

Separation of Creatine Kinase isoenzymes in the serum and white blood cells and detection it's activities in the white Rabbits with induced Diabetes

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Abstract

Diabetes Mellitus is a common disease caused by either a deficiency in the secretion of insulin from β -cells or abnormal insulin receptor will lead to physiological and biochemical changes in different part of the body including the blood, there fore this study was included 22 rabbits induced diabetes mellitus (DM) in which injuction the alloxan under skin. Isoenzyme of creatine kinase (MM-CK, BB-CK and MB-CK) were separated by column of DEAE-cellules gel on serum of 22 normal rabbits and the same number with induced DM.

The aim of this project is seperation Isoenzyme of creatine kinase (MM-CK, BB-CK and MB-CK) from serum and (WBCs) and determination the effected level influenced by oxidative stress rabbits induced (DM),and we showed that the activity of MM-CK and MB-CK were influenced with DM. The BB-CK was resistance the changes in concentrations with the control group.

Before starting the evaluation, it was prepared the (WBCs) from the rabbits (22 control and 22 patients).

In isoenzyme of creatine kinase (MM-CK, BB-CK and MB-CK) results showed that the diabetes mellitus as more effective on MM-CK and MB-CK(74.6 \pm 14)and,(6.3 \pm 1.2), respectively, than on the BB-CK (2.6 \pm 0.5).

The results of the study showed that there are significantly decreased in level of activity of creatine kinase in patients (for serum 80.6 \pm 22) IU/L compared with controls(105.5 \pm 38) IU/L in both serum and, (WBCs) .

Finally, this study was illustrating that there were no significant differences ($P \leq 0.01$) between the levels of serum and (WBCs) in each groups for the parameters studied (isoenzyme creatine kinase)

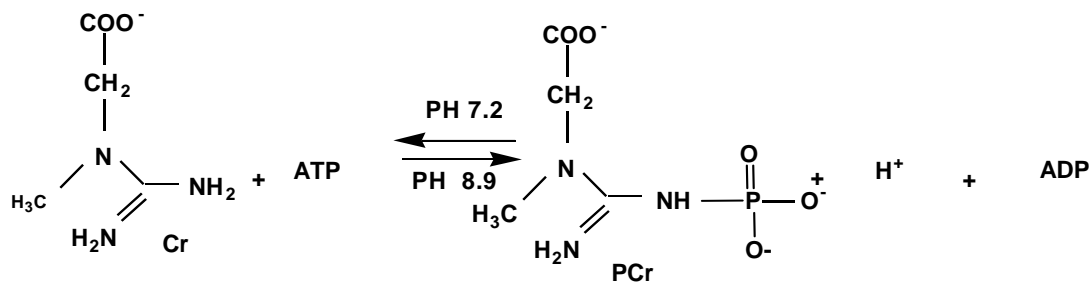
		(CK)	
	(105.5±38)		(80.6 ± 22)
(79±12)		(100±36.7)	
	BB-CK	MB-CK	MM-CK
BB-CK	(6.3±1.2)	(74.6±14) IU/L	
			(2.6±0.5)
(P			"
			≤ 0.01)

Introduction

Creatin kinase(CK)(EC.2.7.3.2) is classified as one of transferase family of enzymes that transfer a phosphate group to nitrogen group as a acceptor also, it was called creatine phospho kinase. ⁽¹⁾

Creatine was reported to be more sensitive indicator than blood urea ⁽²⁾ in the testing of kidney function, cratinine is anhydrous creatine formed from elaboration of

phosphate group of phaspho -ration , then pass to kidney through blood to exerted with urine. the concentration of creatine in the blood and urine is proportion to the muscle size and it is not affected by diet-courante. It is concentration found to be constant along 24 hrs, for this reason it was consider to be the best indicator of kidney function as shown in equation below. ⁽³⁾



CK widely distributed in human body. It has three isoenzymes, that located in deferent tissues, (CK-BB), (CK-MM) and (CK-MB).⁽³⁾

Material and methods

Fourty four experimental rabbits was grouped into two groups 22experimental rabbits as a control group and the other 22 rabbits as a test group.

Animals subject to present study were males with 4 – 9 months age its weight was (750-1750) gm.

Test group was fasted for 18 hrs, then injected under skin with 150 mg/kg body weight of alloxan dose freshly prepared ,this process was repeated for 3dayes to 450 mg /kg body weight as a total dose.

The experimental rabbits was feeding with 50% glucose solution given with drinking water in the first day after alloxan injection , the level to set and take enough of eating and drinking .⁽⁴⁾

The rabbits of control group was anaesthetized (that live in animal house for (10-15)days and eat a plant) by chloroform smelling ,the blood was with drawn by fine needle .

The amount of with drawn blood was (10-17)ml, also blood was with drawn from rabbits with DM after a week from alloxan injection .⁽⁵⁾

Isolatio process of leucocyte cells:

With drawn blood leave at room temperature for30-60 win to precipitation of RBC, a buff coat was formed between serum (the upper) and RBC (the lower).

The RBC was separated after the addition of dextrin by formation an upper ring with a plasma and buffy coat .

Upper layer was isolated that contain WBC in a test tube then kept in refrigerator until use.⁽⁶⁾

Purification and digestion process of leukocyte cells :

10 ml of blood was taken and put in centrifuge tube (10ml size)2 ml of freshly prepared dextrin and well mixed by vertex leave to 45 min to precipitate the cells and RBC.

Supernatant was drain in another centrifuge tube and centrifuged at (500 x g) for 10 min. The precipitate is represent the WBC.

Washing process was repeated to washing WBC by adding 1ml of 0.1 g/100ml Nacl and 3 ml of iced distilled water then mixed well, after this 3ml of cooling solution of 1.8 g/100ml Nacl and centrifuged at (500 x g)for 10 min, then supernatant was isolated and drained. The forming precipitate is WBC.

WBC was broken by use of (1.5ml) triton x-100 solution of precipitate with mixing ,then put in beaker contain iced water for five time to get the broken WBC.

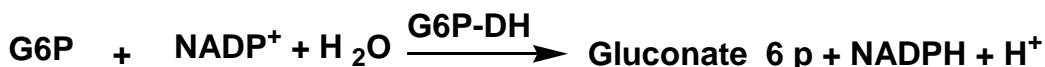
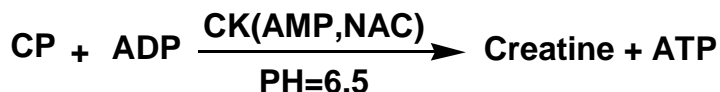
Separation of CK isoenzymes from sera by ionexchange chromatography:

Mercers method was used to separation CK isoenzyme using EDAAE-Cellulose instead of DEAE-Sephadex-A-50 due to high sensitivity and specificity to CK isoenzym. ^(7, 8)

Measurement of CK activity in seram and WBC:

CK catalyzed the reaction between creatine phosphate with ADP formation of creatin and ATP. Glucose present in the kit phosphorate oxidized to glucorate-6-phosphate by the action of 6-phosphate dehydrogenase and in the presence of NADP as a coenzyme that convert to NADPH.

The increase absorption of NADPH at340 nm was measured which proportion to the CK activity



Results and Discussion

CK activities found to be significantly decreased in sera and

WBC of rabbit with DM than those of control group as shown in table 1

Table (1) CK activity (V/L) in sera and WBC of diabetic rabbits and control

		Mean	SD	CK U/L		SE	95% C.I.		P	Sign
				Upper	Lower		Upper	Lower		
Control (22)	Serum	105.5	38	169	54	8.1	-----	-----	-----	-----
	WBC	100	36.7	176.4	66	7.8	-----	-----	-----	-----
Rabbit with DM (22)	Serum	80.6	22	120	54	4.7	43.7	6.2	0.001	Sign
	WBC	79	12	107.7	66	2.7	36	4.7	0.001	Sign

The notice increment in the standard deviation may attributed to the anesthesia are injection of fine needl. ⁽⁹⁾

The symptoms of Dm was appear of Rabbits after 7 days from alloxan injection, by using indicator table on animal urine.

Then the conformation of DM is occur by measuring the blood glucose .The rabbits that reveal >300 mg/100ml as concentration of blood glucose were consider to be diabetic and show sever weakness and multi urination. ⁽¹⁰⁾

CK activity always almost consider to be a biomarker in diagnosis the abnormalities in skelatl and cardiac muscles. ⁽¹¹⁾

Several studies had been reported that CK activity decreases in the sera of patients with DM due to complication of abnormality of cardiac muscle ^(12,13,14) uted this depletion in to

the CK activity to reaction of enzyme with ROS. ⁽¹⁵⁾

The elevation in ROS in patients with DM cause a depilation in CK activity in both serum and WBC . ^(15,16,17) where :

1. ROS react with enzyme and other biomolecules and effected on their stricture and function that due to deference in their pathological condition .Where the site effect of CK contain cystine which consider to be key of enzyme activity and substrate binding site . ⁽¹⁸⁾

Cystiene reports to be the target or ROS which generated in the status of hyperglycemia causes an oxidation in thiol group of active site due to impairment of CK.

Several studies found O₂· and OH· To have reversible inhibition on enzyme activity , whereas the inhibition impact found for ONOO· To be irreversible. ^(19,20,21)

The enzyme super oxide dismutase (SOD) has a reversible action to the O_2^- and OH^- Radicals where acts to scavenging and convert them to H_2O_2 , this process consider to have protective role to CK. ⁽¹⁵⁾

This protection came for two reason; First SOD reduce the oxidized thiol group of active site, second SOD can react directly with ROS which mean give rise to active site to do his role. ^(15, 22)

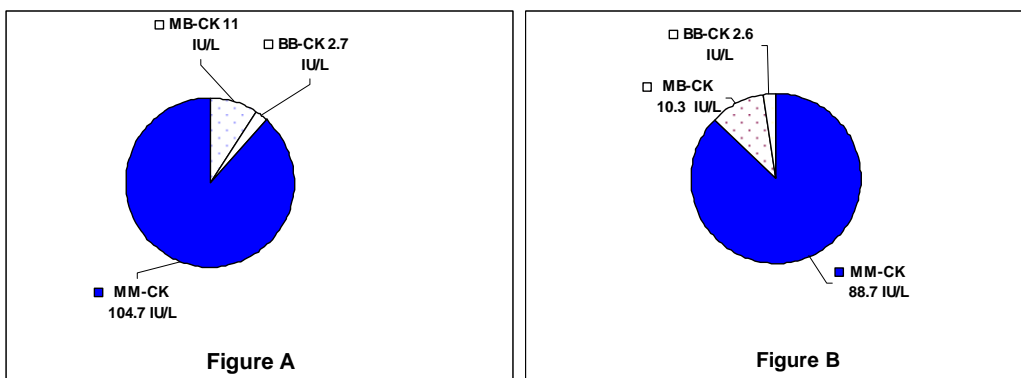
2. Alloxan and streptozotocin widely use in induce of DM in experimental animal by reacting the nitric oxide with Bcell, this was suggested by ⁽²³⁾ and ⁽²⁴⁾, where they notes that the rise in RNS in DM patients lead to rise nitric

oxide which is act against CK action by decrease CK activity by nitrosylation of the thiol groups that CK have eight of them. ⁽²⁵⁾

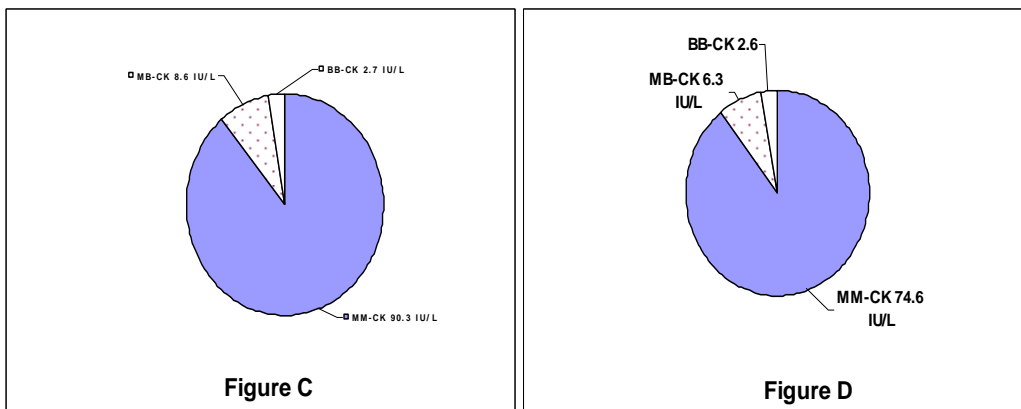
3. Hassan Mekhfi et. al. Show that CK consider to be the main target of RNS. ⁽²²⁾

All previos notes confirms the decrease of CK activity as a result of ROS and RNS on thiol group but its quantity seem to be an changeable. ⁽¹⁸⁾

In the present study, the activity of CK-MM RNA found to be decrease 61.1% when compared with control due to decrease in CK-MM (patient depletion) ⁽¹²⁾ where as ,CK-BB was constant as show in figure 3 and 4.



Figure(1)A. CK activity (V/L)in sera of control group of rabbits and B. CK activity (V/L)in sera of diabetic rabbits



Figure(2)C. CK activity (V/L)in WBC of control group of rabbits and D. CK activity (V/L)in WBC of diabetic rabbits

Conclusions

1. The differences in CK activity can be used to conclude the effect of DM on rabbits.
2. The effect of DM on CK isoenzyme found to be high in CK-MM and CK-MB were than CK-BB.

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