Cu\Zn SOD enzyme activity, partial purification from patients with diabetes type 1

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Abstract

Antioxidants are agents that prevent or reduce the level of oxidative damage to biomolecules in the living organisms. Antioxidants can be enzymatic or non-enzymatic. An enzymatic antioxidants such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) predominantly have metal chelators. Cu/Zn SOD enzyme, reacts with superoxide radical to form H2O2, which will get converted to H2O. In this study, (Cu\Zn)SOD was isolated from human RBCs using DEAE-cellulose chromatography. The enzyme was purified from patients with diabetes type 1 and normal people as well .The (Cu\Zn)SOD activity was measured in the serum of 20 samples of patients with diabetes and 20 normal samples

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Keywords: Antioxidant enzymes; superoxide dismutase (SOD), DEAE-cellulose chromatograph.

Chemistry Classifiication QD415 -436

Introduction

Diabetes is one of the most common chronic diseases . There is a growing scientific and interest in linking of oxidative stress with so many clinical conditions including diabetes mellitus (DM), oxidative stress plays a major role in the development of complications of both types of DM. Hyperglycemia induces free radicals as well as impairs the endogenous antioxidant defense system in patients with diabetes. Endogenous antioxidant defense processes include both enzymatic and non-enzymatic pathways. Their functions in human cells are to counterbalance toxic materials such as reactive oxygen species (ROS). Antioxidants may include vitamins A, C, and E, glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRx). {1}

The enzyme Cu\Zn SOD catalyzes the dismutation of the superoxide radical to oxygen and hydrogen peroxide {2}, Cu/Zn SOD of human RBCs has a molecular mass of 32000 Da and contains two equal subunits each containing one Cu2+ and one Zn2+ in their active site. Superoxide radical can result in many harmful physiological effects and many studies focused on the therapeutic value of Cu\Zn SOD on those effects. {3}.

Methods:

- **Samples**

This study included two groups of samples. Group (1) contained (20) patients with diabetes 10 samples for each gender. Group (2) consisted of (20) healthy persons as control (10 males, 10females). Serum was used to assay the enzyme activity. Cu\Zn superoxide dismutase enzyme was isolated and purified from erythrocytes.

- **Determination of Enzyme Activity**

Enzyme activity was determined according t the ability of the enzyme to inhibit and reduce the autoxidation of pyrogallol. {4}.

- **Estimation of Total Protein**:

The total protein of samples was estimated by Lowry method {5}.

- **Separation of Enzyme from Erythrocytes:**

Erythrocytes were separated using Djalali method {6}.

- **Statistical analysis**

The findings were expressed as the mean ± standard deviation. The data were analyzed with student's independent *t* test then performed with the program Statistical Package for the Social Science (SPSS). A *P* value of <0.05 is accepted as a significant results {7}.

Results:

- **Antioxidant Enzymes:**

The mean values ±SD of sera superoxide dismutase (SOD) activity in control and patients were explained in table (1).

Groups	Sex	No.	Mean	±SD
No. = 20			Control	
SOD (U/ml)	M	10	5	1.23
	F	10	6.8	2.3
No. =20			Patient	
SOD (U/ml)	M	10	26.32**	18.12
	F	10	33.12**	13.96

Table(1):Superoxide Dismutase (SOD) activity

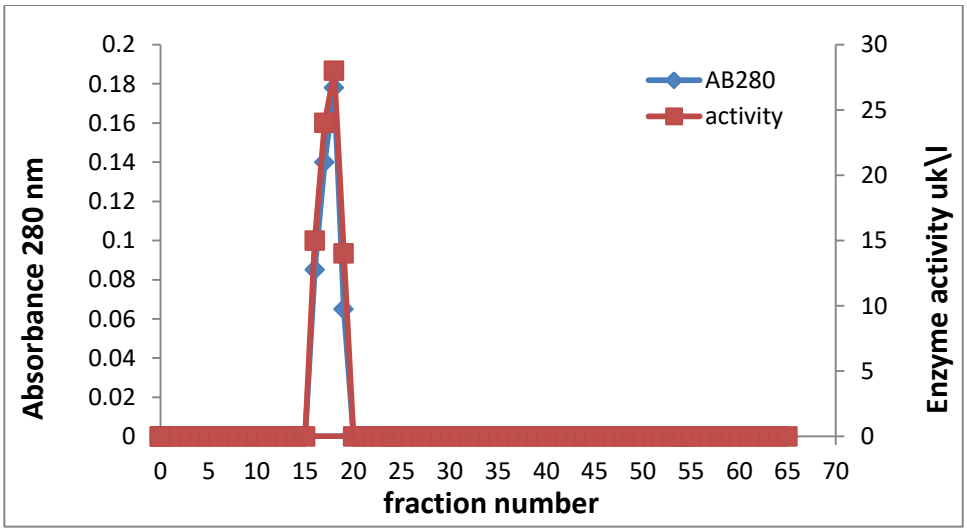
in Sera of Controls and patient with both hypertension and diabetes (M = male , F = females , significant (p <0.05).**

- **Separation and Purification of Enzyme from Erythrocytes**

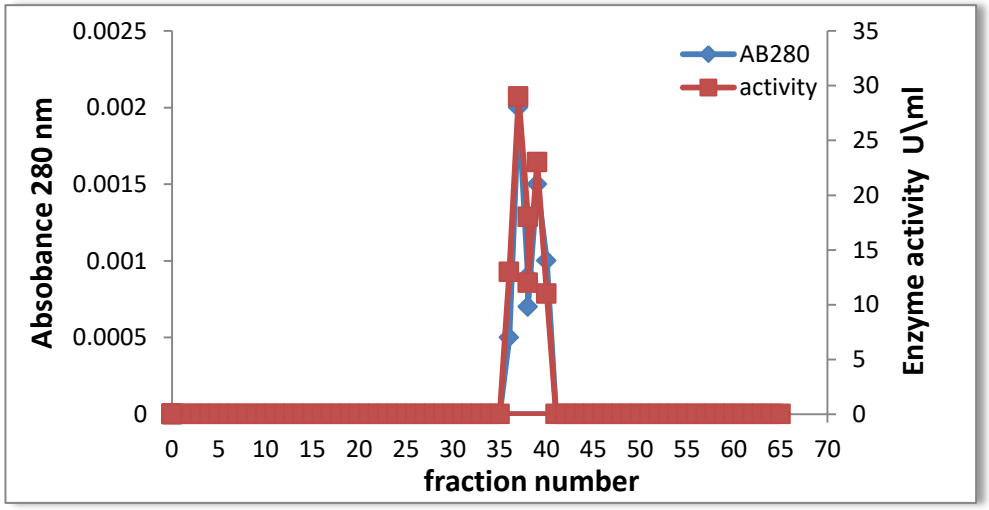
Superoxide dismutase was separated and purified from human erythrocytes samples of both control and patient with diabetes . The purification steps of enzyme from control and patient with diabetes are mentioned in table (2) and (3) respectively. (figure 3, 4)

Sample	*Vol. ml*	*Total SOD (U)*	*Tota0l Protein (mg)*	*Specific Activity (U/mg)*	*Yield%*	*Fold of Purification*
Hemolysate	**14**	**35**	**1.32**	**1.89**	**100**	**1**
After ethanol-chloroform addition	**5**	**15**	**0.80**	**3.75**	**42.8**	**1.9**
After K2HPO4 addition	**3**	**18**	**1.31**	**4.59**	**51.4**	**2.5**
After acetone addition	**3.5**	**12**	**0.70**	**6.89**	**34.3**	**3.7**
Ion exchange wash step	**3**	**24**	**0.30**	**26.6**	**68.6**	**14.08**
Elution step Peak 1	**3**	**18**	**0.120**	**50**	**51.5**	**26.5**
Peak 2	**3**	**13.5**	**0.113**	**39.8**	**38.5**	**21.06**

Table(2):Purification Steps of Human Erythrocytes SOD from Control



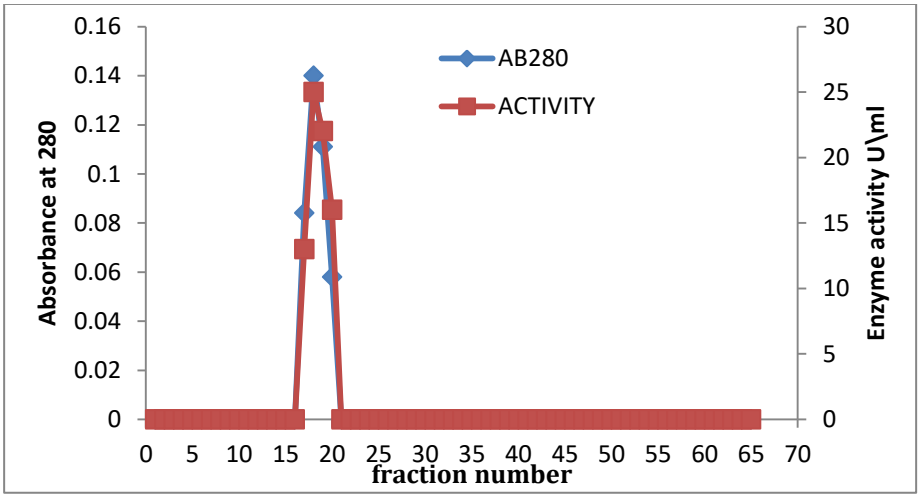
Figure(1): Ion exchange chromatography using DEAE-cellulose column (2.6×10.5)cm, for the purification of Cu\Zn SOD from human erythrocyte of control equilibrated with 1.5 mM and pH 6.8 phosphate buffer in wash step , flow rate 60ml/hr and fraction volume 3ml.



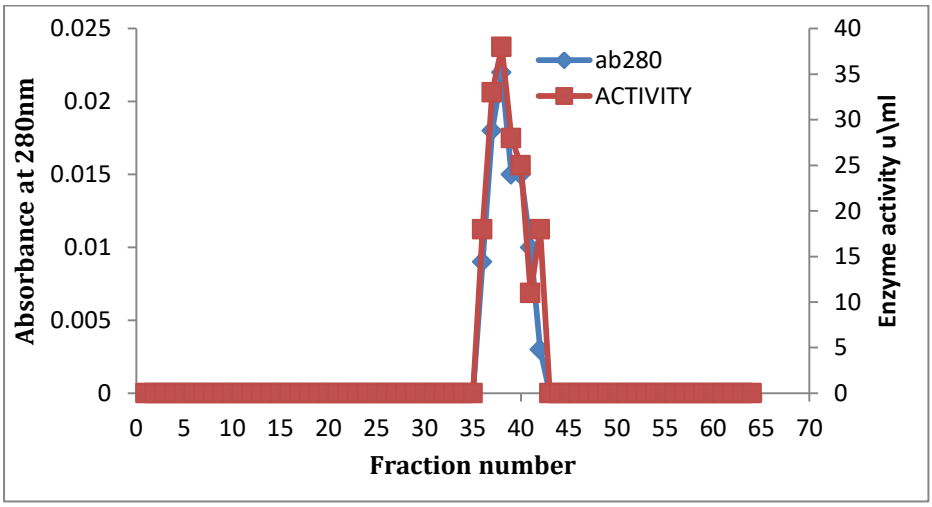
Figure(2): Ion exchange chromatography using DEAE-cellulose column (2.6×10.5)cm, for the purification of Cu\Zn SOD from human erythrocyte of control equilibrated with 20 mM in and pH 6.8 phosphate buffer elution step, flow rate 60ml/hr and fraction volume 3ml.

Sample	*Vol. ml*	*Total SOD (U)*	*Total Protein (mg)*	*Specific Activity (U/mg)*	*Yield%*	*Fold of Purification*
Hemolysate	**12**	**36**	**2.2**	**1.36**	**100**	**1**
After ethanol-chloroform addition	**6**	**27**	**0.8**	**5.7**	**75**	**4.20**
After K2HPO4 addition	**4**	**28**	**1.011**	**6.93**	**77.8**	**5.09**
After acetone addition	**3.5**	**21**	**0.520**	**11.54**	**58.4**	**8.48**
Ion exchange wash step	**3**	**24**	**0.330**	**18.19**	**66.7**	**13.4**
Elution step Peak1	**3**	**18**	**0.112**	**53.57**	**50**	**39.4**
Peak2	**3**	**15**	**0.118**	**42.38**	**41.7**	**31.17**
Peak3	**3**	**12**	**0.112**	**35.72**	**33.4**	**26.3**

Table(3): Purification Steps of Human Erythrocytes SOD from patient



Figure(3):Ion exchange chromatography using DEAE-cellulose column (2.6×10.5)cm, for the purification of Cu\Zn SOD from human erythrocyte of patient with both hypertension and diabetes equilibrated with 1.5 mM and pH 6.8 phosphate buffer in wash step , flow rate 60ml/hr and fraction volume 3ml.



Figure(4):Ion exchange chromatography using DEAE-cellulose column(2.6×10.5)cm, for purification the Cu\Zn SOD from human erythrocyte of patient with hypertension and diabetes equilibrated with 20 mM and pH 6.8 phosphate buffer in elution step , flow rate 60ml/hr and fraction volume 3ml.

Discussion :

The superoxide dismutase activity was measured by the pyrogallol assay. The values of the enzyme activity are demonstrated in table (1). There was an increment in the activity of serum SOD in patients with diabetes. The superoxide ion radicals might affects the enzyme activity only in predisposed organs. Therefore; this suggests that the enzyme activity in the blood may replicate the anti-oxidative defense of the whole organism. The enzyme SOD breakdowns of superoxide anion and it acts as a first line of defense against free radical toxicity. {8}.

Diabetes mellitus was a group of metabolic diseases. Hyperglycemia is explained as a defects of insulin action, insulin excretion or both. Hyperglycemia leads to many diseases such as the development of microvascular complications, and diabetic patients are more disposed to accelerated atherosclerotic macrovascular disease. {9}

oxidative stress plays an important role in the pathogenesis of DM and its complications which lead to the impairment of the main processes that fail during diabetes, insulin action and insulin secretion. In addition, antioxidant mechanisms are decreased in diabetic patients {10}.

Many studies was showed the presence of oxidative stress in DM patient as increase free radical creation, free radicals have short lifetime in plasma, so their presence is determined by their effects on other molecules or enzymes status. Increasing of SOD enzyme activity is a

clear indicator of the presence of oxidative stress {11}.

Also, as shown in the table (1) males have a lower SOD activity level compared with females these differences may be related to the sex hormones, especially those which developed and reached the onset of maturity and work effectively {12}.

Figure (1) showed one peak for protein and the other one for enzyme activity in wash step, (Cu\Zn) SOD enzyme carries a positive charge similar to that of ion exchange in the experimental conditions. Figure (2) showed appearance of two peaks of protein when it was eluted by the phosphate buffer (20mM, pH 6.8) and two peaks of enzyme activity with different specific activities but higher than the specific activity for enzyme in wash step.

Purification of enzyme of patient samples in wash step shows appearance of one peak for protein and one peak for enzyme activity, while in the elution step, as in Figure (4), showed appearance of three peaks of protein when it was eluted by the phosphate buffer (20mM, pH 6.8) and three peaks for enzyme activity with different specific activities as in table (3), which is ignored, results showed another isoenzyme of SOD enzyme, many researches show that erythrocytes have up to seven isoenzymes of Cu\Zn SOD {13}.

Conclusions

1-patients with diabetes incited increase in SOD activity.

2-It may be possible that oxidative stress plays a larger role in DM patients.

3-The purified (Cu\Zn) SOD enzyme from control and patients had isoenzymes because the two forms of enzyme are formed during the purification by Ion exchange chromatography.

4-The (Cu\Zn) SOD enzyme that purified from patient was demonstration different result than control in the elution step of ion exchange chromatography which suggest Changed in the charge of patient enzyme

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دراسة فعالية انزيم Cu\Zn SOD، تنقية جزئية من مرضى السكري النوع الاول

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الخلاصة

مضادات الاكسدة عبارة عن مركبات تقوم بمنع او التقليل من الاضرار الناتجة عن تأكسد الجزيئات الحياتية في الكائنات الحية. تكون هذه المركبات انزيمية او غير انزيمية. مضادات الاكسدة الانزيمية مثل catalase او superoxide dismutase (SOD) اوglutathione peroxidase (GPx) غالباً ما تحتوي على كلابات فلزية. انزيم Cu/Zn SOD يتفاعل مع O_2^- لتكوين بيروكسيد الهيدروجين والذي يتم تحويله لاحقاً الى ماء. في هذه الدراسة تم عزل انزيم Cu\Zn)SOD) من كريات الدم الحمراء من الانسان باستخدام كروموتوغرافيا DEAE-cellulose. ثم تمت تنقية الانزيم الذي تم عزله من اشخاص يعانون من السكري النوع الاول واشخاص سليمين. بعد ذلك تم قياس فعالية الانزيم في 20 عينة للأشخاص المصابون و20 عينة اخرى للأشخاص السليمين.

الكلمات المفتاحية: مضادات الاكسدة، Cu/Zn SOD، كروموتوغرافيا.