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Article · January 2016

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Tikrit Journal of Pure Science 21 (6) 2016 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

Study of Tryptase Activity and Selenium Concentration in Cardiovascular And Diabetic Patients

Nagham Qasim Kazim[1], Dakheel Hussein Hadree[2] , Fawzi Hassan Zayr[3]

[1] *Department of Chemistry , College of Science , University of Tikrit , Tikrit , Iraq*
[2] *branch of physiology and life of Chemistry , College of Veterinary Medicine, University of Tikrit , Tikrit , Iraq*
[3] *Clinical Chemistry , College of Medicine, University of Wasit , Al Kut , Iraq*

Abstract

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among patients with diabetes mellitus (DM). DM can lead to multiple cardiovascular complications, including coronary artery disease (CAD), cardiac hypertrophy, and heart failure (HF). HF represents one of the most common causes of death in patients with DM and results from DM-induced CAD and diabetic cardiomyopathy. selenium concentration and tryptase activity were obtained from 52 samples: 30 sample with cardiovascular and diabetic patients, and 22 normal healthy. A highly significant decrease ($p<0.0001$) in the levels of selenium, and a significant increase ($p<0.05$) in the levels of tryptase in serum of patients with cardiovascular and diabetic patients compared with normal individuals. selenium concentration was affected by cardiovascular and diabetic patients and tryptase has higher diagnostic validity values in the current study.

Keywords: Cardiovascular disease, Diabetes mellitus, Selenium ,Tryptase.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, dyslipidemia, and disturb protein metabolism that results from defects in both insulin secretion and/or insulin action. Diabetes mellitus is a disorder with late complications including cardiovascular disease, nephropathy, neuropathy and retinopathy which severely affect the quality of life[1]. DM is thought to cause not only microangiopathy (associated with the three major diabetic complications, namely diabetic retinopathy, nephropathy and neuropathy) but to constitute a major risk factor for macroangiopathy, such as coronary artery disease (CAD) and cerebrovascular disease [2,3]. It is also known that oxidative stress and inflammation play a key role in the pathogenesis and progression of diabetes-induced CVD, where the increased expression of inflammatory proteins or cytokines such as C-reactive protein (CRP) or oxidative stress-related proteins is shown to serve as a biomarker for the onset of CVD and heart failure (HF) [4]. Chronic inflammatory process induced by pro-inflammatory cytokines and chemokines may also contribute to the pathogenesis of diabetic cardiomyopathy [5-7]. In addition more than 70% of people with diabetes have high blood pressure or are being tested with medications for hypertension. It is imperative, therefore, for at risk community members to not only be aware of their potential diabetes status, but to be aware of the increased risk for cardiovascular disease incurred in combination with other medical conditions[8].

Selenium is an essential trace mineral involved in protection against oxidative damage via selenium-dependent glutathione peroxidases and other selenoproteins. Current recommendations on dietary intake of selenium are based on optimizing the activity of plasma glutathione peroxidases. The recommended dietary allowance for selenium that is estimated to be sufficient to meet the nutritional needs of nearly all healthy adults is 55 µg/d [9]. Because of its antioxidant properties, it has long been hypothesized that selenium may prevent cardiovascular and other chronic diseases [10].

Tryptase is a predominant protease of human mast cells and presents in three isophorms: α-tryptase, pro-ß-tryptase and ß-tryptase. α- and pro-ß-tryptase are enzymatically inactive and released continuously. A persistent rise in serum α-tryptase is an indicator of an elevated number of mast cells, and thus may indicate mastocytosis . ß-tryptase is stored in mast cell granula and is released under conditions of extensive mast cell degranulation. Tryptase is an indicator of mast cell involvement in a variety of clinical conditions. It has been reported to be increased in bronchoalveolar lavage during allergen challenge and in nasal fl uid during allergic rhinitis[11,12].

The troponin complex is protein components in striated muscle tissue (such as cardiac tissue) and consists of a cluster of three distinct polypeptides: Troponin C, Troponin T and Troponin I. The function of the troponin complex is to regulate sarcomere (the basic unit of a muscle cell) contraction[13].

Materials and methods

Thirty patients suffering from of diabetes mellitus and cardiovascular disease and 22 normal healthy were participated in the present study. Their ages ranged from 40 to 66 years. Samples were collected from the medical city hospital in baghdad during the period from august to november 2014. All patients had blood glucose level > 200 mg/dl and positive troponin test. Five milliliters of venous blood were drown from each patient and healthy control individuals. Serum was obtained and kept into small eppendorf tubes capacity 1.5 ml at -20C° until time of analysis. The tryptase assay employs the quantitative sandwich enzyme immunoassay technique by kit supplier by Cusabio company [14]. Atomic absorption spectrophotometer method was used to determine selenium in serum samples:- The serum samples were

digested by transferring (1 ml) of whole blood into a pyrex test tube, adding (1 ml) of conc. HNO3 and (1 ml) of conc. H2SO4, placing in oil bath at 1300C for 45 minutes , until nitric acid (brown fumes) boiling away. After that the tubes removed from bath and cooled to room temperature, added (1 ml) ml of 5M HClO4 , then replaced in oil bath at 1300C for 45 minutes , the tubes removed again from bath and cooled to room temperature , added (1 ml) of 6M HCl , replaced the tubes in bath at 950C for 30 minutes then cooled to room temperature and diluted to (10 ml) by 6M HCl [15,16] , and then stock standard solutions of selinum were prepared (1000 µg/ ml)[17] .

Statistical analysis: The significance of difference between mean values were estimated by student T-test. The probability is considered as significant when $p<0.05$. The data were processed with the software package XLSTATE (2015) and Microsoft Excel 2010 to estimate the receiver operating characteristics (ROC) curves and cut off values.

Receiver operating characteristics (ROC): curves were used to compare the performance of the biochemical diagnostic methods of disease in this study and to determine the appropriate cut off values for the different markers. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to analyze the diagnostic value of each hormone. The area under the curve (AUC) is commonly used as a summary measure of diagnostic accuracy.

Results and discussion

The mean (±SD) concentration of selenium and tryptase, in serum of control group (healthy individuals) and serum of patients with cardiovascular and diabetic group are shown in table (1). There are a highly significant decrease ($p<0.0001$) in the serum levels of selenium in cardiovascular and diabetic patients group when compared with control group, and a significant increase ($p<0.05$) in the serum levels of tryptase in cardiovascular and diabetic patients group when compared with control group .Table (2) shows the criteria of diagnosis validity (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Accuracy tests) of tryptase level in patients group compared with healthy control by using 23.00 ng/ml as cut-off value . The optimal cut-off value for tryptase estimated from ROC curves, according to these results , test is positive if test > threshold value (cut off values). Figure (1) explained the ROC curves for tryptase concentration, sensitivity and specificity shown in figure (2).

Table (1): Mean ±SD selenium and tryptase concentration in groups

parameters / Groups	Selenium µg/L	Tryptase ng/ml
Control group n=22	87.2 ± 3.1	16.6±3.19
Patients group n=30	61.7 ± 2.7	27.1±4.24
P value	P< 0.0001	P< 0.05

Table (2): Predictive values of serum tryptase level in studied groups using 23.00 ng/ml as cut-off value.

Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
69%	81%	88 %	56 %	73%	0.700

- Sensitivity = positive in disease , expressed as percent.
- Specificity = negative in healthy , expressed as percent.
- Predictive value of positive test = percent of patients with positive test results that are diseased.
- Predictive value of negative test = percent of patients with negative test results that are non-diseased.
- accuracy test = percent of patients correctly classified as diseased and non diseased.

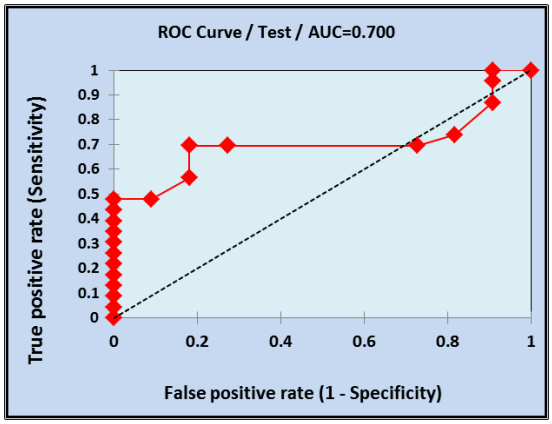

Figure 1: ROC curve for tryptase concentration in in patients group

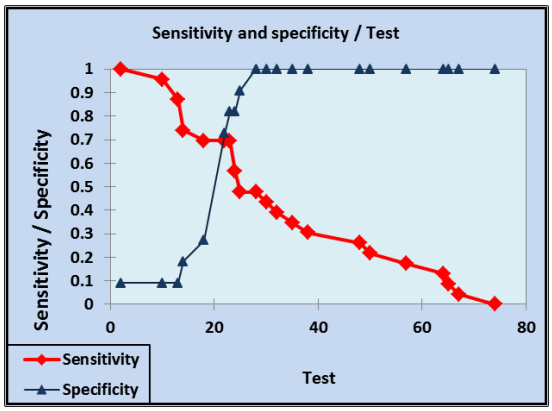

Figure 2: Sensitivity / Specificity test for tryptase concentration

Elevated extra and intra-cellular glucose concentration results in an oxidative stress. When diabetic complications are developed, an increase in oxidative damage and subsequently emaciation of antioxidant defence systems is observed . Changes in oxidant and antioxidant systems are related with duration of disease and become more important as complications develop. Finding of several studies demonstrated that overproduction of peroxides along with emaciation of antioxidant defense systems cause oxidative damage and these events in type 2 diabetic patients are observed in an earlier stage, before diabetic complications develop. Although glucose itself can initiate oxidative stress, deficiency of

essential trace elements such as selenium may exacerbate this oxidative stress in diabetic rats[18].

Some studies reported serum selenium levels of diabetic patients to have increased, decreased or remained unchanged compared to control. Significant reductions in the levels of selenium are indicators metabolic response to oxidative stress in patients with diabetes[19]. Othor studies showed that sodium selenate improved glucose tolerance in the streptozotocin model of diabetes in rats. In mice, selenate reduced gluconeogenesis and inhibited phosphotyrosine phosphatases by 50%.88 In type 1 diabetic rats[20]. However, evidence from other studies suggests that selenium could enhance insulin sensitivity and improve blood plasma glucose levels by mediating insulin-like actions and by acting via several other mechanisms, including detoxifying liver enzymes, exerting anti-inflammatory effect, and providing antioxidant defense to elicits its anti-diabetic effect, however, the mechanisms by which Se exerts these beneficial effects are still not yet fullyUnderstood[21]. The beneficial effect of selenium administration on plasma glucose level has several systemic consequences. Firstly, selenium includes stimulation of glucose uptake and regulation of metabolic process such as glycolysis, gluconeogenesis, fatty acid synthesis, and pentose phosphate pathway. Secondly selenium does activate a key proteins involved in the insulin signal cascade. Also selenium cause partial restoration of mRNA levels and activities of two key glycolytic enzymes (glucokinase and pyruvate kinase)[22].

Various epidemiological and clinical researches have been carried out in order to find out the relationship between selenium deficienc and other diseases, mainly cardiovascular diseases and certain types of cancer.

A number of factors are found to be associated with increased risk for cardiovascular diseases (CVD). Among these factors the trace element selenium is suggested to be associated with CVD. Reportedly, patients with coronary atherosclerosis, myocardial infarction or cardiomyopathy have a significant lower selenium concentration in their serum than do healthy control groups. A small number of studies have also examined the association of blood selenium levels with heart failure in the general population[23]. Another study showed that, dietary intake and blood levels of selenium were found to be lower in heart failure patients: interestingly, the selenium level was correlated with peak oxygen consumption, a major determinant of symptoms and prognosis, but not with left ventricular ejection fraction. Similarly, African Americans with heart failure have been shown to have multiple micronutrient deficits, including that of selenium[24]. The most striking association of selenium deficiency with heart failure is that seen in patients receiving parenteral nutrition[25]. Multiple case reports have shown pathological changes similar to those observed in Keshan disease, and that selenium supplements can reverse the cardiomyopathic process[26-28] .

Some studies found a positive association between circulating tryptase levels and insulin resistance parameters, suggesting a glucose homeostasis impairment in those individuals with higher tryptase levels. In line with our results, two Chinese studies, have considered circulating tryptase levels as an independent risk factor of pre-diabetes and diabetes mellitus, reinforcing the close association between the immune system, obesity, and vascular diseases[29,30]. Although not statistically significant, plasma tryptase levels were also higher in Type 2 diabetes patients than in those with pre-diabetes or with normal glucose levels. More sophisticated studies are required to test a role of mast cells (MCs) in diabetes or obesity, but this human study inspired us to explore MC participation in these metabolic diseases[31].

On the other hand, epidemiology, histopathology and experimental studies point toward a proatherogenic role for mast cells, involving tryptase[32]. There are many reports of several elevated acute phase inflammatory mediators in unstable coronary syndromes. Elevations of inflammatory markers including C-reactive protein, IL-6, serum amyloid protein A and fibrinogen have been reported in patients with angiographically proven stable coronary artery disease. In contrast, only a few studies have measured elevated serum levels of tryptase with patients with acute coronary syndrome. Others observed elevated serum tryptase levels with acute coronary syndrome with ST-depression both in the acute phase and 3 months later, but other researches showed no elevation of serum tryptase with acute coronary syndrome[33,34]. In addition to this, pathologic studies in cardiomyopathy have demonstrated higher MC numbers in failing hearts secondary to ischemic compared to a nonischemic etiologies and higher numbers in akinetic rather than nonakinetic segments[35,36]. In acute coronary syndrome patients, serum tryptase levels at admission may predict patient cardiovascular complexity more reliably than currently known biomarkers. Further studies are needed to demonstrate the long-term prognostic role of this biomarker[37] .

In conclusion, this study demonstrates that Selenium is known to act to prevent free radical damage to cells and elevated tryptase levels could be due to ongoing mast cell activation, degranulation and possibly inflammation.

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دراسة فعالية انزيم التربتيز وتركيز السيلينيوم في مرضى القلب والأوعية الدموية المصابين بالسكري

نغم قاسم كاظم[1] ، دخيل حسين حدري[2] ، فوزي حسن زاير[3]

[1] قسم الكيمياء ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق
[2] فرع الفسلجة والكيمياء الحياتية ، كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق
[3] فرع الكيمياء السريرية ، كلية الطب ، جامعة واسط ، الكوت ، العراق

الملخص

أمراض القلب والأوعية الدموية هي السبب الرئيسي للاعتلالات والوفيات بين المرضى الذين يعانون من داء السكري. داء السكري يمكن أن يؤدي إلى مضاعفات قلب وأوعية دموية متعددة، بما في ذلك مرض الشريان التاجي، وتضخم القلب، وفشل القلب. فشل القلب يمثل واحد من أكثر الأسباب شيوعا للوفاة في المرضى الذين يعانون من مرض السكري، وينتج من السكري الذي يحث حدوث مرض الشريان التاجي واعتلال العضلة القلبية السكرية. تم الحصول على تركيز السيلينيوم وفعالية انزيم التريتيز من 52 عينة: 30 عينة مصابين بمرض القلب الوعائي والسكري، و22 عينة من الاصحاء ظاهريا. وجد ان هناك انخفاض معنوي عالي ($p < 0.0001$) في مستويات السيلينيوم، وارتفاع معنوي ($p < 0.05$) في مستويات انزيم التريتيز في مصل الدم من المرضى الذين يعانون من مرضى القلب الوعائي والسكري مقارنة مع الأشخاص الاصحاء.

نستنتج من هذا البحث ان تركيز السلينيوم قد تأثر بامراض القلب الوعائية في المرضى المصابين بداء السكري وان انزيم التريتيز له قيم تشخيصية عالية في الدراسة الحالية.

الكلمات المفتاحية: مرض القلب والأوعية الدموية وداء السكري، السيلينيوم، انزيم التريتيز.