Biochemical and Kinetic Studies on Alkaline Phosphatase and other Biochemical Features in Sera of Patients with type 2 Diabetes

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Abstract:

Background :Alkaline phosphatase (ALP) was a widely used marker for skeletal and hepatobiliary disorders, but its activity was also increased in atherosclerosis and peripheral vascular disease. Several study has showed that ALP activity was increased in the sera of diabetic patients. The current study was conducted to evaluate ALP activity in type 2 diabetic patients and optimum conditions for enzyme activity in their sera.

Methods: This study was carried out at in AL-Yarmok hospital(diabetic center) between February /2009 and April /2009. Fifty two patients with type 2 diabetes have been enrolled. Besides BMI, WHR, serum fasting blood glucose, ALP, HbA_{1C},uric acid and lipid profile levels have been performed .The relationship between ALP and other biochemical factors have been studied.

Results: From a total 52 cases, FBG, HbA_{1C} and ALP were significantly elevated P value < 0.01 while Uric acid, Cholesterol, TG, HDL, LDL,VLDL and LDL/HDL were significantly different P value < 0.05 in diabetic patients when compared with that found in control group . ALP was significantly associated with LDL ($P < 0.05$) and significantly negative correlation with HbA_{1C} ($P < 0.05$) in diabetic patients. There was different in pH optimum, Incubation time, Temperature, when determination of them in diabetic patients and control.

Conclusions: The current study suggested that the different in ALP kinetic may be referred to another isoenzyme in sera of diabetes patients, and the present study suggested to seperate and characterize of ALP isoenzyme by using electorphoretic purification of enzymes.

Key words: Alkaline phosphatase, insulin-independent diabetes mellitus (T2DM), Lipid profile.

Introduction:

Type 2 Diabetes mellitus (T2DM) consists of heterogeneous conditions responsible for approximately 90% of all individuals with diabetes. It is often associated with central or visceral obesity, as well as other cardiovascular risk factors such as hypertension, and abnormalities of lipoprotein metabolism with the characteristic dyslipidemia of elevated triglycerides and low high-density lipoprotein cholesterol[1]. The (T2DM) is characterized by complex metabolic derangements, with two main abnormalities: insulin resistance and β-cell dysfunction [1]. Circulating insulin levels are higher than early in the disease to compensate for insulin resistance, but eventually, insulin production becomes less sufficient and hyperglycemia develops. This is

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illustrated by the typical progression of the disease that exhibits impaired insulin-mediated glucose utilization with postprandial hyperglycemia in its early stages[2]. Fasting hyperglycemia, the hallmark of T2DM, ensues at a later stage, secondary to the excessive and inappropriate hepatic glucose production. The capacity of insulin secretion in these patients is often enough to prevent ketosis and ketoacidosis, but still manifest during periods of severe stress or acute medical illness. This disease is closely related to obesity.[2,3]

Alkaline phosphates (ALP) [(E.C.3.1.3.1) orthophosphoric-monoester phosphor-hydrolase**]** orthophosphoric monoester phosphor-orolase] is a glycoprotein enzyme that hydrolyzed organic phosphate esters in alkali media. Optimal pH levels of these enzyme is generally about 10. [4].

It is a Zn metaloenzyme which is a glycoprotein, present in most body tissues, especially at or in the cell membranes, and it occurs at particularly high levels in intestine, kidney, bone (osteoblasts), liver, and placenta [5,6]. Although ALP displays a considerable intertissue and intratissue heterogeneity. Rarely more than two or three forms are found in any one serum sample, which are probably originates mainly in the liver, with up to half of the total activity coming from the skeleton[7].

The forms present in sera from patients with various disease have the characteristic of the specific forms present in liver, bone, intestine , plascenta, and very rarely, renal tissue[6]. In certain disorders of the liver and osteoblas bone diseases, the activity of serum ALP is reported to be increased[4,6].

An increased serum ALP may be due to : Congestion or obstruction of the biliary tract which may occur within the liver ,the ducts leading from the liver to the gallbladder ,or the duct leading from the gallbladder through the pancreas that empty into the duodenum (small intestine),any of these organs (liver ,gallbladder , pancreas ,or duodenum) may be involved [6] .

ALP activity is increased in the serum of diabetic patients [8,9,10], In contrast to diabetes mellitus (DM), starvation in rat is associated with a decrease in ALP activity which is reversed by re-feeding. [11,12,13].

The aim of study is to measure ALP activity in T2DM patients and determine the optimum conditions for ALP activity in T2DM patients.

Materials and Methods:

1.Subjects

Five ml have been collected from each subject by vein puncture, centrifuged at 3000 rpm for 5 min after allowing the blood to clot at room temperature.

Fifty two serum sample obtained from type II diabetic (26) males age (40-60) years (M±SD: 51.57 ± 6.88) and (26) females age (40-60) years (M±SD: 52.77 ± 7.2). The medical history has been taken, body weight and height have been measured and body .mass index (BMI) has been calculated[mean BMI 28.83 ± 4.66 Kg/m^2)].

The patient has been diagnosed by specialist doctors in AL-Yarmok hospital National Diabetes Center) .

For comparison, twenty seven apparently healthy men and women who were matched for age, weight, and BMI [n=27; age= (40-65) years (M±SD: 40.92± 5.77) ; BMI = (25.69 ±2.88)(kg/m^2); mean ± SD].

2. Protocol

Clinical variables, including , BMI are calculated as kg/m2.and waist-to-hip ratio (WHR), were

determined in all the subjects. Fasting serum glucose, uric acid ,cholestrol ,TG, HDL-cholesterol and LDL-cholesterol, level were measured by enzymatic method supplied by human Diagnostic. The activity of Alkaline phosphatase was measured in sera according to the method of king and Armstrong [14]. 4-amino antipyrine react with phenol in the presence of alkaline oxidizing agent to produce quinolol substitution product .This product give red color whose intensity was proportional to the phenol liberated.

pH optimum

Determination ALP activity with different pH(8 , 9 ,10 ,11 , 12) at 37 C^o, according to the procedure have been described by method of king and Armstrong [14] .

Incubation Time

ALP activity have been determined in different incubation time (0, 30, 60.90, 120, 150) second, according to the descriptive method of king and Armstrong [14] .

Temperature

The ALP activity have been determined in different Temperature (17, 27, 37, 47, 57) C^o according to the descriptive method of king and Armstrong [14].

Different substrate concentration

Different concentration have been prepared (5, 7.5 ,10 ,12.5 ,15) mM /L of substrate (p.nitrophenylphosphate) in buffer, according to the descriptive method of king and Armstrong [14].

Results and discussion:

The male group and female group with Diabetes mellitus were similar regarding to age ,BMI,WHR with no significant difference [P value >0.05] ,the characteristics of all subjects are shown in table1 .

Table 1 : Age, BMI, WHR in males and females with Diabetes .

	Male[Mean ± SD] [n=26]	Female [Mean ± SD] [n=26]	P value
Age	51.57 ± 6.88	52.77 ± 7.2	N.S.
BMI	28.83 ± 4.66	30.203 ± 2.33	N.S.
WHR	0.95 ± 0.05	0.96 ± 0.03	N.S.

Fasting blood glucose, HbA_{1C}, ALP, Uric acid, Cholesterol, TG, HDL- Cholesterol, LDL- Cholesterol, VLDL and LDL/HDL in patient and control are summarized in table 2.

Table 2: Biochemical variables in patients and control .

Biochemical variables	Patients [Mean ± SD] [n= 52]	Control [Mean ± SD] [n=27]	P value
FBG [mg/dl]	203.19 ± 75.16	90.59 ± 6.7	< 0.01
HbA_{1C} %	9.72 ± 1.70	3.98 ± 0.68	< 0.01
ALP [IU/L]	96.81± 44.69	70.68 ± 16.93	< 0.01
Uric acid[mg/dl]	5.29 ± 1.31	4.24 ± 0.044	< 0.05
Cholesterol[mg/dl]	218 ±16.56	187.11 ± 7.28	< 0.05
TG[mg/dl]	143.65 ±26.88	99.44 ± 25.44	< 0.05
HDL[mg/dl]	45.92 ± 3.17	60.00 ± 5.00	< 0.05
LDL [mg/dl]	143.67 ± 12.89	114.15 ± 13.14	< 0.05
VLDL[mg/dl]	28.75 ± 5.36	18.52 ± 2.15	< 0.05
LDL/HDL	3.14 ± 0.44	2.40 ± 0.31	< 0.05

Table 2 showes a significant difference in FBG, HbA_{1C} and ALP with P value < 0.01, while Uric acid, Cholesterol, TG, TG, HDL, LDL.VLDL and LDL/HDL were found to be significantly different with P value < 0.05 .

There was a significant positive correlation between ALP and LDL [P< 0.05] only in patient as shown in figure 1, While there was no significant correlation in control, this may be explained by the metabolic effect of DM on the liver .

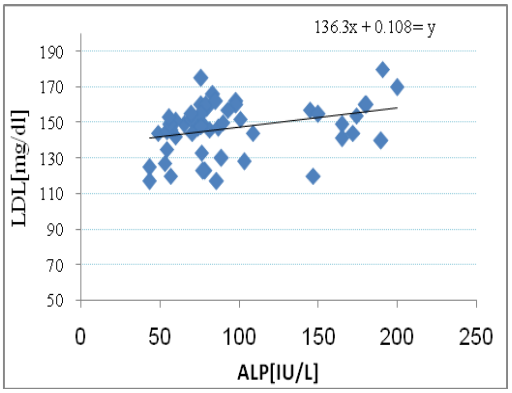


Fig 1: Correlation between ALP and LDL

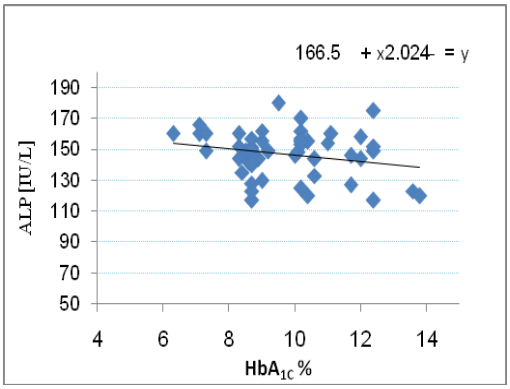
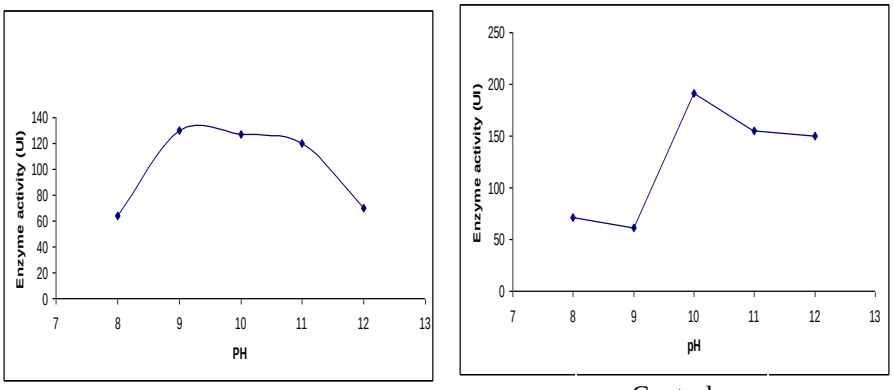


Fig 2: Correlation between ALP and HbA_{1C} %

There was a significant negative correlation between ALP and HbA_{1C}[P< 0.05] only in patient as shown in figure 2 ,While there was no significant correlation in control, this correlation may be refer to the effect of hyperglycemia on metabolism of liver in diabetic patients.

pH optimum

The pH (8 , 9 ,10 ,11 , 12) effect have been studied on ALP activity . Figure 3 showed that highest enzyme activity in diabetic patients was at PH 9, while in control was PH 10.



Patients Control

Fig. 3: Activity of ALP with different pH in diabetic patients and control

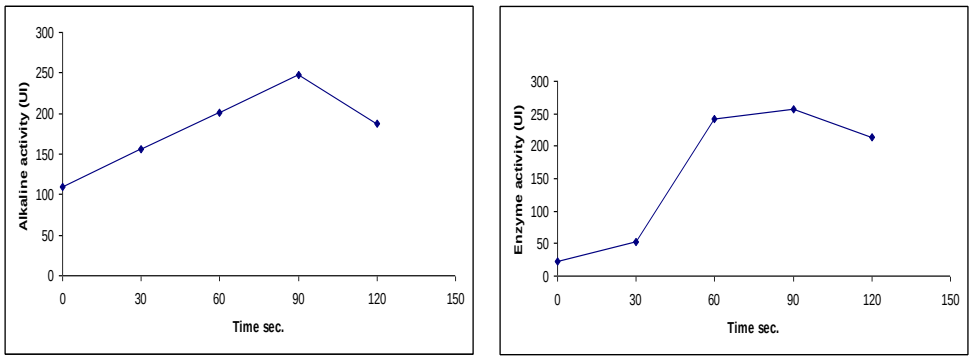
The decrease in ALP activity at acidic pH due to effect of PH environment of reaction in ionic groups which found in active site or changing in ionic state for substrate or complex enzyme-substrate when the concentration of substrate over than Michaelis constants (K_m) ,if the substrate concentration is little, it will depend on enzyme [15] . Other study refers to pH optimum of ALP is 9 at 37 C^o[16] .

The ketone bodies cause different pH in serum patient with type 2 diabetes [17] .The concentration of H^+ affects velocity in several ways .First, the catalytic process usually requires that the enzyme and substrate have in order to interact .The current study suggested that these deferent in PH optimum of ALP may be referred to another isoenzyme of ALP in sera of diabetes patients [18] .

Incubation Time

The enzyme has been incubated in different time (0, 30 ,60 .90,120, 150) second and determination of enzyme

activity was shown in figure 4 .Both diabetes patients and control have produced the highest activity of ALP when incubate for 90 second at 37C^{o}.

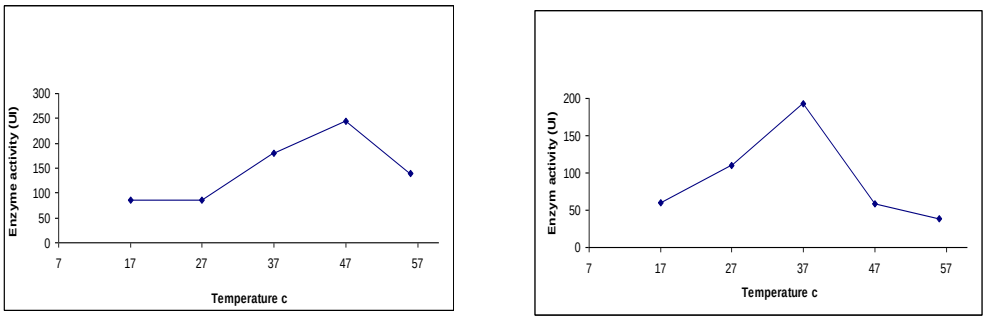


Patient Control

Fig 4: Activity of ALP with different time in diabetic patients and control

Temperature

In diabetes patients ALP activity increases according to the incubation temperature until it reaches maximum at 47 C^{o}, while ALP activity begins to increase until it reaches maximum at 37C^{o} in control as shown in figure 5.



Patient Control

Fig.5: Activity of ALP with different temperature in diabetes patients and control

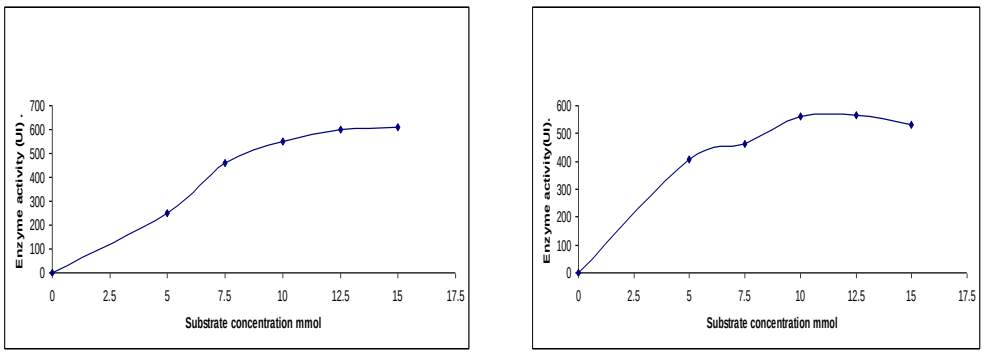
The rapid of reaction with temperature due to increasing kinetic energy of enzyme ALP and substrate which cause making complex – enzyme –substrate [19]. These results are in agreement with [20] in its report optimum temperature of ALP is 37C^{o}.

The reaction velocity increases with temperature until a peak velocity it results from increased number of molecules having sufficient energy to pass the energy barrier and from the products of the reaction .Further elevation of the temperature results in a decrease in reaction velocity as result of temperature – induced denaturation of the enzyme [21,22]. The current study suggested that these deferent in PH optimum of ALP may be referred to another isoenzyme of ALP in sera of diabetes patients, and the present study suggest separation and characterization of ALP isoenzyme by using electorphoretic purification of enzymes.

Different Substrate Concentration

Determination of ALP activity with different substrate concentration (5,7.5, 10, 12.5, 15) mM p-nitrophenylphosphate, and studying this concentration on rate of ALP reaction.

In diabetic patients ALP activity increases according to substrate concentration until it reaches maximum at 47 C^o to 3.7 mM, while ALP activity begins to increase until it reaches maximum at 37C^o in control in 3.4 mM of substrate concentration as shown in figure 6. By using Lineweaver-Burk plot ,the K_m and V_{max} has been found as shown in table 3 and figure 7.



Patients Control

Fig. 6: Activity of ALP with different concentration of substrate in diabetic patients and control

Table3:The K_m and V_{max} for diabetic patients and control.

	K_m	V_{max}
Diabetic patients	3.7 mM	714.6 mM/min
Control	3.4 mM	689.2 mM/min

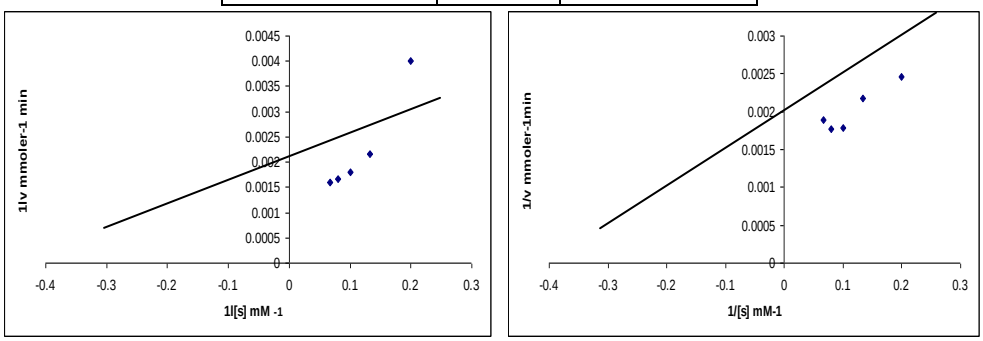


Fig 7: Li neweaver-Burk plot.

There are many studies that deal with K_m and V_{max} for ALP from different sources, since report refers to ALP which was taken from raw milk sources it has got K_m 0.0034 mM and 0.0056 mM for disodium phenal phosphate and B-glycerophate respectivety [23] .

The K_m values for healthy serum ALP were (0.11mM)from bone sourse ,(0.9 mM) from intestinl mocose sourse , and K_m values for cancer patients were (0.11mM)from intestinl mocose sourse , 0.074 from liver sourse [24].

The K_m value of ALP for PNPP has been estimated to be 0.036 mM From *T.caldophius A.pass* [25]. The current study suggested that these different in PH optimum ,Incubation Time, temperature and optimum concentration of substrate of ALP

may be referred to another isoenzyme of ALP in sera of diabetes patients, and the present study suggested separation and characterization of ALP isoenzyme by using electorphoretic purification of enzymes.

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دراسة كيمائية حياتية حركية لانزيم الالكلاين فوسفتيز وبعض العوامل الحياتية في امصال المرضى المصابين بالداء السكري النوع الثاني

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الخلاصة :

خلفية البحث: يعتبر الالكلاين فوسفتيز ALP من الانزيمات المستعملة بشكل واسع كمؤشر للاضطرابات الكبدية والعضلات ايظا قد تحصل زيادة في مستواه عند الاصابة بتصلب الشرايين وامراض الاوعية الدموية المحيطة . اشارت الدراسات الى زياده في فعالية انزيم ALP عند المرضى المصابين بالداء بالسكري . تهدف الدراسة الحالية الى قياس فعالية ALP وبعض العوامل الكيميائية الحياتية عند المرضى المصابين بالسكري النوع الثاني فضلا عن دراسة الظروف المثلى لفعالية الإنزيم عند المصابين بالمرض

طريقة العمل :اجريت هذه الدراسة بعد اخذ نماذج الدم من مركز الداء السكري في مستشفى اليرموك للفترة من شهر شباط 2009 ولغاية نيسان 2009 ،وقد تم دراسة مستوى سكر الدم الصيامي عند 52 مريض وكذلك تم قياس ALP، HbA_{1C} و Uric acid ومستوى الدهون الكلي إضافة الى حساب BMI,WHR ومقارنتها بمجموعة الضبط المكونة من 27 شخص من الاصحاء وكذلك تم دراسة العلاقة بين الانزيم وبقية العوامل المقاسة في هذه الدراسة .

النتائج : لوحظ وجود زيادة مقبولة احصائيا في فعالية ALP فضلا عن HbA_{1C} و Uric acid ونسبة الدهون الكلية. وقد لوحظ حدوث تغير بالظروف المثلى للفعالية الانزيمية عند المصابين عند مقارنتها بالاصحاء. وقد اشارت الدراسة الى وجود علاقة خطية طردية مقبولة احصائيا بين ALP و LDL في حين ارتبط ALP, HbA_{1C} بعلاقة خطية عكسية مقبولة احصائيا عند المصابين بالمرض.

الاستنتاج : الاختلاف في حركيات انزيم ALPعند المصابين بالداء السكري النوع الثاني ربما تعود الى وجود متناظر اخر للانزيم عند المصابين بالمرض وتقترح الدراسة الحالية الى فصل متناظرات الانزيم عند المصابين بالمرض ودراسة خواص كل متناظر اضافة الى اجراء الترحيل الكهربائي لمتناظرات الانزيم عند المصابين بالمرض.