Detection of diabetic autonomic neuropathy in diabetes mellitus type 2

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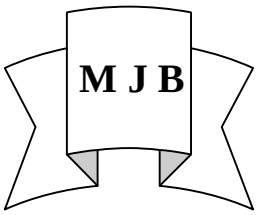

Abstract

Fifty seven subjects (40 diabetic patients and 17 control subjects) were used in this study in Marjan Teaching Hospital/ Hilla between Dec. 2005 to Nov. 2006. The main measures were used; assessment of autonomic nervous system, Doppler ultrasonography and some laboratory tests.

The FMD% was significantly less by 64% in diabetic patients in comparison to control subjects. All autonomic nervous system functions tests were impaired in diabetic patients. An increase in duration of illness, there was a significant proportional increase in HbA1c and plasma nitrite concentration. Duration of diabetic illness affected all autonomic function testes.

The reported lipid profile in this study was a significant high in diabetic patients except high density lipoprotein (HDL) which showed a significant deceased 20% in comparison to control subjects. Plasma nitrite concentration was significantly increased 53.4% in diabetic patients in comparison to control subjects.

الخلاصة

شمل البحث 57 شخصا (40 مريضا بداء السكر و 17 شخصا سليما للمقارنة). اجري البحث في مستشفى مرجان التعليمي في الحلة من كانون الأول 2005 إلى تشرين الثاني 2006. القياسات المهمة في البحث شملت تقييم الجهاز العصبي الذاتي وقياس توسع الشريان العضدي بجهاز الدوبلر وبعض التحليلات المختبرية.

أن توسع الشريان العضدي اظهر قلة معنوية مقدارها 64 % مع اختلال وظيفة الجهاز العصبي الذاتي لدى مرضى السكري مقارنة بالأشخاص السليمين. الزيادة في فترة المرض يصاحبها زيادة طردية ومعنوية في الهيموغلوبين التراكمي وتركيز بلازما النترايت. فترة الاصابة بداء السكري اثرت في جميع اختبارات وظيفة الجهاز العصبي الذاتي.

صورة الدهون المسجلة في هذا البحث اظهرت زيادة معنوية لدى مرضى السكري عدا البروتين الدهني عالي الكثافة الذي اظهر قلة 53 % لدى مرضى السكري مقارنة 4,معنوية مقدارها 20% مقارنة بالاشخاص السليمين. تركيز بلازما النترايت اظهر زيادة معنوية مقدارها بالاشخاص السليمين.

Introduction

Diabetes mellitus (DM) is a syndrome of impaired carbohydrate, fat and protein metabolism caused either by lack of insulin secretion or decreased sensitivity of tissues to insulin (1). Diabetes mellitus type 2 is a disease of adult, which may originate from insulin resistance. The cardiovascular autonomic function tests, that mediated mainly by parasympathetic nervous system (e.g. heart rate response to deep breathing) are abnormal before those mediated by sympathetic nerves (2). In general, abnormal autonomic function tests are found in 20 – 40 % of diabetic patients (3). Some manifestation of autonomic neuropathy may be preceding the diagnosis of diabetes by several years (4). Autonomic function testing is not affected by obesity (5).The FMD% of brachial artery has been used to detect endothelial function (6). The plasma lipid peroxidation activity (L.P.A) was detected by determination of plasma malondialdehyde (MOA) level. The L.P.A is a marker of oxidative stress (7). Abnormal lipid pattern could be contributed in abnormal vascular wall

because oxidized LDL within vascular wall provokes atherosclerosis and ischemic heart disease (8). A low level of HDL-c is an important cardiovascular risk factor (9). Cardiovascular autonomic neuropathy means damage to autonomic nerve fibers that innervate the heart and blood vessels (10).The significant of DAN has not been fully appreciated (11).

Diabetic autonomic neuropathy (DAN) is a serious and common complication of diabetes. It is occur secondary to metabolic disturbance and is related to the duration of diabetes and degree of metabolic control. Exercise allows non insulin dependent glucose uptake into muscle reducing the demand on the pancreatic cells to produce insulin (12). Exercise is vital for weight loss. At least 30 minutes of moderate exercise, 7 days per week should be recommended to achieve acceptable weight loss (13).DAN is frequently coexisting with other peripheral neuropathy and other diabetic complications, but it may be isolated (11). Sympathetic autonomic neuropathy is a marker of poor prognosis. Silent myocardial infarction and sudden death are common causes of death among diabetes with autonomic neuropathy. The major clinical manifestation of DAN includes resting tachycardia, exercise intolerance, orthostatic hypotension, constipation, weakness, gastroparesis and erectile dysfunction. The mortality risk among diabetic individuals of type 2 DM with cardiovascular autonomic neuropathy (CAN) may be high compared with individuals without CAN (11). The diagnosis of neuropathies is based on the following criteria 1- Autonomic function tests. 2-Neuropathic symptoms (11).

Oxidative and nitrossative stress play a key role in the pathogenesis of DAN but the mechanism remains unidentified (14). How ever, it was suggested that the peroxynitrite is the oxidizing agent that impair ANS (15). The prevalence of DAN depending on type of study, number of tests performed, age, duration of diabetes, glycemic control and other factors (16). The pathogenesis of DAN is multifactor etiology, including metabolic insult to nerve fibers, neuropathy, neurovascular insufficiency, autoimmune damage and neurohormonal growth factor deficiency (17).

HbA1c test measures the amount of glucose attached to the hemoglobin in red blood cells. This value gives an average of blood glucose levels over three month period and is measured as a percentage (18). HbA1c% can be used to evaluate diabetic control and evaluate the result of treatment (19). Poor glycemic control plays a central role in development and progression of autonomic dysfunction. Intensive therapy can slow the progression and delay the appearance of abnormal function tests (20). Nitric oxide reacts with superoxide anion to yield peroxynitrite which is a powerful oxidant and nitrosating agent (21). Oxidative and nitrosative stress plays a key role in the pathogenesis of diabetic autonomic neuropathy but the mechanism remains unidentified (14). However, it was suggested that peroxynitrite is the oxidizing agent that impair ANS (15). In diabetes, mesengial cells whose glucose transport rate does not decline rapidly, leading to high glucose inside the cells. These cells push more electrons to molecular oxygen to generate superoxide anion which is a companied by increase generation of iNO. These favor the formation of pyroxynitrite which is strong oxidant and nitrosating agent (22). The peroxynitrite damage perineum (23). Excess accumulation of free radicals had a direct neurotoxic effect (24).The aim of study is to detect the autonomic neuropathy in diabetic patients type 2.

Subjects and methods

The subjects were recruited from outpatient's clinics. Fifty seven subjects (40 diabetic patients and 17 control subjects) were included in the present study. Their ages were between 40 – 60 years. They had no history of smoking, alcohol, hypertension and overt ischemic

heart disease. Diabetic patients with duration 0.5 – 15 years. Sample of 5 ml was obtained from anticubital vein for laboratory tests. The main measurements were: 1- Assessment of autonomic nervous system which was achieved according to Ewing battery (3).
2- Doppler ultrasonography for flow mediated dilatation (FMD) of brachial artery which was based on that recorded by Hashimoto et al, 1999 (6).
3- Plasma total cholesterol with Linear Kit (Span) and plasma triglyceride with Biomaghreb (Tunisia).
4- Plasma high density lipoprotein (HDL) with direct method.
5- Fasting plasma glucose level with Biocon Kit (Germany) by standard enzymatic methods.
6- HbA1c was done in Diabetic National Center/ Baghdad. The method was achieved by Variant HbA1c program as described by Drive, 2003 (25).
7- Plasma nitrite concentration; specific method for determination of plasma nitrite include Gross Reagent System Kit but, because failure in obtaining this Kit, the method mentioned in clinical chemistry by Navarro-Gonzalvez et al 1988 (26) used.
8- Lipid peroxidation activity which was achieved via determination the by product malondialdehyde (27).

Statistical analysis

All data were expressed as mean ± SD. The difference was assessed by unpaired student's t-test. Correlation between variables and linear regression analysis were computed by Microsoft program which runs and windows operating system. A value of $P < 0.05$ was considered to be statically significant (28).

Results

1- No significant difference was observed between male and female neither in control subjects nor in diabetic patients. Therefore the data of both sexes were polled together and considered as one group.
2- The measured biochemical parameters and their differences between diabetic patients and control subjects were recorded in **table 1**. HbA1c, total plasma cholesterol, plasma triglyceride, low density lipoprotein (LDL), lipid peroxidation activity (LPA) and plasma nitrite concentration were all significantly higher in diabetic patients over their values recorded in control subjects. High density lipoprotein (HDL) was found to be lower in diabetic patients in comparison to control subjects.
3- The normal, borderline and abnormal values of cardiovascular autonomic function test values were shown in **table 2**.
4- The clinical signs and symptoms related to diabetic autonomic neuropathy were shown in **table 3**.
5- All autonomic function test values were significant decreased in diabetic patients relative to control subjects (**table 4**). The HRV response to Valsalva maneuver was a significant ($P<0.004$) decreased by 10% in diabetic patients in comparison to control subjects. HRV during deep breathing was a significant ($P< 0.002$) less by 40%in diabetic patients relative to control subjects. Moreover, immediate HR response to standing was significant ($P<0.04$) less by 6% in diabetic patients relative to control subjects. The BP response to standing was significantly ($P<0.001$) decreased by 90% in diabetic patients comparison to control subjects, the most affected test (**table 4**). 6- The FMD% in diabetic patients was significantly correlated with all autonomic function tests values. In contrast, all these parameters were not significantly correlated with other in control subjects (**table 5**).
7- FMD% was significantly ($P<0.001$) less 64% in diabetic patients in comparison to control subjects (**figure 4A**). Furthermore plasma nitrite concentration was significantly ($P<0.001$) increased by 59%in diabetic patients to control subjects (**figure 1B**). 8- There are significant ($P < 0.001$) and negative ($r = -0.76$) correlation between plasma nitrite

concentration and FMD% in control subjects. Again the correlation was sustained in diabetic patients in which FMD% was a significant ($P < 0.001$) and negative ($r = -067$) correlated with plasma nitrite concentration (**figure 1**).

9- There was no significant correlation between the FMD% and HbA1c% in control subjects. In contrast, there was a strong significant ($P < 0.001$) and negative ($r = -0.56$) correlation between these parameters in diabetic patients **(figure 2**).

10- An increase in duration of illness was associated with significant proportional increase in HbA1c%, and plasma nitrite concentration.. The duration of diabetes was associated with significant negative correlation with FMD% (**figure 8**) and autonomic function tests **(figure**5).

Discussion

Individuals diagnosed diabetes with cardiovascular autonomic neuropathy (CAN) have five times higher mortality rate than individuals without CAN involvement (29). So the goal of public health is the prevention of diabetes and its complications. Primary prevention of diabetes is the absolute goal, unfortunately that goal has not yet obtained. However it has been shown that life style intervention can reduce the incidence of type 2 diabetes (31).

The data of present study demonstrated that in comparison to the base level, the diameter of brachial artery of control subjects increased during reactive hyperemia. This increase in FMD% was impaired in diabetic patients (**see table 1**). This result is in agreement with previous published studies (30) who suggested that endothelial dysfunction (ED) in diabetic patients may result from a decreased bioavailability of endothelial nitric oxide (eNO) and with exaggerated production of endothelin-1 induced by hyperglycemia.

The present study showed that the plasma nitrite concentration is significantly higher in diabetic patients relative to control subjects (**table 1**). This finding is supported by Ferlito and Gallina, 1998 (31). Holdtke et al, 2002 (23) showed that hyperglycemia stimulates the production of a nitric oxide by enhancing inducible nitric oxide synthase (iNOS) which then react with superoxide anion to form peroxynitrite. Despite the high level of plasma nitrite concentration in diabetic patients, the FMD% was significantly lower in comparison to control subjects (figure). This may open the way to suggest that the plasma NO level may not necessary reflect the bioavailability of local release. It is possible to suggest that the available plasma nitrite in healthy subjects (low concentration) or in diabetic (high concentration) affect adversely the endothelial function. This was shown quite clearly in **figure 1** where the FMD was inversely correlated with the plasma level of nitrite in both groups.

The present study showed that diabetic patients had high level of plasma lipid peroxidation activity (L.P.A) relative to control subjects (**table 1**). The plasma L.P.A was found to affect FMD% significantly and inversely in both groups **(figure 3)**. It is possible to suggest that free radicals are formed in diabetic patients lead to increase L.P.A. by glucose oxidation with declined antioxidative enzyme levels. This result was in agreement with previous reported study by Ziegler et al, 2004 (32). Present study showed that there was a significant positive correlation between L.P.A and HbA1c% in diabetic patients (**figure 6)**. This is possibly because diabetic patients were poor glycemic control, more liable to oxidative stress, with consequent high level of L.P.A. Also there was a significant positive correlation between L.P.A and plasma nitrite concentration in control subjects and diabetic patients (**figure 7)**. This can be explained that the common underlying pathology hyperglycemia induce overproduction of iNO and oxidative stress state.

The present study showed high level of HbA1c% in diabetic patients in

comparison to control (**table 1**). This indicated that diabetic patients have had a poor glycemic control. The current study showed that metabolism of lipid is disturbed in pattern, total plasma cholesterol, plasma triglyceride, LDL are significantly higher in diabetic patients in comparison to control subjects. While plasma HDL is found to be low (**table 1**). The lipid profile in present study was in general, in agreement with report else where Nakano et al, 2003 (33). Our study showed that all autonomic function tests were significantly decreased in diabetic patients in comparison to control subjects (**Table 5**). This result was in agreement with result obtained by Stevens, 2005 (34). The present study showed significant correlation between autonomic function tests and FMD% in diabetic patients as mentioned in **table 5.** This result may indicate that ANS and ED are affecting each other.

In present study, an increase in the duration of diabetic illness is associated with significant proportional increase in HbA1c% (**figure 8B),** due to poor glycemic control of progressive diabetic patients. Again an increase in the duration of diabetic illness was associated with significant proportional increase in plasma nitrite concentration (**figure 8C**) due to overproduction of iNO in diabetic patients. In contrast, the duration of diabetes was associated with a significant negative correlation with FMD% (**figure 8A).** This result was in agreement with Clarkson et al, 1996 (35). The duration of diabetic illness was correlated significantly with all autonomic function tests (**figure 5**). A comparable result was obtained by Chessa, 2002 (36).

Conclusion

1- The plasma nitrite concentration was significantly higher in diabetic patients relative to control subjects.

2- All autonomic function test values were significant decreased in diabetic patient relative to control subjects.

3-The possible common cause of autonomic nervous system dysfunction was peroxynitrite due to oxidative stress.

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Table (1): Comparison of the measured parameters between control subjects anddiabetic patients

Parameters	Control subjects (n = 17)	DM patients (n = 40)	Propability	Normal range
FBS (mmol/L)	5.1 ± 0.6	10.9 ± 3.6	P<0.001	● 4.2 – 6.4
HbA1c%	5.0 ± 0.6	8.9 ± 2.0	P<0.001	● 3.8 – 6.4
Total plasma cholesterol (mmol/L)	4.1 ± 0.4	4.9 ± 0.5	P<0.001	● <5.18
Plasma triglyceride (mmol/L)	1.4 ± 0.2	1.9 ± 0.2	P<0.001	● <1.7
Plasma HDL (mmol/L)	1.2 ± 0.1	1.0 ± 0.2	P<0.001	● 1.2 – 1.3
Plasma LDL (mmol/L)	2.3 ± 0.4	3.0 ± 0.5	P<0.001	● < 2.6
L.P.A (nmol/ml)	2.7 ± 0.30	6.2 ± 1.6	P<0.001	……
Plasma nitrite concentration (µmol/L)	33.9 ± 6.5	52 .5 ± 6.3	P<0.001	……..

● Kasper et al, 2005.
♦ Ganong, 2001.

Table (2): Clinical signs and symptoms related to the diabetic autonomic neuropathy.

Clinical signs and symptoms	Number of diabetic patients
Blurring of vision	1
History of diabetes mellitus	4
Erectile dysfunction	16
Hypoglycemia	3
Polyuria	20
Diarrhea	1
Constipation	10
Hypotension	5
Resting tachycardia	2
Loss of weight	7
Blurring of vision	1
History of diabetes mellitus	4
Erectile dysfunction	16
Hypoglycemia	3
Polyuria	20
Diarrhea	1
Constipation	10
Hypotension	5
Resting tachycardia	2
Loss of weight	7

Table (3): Normal, borderline and abnormal values of cardiovascular autonomic function tests.

Test	Normal value	Borderline value	Abnormal value
1- HR response to Valsalva maneuver	> 1.21	1.20 – 1.11	< 1.10
2- HR variation during deep breathing.	> 15 beats/minute.	14 – 11 beats/ minute.	< 10 beats/minute.
3- Immediate heart response to standing.	> 1.04	1.03 – 1.01	< 1.00
4- BP response to standing.	< 10 mmHg.	11 – 29 mmHg.	> 30 mmHg.
5- BP response to sustained handgrip.	> 16 mmHg.	11 – 15 mmHg.	< 10 mmHg.

Table (4): Comparison of autonomic function test between control subjects and diabetic patients.

Test	Method	Control subjects	Diabetic patients	P
1- HR response to Valsalva maneuver	Valsalva ratio	1.2	1.12	P<0.004
2- HR variation during deep breathing.	Maximum – minimum of heart rate.	13.1 beats/ minute.	9.2 beats/minute.	P<0.002
3- Immediate heart response to standing.	R-R interval ratio of 30^{th} : 15^{th} beat	1.03	1.02	P<0.04
4- BP response to standing.	Fall in SBP	13.9 mmHg	26.05 mmHg	P<0.001
5- BP response to sustained handgrip.	Increase in DBP	16.1 mmHg	13.46 mmHg	P<0.001

HR**:** Heart rate.
BP: Blood pressure.
SBP: Systolic blood pressure.
DBP: Diastolic blood pressure.
P**:** Probability.

Table (5): Correlation coefficient between FMD% and the autonomic function tests in healthy subjects and in DM patients.

Autonomic function test	FMD (%)	
	HS	DM
1- Heart rate response to Valsalva maneuver	0.09 NS	Ω 0.48 P<0.01
2- HR variation during deep breathing (beat/min)	-0.19 NS	Ω 0.59 P<0.001
3- Immediate HR response to standing	0.13 NS	Ω 0.37 P<0.02
4- BP response to standing. (mm Hg)	0.34 NS	Ω 0.64 P<0.001
5- BP response to hand grip (mm Hg)	0.19 NS	Ω 0.41 P<0.01

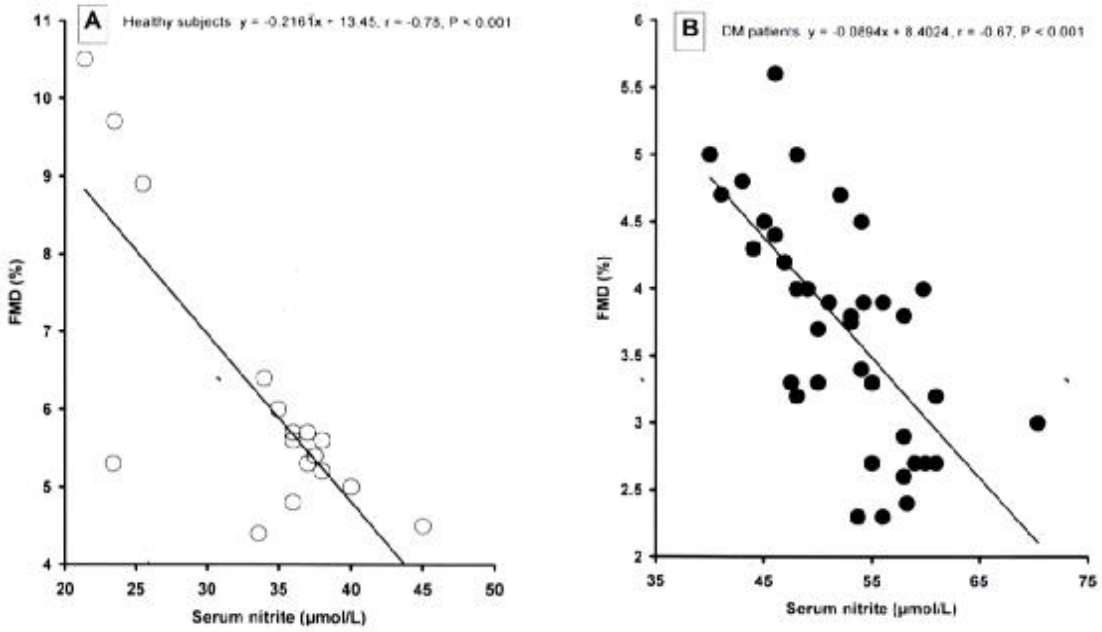


Figure (1): Correlation between serum nitrite concentration and FMD % in control subjects (A) and diabetic patients (B).

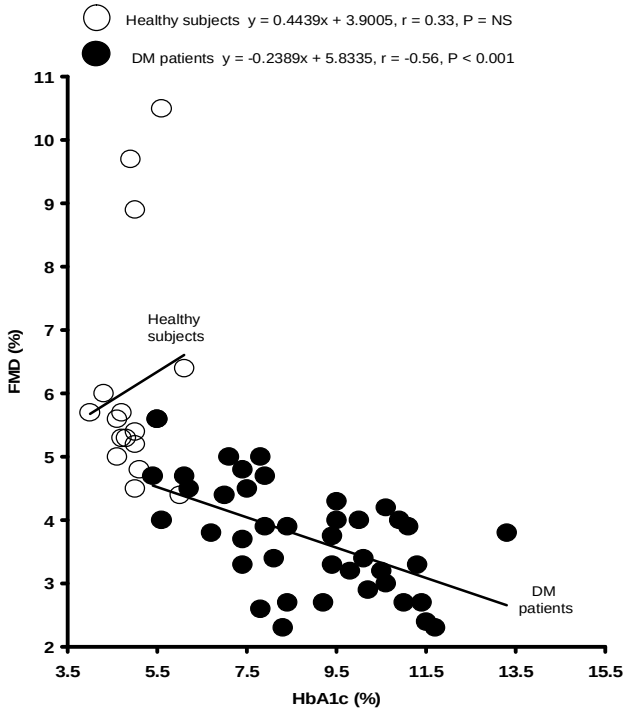


Figure (2): Correlation between HbA1c% and FMD% in control subjects and diabetic patients

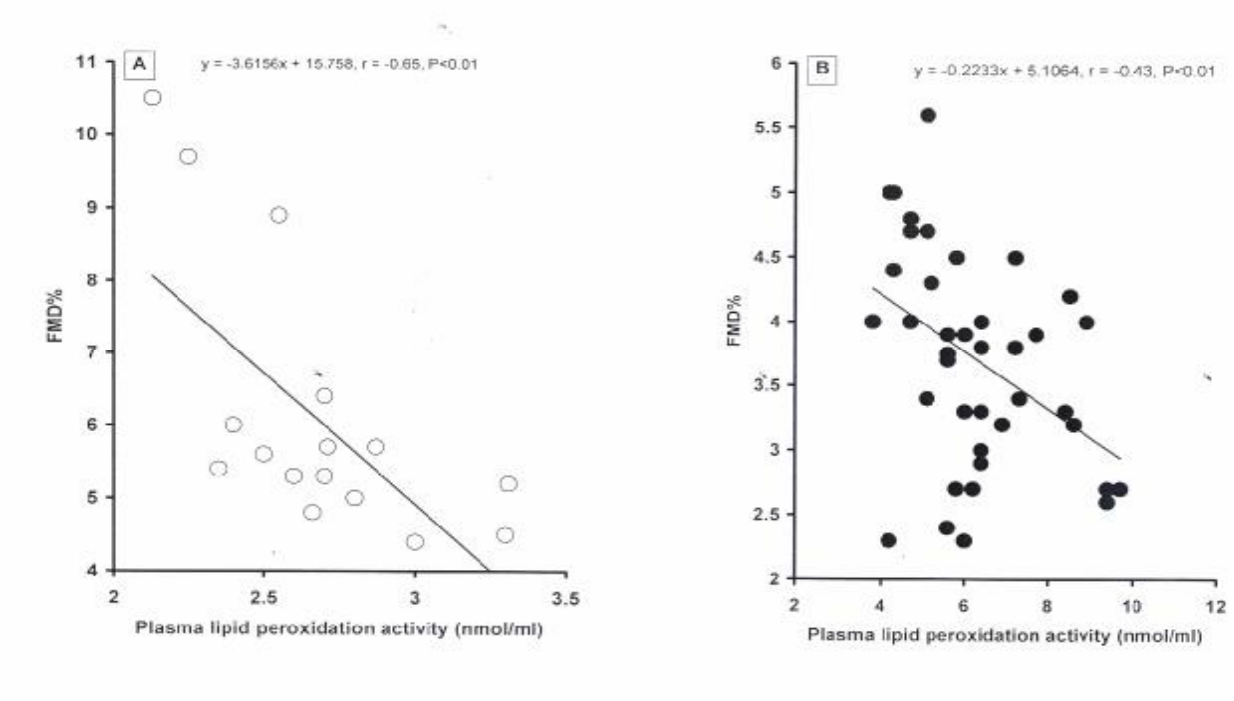
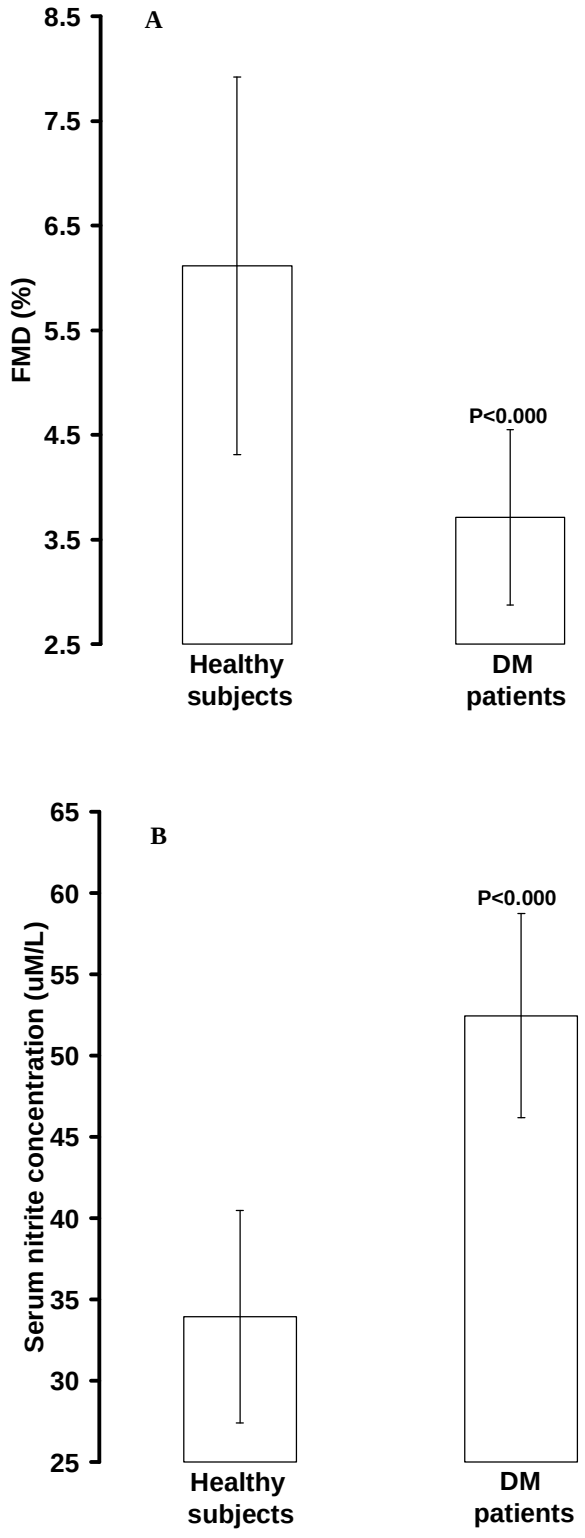


Figure (3): The effect of plasma L.P.A on FMD%



Figure(4): Comparison of FMD (A), and serum nitrite concentration (B) between control subjects and DM patients.

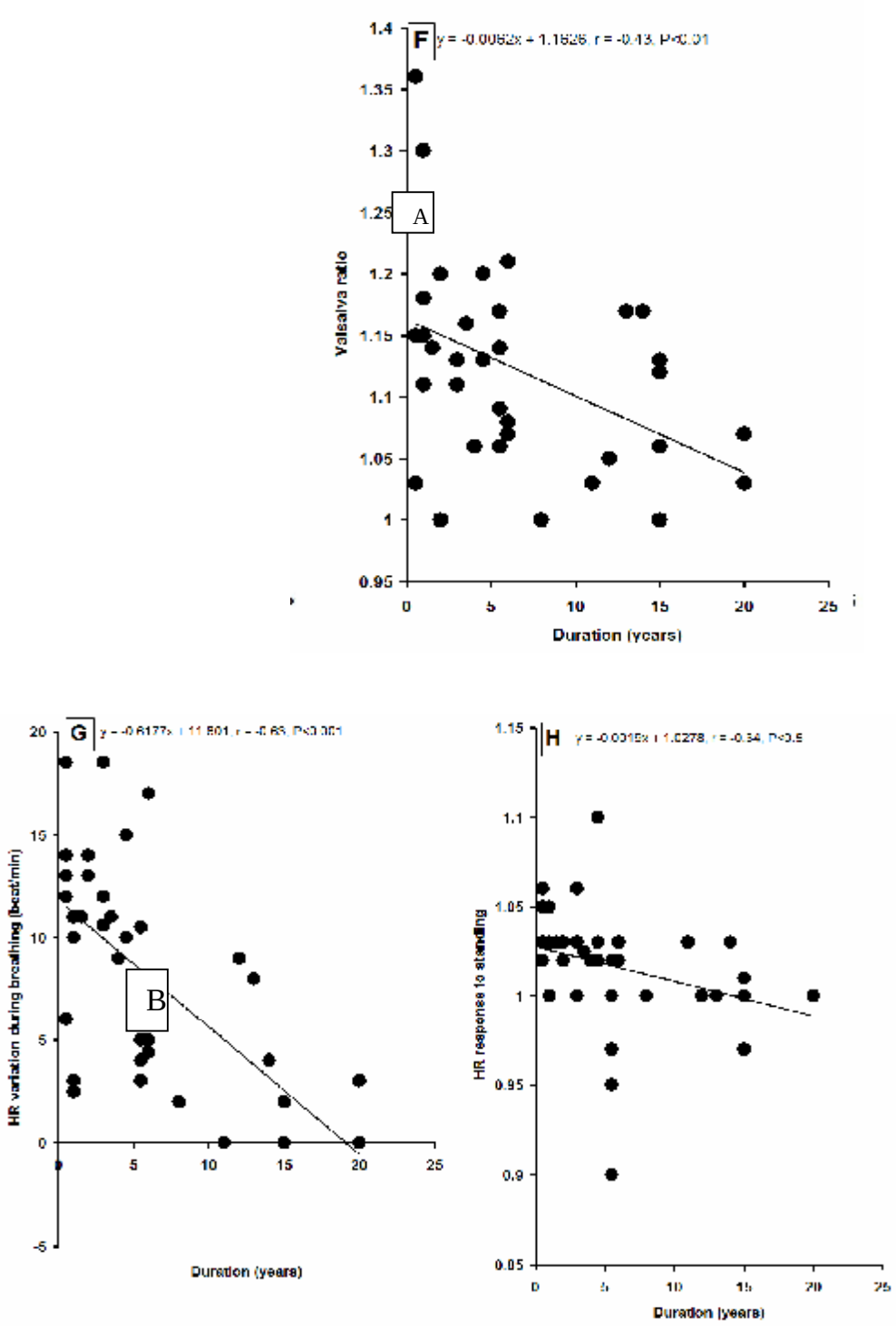


Figure (5): Correlation of the duration of diabetes mellitus with autonomic function tests.

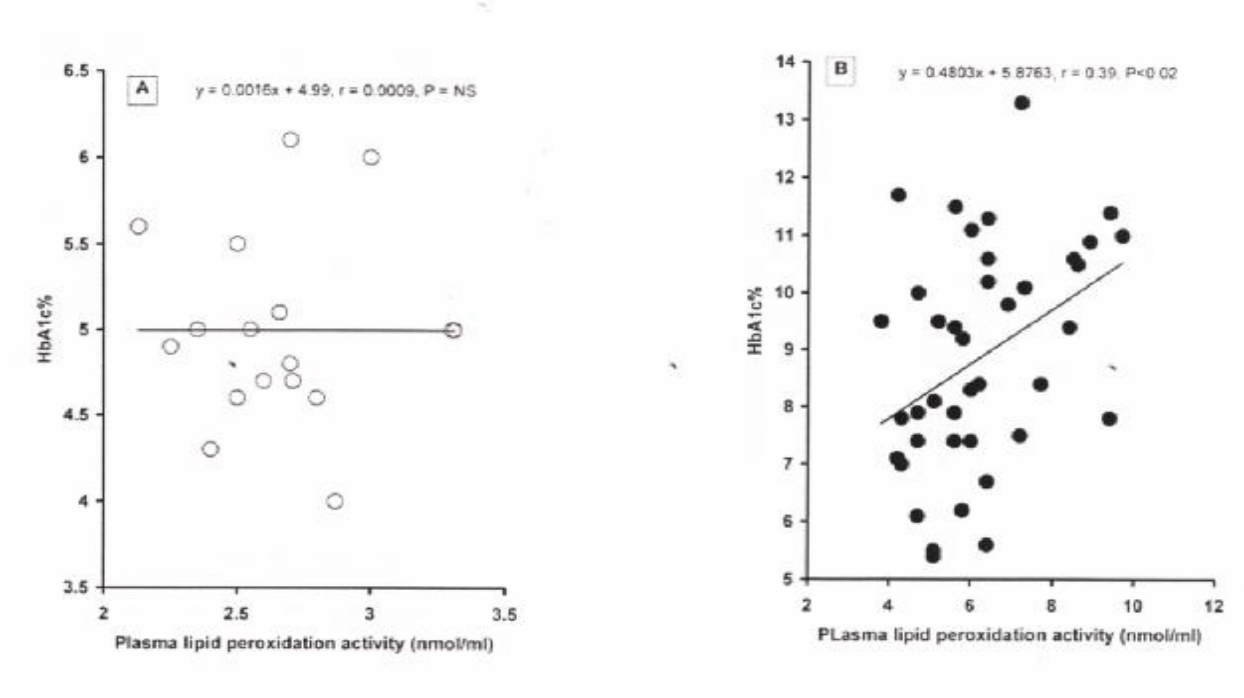


Figure 6: Correlation between plasma lipid peroxidation activity and HbA1c% in control subjects diabetic patients (A) and diabetic patients (B).

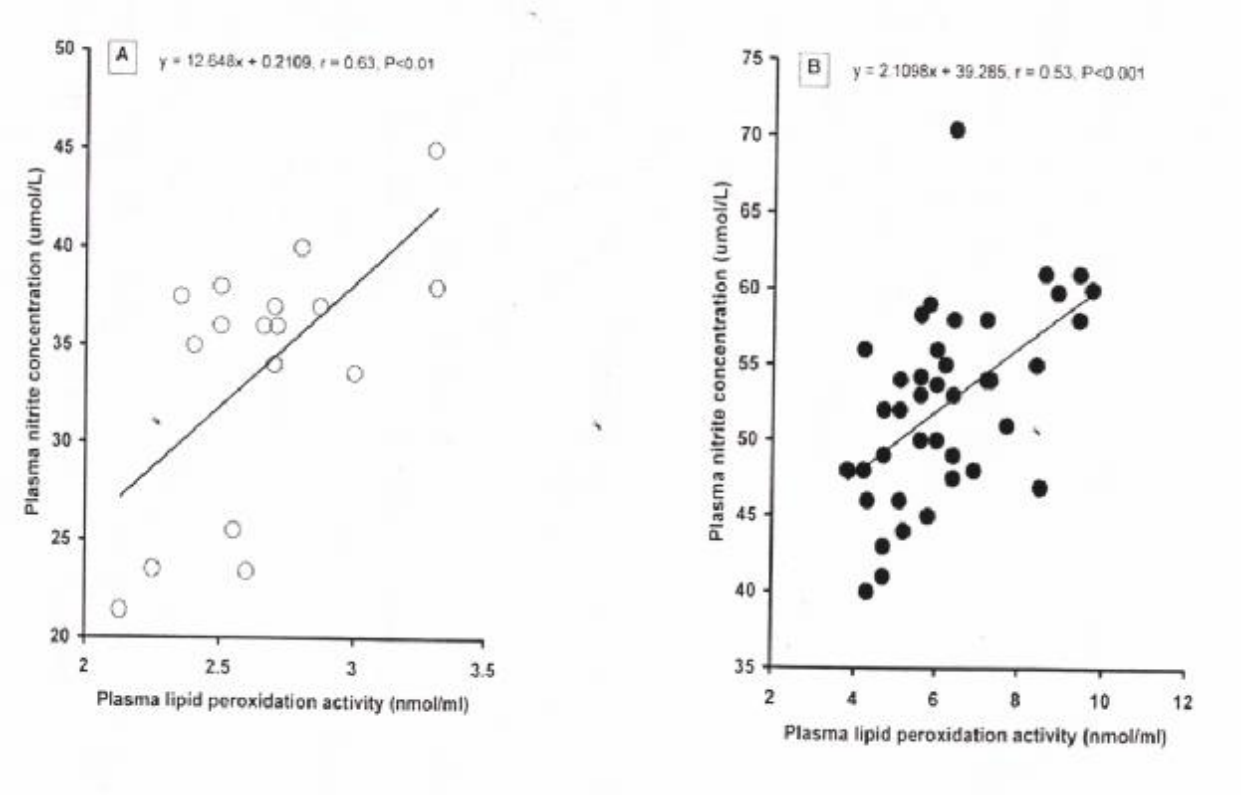
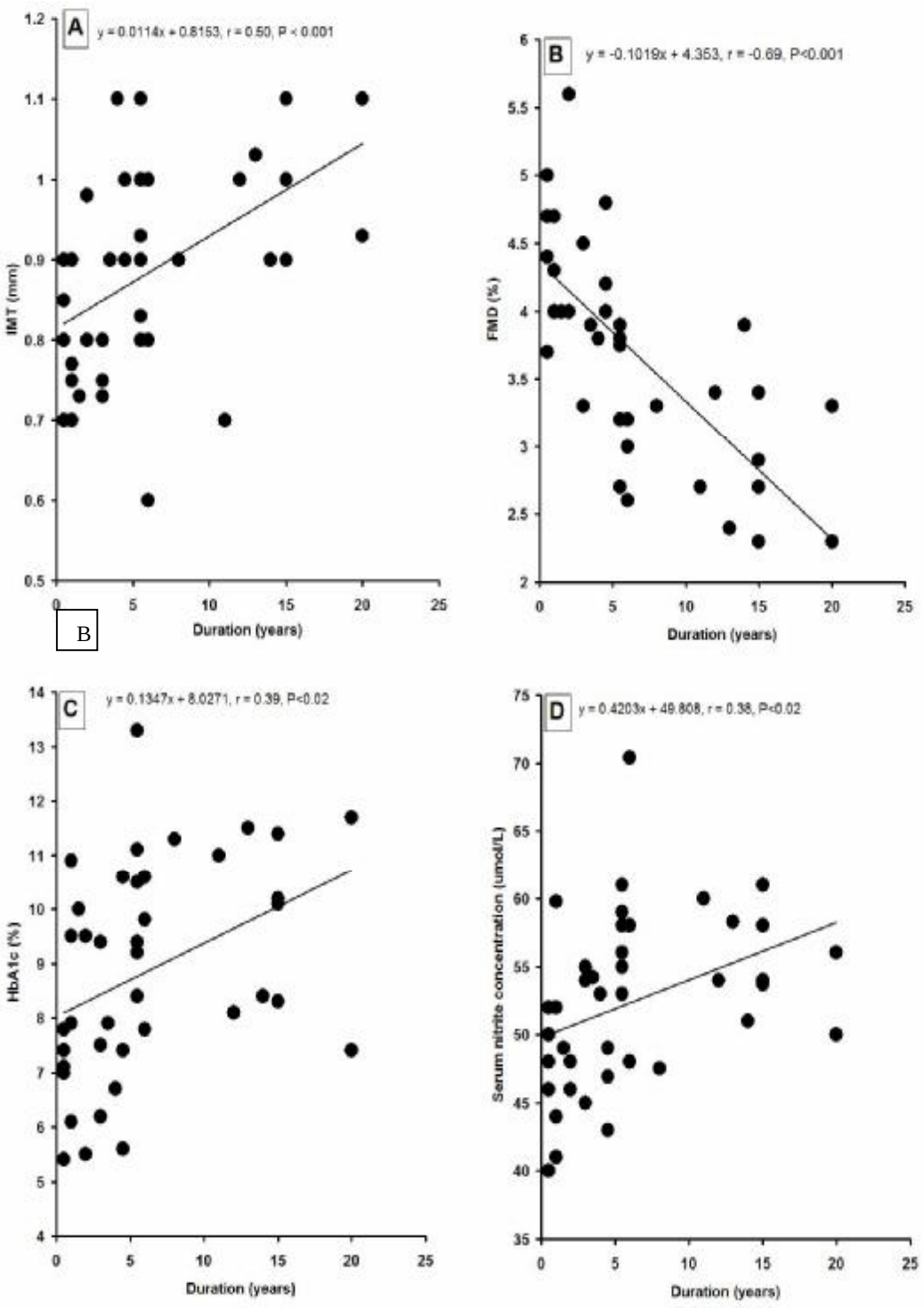


Figure 7: Correlation between plasma lipid peroxidation activity and plasma nitrite concentration in control subjects (A) and diabetic patients (A).

A



B

Figure 8: Correlation of duration of diabetes mellitus and FMD% (A) HbA1c% (B) and serum nitrite concentration (C).