

## Associations between self-reported anxiety and serum lipid, lipoprotein concentrations and platelets in healthy men

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### Abstract

**Objective:** High blood cholesterol is one of the significant risk factors for cardiovascular diseases. Increased cholesterol levels contribute to atherosclerosis, which causes platelet aggregation and increases the risk of blood clots in the arteries. Previous research has investigated relationships of elevated serum cholesterol with anxiety disorders. The current study aims to assess levels of serum lipid, lipoprotein concentrations and platelets in individuals with high and low anxiety. **Methods:** Of a total of 1,038 subjects, 142 healthy men were randomly selected. All participants were asked to complete the Spielberger's self-reported state-trait anxiety inventory (STAI). Participants with scores higher than 46 and lower than 34 were included in high anxiety group (n=28) and low anxiety group (n=69), respectively. Levels of fasting serum lipids, including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and platelets were compared between the two groups. The data were analyzed using independent samples *t*-test and correlation coefficient test. **Results:** The levels of total cholesterol and LDL cholesterol were significantly higher in the high anxiety group ( $P<0.001$ ). There were no significant differences in the levels of triglycerides, HDL cholesterol, and platelets.

**Conclusion:** High anxiety increases total cholesterol and LDL which are risk factors for cardiovascular diseases.

### INTRODUCTION

Elevated levels of low-density lipoprotein (LDL) cholesterol in the blood contribute to the formation of atheroma plaque in the arteries, which can stick to the walls of the blood vessels, make arteries less flexible and increase the risk of blood clots. This process is named atherosclerosis that is one of the main factors of coronary heart disease and other forms of cardiovascular diseases, which causes platelet aggregation and occludes the arteries by the formation of blood clots.<sup>1,2</sup> Prospective epidemiological studies<sup>3,4</sup> leave no doubt of the importance of cholesterol in the development of cardiovascular disease. High levels of cholesterol in the blood can increase the risk of heart disease.<sup>3,4</sup>

Anxiety is a risk factor for heart attack and as an independent factor predicts it.<sup>5</sup> Anxiety and symptoms of anxiety are a risk factor for coronary artery disease in healthy populations.<sup>6-10</sup>

A recent study has found that patients with coronary artery bypass surgery who had high anxiety scores were more likely to experience recurrent atherosclerosis.<sup>11</sup> It is documented that higher anxiety is associated with risk factors for arteriosclerosis, heart attack or coronary mortality.<sup>2-4,11,12</sup>

The number of studies investigating relationships of anxiety with lipid levels are few.<sup>13,14</sup> The current study aims to compare the levels of serum lipid and lipoprotein concentrations including total cholesterol, LDL cholesterol, high-density lipoproteins (HDL) cholesterol, and triglycerides, and platelets, as important predictive risk factors for cardiovascular diseases, in the two groups of participants with high and low anxiety to provide valuable information toward understanding of the nature and clinical implications of links between anxiety disorders and the risk factors associated with them.

## METHODS

### *Study population and design*

This study was carried out from April 2010 to June 2011. The original sample consisted of 1,038 male personnel of an industrial company. Of these, 141 were excluded according to the exclusion criteria. We gave a number from 1 to 897 to each of the 897 remaining individuals and the numbers that were multiples of 6 were randomly selected for enrollment in this study. Thus, the final sample included 142 healthy men, aged 26 to 57 years, who were evaluated in a non-patient situation. We used stratified random sampling technique for sampling from this population. All of the participants consented orally and provided written informed consent after receiving a full explanation of the study.

### *Inclusion criteria*

The inclusion criteria were based on the anxiety scores of the Spielberger's self-reported state-trait anxiety inventory (STAI) questionnaire<sup>15,16</sup> as the participants with scores higher than 46 were included in the high anxiety group and those with scores lower than 34 were included in the low anxiety group. The two groups were adjusted for age, sex, and socio-economic class. Additional inclusion criteria were no history of major illnesses, being free of current or past psychiatric illness, no current medication use for acute or chronic illness or for treatment of hypercholesterolemia, nonsmoker, and no clinical history or treatment for anxiety.

### *Exclusion criteria*

The exclusion criteria were smoking, addiction to alcohol and narcotics, and history of cardiovascular and blood diseases, diabetes, diseases which cause changes in platelets, and taking cholesterol-lowering medications.

### *Anxiety symptoms*

Anxiety symptoms were assessed at enrollment using the Spielberger's self-reported STAI, which is a widely accepted instrument with well established reliability and validity.<sup>15,16</sup> The anxiety scale measures an individual's enduring tendencies to experience anxious moods and anxiety states. The STAI has 20 items for assessing trait anxiety and 20 for state anxiety. All items are rated on a 4-point scale: almost never, sometimes, often, and almost always. Subjects are instructed to respond

to the questions as they feel in general to assess how they feel at the moment or felt in the recent past, or how they anticipate their feelings in a specific situation that is likely to be encountered in the future, or in a variety of hypothetical situations. State-Trait Anxiety Inventory scores range from 20 to 80, with high scores indicating higher trait anxiety. Spielberger's STAI scores of 20-34 indicate no or minimal anxiety, 35-45 indicate mild anxiety, 46-56 indicate the presence of moderate anxiety, and the scores higher than 57 indicate severe anxiety. All participants were asked to complete the Spielberger's STAI questionnaire. Participants with anxiety scores  $\leq 34$  were included in the low anxiety group (n=69) and those with anxiety scores  $\geq 46$  in the high anxiety group (n=28).

### *Laboratory data*

Blood samples were collected in the morning after a 12-hour fast. Standard enzymatic methods (cholesterol oxidase/phenylperoxidaseaminophenozonphenol [CHOD-PAP], Boehringer Mannheim) were used to measure fasting serum lipid and lipoprotein concentrations, including serum total cholesterol, HDL cholesterol, and triglycerides in the laboratory.<sup>17</sup> We calculated LDL cholesterol according to the method of Friedewald and colleagues.<sup>18</sup> Laboratory kit (Pars Azmoon Inc., Tehran, Iran) was used to measure the levels of total cholesterol, triglycerides, HDL, and LDL. Platelets were counted by a cell counter device named Sysmex KX-21N.

### *Statistical analysis*

Independent samples *t*-test was used to compare the means of quantitative variables between the groups. Correlation coefficient analysis was used to evaluate the relationship between anxiety and dependent variables. All *P* values less than 0.05 were considered statistically significant.

## RESULTS

According to the Spielberger's STAI, of a total of 142 subjects, 97 had either high anxiety (high anxiety group; n=28) or low anxiety (low anxiety group; n=69), and the remaining had mild anxiety. In terms of sex distribution, all samples were male. Mean age in the high anxiety group, aged 30 to 56 years, was 45 years and in the low anxiety group, aged 26 to 57 years, was 44 years. There was no significant difference with respect

**Table 1: The laboratory findings of all study subjects**

	Number	Minimum	Maximum	Mean ± Standard Deviation
HDL	97	22	54	38.2 ± 7.8
Cholesterol	97	113	258	183.9 ± 30.9
Triglyceride	97	60	498	163.3 ± 88.0
Platelet	97	198	399	272.6 ± 57.0
LDL	97	54	172	110.6 ± 28.7

to age, sex, and socio-economic class between the two groups.

The Expert Panel of the US National Education Program guidelines<sup>19</sup> classify LDL values below 130 mg/dL and total cholesterol levels below 200 mg/dL as desirable values. Total cholesterol levels above 199 mg/dL and LDL values above 129 mg/dL are classified as borderline-high or high cholesterol ( $\geq 240$  mg/dL), and borderline-high LDL or high LDL ( $\geq 160$  mg/dL), respectively. Table 1 is the laboratory findings of all the 97 study subjects. The mean of serum levels of total cholesterol was measured  $212.1 \pm 28.1$  mg/dL in the high anxiety group and  $172.5 \pm 24.1$  mg/dL in the low anxiety group that showed the levels of serum total cholesterol was higher in the high anxiety group and there was a significant difference between the two groups ( $P < 0.001$ ). Moreover, mean of LDL cholesterol was  $142.9 \pm 22.1$  mg/dL in the high anxiety group and  $97.6 \pm 19.1$  mg/dL in the low anxiety group that showed there was a significant difference between the two groups ( $P < 0.001$ ).

Mean of serum levels of triglycerides was  $160.1 \pm 76.6$  mg/dL in the high anxiety group and  $164.7 \pm 92.8$  mg/dL in the low anxiety group that showed there was no statistically significant

difference among the two groups ( $P = 0.818$ ).

HDL cholesterol was measured in both groups. Mean of HDL cholesterol was  $38.6 \pm 6.9$  mg/dL in the high anxiety group and  $38.1 \pm 8.2$  mg/dL in the low anxiety group. No significant differences were found in HDL cholesterol between the two groups ( $P = 0.776$ ).

The mean of platelet count in the high anxiety group was  $273.9 \pm 58.5 \times 10^3/\mu\text{L}$  and in the low anxiety group was  $272.1 \pm 56.8 \times 10^3/\mu\text{L}$  and there was no significant difference between the two groups ( $P = 0.889$ ).

Table 2 shows the comparison of the means of serum levels of the response variables using independent samples *t*-test between the two groups and Table 3 represents the correlation between anxiety and all the variables.

## DISCUSSION

In this study, serum lipid levels were measured in the two groups of individuals with high and low anxiety and our findings revealed that the levels of total cholesterol and LDL were significantly higher in individuals with high anxiety than those with low anxiety. Both variables are considered to be more specifically correlated with risk of coronary

**Table 2: Comparison of the means of serum levels of lipid profiles and platelets using independent samples *t*-test**

	Low Anxiety Group (n=69)	High Anxiety Group (n=28)	<i>P</i>
HDL (mg/dL)	$38.1 \pm 8.2$	$38.6 \pm 6.9$	0.776
LDL (mg/dL)	$97.6 \pm 19.1$	$142.9 \pm 22.1$	<0.001*
Total cholesterol (mg/dL)	$172.5 \pm 24.1$	$212.1 \pm 28.1$	<0.001*
Triglycerides (mg/dL)	$164.7 \pm 92.8$	$160.1 \pm 76.6$	0.818
Platelet ( $\times 10^3/\mu\text{L}$ )	$272.1 \pm 56.8$	$273.9 \pm 58.5$	0.889

\* Indicates a significant difference between the two groups.

**Table 3: Correlation (r) between anxiety and all the variables**

	HDL	LDL	Total cholesterol	Triglyceride	Platelet
Correlation coefficient (r) with anxiety	-0.006	0.759*	0.629*	-0.033	0.037
P	0.953	<0.001	<0.001	0.751	0.716

\* Correlation is significant.

disease. Several previous studies have found such cholesterol elevations<sup>1,5,20,21</sup>, but most of them have only examined the relationship of anxiety to total cholesterol and few studies have included other lipid and lipoprotein constituents, such as LDL cholesterol, HDL cholesterol, and triglycerides, and platelets. Inclusion of these additional measures may lead to a better determination of the degree to which anxiety is associated with low lipid and lipoprotein concentrations. Some studies found a cholesterol elevation in patients with general anxiety disorder.<sup>22</sup> However, Suarez<sup>14</sup> found that anxiety was associated with lower total cholesterol in a small sample of young women. The mechanisms underlying serum cholesterol elevation in anxiety disorders are still unsettled. Some possible mechanisms have been proposed for the relationship between elevated cholesterol and anxiety.

One possible explanation for the relationship between increased blood cholesterol levels and the physiopathology of anxiety may be that hyperactivity of the noradrenergic system can lead to increased cholesterol levels in individuals with higher anxiety than those with lower anxiety that may be possibly because of a neurochemical or biological mechanism.<sup>23</sup> As noradrenergic hyperactivities have been found in individuals with anxiety disorders<sup>24,25</sup>, cholesterol elevations in anxiety disorders seemed to be most convincingly explained by such a mechanism. High cholesterol level plus increased noradrenergic function would place the individuals at higher risk to develop cardiovascular disease.

The major part of anxiety symptoms, such as shivering, restlessness, palpitation, dyspnea, sweating, is resulting from the action of sympathetic nerves of autonomic nervous system which is under the control of hypothalamus. Hypothalamus is controlled by limbic system. Through the limbic system and hypothalamus, cerebral cortex affects the centers of autonomic nervous system in the brain stem. Therefore, what passes in the cerebral cortex and at the lower parts as emotions and motivation can activate

the automatic nervous system. It is worthy to mention that the action of lipolysis in fat cells, which increases free fatty acids in blood, blood ketones, and cholesterol biosynthesis in the liver, is under the direct control of sympathetic system. Therefore, anxiety can stimulate and activate the sympathetic system and change the metabolism of the lipids.

A second hypothesis is that the action of hypothalamic-pituitary-adrenocortical (HPA) axis increases in anxious subjects.<sup>26</sup> Landén *et al.*<sup>27</sup> reported a significant relationship between anxiety and high triglyceride levels. They evaluated the activity of HPA axis in the individuals with high social anxiety and showed that subjects with high anxiety may have a higher HPA activity in response to stress-induced situations.<sup>27</sup> Condren *et al.*<sup>28</sup> determined HPA axis responsivity to a psychological stressor in patients with social phobia and compared them to healthy controls. Their findings showed that there was no difference in the activity of HPA axis at baseline between the two groups, but when the subjects were evaluated after they were exposed to a stressful situation, the activity of HPA in the anxious group was higher than the control group.<sup>28</sup> Therefore, one possible explanation is that increased atherogenic lipid profile in the high anxiety group is a result of the activated HPA axis in response to stress.

A third explanation is that cholesterol elevation may lead to higher anxiety. Although it is less possible, it should not be disregarded. This hypothesis suggests that serum lipid is effective on behavioral traits such as anxiety, depression, and aggression.<sup>2,29-38</sup> Saliba *et al.*<sup>39</sup> showed that the individuals with impulsivity prefer sweet foods and drinks and a high carbohydrate diet is related to high blood cholesterol.

In this study, the levels of triglycerides were measured in both groups of high and low anxiety. There is a disagreement over the association of hypertriglyceridemia with vascular diseases. Triglycerides levels below 250 mg/dL (120-250 mg/dL) are classified as normal, 250-500 mg/dL are classified as borderline, and above 500

mg/dL are classified as abnormal. Nevertheless, triglycerides levels higher than the normal range are common in the patients with coronary artery and a significant reversal relationship between triglycerides and HDL cholesterol has been found at this range.<sup>40</sup>

In this study, Serum levels of HDL and LDL cholesterol were measured in the two groups of high and low anxiety and a significant difference was found in the levels of serum LDL between the high anxiety group and the low anxiety group. LDL plays a key role in contributing to the development of atherosclerosis by carrying serum cholesterol. Further studies are warranted to explore the cause and mechanisms of elevated LDL in anxious individuals.

This study has investigated blood platelet counts in the two groups of high and low anxiety for the first time. Blood platelet counts were measured and there was no significant difference between blood platelet counts in the two groups. However, previous studies have showed that the number of activated platelets have been found more in the blood of anxious individuals.<sup>41,42</sup>

Elevated levels of cholesterol in the blood contribute to the formation of plaque in the arteries, which make arteries less flexible and increase the risk of blood clots. Thrombosis is the process where a thrombus, or blood clot, forms within a vessel in the body, obstructing the flow of blood through the circulatory system. The development of thrombosis in heart blood vessels (coronary thrombosis) or in brain blood vessels (cerebral thrombosis) is associated with serious complications such as embolus. The embolus lodges in the blood vessels of the lungs and can cause pulmonary embolism. Therefore, elevated levels of cholesterol in the blood vessels can cause an increase in platelet counts and the formation of a blood clot inside a blood vessel. With respect to the findings of the present study, that showed there was no relationship between elevated platelet counts and anxiety, it is suggested that an increase in cholesterol sediments in blood vessels can result in the formation of blood clot in the arteries. These clots require an increase in the number of platelets which can be replaced by bone marrow, but the body itself keeps the number of platelets within a normal range in the blood flow.

Our findings are independent of age, body mass index, physical activity, and other factors known to influence lipid concentrations. Although we detected a significant association between serum cholesterol and anxiety, the relatively small

sample size weakens this study. This study sheds new light on the subject and suggests that further studies with larger samples in different age and sex groups are required to prove such results in the future.

In conclusion, findings from the current study support the general hypothesis that naturally occurring low lipid and lipoprotein concentrations are associated with trait measures of anxiety and high anxiety is related to some risk factors for cardiovascular diseases.

## ACKNOWLEDGEMENTS

The authors would like to thank Ms. Hosseini of Shiraz Neurosciences Research Center (SNRC) and the Vice-Chancellor for Research Affairs of Shiraz University of Medical Sciences for their assistance. We also wish to thank Dr. Vosoughi of the Center for Development of Clinical Research at Nemazee Hospital for providing statistical guidance and Ms. Gholami of SNRC for translation and editing the language of the manuscript.

## DISCLOSURE

Conflict of interest: None

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