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THE KINETIC STUDIES OF ACID PHOSPHATASE IN MALES PATIENTS WITH TYPE II DIABETES MELLITUS

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ABSTRACT
Acid phosphatase (ACP) is basically phosphomonoesterase, used to free attached phosphate group from other molecular during digestion .It is stored in lysosomes and its functions appear when they used with endosomes ; It has an acidic optimum PH : Different forms of ACP are found in different organs ,and their serum level used as a diagnostic for disease in the corresponding organs . The aim of the study is to measure (ACP) activity in type II diabetic mellitus patients ($T_{II}DM$) and determine the optimum conditions for its activity . Fasting blood glucose, ACP, Uric acid, TG, HDL-Cholesterol , LDL , and VLDL, were measured in serum belonged to 30 male patients age (40-70) years and 20 normal subjects age (40-70) years considered as normal healthy controls . Data analysis shows that the levels of FBG (181.18 ±11.66 IU/L), ACP (19.53± 0.83 IU/L) ,Uric acid (5.82 ±0.13 mg /dl), TG(161.33±14.5 mg /dl), HDL(53.86 ±1.85 mg /dl), LDL (136.26 ±12.89 mg /dl), VLDL (30.40 ±2.41 mg /dl) were high ($P < 0.05$) in serum of type II diabetic patients compared with normal control (100.30 ±2.79 mg /dl) , (9.00±2.79 mg/dl), (5.24 ± 0.2 mg /dl), (90.65±2.1 mg /dl),(50.8 ±1.47 mg /dl), (110.5±1.65 mg /dl),(17.05 ±0.45 mg /dl) respectively. Optimum PH, temperature and incubation time were determined in this study for patients and controls.

KEY WORDS: Acid phosphatase, insulin-independent diabetes mellitus (T2DM), Lipid profile.

INTRODUCTION

Acid phosphatase (ACP) (EC 3.1.3.2) is represented by a number of enzymes that can be differentiated according to structural, catalytic and immunological properties, tissue distribution and subcellular location (Yam *et al*., 1980). Acid phosphatase is nonspecific enzyme cleave many different phosphate esters. The exact biochemical role of acid phosphatase is somewhat obscure but is thought to be mainly a digestive enzyme (Waheed *et al*., 1985). Phosphorylated compounds are widely distributed in living systems. They serve as storage forms for energy (e.g., ATP and phosphocreatine), as components of informational macromolecules (*i.e*., nucleotides and deoxynucleotides), as allosteric effectors of certain enzymes (e.g., fructose-1-6- bisphosphate), and as second messengers (e.g., cAMP, cGMP, inositol phosphates).
Phosphorylation-dephosphorylation reactions of proteins, mediated by protein kinases and protein phosphatases, modulate many enzyme activities (phosphorylase, pyruvaten dehydrogenase, etc.). It is therefore not surprising that phosphatases of many kinds can be extracted from many tissues, phosphatase enzymes catalyze the hydrolysis of phosphate esters to produce inorganic phosphate:

R-O-**P** + H_2O ———> R-OH + HPO_4^{2-} (Nakanishi *et al*., 2000)

Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and /or insulin action (Kameswara *et al*., 2003). It is one of the oldest diseases affecting millions of people all over the world (Andullu, 2002). Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes (Sumana and Suryawanshi, 2001). Elevated activity of Acid phosphatase was observed in diabetic. The increased activity of this enzyme in serum may be a result of diabetes-induced damage to the tissues (Prince *et al*., 1997). The aim of study is to measure acid phosphatase activity and the optimum conditions of its activity in TII DM patients.

MATERIALS AND METHODS

All chemicals and reagents used in this study were of purified state and were obtained from fluka (UK), Hopkins and William sigma chemical (NANA) and Riedel Deher form companies. In a period of eight months, thirty type II diabetes mellitus patients of age range (40-70) years were selected from the National Diabetes Center Al-Mustansiryah University; twenty normal controls of age range (40-70) year were taken having no history of diabetes mellitus.

Whole blood was drawn patients and normal controls. The blood was allowed to coagulate at room temperature , and was centrifuged at 3000 rpm for 5min .The resulting sera were placed in test tubes and stored frozen at (-20) until used.

Kinetic studies of Acid phosphatase

The activity of acid phosphatase was measured in sera according to the method of Lin and Clinton (1984). 3 mm of PNPP at in 50 mM-citrate, pH 6.0 (final volume 0.4 ml), was used to determine esterase activity. Incubation was performed at 37 C^o for the time indicated . The

reaction was terminated by the addition of 2.0 ml of 0.1 M-NaOH. The released p-nitrophenol was measured spectrophotometrically at 410 nm and p-nitrophenol standard solution was used to determine the amount of phosphate released.

Effect of pH

Determination ACP activity in different pH(2.6 , 3.6 ,4.6, 5.6 , 6.6 ,7.6) at 37 C^o, according to the procedure have been described by method of Lin and Clinton (1984).

Effect of Incubation Time

ACP activity have been determined in different incubation time (3, 4 ,5 .6,7) second , according to the descriptive method of Lin and Clinton (1984).

Effect of Temperature

The ACP activity have been determined in different Temperature (17, 27, 37, 47, 57,67) C^o according to the descriptive method of Lin and Clinton (1984).

Effect of Substrate concentration

Different concentration have been prepared (15, 22.5, 30, 37.5, 45) mM /L of substrate (p.nitrophenylphosphate) in buffer, according to the descriptive method of Lin and Clinton (1984).

Statistical analyses

Statistical analyses of this study were performed using SPSS version 15.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $P< 0.05$ = significant, $P> 0.05$ = non-significant. Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the differences between variables of differentiated groups.

RESULTS AND DISCUSSION

Fasting blood glucose, ACP, Uric acid, TG, HDL-Cholesterol, LDL, and VLDL in patients and control are summarized in table 1.

Insulin is an important hormone in regulating lipid metabolism in a variety of animal tissues; it can both decrease lipolysis and cause an increase in triglyceride synthesis of adipose tissue (Rosenson, 2005). The mechanisms responsible for hypertriglyceridaemia may be an increased hepatic secretion of VLDL and a delayed clearance of TG-rich lipoproteins, which might mainly be due to increased levels of substrates for TG production, free fatty acids and glucose. Triglyceride enrichment lipoproteins lead to increased production of the small dense form of LDL-C and to depletion of HDL-C (Duell and Orman, 1991; Sabharwal and May, 2008). Another researcher showed similar results in experimental and clinical studies (Gianturco *et al*., 2009).

Optimum PH

The pH (2.6 , 3.6 ,4.6, 5.6 , 6.6 ,7.6) effect have been studied on ACP activity. Figure 1 showed that highest enzyme activity in diabetic patients was at PH 3.6, while in control was PH 4.6 .

The decrease in ACP activity at acidic pH due to effect of PH environment of reaction in ionic groups which found in active site or changing in ionic state for substrate or complex enzyme-substrate when the concentration of substrate over than Michaelis constants (K_m) ,if the substrate concentration is low, it will depend on enzyme (Whitaker and Bernhad, 1972). The ketone bodies cause different PH in patient serum with type II diabetes (Whitby and Smith, 2001). The concentration of H^+ affects velocity in several ways .the concentration of H^+ affects velocity of the reactive because the calalalytic process usually requires that the enzyme and substrate to be in order to react (Rosenthal, 2009).

Incubation Time

The enzyme activity was measured in different incubated time (3, 4 ,5 .6,7) second for diabetic patients and controls the highest activity of ACP when the incubation time 5 second as shown in fig 2.

Temperature

In diabetes patients ACP activity increases according to the incubation temperature until it reaches maximum at 47 C^o, while ACP activity begins to increase until it reaches maximum at 37C^o in control as shown in figure 3.

The increase in velocity of the reaction with increasing temperature is due to increasing the energy of enzyme and substrate which Accelerate the formation of ES complex (Dixon *et al*., 1979).and increase the number of molecules having sufficient energy to puss the energy barrier to form product, Further elevation of the temperature results in a decrease in reaction velocity as result of temperature – induced denaturation of the enzyme (Dennistion and Topping, 2007).

Substrate Concentration

Determination of ACP activity in different substrate concentration (15, 22.5, 30, 37.5 ,45) mM p-nitrophenylphosphate, were studied in diabetic patients and control on rate of ACP reaction.

In diabetic patients ACP activity increases according to substrate concentration until it reaches maximum at 47 C^o to 12.5 mM, while ACP activity begins to increase until it reaches maximum at 37C^o in control in 6.7 mM of substrate concentration as shown in figure 4. K_m and V_{max} has been measured by using Lineweaver-Burk plot as shown in figure 5 and table 3 .

There are many studies show values of K_m and V_{max} of ACP in different sources, since report refers to ACP which was taken from serum sources it has got K_m 1.92 mM for p-NPP (Lin and Clinton 1984). Kinetic study of the acid phosphatase of Baker's yeast showed a linear Lineweaver –Burk plot ,with a Km of 0.45 mM for p-NPP (Schurr and Yagil, 1971), while A Km of 0.30 Mm was found for the acid phosphatase of yeast-like cells of *Sperothrix chenchii* ,and The Km is 0.3 of phosphatase acid barley root in PH 5.5 dependent (Schurr and Yagil, 1971 ; Yasar *et al*., 2004;Wysocki *et al*., 2009). The current study suggested that these difference in optimum it ,Incubation Time, PH5.5 and optimum concentration of substrate of ACP may be PH referred to another isoenzyme of ACP in sera of diabetes patients, and our study conclude purification of these isoenzyme by using electorphoretic methods.

TABLE 1: Biochemical variables in patients and control

Biochemical variables	Patients [Mean ± SE] [n= 30]	Control [Mean ± SE] [n=20]	P value
Age [year]	53.86 ± 1.85	50.80 ± 1.47	N.S
FBG [mg/dl]	181.180 ± 11.66	100.35 ± 2.79	< 0.05
ACP [IU/L]	19.53± 0.83	9.00 ± 0.99	< 0.05
Urea[mg/dl]	40.30 ± 4.83	29.35± 0.83	< 0.05
Uric acid[mg/dl]	5.82 ± 0.13	5.24 ± 0.20	< 0.05
TG[mg/dl]	161.33 ±14.50	90.65 ± 2.10	< 0.05
HDL[mg/dl]	53.86 ± 1.85	50.80 ± 1.47	< 0.05
LDL [mg/dl]	136.26 ± 12.89	110.15 ± 1.60	< 0.05
VLDL[mg/dl]	30.40 ± 2.41	17.05 ± 0.45	< 0.05

Shows a significant difference in FBG, ACP Uric acid, TG, HDL, LDL and VLDL between patients and control P value < 0.05 .

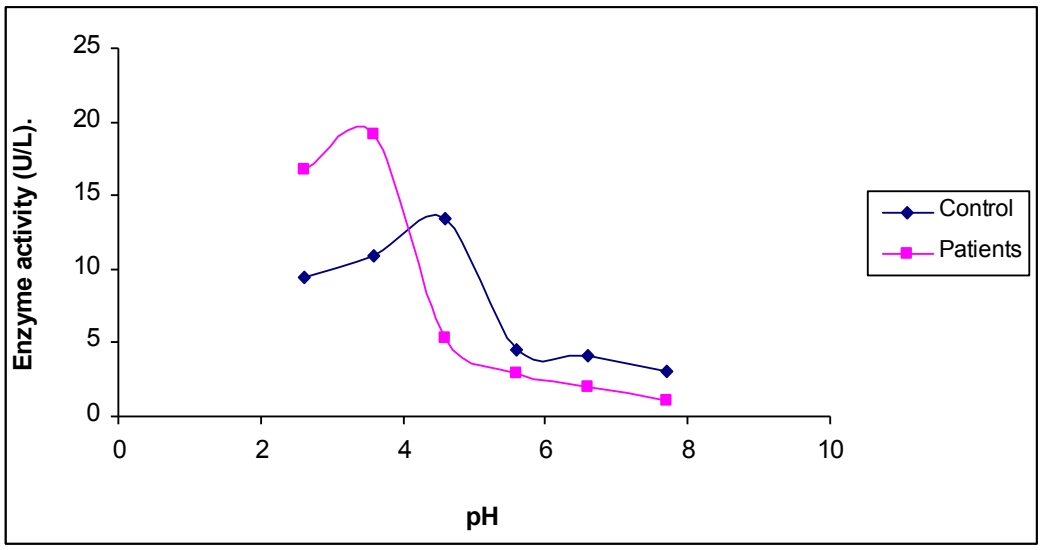

FIGURE 1. Activity of ACP with different PH in diabetic patients and control.

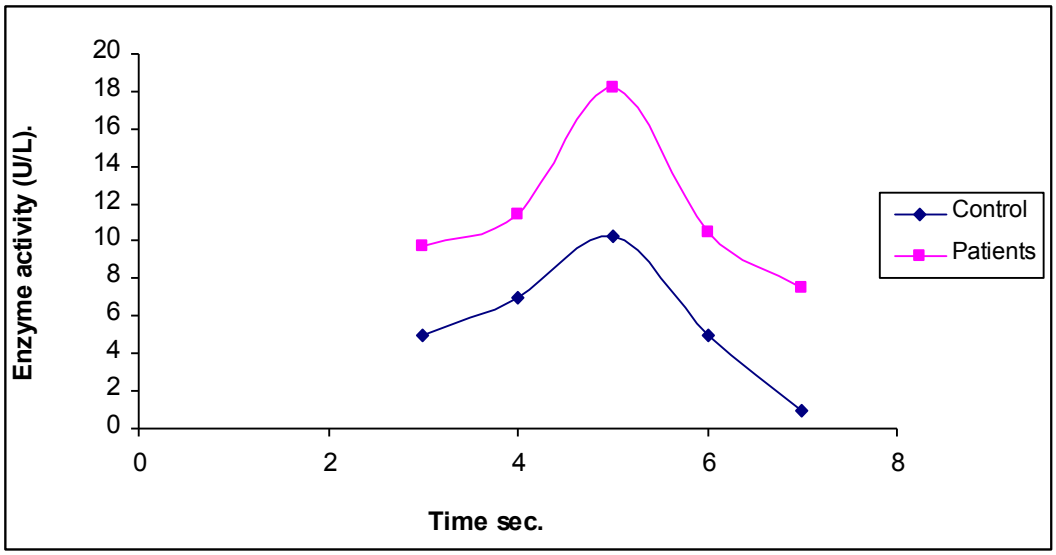

FIGURE 2. Activity of ACP with different time in diabetic patients and control.

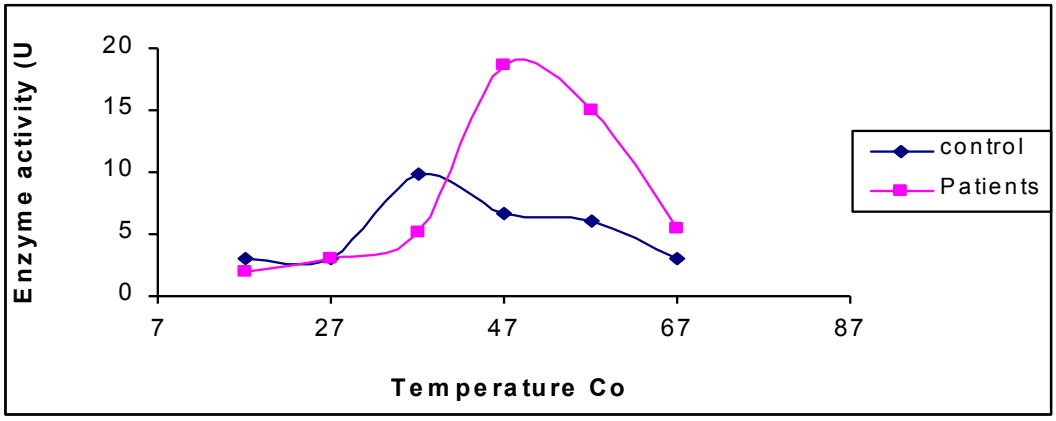

FIGURE 3. Activity of ACP with different temperature in diabetes patients and control.

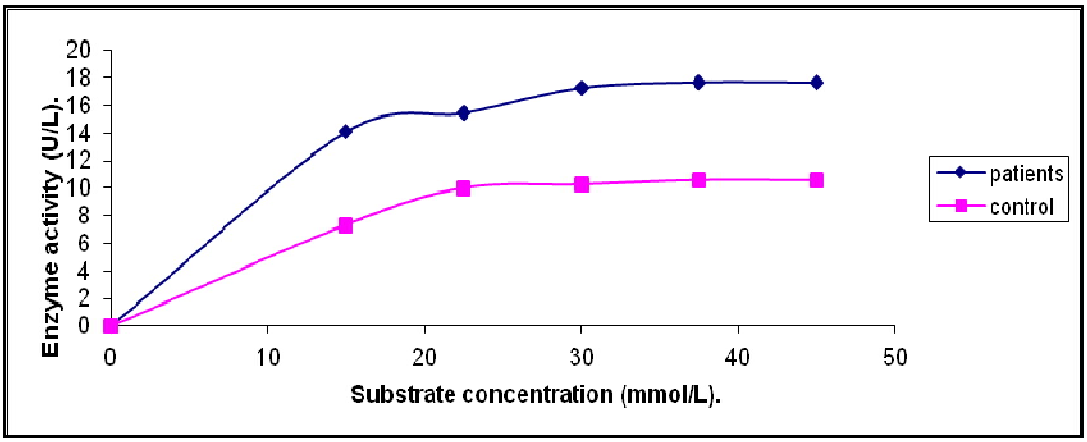

Figure 4: Activity of ACP with different concentration of substrate in diabetic patients and control.

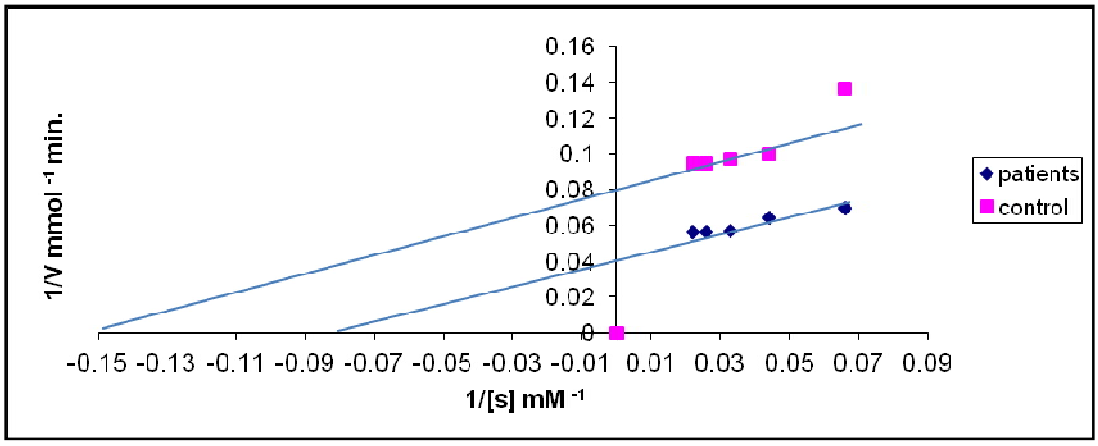

FIGURE 5 : Lineweaver-Burk plot.

TABLE3: The K_m and V_{max} for diabetic patients and control.

	K_m	V_{max}
Diabetic patients	12.5 mM	25 mM/min
Control	6.7 mM	12.5 mM/min

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