Link of Serum Esterase Enzymes with Cognitive Impairment in Diabetic Patients

MAHDAVI P[1], MOKHTARI S[1], IRANPARVAR M[2], AMANI F[3], MAZANI M[4], MOSTAFALOU S[1]

[1]Department of Pharmacology & Toxicology, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran
[2]Department of Internal Medicine, [3]Department of Social Medicine, [4]Department of Biochemistry, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

ABSTRAK

Kecacatan kognitif merupakan salah satu komplikasi utama diabetes, dan pesakit yang mengalami kecacatan kognitif dan dianggap berada dalam peringkat pertengahan penurunan daya kognitif dengan risiko yang lebih tinggi untuk mendapati penyakit demensia dan Alzheimer. Pesakit diabetes juga berisiko lebih tinggi untuk mengalami penyakit Alzheimer. Kajian ini bertujuan untuk menilai aktiviti serum untuk dua enzim esterasa termasuk kolinesterase dan paraoksonase 1 (PON 1) dalam pesakit diabetes serta hubungannya dengan prestasi kognitif dan parameter metabolik. Dalam kajian keratan lintang ini, sejumlah 128 pesakit diabetes telah menyertai. Prestasi kognitif dinilai menggunakan Skala Kecerdasan Dewasa Wechsler yang Dikemaskini (WAIS-R). Indeks jisim badan, glukosa darah puasa, insulin darah puasa, rintangan insulin, dan aktiviti serum kolinesterase dan paraoksonase 1 telah diukur. Kejadian kerosakan kognitif didapati adalah 77% dalam populasi kajian. Aktiviti serum kolinesterase didapati lebih tinggi dalam pesakit diabetes yang mengalami ketakmampuan kognitif berbanding pesakit dengan prestasi kognitif normal (p < 0.04). Selain itu, korelasi negatif didapati antara prestasi kognitif dan aktiviti kolinesterase, walaupun tidak signifikan secara statistik. Perubahan dalam aktiviti enzim kolinesterase telah dikaitkan dengan kerosakan kognitif dalam pesakit diabetes, dan kajian masa depan diperlukan untuk mencari mekanisme patofisiologi dalam disfungsi kognitif diabetes.

Kata kunci: Alzheimer; aryleterase; kecacatan kognitif; kencing manis; keringtangan insulin; kolinesterase; paraoxonase 1

Address for correspondence and reprint requests: Associate Professor Dr. Sara Mostafalou. Department of Pharmacology and Toxicology, School of Pharmacy, Ardabil University of Medical Sciences, 5618953141 Ardabil, Iran. Tel: +98 45 33522437/ +98 9307371247 Email: s.mostafalou@gmail.com, s.mostafalou@arums.ac.ir

ABSTRACT

Cognitive impairment is one of the main complications of diabetes and patients with cognitive impairment are considered to be in an intermediate stage of decreased cognition with higher risk of developing dementia and Alzheimer's disease. This study aimed at evaluating serum activity of two esterase enzymes including cholinesterase and paraoxonase 1 (PON 1) in diabetic patients and association with cognitive performance and metabolic parameters. In this cross-sectional study, 128 diabetic patients were enrolled. Cognitive performance was evaluated using the Wechsler Adult Intelligence Scale-Revised (WAIS-R). Body mass index, fasting blood glucose, fasting blood insulin, insulin resistance, serum cholinesterase and paraoxonase 1 activity were measured. Prevalence of cognitive impairment was found to be 77% in the study population. Serum cholinesterase activity was found to be higher in diabetic patients with cognitive impairment than the patients with normal cognitive performance ($p < 0.04$). Further, a negative correlation was found between cognitive performance and cholinesterase activity, albeit statistically non-significant. Alteration in the activity of cholinesterase enzyme was shown to be associated with cognitive impairment in diabetic patients and future studies are required to find the pathophysiological mechanisms of diabetic cognitive dysfunction.

Keywords: Alzheimer; arylesterase; cholinesterase; cognitive impairment; diabetes; insulin resistance; paraoxonase 1

INTRODUCTION

Diabetes is one of the most common public health problems in the world, characterised by chronic hyperglycemia due to impaired insulin secretion, insulin resistance, and β-cell dysfunction. Genetic and environmental factors as well as lifestyle are involved in the pathogenesis of this disease. The incidence of the disease is increasing and it is predicted that by 2040 the number of patients will reach 642 million people. Death rate of the disease is also increasing due to risky behaviors such as unhealthy diet, inactivity, overweight, obesity, smoking, etc. Diabetes is also associated with numerous health complications such as functional disorders of the central and peripheral nervous system which can decrease the quality of life in the patients. One of the most important complications of diabetes which has been least noticed and studied, is cognitive impairment. Cognitive impairment, which can occur in both early and chronic stages, is known as the fourth complication of the microvascular complications of diabetes. Mild cognitive impairment (MCI) is a cognitive disability level which is evaluated objectively as well as subjectively and is lower

than expected value for patient's age or education. The main symptom of MCI is cognitive performance disorder which includes impairments of mentality, performance capability, language and visual skills, and related clinical signs. MCI is an intermediate stage of decreased cognition resulted from aging or senile dementia and the patients with MCI are at the risk of suffering dementia and particularly, Alzheimer's disease (Ali et al. 2023; Frison et al. 2021).

Accurate pathology of diabetic cognitive dysfunction (DCD) has not been discovered, but hyperglycemia, vascular diseases, hypoglycemia and insulin resistance may have critical role. Experimental evidences have shown that after the onset of diabetes, synaptic plasticity and transmission gradually start to change in the neurons of the hippocampus and cause disorders in neurological abilities like problem solving, memory and learning (Ortiz et al. 2022).

The results of multiple researches in the pathology of cognitive disorders have shown that changes in the neural acetylcholine system are involved in development of the major cognitive impairment disorders. Acetylcholine plays a very important role as a neurotransmitter in the nerves controlling learning, muscle contractions and glandular secretions. The enzyme acetylcholinesterase is responsible for breakdown and inactivation of acetylcholine at the synapses. Inhibitors of the enzyme acetylcholinesterase, which regulates the amounts of acetylcholine in the synaptic space, are one of the main strategic agents for improving cognitive function in patients with Alzheimer's disease (Marucci et al. 2021). In Alzheimer's disease, the level of acetylcholinesterase in some areas of the brain increases, and as the disease progresses, the cortical level of the companion enzyme butyrylcholinesterase (BuChe) is also increased and their enzymatic and molecular properties are changed. Activity of cholinesterase enzymes has been shown to be altered in diabetic state and related metabolic diseases, but the mechanism has not yet been clearly defined (Mushtaq et al. 2014). According to the regulatory role of acetylcholine in systemic inflammation, blood levels of cholinesterase enzyme has also been proposed as a predictive indicator of disease development in Alzheimer and type 2 diabetes (Shenhar-Tsarfaty et al. 2013).

Aside from this, some researchers have demonstrated that high-density lipoprotein (HDL) plays a significant role in maintaining cognitive performance. This may be due to anti-oxidant and anti-inflammatory properties of HDL which can protect neurons and improve cognitive performance (Hottman et al. 2014). Paraoxonase 1 (PON1) is an HDL associated enzyme which has been reported to have a main role in preserving antioxidant and anti-inflammatory properties of HDL (Shokri et al. 2020). Decreased arylesterase and paraoxonase activities of PON1 has been shown to be associated with MCI and Alzheimer incidence (Romani et al. 2020). This can be justified based on the role of altered systemic redox balance and

oxidative stress in development of MCI and Alzheimer's disease (Castellazzi et al. 2016).

Therefore, due to the evidences implicating on the association of diabetes and MCI, and the changes observed in the enzymatic activity of cholinesterase and PON1 in the states of both diabetes and cognitive dysfunction, the present study evaluated the cognitive performance of diabetic patients in association with insulin resistance and enzyme activity of pseudocholinesterase and PON1 in the serum.

MATERIALS AND METHODS

Study Design, Area and Population

This cross-sectional study was conducted on patients diagnosed with diabetes in the Endocrinology clinic, Imam Khomeini Hospital, Ardabil, Iran in 2019-2020. All the patients were inhabitant in the province of Ardabil in the northwest of Iran. The inclusion criteria for participants included being older than 18 years old, having adequate hearing and vision ability, and confirmed diagnosis of type I or II diabetes by Internal specialist. The exclusion criteria for the participants included clinical signs or self-report of disorders like anemia, encephalitis, psychiatric disorders, mental retardation, brain trauma and stroke. All the participants were given information about the project and free procedures. Finally, 128 patients were enrolled in this study with completing predetermined subsets of Wechsler Adult Intelligence Scale-Revised (WAIS-R) test and providing blood samples for biochemical analyses.

This study was approved by the ethics committee of the university by receiving approval codes (IR.ARUMS. REC.1398.096 and IR.ARUMS. REC.1399.043). All the patients signed a written consent to participate in the study. Patients were assured that their identity were kept in privacy, and all costs were borne by the counsel without imposing any cost on the patients.

Evaluation of Cognitive Performance

The Persian version of the WAIS-R was used to evaluate the cognitive performance of patients. Although WAIS-R can measure various abilities, five subsets of this test were recommended to evaluate cognitive performance (Table 1). The subsets of vocabulary and information as the index of verbal comprehension, block design and picture completion as the index of perceptual organisation, and digit-span as the index of working memory were used in this study. At least four subtests were completed for each patient, and an extra subset was done for patients who consent to continue. A trained psychologist conducted WAIS-R test on patients and completed the subsets (Franzen 2002).

Sample Collection

Blood samples were taken from patients while they were 8-hours fasted. The samples were centrifuged to extract serum. The fresh sera were

TABLE 1: WAIS-R subtests' outcome measures and neurologic functions administered
to diabetic patients

Subtests	Outcome measures	Neurologic functions
Information	The general fund of knowledge The ability to access and express this information	Long-term memory functions Working memory
Vocabulary	Word knowledge The ability to access and to effectively communicate that knowledge Expressive and receptive language skills	Long-term memory Working memory
Digit-span	Mental manipulation Attention	Memory span Short-term verbal memory
Picture Completion	Ability to recognize familiar items Ability to identify missing parts Conceptual reasoning skills Visual scanning	Visual memory
Block design	Ability to construct a design to a model	Visual-spatial processing and integration

used to measure concentration of glucose and insulin, and the remained serum samples were kept at 70°C for biochemical assessments.

Assessment of Fasting Blood Glucose

An enzymatic colorimetric kit purchased from Pars Azman Co; Iran, was used to assess the concentration of glucose in serum samples. The protocol was based on the reaction of glucose oxidase-phenol and aminophenazone (GOD-PAP) having an assay range of 5-400 mg/dL (Cat. No: 117500).

Assessment of Fasting Blood Insulin

An ELISA kit purchased from Demeditec Diagnostics GmbH; Germany, was used to assess the concentration of insulin in serum samples (Cat. No: DE2935).

Determination of Insulin Resistance

Homeostatic model assessment of insulin resistance (HOMA-IR) was used as the index of insulin resistance. HOMA-IR was obtained using the following formula: insulin con. (mU/ml) × glucose con. (mg/dL) / 405 (Matthews et al. 1985; Mostafalou et al. 2015).

Assessment of Butyrylcholinesterase Activity

BuChE activity was assessed according to a method developed by Ellman and colleagues in 1961. Acetylthiocholine, was used as the substrate and the rate of production of the enzyme product (thiocholine) was measured by Ellman's reagent or 5,5'-dithiobis-2-nitrobenzoate (DTNB). The reaction of DTNB with thiocholine produces the yellow 5-thionitrobenzoic acid which can be detected at 412 nm (Ellman et al 1961). Briefly, an incubation solution containing DTNB (0.25 mmol/L) in phosphate buffer (75 mmol/L, pH 7.9)

was first prepared. 3 ml of incubation solution was mixed with 10 µl of previously melted serum and the tubes were placed at 37°C to establish a temperature equilibrium. Then 10 µl acetyl thiocoline iodide (3 mmol/L) was added to the test tubes while the blank tube received 10 µl of distilled water instead. Finally, the absorbance was read by spectrophotometer at 412 nm (Abdollahi et al. 2004; Mostafalou et al. 2012).

Assessment of PON1 Activity

Arylesterase activity of PON1 was assessed according to the Beltowski method based on the initial rate of hydrolysis of phenyl acetate as the substrate in the reaction mixture. The assay mixture composed of phenyl acetate (2 mM), CaCl2 (2 mM) and 10 µl of serum in Tris/HCl (100 mM, pH 8.0). The absorbance was monitored at 270 nm for 3 minutes. Blank was also prepared as described above except serum. The activity of PON1 was calculated using the below mentioned formula where = 1310 M^{-1} cm^{-1} is molar absorption coefficient. The results were expressed in U/ml when 1 U of arylesterase hydrolyses 1 µmol of phenyl acetate per minute (Beltowski et al. 2002).

Other Variables

All the patients were personally interviewed in order to obtain sociodemographic information such as sex, age, height, weight, education, occupation, medications in use and duration of having diabetes.

Statistical Analysis

Data were analysed by SPSS 26.0 and reported as frequency, mean and standard deviation (SD). The p-values lower than 0.05 were considered statistically significant.

RESULTS

The demographic, clinical and biochemical characteristics of the patients were shown in the Table 2. The average age of the participants was 57.2 years and the average duration of having diabetes was about 9.7 years. More than half of the patients (57.8%) were female, and almost all subjects were married (98.4%). Half of the subjects did not have education and 85% were unemployed. About two-thirds of the study population had at least one first-degree relative with diabetes. Almost 50% of the participants had cardiovascular and lipid profile disorders and 10% also had thyroid disorders. Data on therapeutic regimen of our study population showed that 75.8% of the patients were using metformin, 37.5% losartan, 28.9% aspirin, 40.6% atorvastatin and 21.9% insulin. The findings of WAIS-R tests indicated that the patients had a mean full-scale intelligence quotient (FSIQ) of 81.12, mean verbal intelligence quotient (VIQ) of 77.62 and mean practical intelligence quotient (PIQ) of 88.53. According to FSIQ data, the frequency of different stages of cognitive impairment in the patients was shown to be 77%, meaning that only 23% of the patients had normal cognitive performance. The average of

HOMA-IR and BMI in the participants were estimated to be 4.8 and 29.3, respectively, and both values were considered to be above the normal range. The mean activity of ChE and PON1 in the serum were estimated to be 93 and 176 IU/ml, respectively (Table 2).

Comparison of mean variables based on gender indicated that men had significantly higher FSIQ than women, and enzymatic activity of ChE in women was shown to be significantly higher than men. There was no difference between the other variables between two gender groups (Table 3).

Comparing tests were conducted between groups with and without DCD and the results were indicated in the Table 4. Fasting blood sugar (FBS), fasting blood insulin (FBI), HOMA-IR and PON1 activity had no statistic differences between the groups with and without DCD, while serum ChE activity which was shown to be higher in the patients with DCD than the patients without DCD ($p < 0.05$) (Table 4).

The Pearson's correlation test between quantitative variables showed that serum PON1 activity was negatively correlated with age and BMI in the patients with diabetes. The correlation between FSIQ and serum ChE activity was shown to be negative which confirmed the previous result albeit this correlation was not statistically significant (Table 5).

DISCUSSION

There have been an extensive

TABLE 2: Characteristics of the study population

Characteristics	n (%)	Mean	SD
Gender			
- Male	54 (42.2)	-	
- Female	74 (57.8)	-	
Marital status			
- Single	2 (1.6)	-	
- Married	126 (98.4)	-	
Occupational status			
- Occupied	43 (33.6)	-	
- Unoccupied	85 (66.4)	-	
Educational status			
- Illiterate	64 (50.0)	-	
- Undergraduate	41 (32.0)	-	
- College education	23 (17.9)	-	
Family history of diabetes			
- At least one first-degree	82 (64.1)	-	
- No first-degree	46 (35.9)	-	
DCD (based on FSIQ)			
-Severe	29 (22.7)	-	
-Moderate	40 (31.5)	-	
-Mild	29 (22.7)	-	
-No	29 (22.7)	-	
Background disease			
-Cardiovascular disorders	66 (51.6)		
-Dyslipidemia	63 (49.2)	-	
-Thyroid dysfunction	14 (10.9)	-	
Medicine			
- Metformin	97 (75.8)	-	
- Losartan	48 (37.5)	-	
- Aspirin	37 (28.9)	-	
- Atorvastatin	52 (40.6)	-	
- Insulin	28 (21.9)	-	
Age (years)	-	57.2 $\pm$ 10.8	
Duration of diabetes (years)	-	9.7 $\pm$ 6.9	
FSIQ	-	81.12 $\pm$ 13.24	
VIQ	-	77.62 $\pm$ 13.20	
PIQ	-	88.53 $\pm$ 16.57	
BMI (kg/m^2)	-	29.3 $\pm$ 5.1	
FBS (mg/dL)	-	173.6 $\pm$ 66.6	
FBI (mU/ml)	-	11.62 $\pm$ 7.97	
HOMA-IR	-	4.8 $\pm$ 3.3	
ChE activity (IU/ml)	-	93.25 $\pm$ 21.4	
PON1 activity (IU/ml)	-	176.51	97.0

BMI: body mass index; FBS: fasting blood sugar; FBI: fasting blood insulin; HOMA-IR: homeostatic model assessment-insulin resistance; DCD: diabetic cognitive dysfunction; ChE: cholinesterase; PON1: paraoxonase 1; FSIQ: full scale intelligence quotient; VIQ: verbal intelligence quotient; PIQ: practical intelligence quotient

TABLE 3: Comparison of the mean variables between gender groups

Variable	Gender	Mean	SD	t	p-value
FBS (mg / dL)	Male	171.51	67.04	-0.306	0.760
	Female	175.17	66.75		
FBI (mU / ml)	Male	11.11	5.98	-0.618	0.538
	Female	11.99	9.17		
HOMA-IR	Male	4.61	3.19	-0.455	0.650
	Female	4.88	3.42		
BMI	Male	29.02	5.42	-0.457	0.649
	Female	29.52	4.92		
ChE	Male	86.75	15.90	-3.082	0.003**
	Female	98.41	23.90		
PON1	Male	164.7	65.47	-1.163	0.247
	Female	184.97	114.08		
FSIQ	Male	84.57	15.61	2.536	0.012*
	Female	78.65	10.69		

FBS: fasting blood sugar; FBI: fasting blood insulin; HOMA-IR: homeostatic model assessment-insulin resistance; ChE: cholinesterase; PON1: paraoxonase 1; FSIQ: full scale intelligence quotient;
*p-value<0.05, ** p-value<0.01

number of studies in effort to identify accessible biomarkers for progression of cognitive impairment toward dementia and Alzheimer's disease. MCI is known as a risk stage for developing Alzheimer's disease and the annual rate of conversion from MCI to Alzheimer's disease has been estimated 12-15%. On the other hand, diabetes has been shown to have higher coincidence with Alzheimer's disease so that the risk of Alzheimer's disease in diabetic patients has been estimated to be three times higher than people without diabetes. In this way, evaluating cognitive performance

TABLE 4: Comparison of the mean of FBS, FBI, HOMA-IR, ChE and PON1 activity between groups with and without DCD (t-test)

	DCD	Mean	SD	F	P value
FBS	Yes	177.81	67.29	0.136	0.171
	No	158.44	64.20		
FBI	Yes	12.13	8.61	1.908	0.232
	No	10.11	5.13		
HOMA-IR	Yes	4.49	2.80	0.002	0.445
	No	3.99	2.44		
ChE activity	Yes	0.12	0.14	7.804	0.046*
	No	0.09	0.02		
PON1 activity	Yes	179.08	102.09	1.004	0.699
	No	171.02	77.86		

FBS: fasting blood sugar; FBI: fasting blood insulin; HOMA-IR: homeostatic model assessment-insulin resistance; ChE: cholinesterase; PON1: paraoxonase 1
* p-value<0.05.

TABLE 5: Correlation between quantitative variables in the patients with diabetes (Pearson's correlations)

		CHE	PON1	FBS	BMI	Age	DoD	FBI	FSIQ	HOMA
ChE	Pearson Corr.	1	-.002	.056	.037	.044	.138	.033	-.034	.053
	Sig. (2-tailed)		.984	.540	.737	.633	.133	.715	.710	.560
PON1	Pearson Corr.	-.002	1	.123	-.232*	-.204*	-.013	.001	.055	.102
	Sig. (2-tailed)	.984		.169	.028	.023	.883	.989	.543	.256
FBS	Pearson Corr.	.056	.123	1	.074	-.075	.087	-.165	-.160	.382**
	Sig. (2-tailed)	.540	.169		.488	.406	.332	.063	.073	.000
BMI	Pearson Corr.	.037	-.232*	.074	1	-.030	-.091	.173	-.175	.199
	Sig. (2-tailed)	.737	.028	.488		.778	.394	.101	.100	.059
Age	Pearson Corr.	.044	-.204*	-.075	-.030	1	.248**	-.028	-.017	-.049
	Sig. (2-tailed)	.633	.023	.406	.778		.005	.757	.853	.584
DoD	Pearson Corr.	.138	-.013	.087	-.091	.248**	1	.080	.091	.137
	Sig. (2-tailed)	.133	.883	.332	.394	.005		.371	.311	.127
FBI	Pearson Corr.	.033	.001	-.165	.173	-.028	.080	1	-.093	.785**
	Sig. (2-tailed)	.715	.989	.063	.101	.757	.371		.296	.000
FSIQ	Pearson Corr.	-.034	.055	-.160	-.175	-.017	.091	-.093	1	-.138
	Sig. (2-tailed)	.710	.543	.073	.100	.853	.311	.296		.121
HOMA	Pearson Corr.	.053	.102	.382**	.199	-.049	.137	.785**	-.138	1
	Sig. (2-tailed)	.560	.256	.000	.059	.584	.127	.000	.121	

FBS: fasting blood sugar; FBI: fasting blood insulin; HOMA-IR: homeostatic model assessment-insulin resistance; ChE: cholinesterase; PON1: paraoxonase 1; FSIQ: full scale intelligence quotient; DoD: duration of diabetes;
*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

of diabetic patients during the course of disease seems clinically important. Both diabetes and Alzheimer's disease share the same pathophysiological mechanisms including inflammation, insulin resistance, accumulation of β-amyloid peptide and abnormal level of antioxidant enzymes such as ChE and PON1. Hence, finding metabolic or biochemical markers in association with cognitive impairment in diabetic patients will be helpful for understanding the disease process and management. This work cross-sectionally evaluated cognitive function of diabetic patients in association with metabolic and enzymatic parameters and found that serum level of ChE activity in the patients having cognitive impairment was higher than the patients with normal cognitive function. Prevalence of cognitive dysfunction in diabetic patients of our study has been estimated about 77%. By comparing the mean variables related to metabolic and enzymatic biomarkers between two groups of patients, with and without DCD, the statistically significant difference was found only for ChE. Although, the mean levels of the FBS, FBI and HOMA-IR were higher in the patients with DCD,

the difference was not statistically significant. In the comparing test for the mean variables between male and female, this finding was also confirmed. The enzymatic activity of ChE in the serum of the women found to be significantly higher than that of men in our study. The mean level of FSIQ in the women was also found to be significantly lower than that of men. In fact, women in our study population had lower FSIQ and higher ChE activity than men implying on the role of enhanced activity of ChE in the association of diabetes with cognitive impairment. In an *in silico* study using BLAST Tool for protein to protein comparison, ChE related proteins were commonly found in both diabetes and Alzheimer's diseases and the authors suggested that esterase enzymes may have an etiological role in the coexistence of these two diseases via affecting insulin sensitivity and lipid metabolism (Sridhar et al. 2006). The mean index of insulin resistance, HOMA-IR, in our study population was estimated as 4.8 which was higher than the normal level and due to the nature of our study population as diabetic patients, this was an expected finding. Involvement of IR in dementia and Alzheimer's disease has already been well studied and the evidence imply on the role of IR in the pathophysiologic mechanism of Alzheimer's disease. In our study, the mean level of IR in the patients with and without cognitive impairment had no statistical difference. According to the literature, increased levels of IR and ChE in both diabetes and Alzheimer's disease have been documented.

However, increased serum activity of ChE of the patients with DCD for about 33% in comparison with the patients without DCD with no changed level of IR indicates that ChE is a much better marker for evaluating the development of cognitive dysfunction.

The other important finding of our study was related to the serum PON1 activity which was shown to have no statistical difference between diabetic patients with and without cognitive impairment. This was in contrary to previous reports on increased level of PON1 in the patients with MCI. However, this difference in the results can be caused due to different study population, tests used to examine cognitive performance and protocols used to assess arylesterase activity of the PON1 enzyme. But our study had shown negative associations of serum PON1 activity siginficantly with both age and BMI of the patients. Since, PON1 is a known antioxidant enzyme which acts in cooperation with HDL cholesterol, thus this finding was expected.

CONCLUSION

Taken together, the results of our study showed that cognitive impairment is a prevalent complication in diabetic patients and considering metabolic and enzymatic parameters, higher incidence of diabetic cognitive dysfunction has been found in association with increased activity of serum cholinesterase enzyme. No change was observed in the activity of PON1 and HOMA-IR in the patients with and without

cognitive impairment. According to the literature implicating on increased activity of serum cholinesterase in both diabetes and Alzheimer's disease, serum cholinesterase activity can be suggested as a candidate biomarker for development of cognitive impairment toward Alzheimer's disease and vascular dementia in diabetic patients.

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