Study of Paraoxonase, Catalase, HDL-Cholesterol and Malondialdehyde Levels in NIDDM

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ABSTRACT

Diabetes Mellitus (DM) is an important cause of morbidity and mortality through its various complications. A decrease in Paraoxonase 1 (PON1) activity in diabetes is associated with occurrence of vascular complications. Our objectives were to estimate PON1 activity, Catalase (CAT) activity and Malondialdehyde (MDA) levels in Non-Insulin Dependent Diabetes Mellitus (NIDDM) cases and controls. We also had an objective to establish possible relationship between PON1, High Density Lipoprotein-Cholesterol (HDL- Cholesterol), CAT, MDA levels and to correlate CAT/PON1 ratio with Glycosylated Hemoglobin (HbA1c) levels.

Keywords: *Diabetes Mellitus, Paraoxonase, Oxidative stress, CAT/PON1 ratio*

INTRODUCTION

Diabetes Mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. The metabolic dysregulation associated with DM cause secondary pathophysiological changes in multiple organ systems. Diabetes Mellitus causes oxidative stress due to overproduction of free radicals or decreased efficiency of inhibitory or scavenging system. These free radicals react with membrane lipids and cause lipid peroxidation which is an important feature of cellular injury and leads to complications.

Lipid peroxidation and consequent degradation yields products like Malondialdehyde (MDA) which is assessed to estimate oxidative stress.[1] It has been known that Paraoxonase1(PON1), an aryl esterase is associated with HDL-Cholesterol (HDL-C) and has a protective effect against oxidative stress.[2,3] Catalase (CAT) is a marker enzyme for peroxisomes[4] which will destroy unwanted peroxide and other free radicals.[5] Glycosylated Hemoglobin (HbA1c) is the best index for long term control of blood glucose level.[6]

Thus, in this present study, lipid peroxidation was measured in terms of MDA along with HDL-C and antioxidant enzymes PON1, CAT. These parameters were correlated with glycemic control and complications arising from poor glycemic control. An attempt was made to know the lipid peroxidation status and CAT/PON1 ratio, if it would help in early detection of vascular complications in patients with poor glycemic control.

REVIEW OF LITERATURE

In 1976, Koeing et al measured the fasting blood sugar and HbA1c concentration of patients before and during treatment and found that the periodic monitoring of HbA1c levels provide a useful way of documenting the degree of regulation of glucose metabolism in diabetic patients and provides a means whereby the relation of carbohydrate control to the development of sequelae can be assessed.[7]

In 1979, Yuzo Sato and his co-workers studied the lipid peroxide levels in plasma of diabetic patients and compared them to levels in normal healthy individuals. They found that the lipid peroxide levels in diabetics was higher compared to normal subjects and the diabetic patients with angiopathy have higher lipid peroxide levels than the diabetic subjects without angiopathy. According to their study, lipid peroxide levels in plasma of diabetics can serve as a useful monitor to judge the prognosis of the patient.[8]

In 1989, Sushil K. Jain and co-workers studied erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes mellitus and found that there was significantly increased membrane lipid peroxidation in diabetic erythrocytes compared to non-diabetic erythrocytes. The degree of membrane lipid peroxidative damage in erythrocytes was significantly correlated with the level of glycosylated hemoglobin.[9]

In 1993, G. Gallou and associates measured malondialdehyde a marker of lipid peroxidation, as thiobarbituric acid reactive substance (TBARS). Results showed that TBARS concentration were significantly higher in diabetics than in control grouped and were still higher in patients with vascular complications. They suggested that increased plasma TBARS in diabetics is due to increased lipid peroxidation which can be due to free

radical hyperproduction or impairment of Intracellular antioxidant systems.[10]

In 1996, Ranjini K Sundaram and co-workers conducted a study in cases of NIDDM vs healthy controls which showed that lipid peroxidation was significantly raised and antioxidant enzymes like catalase were lowered significantly. These changes correlated with the duration of the disease and were of a higher magnitude with the development of complications.[11]

This study was conducted in the year 2000 by Bharti Mackness and co-workers in diabetic patients complicated by retinopathy and it was found that PON1 activity was low in these subjects and the complication may be related to an increased tendency for lipid peroxidation.[12]

In the year 2006, Purnima Dey Sarkar and associates did a study to find out the association between paraoxonase activity and lipid levels in patients with premature coronary artery disease. This study was carried out in angiographically proven premature CAD patients compared with normal subjects. It was found that PON activities towards P-nitrophenyl acetate were lower in subjects with CAD than in control subjects. This study concluded that paraoxonase was an independent risk factor for premature CAD and that PON activity should be evaluated in all coronary artery disease patients.[13]

MATERIALS AND METHODS

A study of PON1, HDL-C, CAT, MDA was conducted in NIDDM patients and healthy controls from Bapuji Hospital and Chigateri District General Hospital, Davangere. Each gave an informed consent and this study was approved by ethical and research committee of J.J.M. Medical college, Davangere, to use human subjects in the research study. The patients and controls voluntarily participated in the study.

A total number of 104 subjects participated in the study. Of 104 subjects, 62 were NIDDM patients (42 patients of NIDDM without complications, 20 patients of NIDDM with complications). The NIDDM patients were identified with thorough history and clinical examination. 42 healthy controls who were age and sex matched (fasting serum glucose levels ≤110.0 mg/dl) were taken for the study.

Inclusion criteria: Controls are healthy individuals, age and sex matched without any major illness.

Cases are NIDDM patient with

1. Fasting blood glucose ≥ 140.0 mg/dl
2. Duration of diabetes ≥ 1 year
3. NIDDM patients with and without complications

Exclusion criteria:

1. NIDDM patients who are alcoholics
2. Smokers
3. Those with clinical evidence of infectious diseases and neoplasia

Collection of blood samples: Selected subjects were asked to fast overnight for collection of blood samples. About 6ml of blood was collected from large peripheral vein with aseptic precautions, out of which 2ml was taken in anti-coagulant bulb (EDTA) for estimation of glycosylated hemoglobin and Catalase, 4ml was taken in a plain bulb and serum was separated after centrifugation and the following parameters were estimated:

1. Serum Glucose by O-Toluidine method[14]
2. Glycosylated Hemoglobin (HbA1c) by Cation-Exchange Resin Method[15]
3. Serum HDL-Cholesterol by CHOD-PAP method[16]
4. Serum MDA by Thiobarbituric Acid Method [17]
5. Serum Paraoxonase 1 (PON 1) activity using p-nitrophenyl acetate as substrate [13]
6. Catalase activity in RBC hemolysate using hydrogen peroxide[18]

ESTIMATION OF SERUM PARAOXONASE1 ACTIVITY USING P-NITROPHENYL ACETATE AS SUBSTRATE[13]

PON1 activity was determined by using P-nitrophenyl acetate as a substrate. The increase in the absorbance at 412 nm due to formation of P-nitrophenol was measured.

REAGENTS

1. Solution A
   0.2M Tris was prepared by dissolving 24.2 gms of Tris in one liter of double distilled water.
2. Solution B
   0.2M HCl was prepared by diluting 17.2 ml of concentrated HCl to one liter double distilled water.
3. Stock Buffer 0.2M of pH 8.0
   50 ml of solution A was mixed with 26.8 ml of solution B and the volume was made upto 200 ml and adjusted to pH 8.0.
4. Working Buffer 20 mM/L
   Prepared by diluting 1 ml of stock buffer to 10 ml double distilled water.
5. For basal PON1 activity
   Tris buffer (20 mM/L) containing 1 mM CaCl2 (11 mg of CaCl2 dissolved in 100 ml of buffer) was prepared.
6. Substrate (5.5mM/L)
   15 mg of paranitrophenyl acetate was dissolved in 0.5 ml of absolute ethanol. It was freshly prepared before use.

Enzyme Assay

1. Basal PON1 activity

The activity was measured at 25°C by adding 50 µl of serum to 3 ml of buffer containing 1 mM/L CaCl2 in a spectro photometric cuvette. The initial absorbance was adjusted to 0.500 in a spectrophotometer at a wavelength of 412 nm. The reaction was started by adding 50 µl of substrate. The rate of increase in absorbance was monitored at 30, 60, 90,120 and 150 seconds.

Non Enzymatic Hydrolysis

3ml of buffer was taken in spectro photometeric cuvette and 50 µl of substrate was added to it. The rate of change in absorbance (A) was monitored at 30, 60, 90,120 and 150 seconds at 412 nm. The ΔA so obtained was deducted from ΔA obtained in the presence of serum.

CALCULATION

Correction for non enzymatic hydrolysis:

Corrected ΔA = Total ΔA – non enzymatic ΔA

PON1 activity was calculated by using molar extinction coefficient of 17000 M^{-1} cm^{-1}

= 0.017/nmol/cm/ml ⇒ 0.00567/nmol/3ml (as total volume was 3 ml).

So enzyme activity per 50 µl of serum under assay conditions:

$$\frac{\Delta A/min}{0.00567} = \Delta A/Min \times 176 nmol/ml/min.$$

The ΔA for 2 min was taken from 30 to 150 seconds for calculating the enzymatic activity. The first 30 seconds was not taken for calculation in order to allow the reaction to reach a steady state.

Enzyme Activity

PON1 activity is expressed as nano moles of P-nitrophenol formed per minute per ml of serum.

ΔA in presence of CaCl2 for basal activity is 0.011.

ESTIMATION OF CATALASE ACTIVITY IN RBC HEMOLYSATE[18]

Catalase catalyses the breakdown of hydrogen peroxide according to the following reaction:

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

In the UV range H_2O_2 shows a continuous increase in absorbance with decreasing wavelength. When H_2O_2 is decomposed by catalase, the absorbance at 230 nm decreases. ΔA/min at 230 nm is measured.

REAGENTS

1. Phosphate Buffer (0.05M, pH 7.4)

4.0827gms of KH_2PO_4 is dissolved in 500 ml deionised water. Then, 10.68gms of Na_2HPO_4. $2H_2O$ was dissolved in one liter. These two solutions are taken in the proportion of 1:1·8 respectively and pH is adjusted to 7.4.

2. Hydrogen peroxide (H_2O_2); 30mM

0.34 ml of 30% H_2O_2 is diluted to 100 ml with phosphate buffer.

3. Preparation of hemolysate

EDTA blood was centrifuged and plasma was removed. RBC's were washed by 0.9% NaCl thrice. 10 volumes of cold distilled water were added to one volume of RBC and contents were mixed and centrifuged at 3000 rpm for 10 minutes. Clear hemolysate was collected.

Above hemolysate was diluted to 1:10 with 0.9% saline. One volume of this hemolysate is diluted with ten volumes of phosphate buffer pH 7.0. 20 µl of absolute ethanol was added per ml of dilute hemolysate.

PROCEDURE

A set of test tubes were prepared as follows:

	Blank	**Test**
Phosphate buffer	3.0 ml	3.0 ml
Diluted Hemolysate	———	0.01 ml
H_2O_2, 30 mM	1.0 ml	1.0 ml

The decrease in the optical density of the system was measured against that of the blank at 1 minute, 2 minutes and 3 minutes at 230 nm.

CALCULATION:

Unit of catalase activity is expressed as mM of H_2O_2 decomposition/min.

Molar extinction coefficient of H_2O_2 is 71.00 L mol^{-1} cm^{-1}.

Catalase activity in units per minute

$$= \frac{\Delta A}{0.071} \times \frac{3}{\text{Vol. of hemolysate used}}$$

$$= \frac{\Delta A/min}{0.071} \times \frac{3}{0.01}$$

$$= \frac{\Delta A/min}{0.071} \times 300$$

= ..... Units/min.

RESULTS

The data was analyzed by SPSS software. Results are expressed as Mean ± SD and range values. One way ANOVA was used for multiple group comparisons and student's t-test for group wise comparisons. Relationship between variables was measured by Pearson's correlation co-efficient.

Table 1: Comparison of FSG, HbA1c, HDL-C, PON1, CAT, MDA AND CAT/PON1 in Healthy Controls and Niddm Cases

Groups	No.	FSG (mg/dl)	HbA1c (%)	HDL-C (mg/dl)	PON 1 (nmol/ml/min)	CAT (Units/min)	MDA (nmol/ml)	CAT/PON
Controls	42	95.2 ± 11.8	6.3 ± 0.6	45.7 ± 7.8	62.3 ± 5.9	178.4 ± 42.6	4.35 ± 0.82	2.90 ± 0.80
Range		(67.6 - 110.6)	(5.0 - 88.0)	(32.0 - 62.0)	(50.6 - 72.2)	(98.0 - 250.1)	(3.0 - 6.8)	(1.51 - 4.48)
NIDDM Cases	62	192.0 ± 61.7	8.1 ± 1.5	35.7 ± 3.7	42.0 ± 8.1	63.1 ± 20.3	7.0 ± 1.4	1.48 ± 0.38
Range		(140.0– 539.0)	(6.3 - 15.9)	(29.1 - 45.1)	(26.3 ± 56.8)	(26.5 - 93.2)	(4.1 - 12.0)	(1.1 - 1.86)
Control vs Cases	t*	12.0	8.6	7.8	14.8	16.4	11.8	10.7
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

* t student's t-test $p < 0.05$ significant
FSG: Fasting Serum Glucose

The mean values in controls were (refer table 1), Fasting serum glucose 95.2±11.8 mg/dl, HbA1c 6.3±0.6%, HDL-C 45.7±7.8 mg/dl, PON1 62.3±5.9nmol/ml/min, CAT 178.4±42.6 units/min and MDA 4.35±0.82nmol/ml. Mean values in NIDDM cases were, Fasting serum glucose 192±61.7 mg/dl, HbA1c 8.1±1.5%, HDL-C 35.7±3.7 mg/dl, PON1 42±8.1nmol/ml/min, CAT 63.1±20.3 units/min and MDA 7.0±1.4 nmol/ml. Statistical analysis by student's t-test showed that the mean levels of FSG, HbA1c and MDA were increased in patients with NIDDM when compared to controls and were statistically highly significant ($p < 0.001$). The ratio of CAT/PON1 in controls was 2.90±0.8 and in NIDDM cases was 1.48±0.38 (decreased). These values were statistically significant ($p < 0.001$).

Table 2: Comparison of FSG, HbA1c, HDL-C, PON1, CAT, MDA AND CAT/PON1 in Diabeticpatients with and without Complications

Groups		No.	FSG (mg/dl)	HbA1c (%)	HDL-C (mg/dl)	PON1 (nmol/ml/min)	CAT (Units/min)	MDA (nmol/ml)	CAT/PON1
I Controls		42	95.2 ± 11.8	6.3 ± 0.6	45.7 ± 7.8	62.3 ± 5.9	178.4 ± 42.6	4.35 ± 0.82	2.90 ± 0.80
IIa Diabetics without Complications		42	188.0 ± 44.4	7.5 ± 0.6	36.4 ± 3.6	46.1 ± 5.9	75.8 ± 9.4	6.33 ± 1.02	1.67 ± 0.30
IIb Diabetics with Complications		20	200.5 ± 88.7	9.3 ± 1.9	34.1 ± 3.4	33.5 ± 4.6	36.4 ± 5.4	8.31 ± 1.24	1.10 ± 0.19
ANOVA*		F	50.6	67.6	40.3	193.9	228.0	114.9	89.8
		P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Difference between groups	I - IIa	t**	13.1	8.70	7.01	12.61	15.25	9.82	9.32
		P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	I - IIb	T	7.62	9.78	6.34	19.3	14.8	15.0	9.9
		P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	IIa -IIb	T	0.75	6.01	2.38	8.41	17.4	6.7	7.8
		P	0.46	< 0.001	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001

* One way ANOVA $p < 0.05$ significant
** Student's t-test $p < 0.001$ highly significant FSG: Fasting Serum Glucose.

From the table 2, it is observed that the mean values of FSG, HbA1c and MDA were higher in group IIb compared to group IIa and the values were statistically significant. The mean values of HDL-C, PON1, CAT and CAT/PON1 ratio were decreased in group IIb compared to group IIa and the values were statistically significant

Table 3: Pearson's Correlation Coefficient

Relationship Between	Diabetic Cases	
	r*	P
FSG and HbA1c	0.16	0.21
HDL and PON1	0.95	< 0.001
HbA1c and PON1	- 0.50	< 0.001
PON1 and MDA	-0.63	< 0.001
CAT and MDA	- 0.62	< 0.001
HbA1c and MDA	0.61	< 0.001
HbA1c and CAT/PON1	- 0.43	< 0.001
CAT/PON1 and MDA	- 0.45	< 0.001

* Pearson's Correlation coefficient $p < 0.001$ highly significant
$p > 0.05$ not significant FSG : Fasting Serum Glucose
$p < 0.05$ significant

It is evident from table 3, that there is a positive correlation between HbA1c and MDA, HDL-C and PON1. If levels of one parameter increases, the levels of associated parameters also increases. HbA1c and PON1, PON1 and MDA, CAT and MDA showed a negative correlation. There was also a negative correlation between CAT/PON1 and MDA. These were statistically significant. The negative correlation between HbA1c and CAT/PON1 was not significant statistically.

DISCUSSIONS

The pathogenesis of complications of diabetes mellitus has long been a subject of controversy and the exact mechanism for its development remains unclear. The role of damage caused by free radical or the ineffective antioxidant mechanisms are said to be responsible.

The goal of our study was to assess the antioxidant activity in terms of PON1 activity, catalase (CAT) activity. Oxidative stress in terms of MDA and the levels of Fasting Serum Glucose (FSG), Glcosylated hemoglobin and HDL-C in NIDDM cases and to evaluate the relationship between these parameters in development of vascular complications in diabetes mellitus.

FSG, HbA1c and lipid profile are the recommended parameters to be measured in diabetes mellitus. Normally, the blood glucose levels are regulated by the hormone insulin. In diabetes mellitus where there is decreased level of insulin or insulin resistance, glucose uptake into the insulin dependent cells is altered resulting in increased serum glucose levels.

The mean values of HbA1c were increased in cases when compared to Controls. Further, the mean values in NIDDM cases with complications were increased when compared to NIDDM cases without complications. These findings are in accordance with the studies done by Boas Gonen et al[19], Ranjini K. Sundaram et al[11], Groop et al[20], Lyons et al.[21]

HbA1c is formed by the post transcriptional glycosylation of hemoglobin A at the amino terminal valine of the beta chain. This is a slow non-enzymatic chemical reaction which occurs throughout the lifespan of the erythrocyte, the prevailing plasma glucose concentration being the most important factor governing the quantity of HbA1c formed. These changes resulting from specific glycosylation reactions explain many of the sequelae of diabetes[7]. HbA1c reflects the mean blood glucose concentration for the previous weeks and not just at the time of measurement. Thus it gives an idea about the metabolic control over the previous weeks and the degree of carbohydrate imbalance better than FSG.[22] It also provides a better index of long-term glycemic control in diabetic patients.

HDL-C levels were decreased in NIDDM cases with complications more than in those of cases without complications and controls. These findings are in accordance with the studies done by H. Surekha Rani et al,[23] N.P. Surayawanshi et al,[24] Madhur Gupta et al.[25] HDL-C is considered to be good cholesterol as its concentration is inversely related to vascular complications. Under oxidative stress, LDL and other serum lipoproteins including HDL are prone to lipid peroxidation. Oxidation of HDL may essentially lower the effective level of this lipoprotein. A reduced level of serum HDL is an independent risk factor for atherosclerosis and oxidized HDL increases the risk. This possibility is related to the fact that native HDL-C functions in the process of reverse cholesterol transport and also as an inhibitor of LDL Oxidation. Studies done by Nagano Y. et al[26] and Morel D.W et al[27] showed that HDL oxidation substantially reduces the ability of this lipoprotein to function as a potent acceptor of cholesterol efflux. Reduced levels of HDL-C may also be due to obesity, increased caloric intake and lack of muscular exercise in patients with diabetes mellitus.[24]

However, HDL-C associated PON1 activity is being used as a risk predictor for diabetic complications which may be of great value for an individual. The results of our study support the concept that reduced PON1 activity is related to the occurrence of complications in diabetes mellitus.[13] Reduced PON1 activity is considered an aetiologic factor in occurrence of CAD.[28, 29]

In our study the PON1 activity was decreased in NIDDM cases when compared to controls and were much more decreased in NIDDM cases with complications when compared to NIDDM cases without complications. These findings are in accordance with the studies done by Purnima Dey Sarkar et al,[13] Bharti Mackness et al,[12] Abbot C.A et al,[3] Ikeda Y. et al.[30] PON1 is a glycoprotein antioxidant enzyme. PON1 activity in diabetes mellitus is decreased as the constant elevation of blood glucose causes glycosylation of the enzymes and their structural and functional properties are altered resulting in decreased activity.

Our study showed a positive correlation between HDL-C and PON1. PON1 has been suggested as the factor largely responsible for the antioxidant role of HDL-C. Serum PON1 activity depends on the number of PON1 molecules in HDL-C. Due to decreased levels of HDL-C in DM, the activity of the associated enzyme PON1 is also decreased. The decreased PON1 activity further reduces HDL-C levels. PON1 can inhibit not only LDL from oxidation but also prevents HDL oxidation.[31] Due to decrease in PON1 activity, lipid peroxidation occurs unchecked thus leading to development of vascular complications. Studies have shown that reduced activity of PON1 is an aetiologic factor in the development of coronary artery disease. [13,28, 29]

PON1 activity also showed a negative correlation with glycemic control. As the glycosylated hemoglobin increased, there is a decrease in PON1 activity. This finding is in accordance with the study done by Bharti Mackness et al.[12]

Our study showed a negative correlation between PON1 and MDA. This finding is in accordance with the studies done by Michael Aviram et al,[31] Watson A.D et al.[32] MDA is a product of lipid peroxidation. Oxidative modification of LDL in the artery wall is believed to be central to the pathogenesis of atherosclerosis. HDL appears to decrease the accumulation of lipid peroxides on LDL. HDL-C associated enzyme PON1 is responsible to prevent accumulation of lipid peroxides on LDL. Under oxidative stress, the inhibitory effect of PON1 on HDL oxidation is associated with preservation of ability of HDL-C to induce cellular cholesterol efflux from macrophages. Thus PON1 protects HDL-C for cholesterol efflux functions.

It is evident that the catalase activity is decreased in NIDDM cases when compared to controls. Further, the values were still more decreased in NIDDM cases with complications when compared to NIDDM cases without complications. These findings are in accordance with the studies done by Ranjini K. Sundaram et al,[11] Kornelia Z et al.[33] Also MDA showed a negative correlation with CAT which is in accordance with the studies done by Ranjini K. Sundaram et al.[11] Hence MDA has negative correlation with CAT/PON1 ratio. Our study also showed a negative correlation between HbA1c and CAT/PON1 ratio. This finding is in accordance with the study done by Sozmen E.Y et al.[34]

In poor glycemic control the mean glucose levels remain elevated in diabetes mellitus, causing reduced activity of CAT and PON1 due to glycation of these enzymes. Catalase also correlates best with poor glycemic control. Thus the ratio of CAT/PON1 can be used as a marker of poor glycemic control.[34]

Thus the decreased efficiency of cellular antioxidant mechanisms in NIDDM with simultaneous enhanced lipid peroxidation may constitute the pathogenic link between hyperglycemia and endothelial dysfunction. Moreover, the extent of oxidative stress and insufficiency of defensive antioxidant mechanism in patients with NIDDM is dependent on the glycemic control in diabetes and the occurrence of complications.

Reduced CAT/PON1 can be used to assess poor glycemic control and as a risk factor for occurrence of complications.[31] Measurement of HbA1c provides information about the glycemic control but does not reflect about the individual's response to combat oxidative stress in diabetes mellitus. Measurement of PON1 and CAT activity gives an idea about the same. Reduced activity of antioxidant enzymes indicates that there will be more peroxidative damage. Thus, this study suggests that measurement of PON1 and CAT activity would help in early detection of complications in NIDDM. CAT/PON1 ratio correlates with HbA1c levels and can be used as a marker of poor glycemic control.

Further studies can be done to estimate CAT/SOD ratio which can also be used as a marker of poor glycemic control.

CONCLUSION

This study suggests that measurement of PON1 and CAT activity would help in early detection of complications in NIDDM and the ratio of CAT/PON1 can be used as marker of glycemic control.

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