Purification of Arylesterase and study the Relationship with some biochemical parameters in type I Diabetic pateints

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Abstract:

The research included determination of Arylesterase activity and its relationship with some biochemical parameters of Type I diabetic patients serum. Significantly decreasing ($p<0.05$) activities of antioxidant enzymes, namely, Arylesterase, Glutathion-S-transferase, Catalase and lipoprotein lipase activities, and non enzymatic antioxidants levels, Glutathione, vitamin C levels, as well as insulin hormone , while non significantly decreasing in Glutathione peroxidase activity, vitaminE and Ceruloplasmin were found in type 1 diabetic patients as compared to control. Our results were demonstrates a significantly increasing ($p<0.05$) in Lypoxygenase, Polyamine Oxidase, Superoxide Dismutase and Xanthine Oxidase activities, in addition to Glucose, Malondialdehyde, urea, creatinine, glycosylated hemoglobin and insulin resistance in patients compare to healthy subjects.

There was a significant positive correlation between ARE and Glutathione peroxidase (r=0.421), Ceruloplasmin (r=0.525), glutathione (r=0.411, vitamins C(r=0.735) and E(r=0.611), while it was a significantly negative with Xanthine Oxidase (r= -0.597), glucose(r= -0.721), Malondialdehyde (r= -0.834) and HbA1c(r=- 0.843).

The partial purification of Arylesterase from serum of Type I diabetic patients was studied by using dialysis, diethylaminoethyl cellulose ion exchange chromatography and electrophoresis techniques. One proteinous peak of ARE activity were obtained with specific activities of 0.944 U\mg protein and with a molecular weight of 42666 dalton.

Abbreviation: Diabetes mellitus: DM, Insulin-dependent diabetes mellitus: IDDM, Non insulin-dependent diabetes mellitus: NIDDM, Arylesterase: ARE, Paraoxnase: PON, Superoxide dismutase: SOD, Glutathione-S-transferase: GST, Glutathione peroxidase: Gpx, Catalase: CAT, Xanthine Oxidase: XO, Lipoxygenase: LOX, Lipoprotein Lipase: LPL, Polyamine oxidase: PAO, Spermine: Spm, Spermidine: Spd, glycosylated haemoglobin: HbA1c, Ceruloplasmin: Cp, Glutathione: GSH, Malondialdehyde: MDA, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis: SDS-PAGE, Diethyl amino ethane-Cellulose: DEAE-Cellulose, Red Blood Cells: RBC, Reactive Oxygen Species: ROS

Introduction

Diabetes mellitus is characterized by fasting hyperglycemia, with both type 1 and type 2 diabetes. Persons are also known to be prone to develop complications related to elevated blood glucose concentrations,including atherosclerosis, retinal damage, cataract, and neuropathy. Hyperglycemia may also result in increased production of the reactive oxygen species within numerous biochemical pathways that have the potential to initiate changes in endothelial function (1). DM is characterized by elevated plasma glucose and episodic ketoacidosis. In insulin-responsive tissues, liver, skeletal muscle and adipose tissue, insulin controls the rate of glucose uptake, intracellular glucose metabolism, lipid metabolism, and the synthesis of proteins at the transcriptional and translational levels. Insulin stimulates lipogenesis, diminishes lipolysis, and increases amino acid transport into cell (2).

The physiological role of enzymatic and nonenzymatic antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. PON1 is a specific antioxidative enzyme with both PON and ARE activities (3). ARE (EC.3.1.8.2) is an enzyme found tightly associated with high density lipoprotein particle in serum. Because of its unique enzyme activity, antioxidant property and its role as an anti atherosclerotic molecule(4). ARE is a calcium dependent esterase exclusively bound to the apolipoprotein A1 containing HDL fraction in plasma (5).

PON1 has been shown to protect lipoproteins from free radical mediated oxidation (6). It can also hydrolyze the oxidized cholesterol esters and lipid peroxides (7). It has been reported that, PON1 activity is significantly decreased in wide variety of human disorders which involve oxidative stress such as cardiovascular disease, DM, obesity, renal disease, liver cirrhosis, non-alcoholic steatohepatitis, and several mental disorders (8, 9). In DM, the PON1 activity has been reported to be decreased (10), as well as significant changes occur in various metabolites in blood (11, 12). There could be several factors responsible for the observed reduction in PON1 activity (13 , 14).

Materials and Methods

Collection of blood samples: Human blood was collected from male type 1 diabetic patients (n=40; age group 25-35 years), and healthy volunteers (n=35; age group 25-35 years). Venous blood samples were drawn by a range 5-10 milliliter to one sample.

Blood serum isolation: The serum is isolated by putting tubes in a water bath at 37°C for 10 min, and centrifuged at 13000xg by refrigerated centerfuge for 10 min. The supernatant was taken to conserve in freezing (15).

Biochemical Analysis:

The levels of serum glucose (16), urea (17), creatinine (18) concentration was measured spectrophotometrically in all samples using enzymetically methods by instruments analysis Fortress Kit, United kingdom.

Two milliltre of blood was collected in a tube containing ethylenediamine tetra acetate as an anticoagulant for determination of HbA1c for assessment of glyceamic state. Concentration of HbA_{1c} was measured after hemolysis of the anticoagulated whole blood specimen, assay techniques for HbA1c include affinity chromatography by using Stanbio-laboratory Kit, USA (19).

Serum Cp, GSH, MDA, Vit C and vit E were estimated by methods (20,21,22,23,24) respectively.

Insulin was assayed by ELISA using monobind, USA Kit which depending on the high affinity and specificity, TREA Co. China. (25), and the insulin resistance was calculated by the following equation:

Insulin Resistance = [fasting insulin (μIU/mL) × fasting glucose (mmol/L)] / 22.5 (26).

Gpx assay: Gpx activity was estimated spectrophotometrically by measuring unreactant GSH by using hydrogen peroxide as substrate (27).

GST assay: GST activity was determined by using the method of Habig *et al* (28), which based on catalyzing the conjugation of glutathione with 1-Chloro-2,4-dinitrobenzene (CDNB) to form Glutathione-2,4-dinitrobenzene (GS-DNB). The rate of increase in the absorption is directly proportional to the GST activity in the sample.

CAT assay: CAT activity was determined spectrophotometrically by monitoring the decrease in absorbance caused by the disappearance of hydrogen peroxide (29).

SOD assay: SOD was assayed by the modified method of Brown and Goldstein (30), based on the ability of SOD to inhibit the formation of formazan by reducetion of NBT by superoxide.

XO assay: XO activity was determined spectrophotometrically by measuring uric acid formation xanthine was used as substrate (31).

LOX assay: LOX activity was monitored spectrophotometrically as the increase in absorption of the conjucated dienes formed when linoleic acid (substrate) was oxidized (32).

LPL assay: LPL activity was determined spectrophotometrically by measuring the release of para nitrophenol from para nitrophenylacetate as substrate (33).

PAO assay: PAO activity was determined spectrophotometrically method reported in refrence (34). The oxidation of spermine by the enzyme is carried out with potassium ferricyanide as electron acceptor. The decrease in absorbance due to the reduction of potassium ferricyanide.

Purification of ARE:

Determination of protein: The protein content of soluble enzyme preparation was measured by the modified Lowry method (35), using bovine serum albumin as standard.

Determination of ARE activity: ARE activity was measured spectrophotometrically at 270 nm in an automated shimadzu UV- 1800 UV Spectrophotometer, Double Beam. Buffer substrate was prepared by adding 50 mM Tris-HCl buffer pH 8.0 containing 2 mM Calcium chloride. Phenyl acetate concentration was 5mM in Isopropyl alcohol. The reaction was initiated by the addition of 10 μl of the sample to 2.90 ml of buffered substrate, and the increase in absorbance at 270 nm was recorded at intervals of 30 sec for 3 min. Blank sample without enzyme was used.

One unit of activity was defined as that amount of enzyme which produced 1 micromole of phenol per min. The molar extinction coefficient of phenol was 1310 M-1 cm-1 (36).

Dialysis: Eight milliliter of serum was dialyzed in a dialysis bag with 10 KDa cut-off. Dialysis was carried out about 12hrs against 50 mM of Tris-HCl buffer pH 8.0 at 4 °C with 3 changes.

Fractionation on DEAE-Cellulose column: The dialyzed enzyme solution was applied on DEAE-Cellulose anion exchanger column 2.5×40cm, which has been equilibrated with 50 mM Tris-HCl buffer pH 8.0. Fractions of 6 ml volume were collected. Flow rate was 60 ml / hr.

Determination of molecular weight

The fractions 114-186 showing ARE activity were pooled together and lyophilized, then subjected to the SDS-PAGE using the method of Laemmli (37) on a Tris-HCl buffer 1M, pH 8.9, 30% polyacrylamide containing 10% SDS. For electrophoresis in the presence of SDS, samples and control were boiled for 3 min in the sample buffer, which contained 5% 2-mercapto ethanol and 1% bromophenol blue. Electrophoresis was performed horizontal slab mini-gel apparatus (Labnet, USA) at 1000 V for 5-6 hrs.

A Standard curve for SDS-PAGE determination of reference proteins molecular weight were explained by Fig.1.

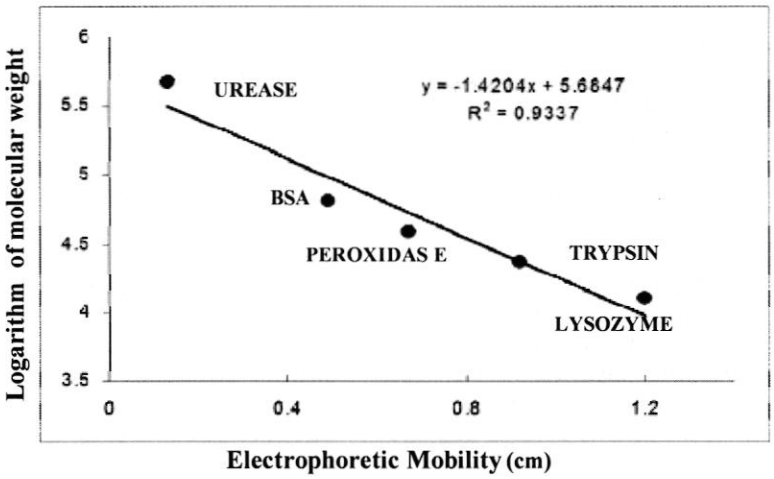

Fig. 1: Standard curve for SDS-PAGE determination of protein molecular weight

Statistical analysis

All values are reported as mean ± SEM. Statistical significance was assessed using Student's t-test . P value less than 0.05 was accepted as the significance level.

Results and Discussion:

PON1, an HDL-associated enzyme, prevents lipoprotein oxidation. PON1 enzymatic activity has been shown to decrease in patients with diabetes (38) and lipid peroxide products (7).

The data presented in table 1 indicates, ARE activity was significantly lower in patients with type 1 diabetes when compared to the controls. These results corresponded with the results of Wegner *et al* (39), which observed that ARE activity was lower in type 1 diabetic patients than healthy people. The results showed that serum ARE activity was significantly lower in patients with metabolic syndrome than healthy subjects (40).

Serum PON1 was found to decrease macrophage oxidative stress (41) and to be decreased under oxidative stress in atherosclerotic process (42). Low PON1 activity increases lipid oxidation in patients with type 1 diabetes (43,44). Therefore, low activity of PON1 could contribute to vascular dysfunction and late diabetic complications in patients with type 1diabetes (45)

The absence of insulin in patients with IDDM results in uncontrolled rate of lipolysis in adipose tissue. This increases blood levels of fatty acids and results in accelerated ketone body production by liver (2). In cases of DM, impaired lipid profile occured (46, 47) and the levels of glucose and ketone bodies are elevated substantially. The higher concentration of ketone bodies may be responsible for the decreasing of ARE activity (48).

LPL plays a central role in lipoprotein metabolism by catalyzing hydrolysis of triglycerides in chylomicrons and very low density lipoprotein particles (49). An imbalance of LPL activity may alter the partitions of plasma triglycerides between muscle and adipose tissue, and thus influence insulin resistance and obesity (50).

The results showed a significantly decreasing in LPL activity in patients with type 1 diabetes compared to healthy subjects (Table 1). Bos *et al* (51) observed decreasing of LPL activity in subjects with impaired glucose metabolism and diabetes compared to normal. Reduction of LPL is observed in patients with type 2 diabetes and individuals with insulin resistance (52).

In adipose tissue the decrease in insulin and increase in glucagon results in inhibition of lipogenesis, inactivation of LPL and activation of intracellular hormone-sensitive lipase. This leads to release from adipose tissue of increased amounts of glycerol and free fatty acids, which are used by liver, heart, and skeletal muscle as their preferred metabolic fuel, therefore sparing glucose (53).

GST, along with other antioxidant enzymes, such as Se-dependent GPx, provides the cell with protection against a range of harmful electrophiles produced during oxidative damage to membranes (54).

Our present study indicates significant decreasing of GST activity was observed in patients than control group (Table 1). GST activity was also significantly decreased in diabetic group compared to controls **(55)**.

Oxidative denaturation of hemoglobin leads to the release of hemin into the RBC membrane and the released hemin is capable of oxidizing membrane proteins via a thiyl radical intermediate. GST can bind free hemin that is released during hemoglobin oxidation presumably reducing damage to RBC membrane. Therefore, a significant decrease in the levels of GST indicated protection against oxidative stress (56).

CAT is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is responsible for the degradation of hydrogen peroxide

as a substrate. (53). It is a protective enzyme present in nearly all animal cells and plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (57).

Table 1 indicates greatly decreasing of CAT activity was observed in patients than control group. In RBC the antioxidant enzyme CAT activity showed a significant lower in NIDDM patients compared to normal subjects (58).

Cojocaru *et al* (59) conclude that in diabetic patients the decrease in plasma CAT activity is the consequence of oxidative modifications, and suggest that diabetic patients have significantly increased oxidative damage.The increase in superoxide radical in diabetes may inhibit the activity of CAT and Gpx (60).

Table 1: Activity of enzymes in the serum of healthy controls and diabetic patients (Values are expressed in mean ± SD)

Parameters	Control	Diabetic subjects	
ARE(U/ml)	0.239±0.024	0.205±0.031	S
Cat	0.137±0.026	0.069±0.015	S
GPx(µmol/min)	0.715±0.033	0.684±0.044	NS
GST(U/l)	11.4±3.38	7.7±1.69	S
LPL(U/ml)	0.176±0.013	0.127±0.011	S
LOX(U/ml)	0.445±0.086	0.622±0.13	S
PAO(U/ml)	0.045±0.0133	0.079±0.018	S
XO(U/l)	0.066±0.017	0.08±0.02	S
SOD	0.018±0.01	0.037±0.022	S

Data are reported as mean ± SD. *P<0.05 compared to control (Student t-test).S: significant, NS: Not significant.

GSH is a tripeptide containing a sulfhydryl group that not only protects the cells against
oxidative damage ; it is also acts as body's immune system boosters. It is present in high concentrations in the cells (57). GSH plays a key role in detoxification by reacting with hydrogen peroxide and organic peroxides.(61).

The results in table 2 showed a significantly lowering in GSH levels in patients compared to controls. Al-Bajari (62) study showed a significant decrease of antioxidant, GSH in patients of both sexes suffering from NIDDM.

Decreased GSH levels in diabetes have been considered to be an indicator of increased oxidative stress, Lowered levels of GSH may also be due to the utilization of GSH by the GPx and GST as their substrate (63), or may be caused by the increased sorbitol synthesis causing NADPH depletion and deficiency of this limits the reduction of GSSH to GSH catalyzed by glutathione reductase.(64).

Ascorbic acid (vit.C) is an oxidation-reduction coenzyme. It also plays a role in free radical defense (65).Vit.C levels were showed significant decrease in patients compared to controls (Table 2). Recently, vit.C levels was determined in RBC and plasma of type I, II and non treated diabetic females and found to be significantly lower than that of normal (66).

The decrease of vit.C refers to its utilization which prevents the cellular damage from free radicals (67). Thus, the elevation in glucose concentration may depress natural antioxidant like vit. C or due to decrease in GSH levels, since GSH is required for recycling of vit. C (68).

Table 2 elucidates a significantly decreasing in insulin hormone levels in patients compared to controls. So, Naseem *et al* (69) found a significantly decreasing in serum fasting diabetic male and female insulin levels compared to healthy control .

During pathogenesis of DM, oxidative and nitrosative stresses contribute to the destruction of insulin-producing beta cells. Moreover, it is believed that increased oxidative stress is one of the main factors in the etiology and complications of DM (70)

Additionally, there was no statistically significant reduce of Gpx activity (Table 1), Cp and vit. E levels (Table 2) in patients compared to controls.

Decreased activity of GPx may result from radical induced inactivation and glycation of the enzymes, which is known to occur during diabetes (71). A negative correlation of fasting plasma glucose with ceruloplasmin may possibly indicate increased glycation of proteins that may damage antioxidant proteins like Cp (72), and/or lower Cp levels in diabetic may be due to the adaptive response of Cp as an antioxidant. (73).

Vit. E reduces due to regulates mitochondrial hydroxyl radical generation and ROS production (73). Lowered vit. E levels is presumably due to its use in preventing free radical damage that seems more extensive in diabetic patients (67).

Table 2 Biochemical parameters in the serum of healthy controls and diabetic patients (Values are expressed in mean ± SD

Parameters	Control	Diabetic subjects	
Glucose(mmol/L)	4.189±0.809	11.697±3.77	S
MDA(µmol/L)	1.156±0.187	2.668±0.682	S
GSH(µmol/L)	17.636±2.78	10.225±1.958	S
Urea(mmol/L)	4.049±1.532	6.312±1.642	S
Creatinine(mmol/L)	66.842±14.18	80.96±18.66	S
Vitamin.C(µmol/L)	86.89±11.22	63.906±18.107	S
Vitamin.E(µmol/L)	39.217±9.937	37.464±11.20	NS
Cp (µmol/l)	223.7±61.9	192.5±54.1	NS
HbA1c(%)	4.987±0.673	8.802±1.288	S
Insulin µlU/ml	32.726±3.61	14.114±1.73	S
Insulin resistance	1.80±0.177	2.53±0.258	

Data are reported as mean±SD. P<0.05 compared to control (student t-test). S: significant, NS: not significant

LOX is a non-heme iron dioxygenases which catalyze the incorporation of dioxygen into 1,4-cis,cis-pentadiene-containing fatty acids to form hydroperoxide products, and widely distributed throughout the plant and animal kingdoms (74). LOX has been implicated in the pathogenesis of diabetic macrovascular complications. LOX products, hydroxyei- cosatetraenoic acid has a role in various biological processes, including LDL oxidation in atherogenesis, cancer cell growth, and neuronal apoptosis after oxidative stress (75).

We noticed a significantly increasing in LOX activity in patients with type 1 diabetes compare healthy subjects (Table 1). In type 2 diabetic mice, Hatley *et al* (76) showed that glucose increases production of endothelial hydroxyeicosatetraenoic acid and significant elevations in levels of urinary LOX products *in vitro*. On the other hand, LOX significantly higher in alloxan diabetic mice, and the orally treatment by quercitine rutinoside showed significant lowering in activity (77).

Insulin has ability to depress the level of intracellular cAMP which inhibits lipolysis in adipose tissue and thereby reduces the concentration of plasma free fatty acids (which are substrate of LOX) (53). On the other hand the elevated levels of glucose supports increasing LOX activity in many cells such as vascular smoth muscle and pancreatic ß-cells (78).

XO catalyzes hypoxanthine to xanthine, and can further xanthine to uric acid, and play an important role in the catabolism of purines in some species including human (79).This enzyme is involved in free radicals and responsible for superoxide production (80).

As shown in Table 1, a significantly increasing in XO activity in patients with type 1 diabetes compare healthy subjects. A significant increase in the activity of XO was observed in diabetic men and women below 30 years when compared to nondiabetic controls (81). Serum XO and conjugated dienes were significantly higher in diabetic than in nondiabetic group (82). However, the inhibition of xanthine oxidase can improve endothelial function in subjects with type 2 diabetes compared with control subjects of a similar age (83).

The tissues that express the highest activity of XO are liver and intestine. The increase in XO activity in diabetes could be attributed to the release of XO from liver which has been observed not only in diabetes but also in other pathological states, such as morrhagic shock (81)

SOD occurs in all major aerobic tissues in the mitochondria and the cytosol and protects aerobic organisms against the potential deleterious effects of superoxide.

There was a significantly higher of SOD activity in patients compare healthy subjects (Table 1). Our result is consistent with the result of Chugh *et al* (84), who reported an increase in antioxidant enzymes such as SOD in type 2 DM which gives an evidence of increased reactive oxygen species production. Significantly higher activity of serum SOD was found in type 2 diabetic patients as compared to control (1). The elevated activity of SOD due to Superoxide formation in diabeties, because of oxidative stress increasing or by the action of xanthine oxidase.

PAO catalyzing the oxidative deamination of Spm and Spd, resulting in production of amonia, corresponding amino aldehydes and H2O2.

PAO activity was elevated significantly in patients with type 1 diabetes compare healthy subjects (Table 1). PAO activity in children with DM 1 was reported very high (85). In fact, the pancreas is the organ containing the highest concentration of intracellular Spm and Spd which have a role in insulin secretion (86). Spm and Spd are localized in the secretory granules found in the beta-cell cytoplasm (87), and degraded by PAO, which modulates membrane fusion processes in the beta-cells (88). Moreover, they seem to be directly involved in the transcriptional control of the insulin gene (89).

Glucose, MDA and HbA1c were greatly increased in patients with type 1 diabetes compare healthy subjects (Table 2). Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that result from defects in insulin secretion, or action, or both (90). Plasma glucose, MDA and HbA1c in RBC and plasma were determined, and found significantly higher in female diabetic patients type I,II and non treated diabetic females than that of normal (66). Bjelakovic *et al.*(85) found glycemia, MDA and HbA1c were significantly increased in diabetic children compared to the control subjects.

MDA and acrolein, potentially toxic agents which spontaneously formed from aminoaldehydes, and induce oxidative stress in mammalian cells, PAO activity may participate in these circumstances.

Hypoinsulinaemia in diabetes increases the activity of the enzyme fatty acyl coe nzyme A oxidase, which intiates β-oxidation of fatty acids, resulting in lipid peroxidation and increase of MDA (71).

However, The classical factor known to initiate the glycation of proteins in vivo is glucose concentration. MDA can enhance the glycation of hemoglobin (91). As well as, Nalini (56) hypothesized that as oxidative stress and hyperglycemia are major etiologic and pathologic factors of DM, then the antioxidant enzyme, GST in RBC, are likely to have a role in the glycation of hemoglobin in diabetic patients.

Table 2 explains a significantly higher in insuline resistance levels in patients with type 1 diabetes compare healthy subjects. Al-Bajari (62) showed a significantly increasing in insulin resistance in patients of both sexes suffering from NIDDM.

Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense

mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance leading to diabetic complications (92,93).

The results in table 2 showed a significantly increasing in urea and creatinine levels in patients with type 1 diabetes compare healthy subjects .

Our observations were in accordance with various studies which showed raised plasma creatinine and urea levels in diabetic patients (94). Also, urea and creatinine levels were shown significantly increase for patients a long time infected with type II diabetic mellitus (62).

In diabetes, chronic hyperglycemia sustains the oxidative stress by excessive generation of ROS in glomerular and tubular cells, via overexpression of NADPH oxidase and contributes to *renal* tissue injury. And this effective on urea and creatinine levels. (95).

Correlation between ARE and biochemical parameters

A significant positive correlation was found between ARE and Gpx (r=0.422), Cer (r=0.525), GSH(r=0.411), vitamins C (r=0.735) and E(r=0.611), and non significantly positive correlation with GST, LPL, urea, and insulin hormone. The results also indicated a significant negative correlation between ARE and XO(r= -0.597), glucose(r= -0.721),HbA1c(r= -0.841) MDA(r= -0.834) and I.R(r= -0.430), whereas non significantly negative correlation with CAT, LOX, SOD, PAO and creatinine.

Purification of ARE:

Table (3) shows purification steps of ARE. The specific activity of crude enzyme was 0.0219 unit/mg protein, after dialysis became 0.5 unit/mg protein. The elution profile of ARE activity from anion exchange chromatography column is shown in Figure (2). Protein was eluted in one protein peak. So enzyme activity was eluted in only one peak at elution volume 114-186 ml with specific activity 0.944 unit / mg protein and with purification fold of 43.1 compared to crude enzyme .

ARE was first purified by Main (96) by methods involved a series of ethanol precipitation and ammonium sulphate fractionation steps. ARE was purified from human serum by two step methods of purification involving affinity chromatography followed by gel filtration on sephadex G50.The final preparation was 27.7 fold purified and with Specific activity 4.6 mU/mg compared with the serum (4). By Gel filtration chromatography resulted one peak of ARE activity was pooled and retained (samples 69–100) with Sp. Act. 402.8 and yeild 2.1% from rat liver (97). Human serum contains a single enzyme with arylesterase activity throughout the steps of purification and after obtaining over 600 fold (36).

Table 3: ARE purification steps from normal individuals serum

Purification steps	Volume (ml)	Protein conc. (mg/ml)	Total protein (mg)	Activity (U/ml)	Total activity U*	Sp. Activity (U/mg)	Yield %	Purification fold
Crude	8	14.244	113.95	0.313	2.504	0.021	100	1
Dialysis	8.3	9.544	79.21	0.476	3.95	0.5	157.74	22.83
Ion exchange	80	1.08	8.64	0.102	8.16	0.944	325.87	43.1

***U: A unit is defined as that amount of enzyme which produced 1 micromole of phenol per min.**

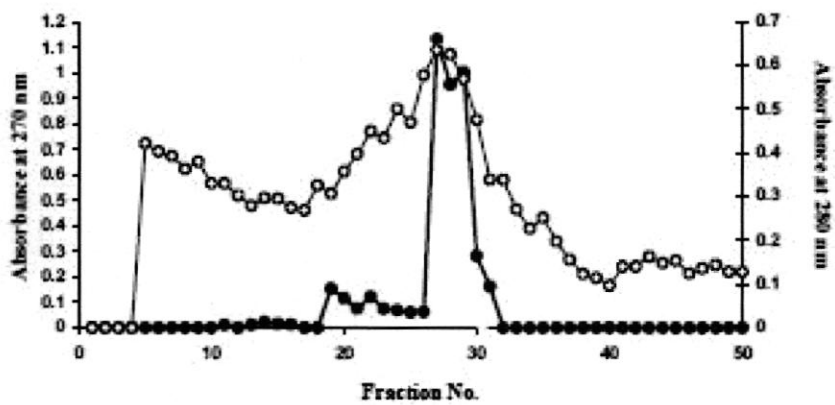

Figure 2: Elution profile of ARE purification enzyme activity of Type I diabetic patients by DEAE-cellulose chromatography column (40×2.5 cm). ARE activity at 270nm, Protein at 280 nm

Sodium dodecyl sulfate electrophoresis: On sodium dodecyl sulfate electrophoresis, The purified enzyme showed a single band on SDS-PAGE (Fig. 3 , lane 6). The molecular weight of the enzyme was estimated to be 42666 Da.

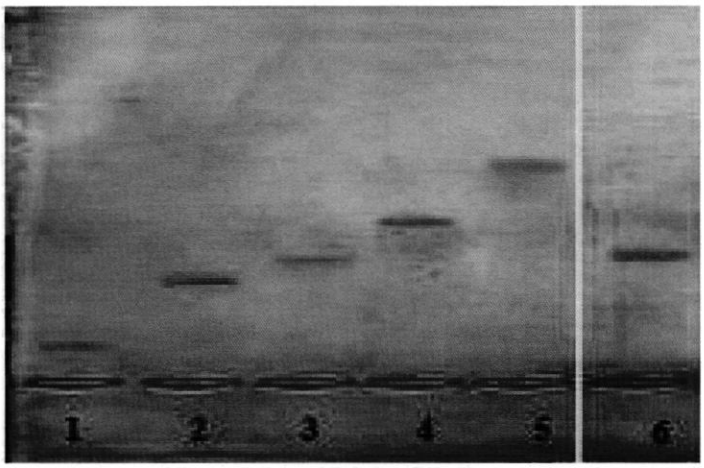

Figure 3: Analysis of partially purified ARE by SDS-PAGE. Protein bands were visualized by staining with Coomassie brilliant blue R250. Molecular mass markers: Lane 1 cytocrome oxidase 13 KDa , Lane 2 Trypsine 23.8 KDa , Lane 3 Peroxidase 40 KDa , Lane 4 BSA 67 KDa, Lane 5 Urease 480 KDa and Lane 6 partially purified ARE approximately 42666 Da.

The purified enzyme had a molecular weight ranging from 35-50 KDa (96). Purified ARE is a glycoprotein with a minimal molecular weight of about 43 KDa. It has up to three sugar chains per molecule, and carbohydrate represents about 15.8% of the total weight (36). Huang *et al.*(98) reported purification of an ARE from mouse hepatic microsomes has a molecular mass about 40 KDa. Purified human serum ARE was analysed by SDS-PAGE and single band migrated with a mobility corresponding to an apparent 45 KDa by Coomasie Blue staining (99) and 43 KDa by silver staining (4).

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تنقية انزيم الارايل استريز ودراسة علاقته مع بعض المتغيرات الكيموحيوية في مرضى النوع الاول من داء السكر

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الملخص

تضمن البحث تحديد فعالية انزيم اريل استريز وعلاقته ببعض المتغيرات الكيموحيوية في مصل دم المصابين بالنوع الاول من داء السكر. وجد انخفضا معنويا ($p<0.05$) في مستوى فعالية مضادات الاكسدة الانزيمية اريل استريز، كلوتاثيون ترانسفيريز، الكاتاليز واللايبوبروتين لايبيز، ومستويات مضادات الاكسدة غير الانزيمية الكلوتاثايون، وفيتامين C، فضلا عن هرمون الانسولين، بينما كان الانخفاض غير معنويا بالنسبة لأنزيم كلوتاثيون بيروكسيديز، فيتامين E والسيريلوبلازمين في المصابين بالسكري نوع I مقارنة بالسليمين. بينت النتائج وجود ارتفاعا معنويا ($p<0.05$) في مستو ى فعالية لنزيمات اللايبأوكسيجينيز، البولي امين اوكسيديز، السوبراوكساید ديسميوتيز والزانثين اوكسيديز، بالاضافة الى الكلوكوز، المالونداياالديهايد، اليوريا، الكرياتينين، الهيموكلوبين المتسكر ومقاومة هرمون الأنسولين، في المصابين بالسكري النوع I مقارنة بالسليمين.

وجد ان العلاقة طردية معنويا بين انزيم اريل استريز وانزيم كلوتاثايون بيروكسيديز ($r=0.421$)، السيريلوبلازمين ($r=0.525$)، الكلوتاثايون ($r=0.411$)، فيتامين C ($r=0.735$) وفيتامين E ($r=0.611$). بينما وجدت العلاقة عكسية معنويا مع انزيم زانثين اوكسيديز ($r=-0.597$) ،الكلوكوز ($r=-0.721$)، المالونداياالديهايد ($r=-0.834$) والهيموكلوبين المتسكر ($r=-0.841$).

نقي أنزيم ايل استريز جزئياً من مصل دم المصابين بالنوع الاول من داء السكر، باستخدام تقنيات الفرز الغشائي، وكروماتوغرافيا التبادل الأيوني بواسطة المبادل DEAE-cellulose والترحيل الكهربائي. تم الحصول على قمة بروتينية تمتلك فعالية أنزيم اريل استريز بفعالية نو عية 0.944 وحدة انزيمية/ملغم بروتين، وبوزن جزيئي 42666 دالتون.