C (Vit. C): vitamin C was determined using 2,4-dinitro phenyl hydrazine (DNPH) method [13, 14].

C. Vitamin E (Vit. E): was determined using colorimetric method [15].

3.Malondialdehyde (MDA): was measured using colorimetric method [16].

Statistical Analysis: T-test was used to determine the probability (P- value).

Results and Discussion

Results shown in table (1) demonstrate a significant increase in serum levels of (total cholesterol, triglycerides, VLDL-C and LDL-C) and a significant decrease in HDL-C level in patients with gallstones compared to control group for both sexes. This agrees with many studies [17,18]. Other studies focus on the elevation of triglycerides and the decline of HDL-C levels only. These studies demonstrates that high level of triglycerides may be the obstacle that retards cholesterol to be excreted from the bile and then its stagnation and crystallization to form the stones [19].

Table (1): Serum lipid profile levels for patients with gallstones and the control group

Parameters	Sex	Patients with gallstones	Control group
Total Cholesterol (mg/100ml)	Male	257.25*** ± 15.0	193.18 ± 9.8
	Female	229.27*** ±9.1	189.3 ± 11.1
Triglycerides (mg/100ml)	Male	146.29 *** ± 29.7	101.9 ± 11.5
	Female	158.68*** ±14.1	105.8 ± 9.8
HDL-C (mg/100ml)	Male	23.70 *** ± 4.5	42.27 ± 3.6
	Female	25.97*** ±4.4	42.6 ± 5.5
VLDL-C (mg/100ml)	Male	29.25*** ± 5.9	20.38 ± 2.3
	Female	31.73*** ± 2.8	21.16 ± 1.9
LDL-C (mg/100ml)	Male	204.28***± 17.2	130.5 ± 9.5
	Female	171.74*** ± 11.7	125.54 ± 9.0

The data is expressed by mean ± standard deviation
(* P-value ≤0.01, ** P-value ≤0.001 , *** P-value ≤0.0001)

Further more evidence was obtained by the measuring serum antioxidant vitamins levels (vitamin A, vitamin C and vitamin E) as well as malndialdehyde as shown in table (2). The results show a significant decrease in antioxidant vitamins levels and an increase in malondialdehyde concentration for the patients compared to control group for both sexes, this agrees with the findings of many researchers[20], who revealed a lack of these vitamins in the sera of patients with gallstones.

The researchers theorize that oxidant stress within the liver leads to unfavorable changes in bile composition that promote precipitation of both cholesterol and bilirubin (thus favoring the formation of both cholesterol and pigment stones).[21,22] These findings corroborate earlier studies showing that antioxidant deficiencies can induce gallstone formation in animals [23,24].

Table (2): Antioxidant vitamins levels in the sera of the patients and the control group

Parameters	Sex	Patients with gallstones	Control group
Vitamin A (IU/ 100ml)	Male	163.7* ± 8.8	171.45 ± 5.2
	Female	163.37* ± 8.5	174.8 ± 7.7
Vitamin C (µmol/L)	Male	45.41**± 3.0	53.09 ± 4.6
	Female	38.2** ± 5.4	50.1 ± 6.3
Vitamin E (µmol/L)	Male	10.22***± 2.15	17.82 ± 2.85
	Female	12.76*** 3.5	19.29 ± 3.0
Malondialdehyde (µmol/L)	Male	2.43***±0.30	0.16±0.07
	Female	2.33***±0.18	0.15±0.08

The data is expressed by mean ± standard deviation
(* P-value ≤0.01, ** P-value ≤0.001 , *** P-value ≤0.0001)

From another aspect, vitamin C may be involved in the transformation of cholesterol to bile acids and thus prevent it from accumulation and precipitation in the bile to form stones [19].

The decline of antioxidant vitamins in the sera of the patients may explain the rise in malondialdehyde, total cholesterol, triglycerides, LDL-C and VLDL-C as well as decrease of HDL-C and this corroborates many such findings [25,26,27]. This can be more clarified as shown in table (3).

Table (3): Pearson correlation between malondialdehyde and lipid profile parameters

Pearson correlation	Total cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
P-values	0.0003**	0.007*	0.005*	0.0009**	0.007*

*Correlation is significant at P≤0.01
**Correlation is significant at P≤0.001

The results demonstrate that malondialdehyde, (which is considered as a marker for the oxidation stress) [28], is directly proportional to the total cholesterol, triglycerides, LDL-C and VLDL-C. whereas it is inversely proportional to HDL-C levels.

The lack in antioxidant vitamins may lead to the oxidation of lipoproteins in which these particles may lose their function as a carrier of lipids[29]. This oxidation can take place on their apoproteins. LDL particles undergo oxidation on their apoprotein E and so they become unidentified by their receptors on the cells leading to their aggregation and finally the elevation of serum LDL and its content of cholesterol [30].

The oxidation of HDL particles can occur on their apo A-I and Apo A-II, or on lecithin: cholesterol acyl transferase (LCAT) enzyme and this leads to the decline in HDL-C levels [5].

The rise in the levels of triglycerides and VLDL-C concomitant with the decrease of antioxidant vitamins may be attributed to the oxidation of lipoprotein particles, or to the oxidation of cellular lipoprotein lipase (LPL) which is responsible for triglycerides hydrolysis to free fatty acids and glycerol [1,5].

Finally antioxidant vitamins play their role in the prevention of the formation of gallstones in two ways, the first is direct, by preventing the oxidation stress within the liver leading to unfavorable changes in the bile composition and the second way is indirect through their influence on lipid profile.

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