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Development of Chronic Diabetic Complications and Role of Porphyrin Derivatives in Reducing Inflammation in Type 2 Diabetes Mellitus

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Abstract: *The development of chronic complications of type 2 Diabetes Mellitus can cause premature death mainly via cardiovascular disease and other chronic complications. It is known from previous studies that Acetylcholinesterase activity of the erythrocyte cell membrane increases in Diabetic patients. High levels of Acetylcholinesterase enzyme concentration can lead to a decrease in the levels of Acetylcholine that can result in the development of inflammation in type 2 Diabetes Mellitus. This twofold study first aimed to investigate the prevalence of chronic complications of type 2 Diabetes Mellitus in Arar (Saudi Arabia).It found that the overall prevalence of neuropathy, nephropathy, retinopathy and diabetic foot ulcer were 28.4%, 36.6%, 15.9% and 10.4% respectively. The second part of the study aimed to test the effect of Porphyrin derivatives, Tetraphenylporphine sulfonate (TPPS), 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) nitrosyl Chloride (FeNOTPPS), on Acetylcholinesterase activity by a laboratory-based investigation. The results showed that all of the compounds, TPPS, FeTPPS and FeNOTPPS, are inhibitors of Acetylcholinesterase (AChE) in vitro.*

Keywords: *Type 2 Diabetes Mellitus, chronic complications, glycemic control, Acetylcholinesterase activity.*

1. INTRODUCTION

1.1. Cataloging Chronic Complications in Type 2 Diabetes

The complications of diabetes mellitus are less common and less severe in people who have good glycemic control [**1**].Some studies have pointed to the fact that diabetic patients have a greater likelihood of developing micro- as well as macro-vascular complications (Figure 1) [**2,3**]. Macrovascular complications include: (i) Coronary artery disease, (ii) Peripheral vascular disease, which contributes to diabetic foot ulcers [**4**], (iii) Stroke and (iv) Micro and macrovascular complications that can involve various organs and tissues and can result in death.

It has been estimated that about 50-80% of diabetic patients die of cardiovascular disease [**5**]. Epidemiological studies have shown that foot ulcers, one of the complications of diabetes, account for more than 85% of non-traumatic lower extremity amputations in Diabetic patients [**5**]. Another chronic complication, Retinopathy, has been attributed to be a major cause of blindness throughout the world [**6**].Very few studies have, however, been done about the extent of diabetic complications in Saudi Arabia. The information about chronic complications of Diabetes Mellitus complications is useful for designing health care policy. The first half of this study investigated the extent of glycemic control of Diabetic patients and cataloged the chronic complications of type 2 Diabetes.

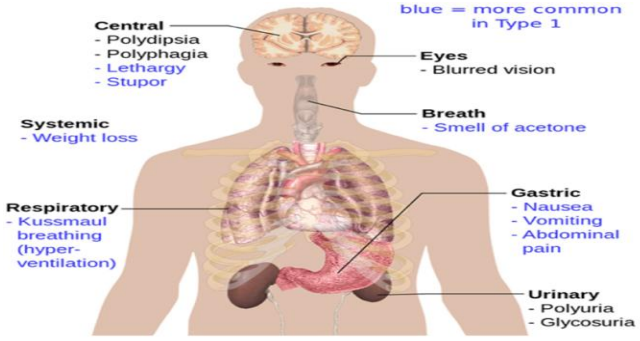

Figure1. *Some of the chronic complications produced in type 2 Diabetes Mellitus*

1.2. Laboratory Investigation to Alter Membrane Fluidity in Diabetics by Inhibiting Acetylcholinesterase Enzyme Activity

Acetylcholinesterase is a choline esterase enzyme. It hydrolyzes acetylcholine and is present in high concentrations in the nervous system and erythrocytes. Acetylcholinesterase (AChE) hydrolyzes Acetylcholine by a nucleophilic attack on the carbonyl carbon followed by the hydrolysis of the acylated enzyme intermediate to produce acetic acid. Acetylcholinesterase activity has been found to be higher in the islets of Langerhans in pancreas of Diabetic rats [**7**]. Some studies have suggested that Diabetes can affect specific subsets of cells and isoforms of Acetylcholinesterase that can lead to an increase in Acetylcholinesterase activity and cause Diabetic complications [**8,9**]. Some recent studies have shown that plasma and tissue concentrations of Acetylcholinesterase are increased in type 2 Diabetes Mellitus. Since Acetylcholine has anti-inflammatory action, therefore, high level of Acetylcholinesterase concentration can decrease the level of acetylcholine available to trigger systemic inflammation [**10**]. Hence, prevention of over-expression of AchE is an ideal strategy to inhibit the development of chronic complications of Diabetes.

Porphyrins are aromatic compounds**.** They form penta-coordinated complexes with ligands that have square-pyramidal geometry [**11**]. In the second part of this study, Porphyrin derivatives, TPPS, FeTPPS and FeNOTPPS, were synthesized and used to investigate inhibition of Acetylcholinesterase enzyme of erythrocyte cell membrane by using enzymatic assays.

2. MATERIALS AND METHODS

2.1. Materials

The Porphyrin compounds, TPPS, FeTPPS and FeNOTPPS were synthesized using established protocols [**12**]. Commercially availablepyrrole, benzaldehyde and acetone were distilled before use. A stock solution of BF_3-ether (2.5 M) was prepared in CH_2Cl_2.

2.2. Methods

In laboratory-based investigation, ^{1}H NMR were recorded in $CDCl_3$ using a 400 MHz. The UV-Vis. spectra were recorded on a UV-Vis. spectrophotometer. Erythrocyte hemolysate was prepared by the method of Beutler [**13**]. RBCs were added to 0.154 M NaCl solution and β-Mercaptoethanol-EDTA was added as a stabilizer. The membrane-bound AChE activity in RBCs cell was analyzed by established procedures [**14**].

The clinical part comprised a retrospective study conducted at the affiliated hospital in Arar, Saudi Arabia. It employed 1199 type 2 Diabetic subjects. Their HbA_{1c} level was measured by DiaSTAT Hemoglobin A1c Program (Biorad).The fasting plasma glucose (FPG) level was measured by the Glucose Oxidase method. HbA_{1c}values of 7%, 7-8.5% and > 8.5% while FPG values of < 126 mg/d1, 126-144 mg/d1, and <144 mg/d1 were taken as representing good, fair and poor control glycemic control respectively. The enzymatic methods (GPO-PAP and CHOD-PAP) were used for measurement of HDL, total cholesterol and triglycerides. The values of low density lipoproteins (LDL) were calculated. Abnormal values were defined as being: total cholesterol > 200 mg/d1; triglycerides > 150 mg/d1;LDL> 130 mg/d1 and HDL< 40 mg/d1 for males and < 50 mg/d1 for females. Body mass index (BMI) was calculated by the use of standard formula and obesity was defined by the use of criterion developed by the International Obesity Task Force (BMI > 25 $kg/m^2$is considered indicative of obesity) [**15**].The data was analyzed by the use of SPSS version 10.

3. RESULTS AND DISCUSSION

The frequencies of complications arising in type 2 Diabetes Mellitus are shown in Tables 1 and 2. The study found that the most frequently occurring complication was hyperglycemia (high FPG level was found in 88.8% subjects), high HbA_{1c}level (81.3% subjects), followed by obesity (59.2% subjects) and hypertriglyceridemia (54.5% subjects). Among microvascular complications, the occurrence of neuropathy was 36.6% and nephropathy 28.4%. About half (50.4%) of the subjects were hypertensive. The frequency of obesity, low HDL and hypertension was significantly higher among females (29.1%). The frequency of retinopathy (17.9%), nephropathy (32.2%), neuropathy (40.1%) and Diabetic foot ulcer was higher among males (14.1%) than females (6.9%).

When *in vitro* effect of TPPS, FeTPPS and FeNOTPPS on AChE activity was investigated by enzymatic assays, it was found that FeNOTPPS forms the most stable complex with AChE. In 10^{-10} M

FeNOTPPS solution, the activity of AChE dropped by almost 20% at (Table 3). Whereas, upon addition of 10^{-10} M FeTPPS, the activity dropped by only 15%. The 10^{-10} M TPPS solution had no effect on AChE activity.

Table1. *Mean values for metabolic parameters of the subjects*

Differences between group mean and target value indicator of metabolic risk							
Sex of patients							
	Males				**Females**		
	Reference value	**Mean**	**SD**	**p-value**	**Mean**	**SD**	**p-value**
Body Mass Index (kg/m2)	25	25.2	3.9	0.120	27.4	5.1	0.000
HbA_{1c} (%)	8.5	9.1	2.3	0.001	9.1	2.3	0.001
Fasting Plasma glucose (mg/dl)	144	195.4	77.0	0.001	198.5	76.9	0.001
Random plasma glucose (mg/dl)	200	263.5	98.1	0.001	258.3	98.6	0.001
Diastolic blood pressure (mmHg)	85	82.5	11.4	0.001	82.7	12.2	0.001
Systolic blood pressure (mmHg)	130	131.3	21.1	0.001	137.1	23.3	0.001
Cholesterol (mg/dl)	200	263.5	98.1	0.001	258.3	98.6	0.001
Triglycerides (mg/dl)	150	197.4	130.2	0.000	189.6	109.4	0.000
Low density lipoproteins (mg/dl)	130	123.3	36.1	0.001	130.7	38.9	0.767
High density lipoproteins (mg/dl)	40m 50f	36.1	13.9	0.000	41.3	29.1	0.000

Table2. *Frequency of diabetic complications*

	Sex			
Metabolic parameters	**Overall %**	**Male**	**Female**	**p-value**
Hyperglycemia on the basis of FPG	88.8	88.8	88.8	0.500
HbA1c > 7%	81.3	83.1	79.6	0.100
Hypercholesterolemia	46.1	43.4	48.7	0.056
Hypertriglyceridemia	54.5	53.6	55.5	0.570
LDL	39.6	38.9	40.4	0.370
Obesity	59.2	52.2	65.6	<0.001
Microvascular complications				
Retinopathy	15.9	17.9	14.1	<0.018
Nephropathy	28.4	32.2	23.9	<0.005
Neuropathy	36.6	40.1	33.3	<0.005
Diabetic foot ulcer	10.4	14.1	6.9	<0.001
Macrovascular complications				
Hypertension	50.4	46.1	54.5	0.000
Coronary artery disease	15.1	16.0	14.3	0.600
Stroke	4.4	4.3	44.4	0.400
Peripheral vascular disease	5.5	7.0	4.0	0.002

Table3. *Human erythrocyte membrane Acetylcholinesterase activit*

Compound	**Control**	**Enzyme Activity***
		32.00 ± 2.39
	Concentration in M	
TPPS (Na_4TPPS)	10^{-10}	34.00 ± 2.11
	10^{-9}	22.37 ± 3.14
	10^{-8}	20.89 ± 4.37
	10^{-7}	18.00 ± 1.74
	10^{-6}	16.02 ± 1.76
	10^{-5}	13.80 ± 3.71
	10^{-4}	11.80 ± 1.48
	10^{-3}	09.88 ± 2.14
FeTPPS	10^{-10}	27.68 ± 2.66
	10^{-9}	25.68 ± 2.92
	10^{-8}	23.42 ± 0.63
	10^{-7}	20.20 ± 2.64

	10^{-6}	18.70 ± 1.07
	10^{-5}	16.20 ± 3.62
	10^{-4}	13.90 ± 1.54
	10^{-3}	11.48 ± 1.07
FeNOTPPS	10^{-10}	26.40 ± 2.08
	10^{-9}	24.30 ± 1.73
	10^{-8}	22.50 ± 1.61
	10^{-7}	19.40 ± 2.55
	10^{-6}	17.70 ± 1.87
	10^{-5}	15.00 ± 4.04
	10^{-4}	13.90 ± 3.64
	10^{-3}	11.20 ± 2.23

y after in vitro treatment with the synthetic compounds: TPPS, FeTPPS and FeNOTPPS

**Acetylcholinesterase activity is expressed in terms of μ mole of acetylthiocholine iodide hydrolyzed/minute/ gram of hemoglobin 37℃.*

4. CONCLUSION

The clinical part of this study has provided a detailed estimate of the prevalence of chronic complications of type 2 Diabetes Mellitus in Arar, Saudi Arabia. Glycemic control, serum lipids level, blood pressure and BMI control have beenfound to be poor in most of the research subjects. The results show that only 49.6% of the subjects had normal blood pressure (130/85 mmHg) and only 18.7% had HbA_{1c} level <7.0%. The laboratory part of this study found that TPPS, FeTPPS and FeNOTPPS are inhibitors of Acetylcholinesterase (AChE) activity. FeNOTPPS is a superior inhibitor *in vitro* and inhibits AChE activity more effectively than both FeTPPS and TPPS.

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