Serum alpha-1-antitrypsin level in Iraqi patients with coronary heart disease

مستوى بروتين الفا 1 مضاد التربسين في مصل الدم عند مرضى عراقيين مصابين بامراض شرايين القلب الاكليلية

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Abstract

This study was designed to estimate the level of serum alpha -1- antitrypsin in patients with coronary heart disease. Patients were clinically subdivided into stable angina 25 patients, unstable angina 30 patients and myocardial infarction 50 patients. A control sample of 30 individuals was matched with patient for age and sex. Non-significant elevation of serum alpha one antitrypsin were noted in MI cases compared to healthy control individuals, even some MI patients showed decreasing level of alpha one antitrypsin, which may return to hereditary alpha one antitrypsin deficiency in Iraqi patients. The Stable angina patients group showed non-significant decrease in alpha one antitrypsin level compared to healthy control, while unstable angina patients group showed significant decrease in alpha one antitrypsin level that will may facilitate developing disease towards MI. All present results need additional studies to be carried out on larger samples of Iraqi individuals and alpha one antitrypsin deficient patients, furthermore, highlighting on the relationship between alpha one antitrypsin and risk of coronary heart disease.

المستخلص

اجري في البحث الحالي دراسة مستوى بروتين الفا- 1 مضاد التربسين في مصل الدم لثلاثة مجاميع من مرضى شرايين القلب الاكليلية وشملت 25 مريضا مصابا بالذبحة الصدرية المستقرة و30 بالذبحة الصدرية غير المستقرة و50 مريضا باحتشاء العضلة القلبية مقارنة بثلاثين فردا من الأصحاء , وقد سجل بروتين الفا - 1 مضاد التربسين ارتفاعا غير معنويا في مرضى احتشاء العضلة , ورغم إن بعض مرضى احتشاء العضلة القلبية سجلوا انخفاضا بمستوى البروتين وقد يعزى الى النقص الوراثي له عند هولاء المرضى . المجموعتين الأخريين من المرضى سجلوا انخفاضا غير معنويا لمرضى الذبحة المستقرة ومعنويا للذبحة غير المستقرة في مستوى البروتين . الامر الذي قد يودي الى تفاقم الحلة المرضية وتطورها باتجاه احتشاء العضلة القلبية .

نوصي بضرورة إجراء دراسات مستفيضة تشمل عينات اكبر لإلقاء الضوء على حالات النقص الوراثي لبروتين الفا مضاد التربسين عند الإفراد العراقيين، وتوضيح العلاقة بين التركيب الوراثي للبروتين وعلاقته كعامل خطورة بمرض شرايين القلب الإكليليه .

Introduction

Inflammation is involved in the determining of atherosclerosis and coronary heart disease (CHD)[2]. Increased levels of acute phase proteins (APPs) were found in patients with CHD [2]. Arise in Plasmatic concentration of APPs like alpha 1-

Key word: alpha 1-antitrypsin, myocardial infarction, coronary heart disease, risk factors.

Antitrypsin (AAT), alpha -1 glycoprotein, haptoglobin, ceruloplasmin and C-reactive protein(CRP) were seems to be associated with an inflammatory response featured to acute myocardial infarction and seems to own a short- term prognostic relevance [1, 3,4,5]. Increased level of APPs like CRP and cruloplasmin were found in Iraqi patients of coronary heart disease [6,7].

Alpha 1- antitrypsin (AAT) is an acute phase protein, plasma level rises several flod in acute and chronic active inflammation [8]. AAT belongs to family of serpins, a group of serine protease inhibitors [2]. The serine proteases are a group of closely related proteolytic enzymes, with serine in their active site, which play a key role in coagulation and fibrinolysis and in kinin and complement activation [9]. The activities of these enzymes are controlled at least in part by specific inhibitors known collectively as serine protease inhibitors, or serpins [10]. Elastase cleaves connective tissue structure such as collagen and elastin, this step is necessary for the formation of pus and liquefaction of an inflammatory site [9]. Serpins are inactivated by forming irreversible complexes with serine proteases such as elastase chemotrypsin, trypisn and thrombin, alpha 1-antiteypsin is therefore also referred to as α1-proteinase inhibitor [8].

The serine protease inhibitor found in highest concentration in plasma is AAT, a 51 KDa. Glycoprotein, which occurs in approximately equal concentration in plasma and in interstitial fluid [1,11]. The gene for AAT has been cloned and mapped to the long arm of chromosome 14 [10]. Although the AAT gene is highly polymorphic, only two mutations Z and S cause the great majority of diseases associated with a deficiency of this protease inhibiter [9]. The different forms of AAT, frequently designated as pi for proteinase inhibitor are commonly distinguished by differences in electrophoretic mobility [10]. AAT synthetic sites, besides hepatocytes, include alveolar macrophages and monocytes, Furthermore, synthesis of inflammation sensitive plasma proteins like ceruloplasmin and AAT is regulated by various cytokines which are produced by inflammatory cells at multiple sites [2]. The daily synthetic rate of AAT is 34 mg/kg of body weight. It's the half-life is (6-7) days [9].

The goal of present study was to investigate an individual changes in blood levels of AAT in Iraqi patients with coronary heart disease.

Materials and Methods

One hundred and thirty five Iraqi patients with coronary heart disease (CHD), who admitted to Ibn-AL-Nafis teaching hospital, were studied. The patients were classified into their clinical subgroups, 50 with myocardial Infarction (MI)(31 male and 19 female) with age range (40 – 78) year (mean 54.96), 25 with stable angina (S.A) 20 male and 5 female with age range (36 – 80) year (mean 59.04), and 30 with unstable angina (Un.A) 15 male and 15 female with age range (44 – 73) year (mean 49.83), according to a clinical examination by consultant physicians, electrocardiogram (ECG) and biochemical markers.

The other group is control which consist of 30 Iraqi healthy individuals 21 male and 9 female with age range (33 – 76) year (mean 51.1), were age and sex Matched with patients group .

The laboratory investigations:-

Estimation of serum alpha 1- antitrypsin levels by single radial immunodiffusion (SRID) plates for accurate quantitative determination of human (Biomaghreb-Tunisia), using specific endoplate, with incubation for 48hr at 23C° in case of AAT, the concentration of AAT was determined from the standard curve (reference AAT concentration, versus squares of ring diameter). Subjects were distributed according to their serum alpha 1- antitrypsin Concentration into three groups. Low level (< 0.7 g/L), to normal level (0.7 – 1.8) g/Land high level > 1.8 g/L [8].

The statistical analysis were done using SPSS version 7.5 computer software, further exploration of significance difference were assessed by Duncan test.

Results

Table (1) shows the mean level of AAT concentration in patient groups and healthy control, the MI group had the highest mean (1.367 ± 0.450) with no significant differences compared to healthy control (1.332 ± 0.581) ,While S.A group (1.320 ± 0.379) revealed low mean compared to healthy control with no significant differences, but there was significant decreasing among Un.A group (1.135± 0.370) and all other study groups (P≤ 0.05) .

Table (1): Statistical analysis among the study groups according to the concentration of alpha -1-antitrypsin(g/L).

Groups	No.	Mean ± SD	Duncan*	SE	minimum	maximum
Control	30	1.332± 0.581	a	0.106	0.405	3.200
MI	50	1.367± 0.450	a	0.064	0.315	2.480
S.A	25	1.320 ±0.379	a	0.076	0.405	1.906
Un.A	30	1.135± 0.370	b	0.068	0.405	1.736

***the similar letters mean that there are no significant differences (p≥0.05).**

Patients were distributed to their serum AAT concentration into three groups as shown in table (2). This distribution according the previous study about [8]. Low level (≤0.7) g/L, to normal level (0.7 – 1.8) g/L and high level (≥1.8) g/L [8].

At low level, all patient groups recorded higher % compared to control group, the Un.A groups was the highest one with significant differences according to Chi-square ($p \leq 0.05$). At normal level, Patients group with MI recorded lower % while patients with MI recorded the highest %, 21 (84 %) followed by 24 (80 %) of Un.A patients consequently compared to healthy control 24 (80).

At high level MI patients groups recorded the highest (%)9 (18 %) compared to healthy control 4 (13.33%), while S.A group recorded lower %, at the same time there were no patients with Un,A recorded high serum level of AAT.

Table(2) Distribution of study groups according to the concentration of alpha -1- Antitrypsin

Groups	Level of alpha -1- antitrypsin(g/L)							
	Low level < 0.7		Normal level (0.7- 1.8)		High level > 1.8		Total	
	No.	%	No.	%	No.	%	No.	%
Control	2	6.67	24	80	4	13.33	30	100
MI	4	8.0	37	74	9	18.0	50	100
S.A	2	8.0	21	84	2	8.0	25	100
Un.A	6	20	24	80	0	0.0	30	100

X^2 = 9.548 , df=6 , p> 0.05 [NS.]. Ttotal comparison
X^2= 6 , df=2 , p ≤0.05 [S] . Between Un.A and control

Discussion

Present study supports previous reports [11,12], showing that MI may be followed by rising in the blood levels of AAT, because MI is a dynamic process that begins with the transition from reversible to irreversible injury, leading to cell death [8], all these process make this site as inflammatory site, therefore AAT as serine protease inhibitor is released in a high level to form complexes with the protease to facilitate elimination [13], AAT is found in relatively high concentration in the plaque, which could be enhance fibrosis of lesion because of their inhibitory effects on collagenas and elastase [5,14], but that elevation of AAT in the present study was non significant, while in previous study was significant that difference may be depending on the causative factors [4], especially risk factors of CHD, which are induced inflammation like lipid atheromatous [12]. Furthermore, in a prospective study, plasma levels of inflammatory markers like C-reactive protein and AAT are generally higher in smokers compared with non smokers patients with MI [15,13].

Present result agrees with recent report, that a high level of AAT was observed more often in patients with MI than in those with Un.A [3], while, present data disagrees with the previous study, which recorded a significant elevations of serum AAT in MI and angina cases [4]. Also the % data disagree with previous study which showed a reduction of AAT in MI and Un.A patients [16] .

In another view, present S.A patients group recorded decreasing mean with no significant differences comparing with control and MI group consequently, that may be associated with reversible effect of cell damage which will not cause the cell death, because the course of disease is determined not only by the severity of ischemic events, but also by the response of the acute phase reaction mechanism [9,17]. Myochardial infaction and unstable angina represent unstable phases of ischemic heart disease that is associated with a more sever, short term prognosis than chronic stable angina [16] .

present patients group with Un.A recorded significant decreasing of AAT mean Table (1) which, indicates the progressive of Un.A towards MI, because this low level means absence of the protection activation of AAT, at the same time , present serum AAT level in Un.A individuals not reachs more than 1.8 g\L Table (2), so that low mean level of AAT will allowed Un.A disease to develop towards MI, because

Un.A with low level of AAT frequently evolves towards MI [16], in another words, low serum AAT levels promote atherogenesis [16]. Morover, low level of AAT cannot Inhibit neutrophil and proteolytic damage, which cause artery damage [18], because activated neutrophils elaborate elastase and if elastase is unchecked by the proteinase inhibitor AAT could be caused destruction of tissue, and the damaged cell will release oxygen radicals and chlorinated oxidants, which will can oxidase the methionine at the active site of AAT leading to decrease of the rate of the association of the inhibitor with neutrophil elastase and reducing the ability to inhibit elastase activity [9] .

In present data, the 1.8 g\L of serum AAT is considered the high level, while the study about Sweden CHD patients defined that the AAT ≥ 1.43 g\L as a high level, which it was highly correlated with increasing risk of cardiovascular disease [2]. While, another report about Sweden CHD patients considered the level of AAT ≥ 1.42 g\L as highly levels during the screening examination usually is used in Swedish routine clinical practice to reflect inflammatory activity [15]. On the other hand, another study about Caucasian, defined the AAT ≥ 1.8 g\L as a high level [8]. When our patients with MI were grouped according to the high level of AAT ,which reached to more than 1.8 g\L, some interesting features became evident, such as most MI patients 80% showed normal level ,while a number of MI patients (18%) showed a high level of AAT more than 1.8 g/L, that may be associated with an increased inflammatory systemic activation featured by plasmatic concentration of C-reactive protein and others of acute phase protein like AAT, that might be associated with both coronary atherosclerosis and an impaired coronary micro-circulation [13], so the AAT can inhibit neutrophil super oxide production and exogenous administration of AAT conferred protection against ischemia and reperfusion injury [16].

Moreover, the proteolytic inhibitor like AAT found in high concentrations in the plaque, could enhance fibrosis of the lesion because of their known inhibitory effects on collagenase and elastase [13]. Nnormal level of AAT recorded in present serum patients with MI as acute phase protein may related with the size of damaged tissue because a small size of infarct tissue may be un able to trigger the acute phase response [16], or a very large infarct tissue may consume significant quantities of circulating AAT [17].

In fact our data do not indicate which one of the two possibilities about size of infraction is correct or whether both are, that could be determine by biochemical markers like Creatin Kinase(Ck), which will give a clear idea about the size of infarction [8]. Furthermore, plasma protein map has been identified the presence of seven AAT isoforms in plasma from normal individual, and acute MI associated with a failure to increase some AAT isonforms which, promote atherogenesis and some AAT isoforms associated with increasing risk of CHD [16,19,20].

In present study, some patients from all study groups recorded AAT level less than 0.7 g/L, those patients may have deficient AAT with abnormal regulation of the inflammatory response, which could be manifested by the failure of increasing the AAT level [8,10], that may predispose expansion of the necrotic area with more hazardous clinical course [17,19]. Patients who already have deficien

evolve a greater progression of established coronary artery atherogenesis [20], because of their losing a protective role of AAT (11, 27), that agrees with our data about Iraqi patients who recorded AAT serum level less than 0.7g/L, that may be represented a predict factor, because deficiency of the elastase inhibitor AAT may represent a predisposing condition [18].

A disequilibrium between proteolytic enzymes and protease inhibitors (AAT) may be contributed to the pathogenesis of vessel wall elastic lamina and degradation of elastic fibers, which causes losing blood vessel tone and development of atherosclerosis, that will moderate atherogenesis leading to atherosclerotic lesions in coronary heart arteries [10] .

Another information, the reduction of AAT concentration to less than 0.7g/L is related with heredity AAT deficiency [8]. The level of AAT usually rises up to a maximum of three times in normal individual during acute phase response, so that, the deficiency will facilitate damage of vascular [18]. That may explain why 20% of our patients with Un.A recorded the highest percentage of low level at table two, because most of them had a heredity AAT deficiency. Furthermore, deficient may related with releasing an altered protein [25, 17]. The altered protein is less effective as an inhibitor of neutrophil elastase than the normal form [19] .

Furthermore, low level was found in all patient groups, which it could consider as a failure of the serum AAT to rise during the acute phase response of CHD. The low level of AAT is an important pathogenic factor responsible for development of disease [9], which associated with a poor clinical outcome including cardiogenic shock and mortality [17].

Finally, we need further studies about a heredity deficiency of serum AAT in Iraqi population and detected alleles by using allele –specific oligonucleotid probes for normal and mutant. Also, we need more investigation to clarify the protective role of AAT in the progression of different factors for CHD and wether the AAT is liver-derived or produced locally by macrophages and also study its role in preventing excessive matrix remodeling in the vessel wall. Before all that we need established data for normal range value of serum AAT in Iraqi population, also more investigation by biochemical markers like Creatin Kinase (Ck),_which will give a clear idea about the size of infarction [8]. A large numbers of patients are needed to establish the clinical importance of present finding, that agree with immunological theory which consider the rise level of serum AAT in patients of MI is due to its property of a cute phase protein due to inflammation and necrosis in cardiac muscle and can used as a tools to diagnostic the size of damage to manage the treatment, even some MI patients individually showed decreasing change in serum AAT levels, which may return to hereditary AAT deficiency in Iraqi patients. The two other patients groups showed decreasing in AAT level compared to healthy control that will facilitate developing disease towards MI, especially Un.A patients

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