DETERMINATION OF TESTOSTERONE, INFLAMMATORY PROTEINS AND SEVERAL BIOCHEMICAL PARAMETERS IN SERA OF PATIENTS WITH ATHEROSCLEROSIS

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ABSTRACT

The present study aims to investigate the concentration of testosterone with some inflammatory proteins (Ceruloplasmin and Albumin) and Lipid profile in order to evaluate their role in Atherosclerosis and the relationship between them.

The study included 33 male patients with age range (40-75) years, 25 healthy males to be used as a control group with age range (39-63) years. Testosterone showed significant decreasing level compared to control group, while Ceruloplasmin and total serum protein showed significant increasing levels with non-significant decreasing level of albumin in sera of the same patients compared to control. At the same time, Cholesterol and Triglyceride showed significant increasing levels while decreasing level of high density lipoprotein in sera of patients group compared to control group was observed.

In conclusion, the present results shows negative correlation between Testosterone with inflammatory proteins and between Testosterone with other Biochemical markers which may show a relationship between low level of Testosterone, inflammatory proteins and other markers.

Key Words: Atherosclerosis; Testosterone; Ceruloplasmin; Albumin; Lipid profile.

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تحديد مستوى التيستيرون والبروتينات الالتهابية وبعض الدلائل الكيميائية في مصل مرضى تصلب الشرايين

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الخلاصة
تهدف الدراسة الحالية إلى اختبار تركيز التيستيرون مع بعض البروتينات الالتهابية (السيสัมผريلوبلازمن والألبومين) ونطاق درجة الدهنيات الدم في مرض تصلب الشرايين والعلاقة فيما بينهم. 
شملت الدراسة 33 مريضاً من الذكور؛ وتراوح عمرهم من 40-75 سنة ويشتهر مقارنتهم بـ 25 من الأصحاء وتراوح معدل العمر من 39 إلى 63 سنة. أظهر هرمون التيستيرون نقصاناً معيناً مقارنة بالالمجموعة الضابطة بينما السيريلوبلازمن والبروتين الكلي أظهراً زيادة معيونية مع إخفاق غير معنى للألبومين في مصل المرضى نفسه مقارة بالمجموعة الضابطة. 
كما كانت هناك زيادة معيونية في مستوى الكولسترول والدهون الثلاثية رافقها إخفاقاً في مستوى الدهنيات عالية الكثافة في مصل المرضى مقارة بالسيطرة.

نستنتج من دراسة النتائج الحالية أن هناك إرتباطاً معيونياً سالباً بين التيستيرون والبروتينات الالتهابية وكذلك بين الهرمون والدلائل الكيميائية الأخرى مما قد يشير إلى وجود علاقة بين المستوى المنخفض للهرمون وإرتفاع البروتينات الالتهابية والدلائل الأخرى لدى مرضى تصلب الشرايين.
INTRODUCTION

In men testosterone is released from adrenal cortex and testis. Spermatosis as male sex hormone plays role in the development of secondary sex characters and releases of gonadotropin (1). The released testosterone is carried in blood by binding to albumin. Testosterone is found in body in three forms as free, Sex hormone-binding globulin and albumin or slightly tied to cortisol binding globulin (2).

Cholesterol is the biggest lipid in the body and it is the precursor of steroid hormones and bile acids (3). The effect of Anabolic Androgenic Steroid (AAS) is synthetic derivations similar to testosterone, the effect of (AAS) on cholesterol metabolism have not been defined precisely (4,5). However it has been reported that the increase on the cholesterol level is resulted from common side effects of AAS. The disorder in the cholesterol mechanism can cause (Atherogenic) to Atherosclerosis is characterized by thickening of the Artery walls and the narrowing of the blood vessels as cholesterol is deposited in the lining of the arteries, it is the major cause of cardiovascular disease including ischemic heart disease (heart attacks) and stroke (6).

Several factors such as increasing age, hypercholesterolemia, hypertension, smoking, diabetes mellitus, obesity, and male sex accelerate the rate of progression of atherosclerosis (7). At the same time, atherosclerosis is inflamed disease and inflammation is associated with increasing level of sensitive inflammatory proteins like C-reactive protein, Ceruloplasmin, Albumin, Fibrinogen and Transferrin (8). Ceruloplasmin (Cp) is a glycoprotein which is synthesized mainly in hepatocytes with six atoms of copper incorporated prior to secretion, it is secreted into the plasma as an α 2-glycoprotein (9). It acts as a host defense mechanism by its radical scavenging and copper donor activity (10). Its increased level may cause an early progression of atherosclerosis (7).

Albumin is a globular protein which is synthesized in hepatocytes, it serves as a carrier protein for many insoluble organic substances, also it binds Cu tightly and Fe weakly, present at high level (50-60mg/ml). Cu bound to albumin may still participate in Fenton reactions, but the hydroxyl radical would be formed on the albumin surface and scavenged by it, not allowed to escape into free solution so the albumin is sacrificial antioxidant (11).

MATERIALS AND METHODS

This study was conducted at Ibn-AlNafes Hospital in Bahgdad. Thirty three males patients of an mean aged (56±10.0) years (range 40-75) with history of atherosclerosis, and 25 healthy male volunteers mean aged (52.92±7.55) years (range 39-69) referred to the control group. Body mass index (BMI) was calculated as weight divided by height squared (Kg/m²). Patients were excluded if they take any medication treatment. Blood pressure is commonly expressed as systolic / diastolic.

Blood samples (10 ml) were collected from all subjects by vein puncture and allowed to clot at room temperature for 30 minutes. Serum samples obtained after centrifugation at 3000 rpm for 20 minutes. Serum was removed with micropipette and stored at -20°C and used for the estimation of:
Testosterone evaluation by Radioimmunoassay using a kit from DRG company, it is based on the completion between labeled( 1¹²⁵) –testosterone and the testosterone in the sample against limited number of antibodies site bounded to the solid phase (coated tube), after incubation, the unbounded tracer is easily removed by washing step (12).

Lipid profile: the levels of Triglycerides (TG), Low density lipid cholesterol (LDL), High density lipid cholesterol(HDL) and Total cholesterol(TCH); have been estimated among patients using enzymatic and colorimetric methods. Using kit from Radnox company, UK.

Serum ceruloplasmin (Cp) level estimated by signal radial immunodiffusion(SRID) plates for accurate quantitative determination of proteins in human serum (Biomaghreb-Tunisia ), using specific endplate, with incubation for 48 hours. at 23°C in case of Cp the concentration of Cp was determined from the standard curve (reference Cp concentration, versus square of ring diameter )and expressed as; Normal value for Cp (1.9-5.7)g/dL.

Albumin :Albumin level was determined by dye-binding technique, which using bromo-cresol sulphonphthalin (bromocresgreen, BCG ). The albumin bind to BCG in Phosphate buffer at pH=4.2, yielding a blue complex which is measured spectrophotometrically at 623 nm, albumin acts as a cation to bind the anionic dye (Randox Laboratory Ltd.UK.)

Data were analyzed with statistical software SPSS version 17, results were expressed as the mean ±SD. Statistical analysis of data was performed utilizing unpaired Students t-test. Data with p<0.05 were considered significantly.

RESULTS AND DISCUSSION

The results in table(1) clearly showed that differences in the testosterone levels were(15.91±4.45) and(39.6±11.1) nmol/l of the patients compared with control group respectively. The mean value differences were highly significant increased (p<0.05).

Table (1) show the characteristics of study groups, serum levels of total serum protein and Ceruloplasmin show significant increasing (p<0.05), in patients compared to healthy control, while Albumin level show non significant decreasing (p>0.05).

Table(1):Mean value of Serum levels of Testosterone, total protein, albumin, and ceruloplasmin for both patient and control group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients(n=33) (mean±SD)</th>
<th>Control(n=25) (mean±SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone[nmol/l]</td>
<td>15.901±4.453</td>
<td>39.6±11.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T.protein [g/dl]</td>
<td>8.163±0.835</td>
<td>7.212±0.459</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Alb[g/dl]</td>
<td>4.563±0.631</td>
<td>4.691±0.359</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ceruloplasmin [g/dl]</td>
<td>4.007±1.0365*</td>
<td>3.1756±0.997</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* Significant p<0.05 compared with control group.
Table 2: Mean value of lipid profile in sera of study groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=33) (mean±SD)</th>
<th>Control (n=25) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol [mmol/l]</td>
<td>6.609±1.32</td>
<td>4.624±0.786*</td>
</tr>
<tr>
<td>T.G [mmol/l]</td>
<td>2.238±1.108</td>
<td>1.128±0.4077*</td>
</tr>
<tr>
<td>HDL [mmol/l]</td>
<td>1.007±0.284</td>
<td>1.216±0.274*</td>
</tr>
<tr>
<td>LDL [mmol/l]</td>
<td>5.458±1.783</td>
<td>3.525±0.9981*</td>
</tr>
<tr>
<td>VLDL [mmol/l]</td>
<td>1.0988±2.127</td>
<td>0.2264±0.0844*</td>
</tr>
</tbody>
</table>

*Significant p<0.05 compared with control group.

Figure 1: The mean value of lipid profile in sera of control and patients.

The results of the following data appeared lipid profile in table (2): it showed significant increase (p<0.05) in total cholesterol TC, triglyceride TG, low density lipoprotein LDL and very low density in patients with Atherosclerosis compared with control. High density lipoprotein HDL showed significant decreased in patients (1.007±0.285) compared with control group (1.216±0.275) mg/dl.
Table (3): Correlation coefficients and the significant levels of testosterone levels and other parameters in Atherosclerotic patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Testosterone n mol/l</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.445**</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>T.P</td>
<td>-0.219</td>
<td>0.580</td>
<td></td>
</tr>
<tr>
<td>Alb</td>
<td>-0.141</td>
<td>0.429</td>
<td></td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>-0.085</td>
<td>0.504</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.202*</td>
<td>0.255</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>-0.186</td>
<td>0.301</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>-0.246*</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>-0.119</td>
<td>0.508</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>-0.024</td>
<td>0.894</td>
<td></td>
</tr>
<tr>
<td>Blood presser</td>
<td>-0.102</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.251</td>
<td>0.043</td>
<td></td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Figure (2): Correlation between testosterone level and age in patients with atherosclerosis.
Figure (3): Correlation between testosterone levels and total serum protein in sera of patients with Atherosclerosis.

\[
y = 0.0196x + 7.815 \\
R^2 = 0.0483
\]

Figure (4): Correlation between testosterone levels and albumin in sera of patients with atherosclerosis.

\[
y = -0.0103x + 4.735 \\
R^2 = 0.0131
\]
Figure (5): Correlation between testosterone levels and HDL in sera of patients with atherosclerosis.

\[ y = 0.0072x + 0.8835 \]
\[ R^2 = 0.0607 \]

Figure (6): Correlation between testosterone levels and cholesterol in sera of patients with atherosclerosis.

\[ y = -0.0143x + 6847 \]
\[ R^2 = 0.041 \]

Figure (7): Correlation between testosterone levels and ceroloplasmin in sera of patients with atherosclerosis.

\[ y = -0.0091x + 4.162 \]
\[ R^2 = 0.0073 \]
Calculating correlation coefficient among biochemical changes as well as general characteristics are presented in table (3). The results of testosterone levels was showed a negative significantly correlation in all parameters, There was a negative significant correlation \( r=-0.445 \) at the \( p (0.004^* ) \) level (2-tailed) in testosterone with age in the patients than the control group figure (2). Mean testosterone \( 15.91\pm4.45 \)nmol/l were showed non significant negative correlation with T.P\( (r= -0.219 ) \),Alb\( (r= -0.141 ) \) and Cp\( (r= -0.085 ) \),while cholesterol\( (r=-0.204 ) \) p \( (0.255 ) \),HDL\( (r=-0.246 ) \) p\( (0.086 ) \),shows a negative significant correlation in figures (3,4,5,6,7). However serum testosterone concentrations showed negative significantly correlated to body mass index\( (r=-0.25 , p<0.05 ) \) (kg/m²),and with hypertension \( ( r=-0.102 , p <0.031 ) \). Atherosclerosis is one of the most common diseases with pathological process that could lead to cardiovascular disease, atherosclerotic lesion development is mostly confined to regions of arterial curvature and branch points which are exposed to disturbed blood flow .An association between circulating hormone levels and increasing wall thickness of the artery was related to lower serum testosterone(13). Although it is known that testosterone level influence lipoprotein only a few studies have directly examined the relation between testosterone and markers of Atherosclerosis . The results in the present study show that low plasma testosterone level may be a risk factor for CHD, which may related to the influence of plasma lipoprotein metabolism by endogenous testosterone this agreement (14) in Chinese men. The present study is in agreement with previous report (15) which found an inverse relation between serum testosterone and degree of coronary artery disease. Testosterone has been shown to have immune-modulating effects; testosterone in particular appears to suppress activation of pro-inflammatory cytokines. Present study agrees with previous report which found correlated link between low serum testosterone levels and carotid artery atherosclerosis. In addition, links were found with their age, BMI and higher total cholesterol levels; moreover, low testosterone link to atherosclerosis was present whatever the blood pressure was(16). Several studies show that systemic immune system as the local Vascular immune system involved in the development and progression of atherosclerosis (15,17). In this study, we discuss some of the biochemical markers that may be involved in Atherosclerotic disease and the putative link between testosterone deficiency and these markers with atheroma formation(18), since the inflammatory process in the atherosclerotic artery may lead to increased blood levels of inflammatory cytokines and other acute –phase proteins (19). Present data show non- significant decreasing level of Albumin in patients compared to control group, the results confirm previous finding that decreased level of serum Albumin may be an early marker for Coronary heart disease in an older population (11). Another study consider increasing level of serum albumin may be a marker of susceptibility to the inflammatory response in African –American middle aged men and women patients with atherosclerosis (20). Present patients show albumin level \( 4.563 \) g/dl, these patients may be tended to have a Lower risk for incident coronary heart disease, since patients with albumin level \( \leq 4.3 \) g/dl may increase coronary heart disease rate (11), or they may not have progress disease because lower serum albumin levels in young patients are not associated with an increased odds of prevalence carotid atherosclerosis (21) that may be related with the present study especially age range of
present patients (40-75). In contrast, previous study illustrated that the risk level of albumin is 1.25 g/dl for male and 2.12 g/dl for females which could leads to Coronary heart disease (22). The albumin binds to several ligands (variety of substances), interacts with free fatty acids, and inhibits their promoting effects on platelet aggregation (17). Moreover, serum albumin may act as an indirect and sacrificial antioxidant (11) and inhibits peroxidase, free radical generation (23), also may inhibit tissue damage by reducing vascular permeability and inhibiting human endothelial apoptosis (24). All previous roles for albumin may inhibit the pathogenesis process of atherosclerosis (11), therefore lower albumin levels may be a marker of persistent injury to arteries and progression of atherosclerosis (24), so reduced levels of albumin within the normal range have been associated with preclinical disease and severity of disease (23). That's agree with present data. Present data show significant increasing serum Ceruloplasmin levels in patients compared to control that agree with previous reports which they are emphasize on the relationship between Cp and oxidative stress (11, 19). Ceruloplasmin is abundant in atherosclerosis lesions, which play a role in oxidative processes in pro-atherosclerosis areas and lesion of enlarged intima and fatty streaks of the arterial wall (13). It reacts either as a ferroxidase enzyme by catalyzing the oxidation of Fe²⁺ to Fe³⁺ and thus prevents peroxidation of membrane lipids because this could lead to cell injury, which means Cp acts as an antioxidant through ferroxidase activity and it scavenges superoxide anion radical (25). Cp acts as a host defense mechanism by its radical scavenging and copper donor activity (26). The vast majority of serum copper is transported bound to Cp, the rest is bound to albumin, transcuprein and copper -amino acid complexes (19). At the same time, LDL oxidation process dependent on high concentration of transition metals such as copper and iron in vitro because these free ions can company in oxidation and reduction reactions forming most toxic radical which is hydroxyl (27), so that two sensitive inflammatory proteins play a role as protective mechanisms, Cp as positive and albumin as negative inflammatory protein. All previous could explain the correlation between the testosterone and proteins.

**Recommendation**

The present study about Iraqi atherosclerotic patient's confirms the findings of previous studies by showing that low level of hormone correlated with high level of Cp, low level of albumin, high level cholesterol, Triglyceride and low level of HDL, but further studies must conduct to obtain more clarification about the relationship among risk factors. Moreover, we need a follow up study to establish the association among study parameters and complications of disease.

Early investigate might be useful for detecting patients at risk for developing disease, the albumin and Cp determination in blood are done quickly and the costs are low which could consider as risk factors among the other traditional risk factors like Hyperlipidemia, smoking and body mass index thus serum albumin and Cp.
REFERENCES


