ORIGINAL ARTICLE



# Angiotensin converting enzyme (ACE) gene polymorphism increases the susceptibility of diabetic nephropathy in Western Indian Type 2 diabetic patients

Hitesh Vashrambhai Patel, Kiran Kalia, Jyoti Mannari

Int J Diab Dev Ctries. 2011 ; 31 : 223-229

Abstract Angiotensin-converting enzyme (ACE) gene has been associated with the pathogenesis and progression of chronic kidney diseases. Diabetic nephropathy has become leading cause of renal end stage disease (ESRD). An I/D polymorphism of angiotensin-converting enzyme (ACE) gene has been suggested as one of the risk factors for the progression of diabetic nephropathy. We analyzed the genotype and allele frequency distribution of ACE gene in 166 Type 2 diabetic patients without any complication (T2DM), 61 with diabetic nephropathy (DN), 50 with non-diabetic nephropathy (NDN) and 50 healthy individuals from western Indian population. ACE genotype was analyzed by PCR method. The D allele distribution for the ACE I/D polymorphisms was not significantly different between control group and patients with T2DM without any complication (41.0% vs. 45.2%, P = 0.461) and between control subjects and patients with non-diabetic nephropathy (NDN) (41.0% vs. 44.0%, P = 0.668). Frequency of the D allele (63.9% vs. 45.2%, P < 0.001) and DD genotype (I allele noncarrier) (44.3% vs. 25.3%, P = 0.006) of ACE gene was significantly higher in patients with diabetic nephropathy (DN) than in patients with T2DM without any complication. Results of the present study indicate that ACE gene polymorphism does not have significant influence on development of diabetes mellitus and nondiabetic nephropathy, whereas, the DD polymorphism in ACE gene has been associated with the development of diabetic nephropathy in the Western Indian population.

Keywords Diabetes mellitus · Diabetic nephropathy · ACE gene · Polymorphism

K. Kalia
kirankalia_in@yahoo.com

## Introduction

Diabetic nephropathy (DN) has become the leading cause of end-stage renal disease (ESRD) characterized by persistent microalbuminuria (i.e. albumin levels in the urine, >30 mg/24h). Decreased glomerular filtration rate frequently occurs in patients with T2DM ultimately requiring renal replacement therapy [1, 2]. Various factors including gender, older age, presence of hypertension, moderate or severe proteinuria and severe renal lesions are responsible for the progression of renal damage.

Furthermore, drugs such as angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists (ARA) protect the development and progression of nephropathy and also delimit progression of nondiabetic proteinuric kidney disease [3]. These studies suggest that ACE, the key component of renin angiotensin system (RAS) is implicated in the development of diabetic nephropathy. This system plays an essential role in the regulaton of blood pressure, vasoconstriction, anti-diuretic hormone release and sodium homeostasis. ACE, a dipeptidyl exopeptidase converts angiotensin I to the potent vasoconstrictor angiotensin II and inactivates bradykinin which is a potent vasodilator [4]. Genetic studies have revealed that various environmental and genetic factors affect the status of RAS in individuals due to its highly

polymorphic nature. There is an increasing evidence that genetic predisposition plays a significant role in the development of diabetic nephropathy [5]. ACE level is modulated by ACE gene located on chromosome 17q23 consist of 26 exons and 25 introns. Within intron 16 of ACE gene, the presence or absence of 287-bp fragment called Alu repeat sequence are defined by insertion (I) or deletion (D) polymorphism respectively [6]. A correlation between ACE (I/D) polymorphism and its enzymatic levels was established for both circulating and cellular ACE level [7]. The ACE (I/D) polymorphism has been suggested to be a significant risk factor for various chronic kidney diseases, [8] coronary artery disease, coronary heart disease (CHD) [9] and hypertension [10]. A strong genetic association has also been found between a DD genotype and increased risk of diabetic nephropathy. Recent studies on the role of ACE (I/D) polymorphism on development of progression of diabetic nephropathy gave contradictory results among different ethnic groups [11]. In light of these contrasting findings, we analyzed distribution pattern of ACE gene to investigate the association of ACE gene polymorphism with Type 2 diabetes mellitus, nondiabetic nephropathy and diabetic nephropathy in the Western Indian population.

## Materials and methods

We studied the ACE (I/D) polymorphism in 227 patients with Type 2 diabetes (126 male and 101 female). Fifty patients with nondiabetic nephropathy (NDN) (28 male and 22 female) were recruited from P. S. Medical College and Hospital, Karamsad and Muljibhai Patel Urological Hospital, Nadiad. Nondiabetic subjects were selected from visitors to the health care center of the same hospital and staff of various departments. The patients with Type 2 diabetes were divided in to two groups: with nephropathy (DN, n = 61) and without any complication (T2DM, n = 166). Diagnosis of diabetes mellitus was based on the physical and clinical examination of patients by the doctors followed by appropriate laboratory and other investigations as per the recommendation of American Diabetes Association (ADA). Duration of diabetes was considered as the time from which the patient was diagnosed with diabetes. Nephropathy was diagnosed on the basis of persistent microalbuminuria (UAER 20–200 mg/24 h) or proteinuria (UAER > 200 mg/24 h) in diabetic and nondiabetic subjects by the consulting physician. The detailed medical and clinical demography including height, weight, duration of diabetes, age and medication were obtained. The weight and height were recorded and the body mass index (BMI) was calculated using the formula: BMI = Weight/Height ($kg/m^2$). After explaining the purpose of this study, signed informed-consent forms were obtained from the subjects included in the present study. The study protocol was approved by local medical and research committee of Sardar Patel University. Blood samples were collected in Vacutainer™ tubes and plasma was separated from blood cells by centrifuge at 3,000 rpm for 10 min. The serum creatinine and glucose concentration was determined using commercially available enzymatic kits using the modified Jaffe's and GOD/POD method respectively. Glycosylated hemoglobin was determined by chemical method described previously [12].

### Genomic DNA isolation

Whole genomic DNA was isolated from 2.0 ml whole blood using standard protocol described by Mathew [13]. In brief, whole blood cell lysis was carried out by DNA buffer followed by overnight incubation with DNA buffer, 10% SDS and 40µl of Protease K (20mg/ml). The DNA was extracted from the mixture by PCI (phenol: Chloroform: Isoamyl alcohol; 25:24:1) treatment and precipitated by chilled iso-propenol. Genomic DNA was washed with 70% ethanol, resuspended in TE buffer (pH-7.4), stored at 20°C for the further analysis. The DNA yield and purity was determined by taking absorbance at 260 nm and A260/A280 nm using UV Spectrophotometer (Cary Win, Japan) respectively.

### Determination of ACE genotypes

ACE gene sequence was amplified by polymerase chain reaction (PCR) using mismatch primers (forward primer 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and reverse primer 5′-GATGTGGCCATC ACATTCGTCAGAT-3′) on a Thermal Cycler (My cycler™, Bio-Rad laboratory, USA). The PCR reaction mixture (25µl) consisting of 200ng genomic DNA, 500nM of each primer, 0.2mM of each dNTPs and 1.0 units of Taq polymerase was prepared in PCR buffer containing 1.5mM $MgCl_2$. The ACE gene was amplified after denaturation of the reaction mixture at 95°C for 5 min followed by 35 cycle of 94°C for 1.0 min, 58°C for 1.0 min (annealing) and 72°C for 1.0 min (extension). The PCR product was visualized on 8% polyacrylamide gel electrophoresis (PAGE) stained with silver or 1% agarose gel electrophoresis stained with ethidium bromide (EtBr) and the bands were visualized under white light or UV light respectively. The following genotype pattern were observed as: I/I (490bp), I/D (190bp + 490bp) and D/D (190bp)

### Statistical analysis

Data are shown as mean ± SD. Statistical comparisons between different studied groups were performed by

(ANOVA) SPSS software version 11.0. The allelic and genotype frequencies of ACE gene polymorphism were compared among the studied groups using chi-square ($\chi 2$) test or Fisher's exact test. The recessive genetic model was used to find out its association with progression of nephropathy in diabetic patients. 95% confidence Interval (CI) and odd ratio (OR) were calculated to assess the strength of association between ACE gene polymorphism with DN or NDN. Odds ratios (OR) were calculated with 95% confidence intervals to estimates of relative risk (RR) for disease. A P value of <0.05 was considered significant.

## Results

The clinical characteristics and demographic details of control, T2DM, DN and NDN subjects are summarized in Table 1. The mean age of the control subject (49.8±12.5) was non-significantly lower than the T2DM (58.1±9.6) or DN (58.3±13.5) or NDN (59.6±14.2). There was no significant difference observed in body mass index (BMI), systolic and diastolic blood pressure and hemoglobin levels in all the three studied groups (T2DM, DN, NDN) compared to control subject and also between three different studied groups (T2DM, DN, NDN). However, fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) in Type 2 diabetic patients without any complication and in patient with diabetic nephropathy were significantly ($P<0.05$) higher than control healthy subjects. Serum creatinine level was significantly ($P<0.05$) higher among patients with NDN and DN compared to T2DM and control subjects

Use of mismatch primers for detection of I/D polymorphism in ACE gene results in amplification products of 490 and 190 bp corresponding to I and D alleles respectively are depicted in Fig. 1. The serum creatinine level was found to be associated with ACE genotypes status as evaluated using box and whisker plots, represented in Fig. 2. Serum creatinine was significantly higher in subjects with DD genotype than ID or II genotype individuals.

The genotypic and allelic frequencies of ACE (I/D) gene in T2DM, DN, NDN patients, and control subjects are shown in Table 2. ACE genotypic distribution was consisted with Hardy-Weinberg equilibrium. Genotype II+ID/DD and I/D allele of ACE gene was compared between T2DM verses control, DN verses control, NDN verses control and T2DM verses DN. (Table 3)

I alleles and I/D genotype were found to be most common in healthy control subject. The frequencies of D allele ($\chi 2=0.545$, OR=1.186, 95% CI of 0.755–1.863, RR=1.076, P=0.461) and DD genotypes ($\chi 2=0.591$, OR=1.355, 95% CI of 0.630–2.903, RR=1.071, P=0.442) of ACE gene were not different between control subjects and in Type 2 diabetic patients without any complication (T2DM). There was no significant difference in D allele ($\chi 2=0.184$, OR=1.131, 95% CI of 0.646–1.978, RR=1.054, P=0.668) and DD genotypes ($\chi 2=0.233$, OR=1.263, 95% CI of 0.497–3.206, RR=1.053, P=0.810) between control subjects and patients with non-diabetic nephropathy (NDN). These results suggest that D allele and DD genotype are not associated with development of diabetes and nondiabetic nephropathy. In patients with diabetic nephropathy (DN), D

Table 1 Basic clinical and biochemical characteristics of control and study subjects

| Variables | Control | T2DM | DN | NDN |
|---|---|---|---|---|
| Number (n) | 50 | 166 | 61 | 50 |
| Age (years) | 49.8±12.5 | 58.1±9.6 | 58.3±13.5 | 59.6±14.2 |
| Sex (Male/Female) | 40/10 | 85/81 | 41/20 | 28/22 |
| BMI (kg/m$^2$) | 24.9±2.7 | 25.3±2.4 | 24.6±2.1 | 24.2±1.2 |
| Diabetic duration (years) | N.A. | 7.4±3.5 | 11.5±5.3 | N.A. |
| Hemoglobin (%) | 12.2±2.6 | 12.3±2.7 | 11.4±3.0 | 11.3±2.4 |
| $HbA_{1c}$ (%) | 5.5±0.8 | 8.8±1.4* | 8.4±1.2* | 6.0±0.8 |
| FBG (mmol/l) | 4.4±0.4 | 8.2±1.5* | 8.1±1.2* | 5.2±0.2 |
| SBP (mm Hg) | 125.4±14.2 | 136.4±20.1 | 143.1±21.1 | 142.8±15.2 |
| DBP (mm Hg) | 77.3±7.07 | 82.8±7.5 | 82.4±8.5 | 83.1±10.1 |
| Serum creatinine (mg/dl) | 1.1±0.3 | 1.2±0.8 | 1.9±0.5*† | 2.1±0.7* |

Data are expressed as mean±SD

BMI body mass index; HbA1c glycosylated hemoglobin; FBG fasting blood glucose; SBP systolic blood pressure; DBP diastolic blood pressure; T2DM Type-2 diabetes without any complication; DN diabetic nephropathy; NDN non-diabetic nephropathy

* $P<0.05$ when compared to control

† $P<0.05$ when compared to T2DM

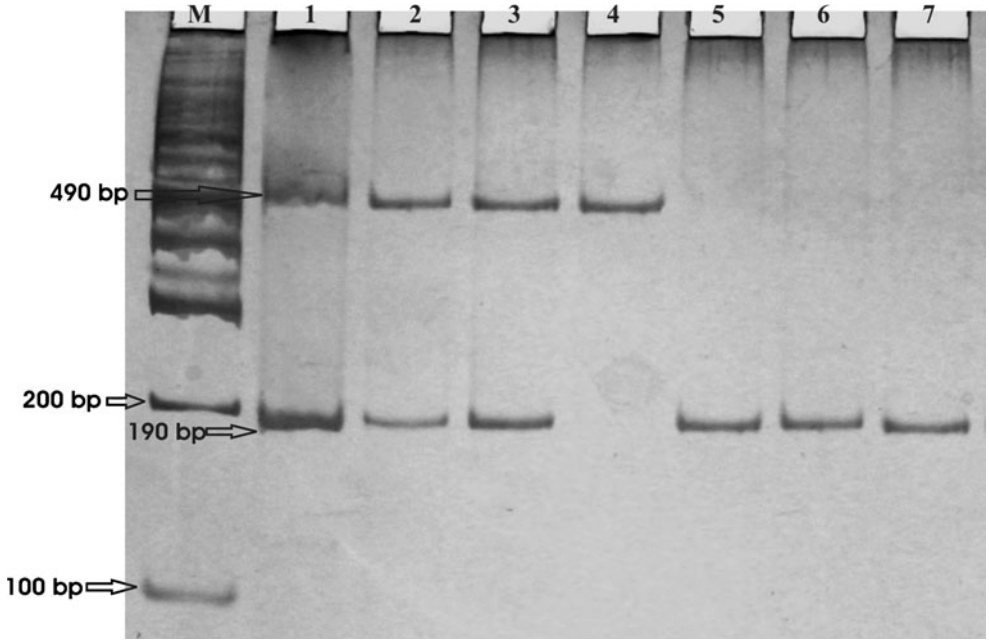



Fig. 1 Gel Electrophoresis demonstrating the different genotype of ACE (I/D) polymorphism after PCR analysis. Lane M shows DNA marker (100bp), Lane 4 shows I/I homozygous (490bp), Lane 1,2,3 shows I/D heterozygous (490bp and 190bp), Lane 5,6,7 shows D/D homozygous (190 bp)

allele ($\chi 2 = 12.551$, OR = 2.151, 95% CI of 1.404–3.295, RR = 1.520, $P < 0.001$) and DD genotype ($\chi 2 = 7.580$, OR = 2.345, 95% CI of 1.273–4.320, RR = 1.340, $P = 0.006$) were significantly higher than in patients with diabetes without any complication (T2DM). Frequencies of D allele ($\chi 2 = 11.632$, OR = 2.551, 95% CI of 1.484–4.385, RR = 1.636, $P = 0.001$) and DD genotypes ($\chi 2 = 7.279$, OR = 3.176, 95% CI of 1.361–7.391, RR = 1.435, $P = 0.007$) were significantly higher in patients with diabetic nephropathy (DN) than control subjects. In contrast, frequencies of I allele and I/I genotypes were significantly lower in patients with diabetic nephropathy (DN) in comparison with Type 2 diabetese without any complication (T2DM), suggests that D allele and D/D genotype are related to higher risk of development of diabetic nephropathy in the Western Indian population.

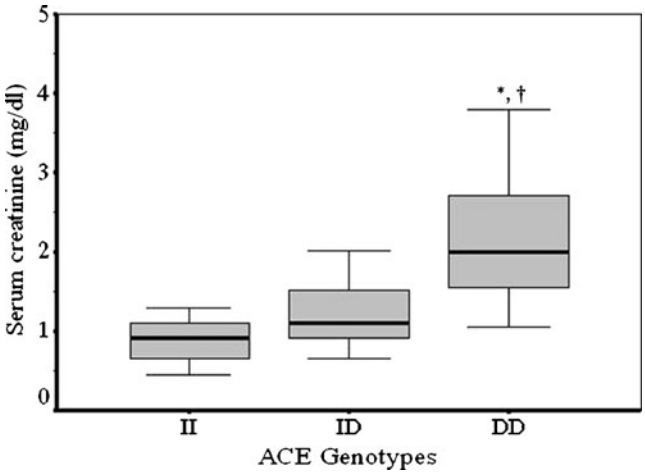



Fig. 2 Serum creatinine level according to status of ACE genotype (II, ID, DD) in total studied subjects. Box and whisker plots show median, 25th/75th percentile and full range. * and † $P < 0.05$, significantly higher than in subject with ID and II genotype

## Discussion

The present study has indicated a significant association of ACE I/D polymorphism with progressive deterioration of renal function and development of diabetic nephropathy located in the intron 16 region of ACE gene in the Western Indian population. Individuals with D allele or DD genotype of ACE gene have shown susceptibility to the development of diabetic nephropathy in Type 2 diabetes. Despite this, the frequency of D-allele and DD genotype among patients with nondiabetic nephropathy was not significantly different with control subjects, indicating that ACE (I/D) polymorphism is not associated with the development of nondiabetic nephropathy, which may be controlled by some other genetic factors. There has been growing agreement from many studies, which reveal the non alliance between the D allele of the ACE gene and the development and progression of nondiabetic nephropathy [14]. A higher serum creatinine level was noted in individual with DD genotype compared to II and ID genotype in the present study, which indicated that individuals with DD genotype are most susceptible for the renal damage followed by ID and II genotypes in the Western Indian population. In agreement with our result, a multiple logistic regression analysis showed that DD genotype was significantly associated with progression of

Table 2 Genotype distribution and allele frequencies of ACE gene polymorphism in control, Type 2 diabetes without any complication (T2DM), diabetic nephropathy (DN) and non-diabetic nephropathy (NDN) groups

| Group | Allele frequency n (%) | | Genotype frequency n (%) | | |
|---|---|---|---|---|---|
| | I | D | I/I | I/D | D/D |
| Control (n=50) | 59 (59) | 41 (41) | 19 (38) | 21 (42) | 10 (20) |
| T2DM (n=166) | 182 (54.8) | 150 (45.2) | 58 (34.9) | 66 (39.8) | 42 (25.4) |
| DN (n=61) | 44 (36.1) | 78 (63.9) | 10 (16.4) | 24 (39.3) | 27 (44.3) |
| NDN (n=50) | 56 (56) | 44 (44) | 18 (36) | 20 (40) | 12 (24) |

Data are expressed as n (%)

diabetic nephropathy that those with II genotype in Korean Type 2 diabetic patients [15].

It has been demonstrated that DD genotype is associated with high activity of ACE compared to ID and II genotype not only in plasma but also in several tissues such as heart and kidney [7, 16]. The deletion polymorphism is associated with elevated serum and cellular ACE levels. DD genotype of ACE gene leads to the development of diabetic nephropathy (DN), probably due to high level of angiotensin II, a vasoactive peptide that increased Glomerular Filtration Rate (GFR) and intraglomerular pressure and also promoted proliferation of mesangial cells and matrix [17] and inactivation of bradykinin rereleased from the variety of tissues effecting arterial vasodialation and venoconstriction. DD genotype or D allele strongly associated with increased serum ACE activity leads to higher ACE expression and its activity and may predispose individuals to diabetic complication [18]. Experimental data suggest that elevated ACE activity is genetically associated with pathogenesis of diabetic renal damage [5]. It has been demonstrated that treatment with Losartan, an angiotensin II antagonist reduces the progression of renal damage in proteinuric Type 2 diabetic patients with D allele [19]. It has been reported that D allele genotype accelerates the progression of chronic renal failure in diabetic nephropathy patients. In a previous study, the presence of DD genotype or D allele in Asian patients with Type 2 diabetes had increased risk for developing diabetic nephropathy [11]. In agreement with our results, Ahluwalia et al. showed that ACE (D allele) and its interaction with RAS polymorphisms associated with increased risk of nephropathy in Type-2 diabetic patients of Asian Indians [20]. Our study has also shown a positive association between the DD genotype of ACE gene polymorphism in patient with diabetic nephropathy. Recently, a research group has reported that DD genotype of ACE gene has been associated with progression of diabetic nephropathy on the basis of renal function impairment in Korean patients with T2DM [21]. The result of the present study are in agreement with previous study reported that ACE I/D polymorphism do not predict the course of idiopathic nephritic syndrome in Swiss children [22]. We have not observed any association between ACE genotypes and the development of Type 2 diabetes, which was previously reported [23]. I/D polymorphism of ACE gene have been implicated as a risk factor for the development of various pathological conditions such as cardiovascular disease, [9] hypertension [24] in patients with Type 2 diabetes. The percentage frequency of D allele of the control group in our study was 41.0%, similar to the earlier report (45.0%) in Asian Indians population [11]. Patients with Type 2 diabetes who have DD genotype or D allele are at higher risk for developing diabetic nephropathy. A positive correlation was found between D allele (DD genotype) of the ACE polymorphism and increased incidence and severity of diabetic nephropathy in south Indian patients [22, 25], but contradictory results were found in the north Indian population [26].

Here, we report a significant and independent association between the DD genotype and diabetic nephropathy in Western Indian Type 2 diabetic patients. Our results suggest that D allele has a co- dominant effect on the development of diabetic nephropathy. This also suggests that the ACE deletion polymorphism is a risk factor for the development of diabetic nephropathy in the Western Indian Type 2 diabetic patients but not associated with nondiabetic nephropathy.

Table 3 Probability value (P value) of ACE genotype and allele frequencies between different studied group comparisons

| | Control vs. T2DM | Control vs. DN | Control vs. NDN | T2DM vs. DN |
|---|---|---|---|---|
| ACE | | | | |
| I/D allele | 0.461 | 0.001 | 0.668 | < 0.001 |
| II+ID/DD | 0.442 | 0.007 | 0.223 | 0.006 |

T2DM Type 2 diabetes without any complication; DN diabetic nephropathy; NDN non-diabetic nephropathy

Acknowledgment We acknowledge the Dean and Medical staff of the Pramukh Swami Medical College and Hospital, Karamsad and Muljibhai Patel Urological Hospital, Nadiad for their assistance in the clinical diagnosis and collection of blood samples for the present study. We are thankful to the University Grant Commission (UGC), New Delhi for the financial support (Sanction no:-F.32-610/2006[SR]) for this work. HVP was a project fellow of this project.

## References

1. Dalla VM, Saller A, Bortoloso E, Mauer M, Fioretto P. Structural involvement in type 1 and type 2 diabetic nephropathy. Diabetes Metab. 2000;26:8–14.
2. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005;28:164–76.
3. Ruggenenti P, Bettinaglio P, Pinares F, Remuzzi G. Angiotensin converting enzyme insertion/deletion polymorphism and renoprotection in diabetic and nondiabetic nephropathies. Clin J Am Soc Nephrol. 2008;3:1511–25.
4. Wolf G, Ziyadeh FN. The role of Angiotensin II in diabetic. Am J Kidney Dis. 1997;2:153–63.
5. Lely AT, Luik PT, Navis G. Angiotensin I-converting enzyme: a pathogenetic role in diabetic renal damage? Curr Diabetes Rev. 2007;3:41–52.
6. Hubert C, Houot A, Corvol P, Soubrier F. Structure of the angiotensin I-converting enzyme gene: two alternate promoters correspond to evolutionary steps of a duplicated gene. J Biol Chem. 1991;266:15377–83.
7. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin 1-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990;86:1343–6.
8. Prasad P, Tiwari AK, Kumar KM, Ammini AC, Gupta A, Gupta R, et al. Chronic renal insufficiency among Asian Indians with type II diabetes: role of RAAS gene polymorphisms. BMC Med Genet. 2006;7:1–9.
9. Emmanuel C, Kanthimathi S, Saibabu R, Cherian KM, Ramesh A. Angiotensin-converting enzyme insertion/deletion genotype is associated with premature coronary artery disease in Indian population. Curr Sci. 2005;88:967–72.
10. Merlo J, Bengtsson-Boström K, Lindblad U, Råstam L, Melander O. Multilevel analysis of systolic blood pressure and ACE gene I/D polymorphism in 438 Swedish families—a public health perspective. BMC Med Genet. 2006;7:14.
11. Movva S, Alluric RV, Komandurc S, Vattamc K, Eppac K, Mukkavalid KK, et al. Relationship of angiotensin-converting enzyme gene polymorphism with nephropathy associated with Type 2 diabetes mellitus in Asian Indians. J Diabetes Complicat. 2007;21:237–41.
12. Chandalia HB, Sadikot S, Bhargava DK, Krisnaswamy PR. Estimation of glycosylated hemoglobin by a simple chemical method. J Assoc Phys India. 1980;29:285–6.
13. Mathew CGP. The isolation of high molecular weight eukaryotic DNA. In Methods of Molecular Biology. Humana Press 1984. Vol. 2. pp. 31–34.
14. Dixit M, Mansur A, Dixit N, Gilman J, Santarina L, Glicklich D. The role of ACE gene polymorphism in rapidity of progression of focal segmental glomerulosclerosis. J Postgrad Med. 2002;48:266–9.
15. Ha SK, Park HC, Park HS, Kang BS, Lee TH, Hwang HJ, et al. ACE gene polymorphism and progression of diabetic nephropathy in Korean type 2 diabetic patients: effect of ACE gene DD on the progression of diabetic nephropathy. Am J Kidney Dis. 2003;41:943–9.
16. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, et al. Angiotensin-converting enzyme in human heart: effect of deletion/insertion polymorphism. Circulation. 1995;92:1387–8.
17. Marre M, Bouhanick B, Berrut G, Gallois Y, Le JJ, Chatellier G, et al. Renal changes on hyperglycemia and angiotensin-converting enzyme in type 1 diabetes. Hypertension. 1999;33:775–80.
18. Wolf G, Ritz E. Diabetic nephropathy in T2D prevention and patient management. J Am Soc Nephrol. 2003;14:1396–405.
19. Parving HH, de Dick Z, Cooper ME, Remuzzi G, Liu N, Lunceford J, et al. ACE gene polymorphism and losartan treatment in type 2 diabetic patients with nephropathy. J Am Soc Nephrol. 2008;19:771–9.
20. Ahluwalia TS, Ahuja M, Rai TS, Kohli HS, Bhansali A, Sud K, et al. ACE variants interact with the RAS pathway to confer risk and protection against Type 2 diabetic nephropathy. DNA Cell Biol. 2009;28:141–50.
21. Park HC, Choi SR, Kim BS, Lee TH, Kang BS, Choi KH, et al. Polymorphism of the ACE gene in dialysis patients: overexpression of DD genotype in type 2 diabetic end-stage renal failure patients. Yonsei Med J. 2005;46:779–87.
22. Sasse B, Hailemariam S, Wuthrich RP, Kemper MJ, Neuhaus TJ. Angiotensin converting enzyme gene polymorphisms do not predict the course of idiopathic nephritic syndrome in Swiss children. Nephrol (Carlton). 2006;11:538–41.
23. Viswanathan V, Zhu Y, Bala K, Dunn S, Snehalatha C, Ramachandran A, et al. Association between ACE gene polymorphism and diabetic nephropathy in south Indian patients. JOP. 2001;2:83–7.
24. Bhavani BA, Padma T, Sastry BKS, Reddy NK, Nausheen K. The insertion I/deletion D polymorphism of angiotensin-converting enzyme (ACE) gene increase the susceptibility to hypertension and/or diabetes. Int J Hum Genet. 2005;5:247–52.
25. Naresh VVS, Reddy ALK, Sivaramakrishna G, Sharma PVGK, Vardhan RV, Siva Kumar V. Angiotensin converting enzyme gene polymorphism in type II diabetics with nephropathy. Indian. J Nephrol. 2009;19:145–8.
26. Kumar A, Mohindru K, Sehajpal PK. Angiotensin I converting enzyme polymorphism and diabetic nephropathy in north India. Int J Hum Genet. 2005;5:279–83.

ORIGINAL ARTICLE



# Asymptomatic peripheral arterial disease in type 2 diabetes mellitus: prevalence patterns and risk factor associations

Uday S. Ghosh, Saumik Datta, Samar Banerjee

Int J Diab Dev Ctries. 2011 ; 31 :229-239

Abstract Aim of the study was to estimate the prevalence of peripheral arterial disease (PAD), by measuring ankle brachial pressure index (ABPI), and evaluate risk factor impacts on asymptomatic PAD in type 2 diabetes mellitus (T2DM) patients in Eastern India. It is a clinic based study of consecutive patients with calculated number for reasonable power. Eighty-four patients were studied. Risk factors assessed were T2DM duration, age, gender, body mass index (BMI), systolic (SBP) and diastolic (DBP) pressure, lipids and smoking status. Risk factor levels and impacts on ABPI was studied in total patients and then by segregation according to gender, ABPI level, smoking status and duration (3 groups: <1 year;1–10 year; >10 year). Student's T Test, Pearson's correlation, Multiple linear regression. Prevalence of mild PAD was 53% and moderate 14%. Mean ABPI was lower in males (0.83 ± 0.09) and smokers (0.84 ± 0.099), but higher in <1 year duration (0.91 ± 0.13). Male gender (p = 0.000) and duration of T2DM (p = 0.016) were prime contributors of ABPI lowering, lipids might play some role. Higher SBP was partially contributory; independent role of smoking, age, DBP was questionable. Higher BMI and female gender seems protective. Asymptomatic PAD prevalence in T2DM is high. Lean male dyslipidemics are more predisposed; duration and SBP adds to it. Risk factor impacts differ at different durations in the various segregated groups compared to the total patients.

Keywords T2DM · PAD · Prevalence · Risk factor · India

U. S. Ghosh
e-mail: nokusank@yahoo.com

## Introduction

Peripheral arterial disease (PAD) may be defined as disease of any artery that is not part of the heart or brain; commonly lower limb vessels are implicated [1, 2]. In type 2 diabetes mellitus (T2DM) it has a chronic, predominantly asymptomatic course [3]. It is fourfold commoner, manifests earlier, progresses rapidly, probably has equal gender distribution [4, 5] PAD has been implicated as an important indicator of increased coronary artery disease (CAD) related mortality [5, 6]. Because of the asymptomatic nature, true prevalence of PAD is difficult to determine [3]. Indian studies report a prevalence of 6.3% to 15.3% [7, 8]. International data reveals prevalence range from 20% to 30% [9, 10]. Indians are supposed to be less predisposed [7]. A cluster of conditions such as metabolic syndrome, hyperglycemia and its duration, hypertension, smoking, advanced age, body mass index(BMI), male gender and dyslipidemia are important risk factors for PAD [11, 12]. Prior cardiovascular event, neuropathy and retinopathy are also recognized risk markers [13, 14]. Early identification of high risk T2DM patients, asymptomatic for PAD may guide preventive strategies, as few options exist for established, symptomatic and advanced PAD; moreover it can also indicate the CAD related risk in the patient [15, 16].

In a country with high prevalence of CAD, PAD cannot lag behind. Despite the increased vascular risk, PAD remains underrecognized, and naturally underdiagnosed

and undertreated. Increased awareness will overcome this barrier to effective secondary prevention of vascular events. Measurement of ankle-brachial pressure index (ABPI) is a simple way of identifying PAD [17, 18]. Studies on asymptomatic PAD status in T2DM vis-à-vis prevalent risk factors are few from India [7, 8, 19]. This clinic based study was planned to find the approximate prevalence of PAD in asymptomatic T2DM patients, as detected by ABPI and its risk factor associations.

ABPI status as well as impacts of risk factors will be studied in total patients as well as patients segregated by gender, high and low ABPI levels, smoking and nonsmoking status and early, intermediate and late duration of T2DM.

## Methods

This study was performed during November 2007–March 2008 at NRS Medical College Kolkata and had approval of the ethical committee of the institution. It was a cross sectional survey based on convenience sampling. Consecutive T2DM patients satisfying inclusion criteria were selected from the Diabetes Clinic of the hospital. Informed consent was taken in accordance with the Helsinki declaration. Inclusion criteria were all cases of clinical T2DM above the age of 30 years without any overt or clinically apparent complications. T2DM with symptomatic foot (painful or painless, swelling, infection, ulcer, gangrene, claudication), heart failure, severe hypertension, recent (less than I year) stroke, myocardial infarction or any vascular event(including retinopathy requiring intervention), dipstick proteinuria and those on medications that might alter vascular morphology(statin, ARBs, ACEIs), were excluded for relevant reasons [1, 13–16].

The assumed prevalence of PAD was 30% (as it is a tertiary referral clinic) and for estimation of the actual prevalence within 10% limits of the true population prevalence the calculated patient number was 84 [1, 16]. Risk parameters studied were age, body mass index (BMI), gender,duration of T2DM, systolic blood pressure (SBP), diastolic blood pressure (DBP), lipids (HDL cholesterol - HD, total cholesterol- TC, LDL cholesterol- LD, triglyceride- TG, and total cholesterol to HDL cholesterol ratio- TC/HD, smoking status(smoker-Sm/nonsmoker-NSm) [15, 16]. Impacts of the risk factors were assessed in total patients as well as by gender, ABPI levels, smoking status and duration of T2DM. Prevalent HbA1c is often not correlated with ABPI and serial data on the preceding levels are sparsely available, so it was not included as a risk variable [20].

The duration stratification was done depending on the time from initial detection, as stated by the patient as well as corroboration from previous treatment documents, into three groups—group A composed of patients having T2DM diagnosed for less than 1 year; group B had patients having T2DM diagnosed for more than 1 year to upto 10 years and group C comprised of patients of more than 10 years duration. Each duration group was then segregated by gender, ABPI levels and smoking status [15]. The segregation of ABPI into low (LAb) and high (HAb) was done by the levels of $\leq 0.8$ and $>0.8$ respectively; an ABPI of $\leq 0.8$ is more often associated with adverse vascular outcomes [15, 21].

Age was assessed from the voter ID cards issued by the Election Commission of India. BMI was measured by the standard formula—weight in kilogram divided by height in meter squared. Resting supine SBP was measured from both the brachial arteries—higher value was the denominator, and in both posterior tibial and dorsalis pedis arteries—the highest value was the numerator; this ratio was the ABPI [15, 21]. A hand held continuous wave Doppler with a 10 MHz probe and an ankle blood pressure cuff was used [7, 15]. The ABPI range of 0.91–1.30 was taken as normal, values below 0.9 indicated obstructive PAD; values above 1.3 were considered false negative [22]. Any amount of persistent smoking for more than 5 years was considered smoker. Lipids were measured by auto analyzer (Ebras 600) and rest of the parameters was decided from clinical assessment. For patients with hypertension a drug washout period of 5 days were allowed with close monitoring of blood pressure.

Statistical analysis: Univariate data for the continuous variables were presented as mean ± standard deviation. The discrete variables were put as percentage and differences assessed by z score. Differences between 2 groups were analyzed by independent sample Student's t-tests. More than 2 groups were compared by one way Anova with post hoc analysis by Bonferroni modification. Bivariate linear correlation between ABPI (of the various patient groups) and the different risk parameters were calculated by Pearson's Correlation. The risk factors for PAD were examined by a multiple linear regression analyses with stepwise removal of the independent variables to find the most significant ones contributing to the ABPI status(the dependent variable) in a particular group; a negative '$\beta$' and 't' value were considered to have a negative impact while a positive value a positive impact. Along with it the adjusted R2 values and Anova were computed. The number of independent variables varied for each group of patients (total patients, males, females, smokers, non smokers, low ABPI, high ABPI, 3 durations –A,B,C) as the number of patients varied- approximately 10 patients per independent variable were considered. These independent variables were selected from the results of Pearson's correlation coefficient values—the best correlating variable was chosen according

to the calculated number of cases in each patient group. Positive smoking status and male gender were given a score of 1 while nonsmoking status and female gender were given a score of 0 while inputting them as variables in the linear regression analysis. Regression analysis was not done in case groups having less than 20 patients as that would lead to much skewed results (duration segments of the genders, ABPI groups and smoker and nonsmoker), the risk factor impact analysis in them was inferred from the univariate and bivariate analysis. A two-tailed $p<0.05$ was considered statistically significant.

## Result analysis

Ninety-one consecutive patients of T2DM satisfying the inclusion criteria and attending for the first time were selected over a period of 5 months; 7 could not complete the total protocol. Ultimately 84 patients were studied with written informed consent. There were 45 males and 39 females with age range of 30 to 76 years. The duration segregated groups (A, B, C.) each had 28 patients. Around 53.6% of patients had an ABPI < 0.9, in 14.3% it was <0.8 and none were below 0.7. In low ABPI group the prevalence of males and smokers was 84.6% and 53.8% and in the high ABPI group it was 37.8% ($p=0.00$) and 33.3% ($p>0.05$) respectively. In the smoker and nonsmoker groups the prevalence of males was 88.9% and 35.4% ($p=0.00$) and an ABPI of <0.9 of 55.6% and 50.1% ($p>0.05$) respectively. The percentage of male was 50%, 67.9% and 53.4% respectively for duration groups A, B and C ($p>0.05$). Prevalence of ABPI ≤0.8 (%) in group A was 42.9%, in group B 60.7% and in group C it was 57.1% ($p>0.05$).

Table 1 outlines the clinical parameters and Table 2 the biochemical parameters of total patients and the segregated broad groups (genders, smokers and non-smokers, low and high ABPI and the 3 duration segregated groups) as mean ± standard deviation. Tables 3, 4 and 5 depict the clinico-biochemical parameters of the genders, smokers and non-smokers, and low and high ABPI levels by duration segregation respectively as mean ± standard deviation. Table 6 enumerates the ABPI correlations of risk factors of total patients and non-duration segregated broad groups. Table 7 enumerates the ABPI correlations and of risk factors of duration segregated groups.

Student's T test: (Tables 1 and 2) Males had higher age, duration and SBP but lower ABPI, BMI and DBP

Table 1 Clinical parameters: total patients, males and females, smokers and non-smokers, low and high ABPI and duration groups (mean ± standard deviation)

| Groups(no.) | Age(yrs) | BMI(kg/m$^2$) | Duration(yrs) | SBP(mmHg) | DBP(mmHg) | ABPI |
|---|---|---|---|---|---|---|
| Total(84) | 52.1±11.2 | 22.7±3.5 | 6.7±5.9 | 144.2±22.3 | 84.5±11 | 0.86±0.11 |
| Male(49) | 53.9±11.4 | 21.9±2.7 | 7.2±6 | 147.2±23.4 | 83.4±11.2 | 0.83±0.09 |
| Female(35) | 49.5±10.5 | 23.8±4.1 | 6±5.8 | 139.3±19.5 | 85.9±10.3 | 0.91±0.11 |
| p-value | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* |
| L-Ab(45) | 52.6±11.6 | 22.1±3.5 | 7.09±5.1 | 148.1±25 | 84.9±12.1 | 0.77±0.04 |
| H-Ab(39) | 51.4±10.8 | 23.3±3.4 | 6.3±6.8 | 139.2±18 | 83.9±9.4 | 0.95±0.06 |
| p-value | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* |
| Sm(36) | 54±11.6 | 22.3±2.8 | 7.3±6.3 | 148.7±24.5 | 84±8.3 | 0.84±0.1 |
| NSm(48) | 50±10.7 | 23±3.9 | 7.4±4.9 | 140±19.9 | 84.8±12.5 | 0.88±0.1 |
| p-value | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* |
| A(28) | 47.8±11.1 | 24.04±3.7 | 0.26±0.27 | 133.5 ± 17.8 | 82.1±7.2 | 0.91±0.13 |
| B(28) | 53.9±10.6 | 22.3±3.2 | 6.6±1.2 | 149.2 ± 20.7 | 86.5±9.5 | 0.84±0.08 |
| C(28) | 54.5±10.3 | 22.6±3.4 | 13.2±4.2 | 149.4±1 3.4 | 84.7±14.1 | 0.83±0.09 |
| Anova- p value | 0.043* | 0.728 | 0.000* | 0.006* | 0.325 | 0.014* |
| p-value: A-B | 0.11 | 1.00 | 0.000* | 0.018* | 0.41 | 0.049* |
| p-value: A-C | 0.07 | 1.00 | 0.000* | 0.016* | 1.00 | 0.024* |
| p-value: B-C | 1.00 | 1.00 | 0.000* | 1.00 | 1.00 | 1.00 |

BMI body mass index; yrs years; A, B, C duration groups (A=<1 year, B=1–10 years, C= >10 years); ABPI ankle brachial pressure index; L-Ab low ABPI; H-Ab high ABPI; SBP systolic blood pressure; DBP diastolic blood pressure

* = significant p value; (no.): number of patients

Table 2 Biochemical parameters: total patients, males and females, smokers and non-smokers, low and high ABPI and 3 duration groups (mean ± standard deviation)

| Groups (no.) | TC (mg/dl) | TG (mg/dl) | HD (mg/dl) | LD (mg/dl) | TC/HD |
|---|---|---|---|---|---|
| Total(84) | 191±82 | 159.6±82.1 | 41.4±12.7 | 116.8±67.1 | 4.9±2.9 |
| Male(49) | 184±73 | 155.6±79.3 | 39.8±13.5 | 112.7±62.9 | 5.1±2.7 |
| Female(35) | 200.7±93.5 | 165.1±86.7 | 43.7±11.4 | 122.5±73.1 | 4.6±1.2 |
| p-value | 0.38 | 0.61 | 0.17 | 0.51 | 0.44 |
| L-Ab(45) | 194.1±100 | 141.3±75 | 41.9±13.1 | 121.6±82.6 | 4.7±1.4 |
| H-Ab(39) | 187.4±54.8 | 180.7±85.8 | 40.9±12.5 | 111.3±43.3 | 5.2±3.9 |
| p-value | 0.71 | 0.03* | 0.71 | 0.48 | 0.000* |
| Sm(36) | 188.5±82.2 | 158.8±81.8 | 38.1±11.8 | 118±71.5 | 5.5±4.1 |
| NSm(48) | 192.9±82.8 | 160.2±83.2 | 43.9±12.9 | 116±64.3 | 4.4±1.1 |
| p-value | 0.81 | 0.94 | 0.04* | 0.89 | 0.000* |
| A(28) | 169.8±42.4 | 159.9±82 | 39.8±10 | 97.5±30.3 | 4.41±1.0 |
| B(28) | 198±90.2 | 163.2±78.1 | 42.1±11.6 | 125.8±72.2 | 4.8±1.4 |
| C(28) | 205.2±100 | 155.7±88.6 | 42.4±16.2 | 127.2±82.3 | 5.6±4.6 |
| Anova p value | 0.237 | 0.945 | 0.719 | 0.174 | 0.324 |
| p-value: A-B | 0.6 | 1.00 | 1.00 | 0.34 | 1.00 |
| p-value: A-C | 0.33 | 1.00 | 1.00 | 0.29 | 0.43 |
| p-value: B-C | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 |

Sm smoker; NSm non-smoker; A, B, C duration groups (A=<1 year, B=1–10 years, C= >10 years); L low; H high; Ab ABPI; TC total cholesterol; TG triglyceride; HD HDL cholesterol; LD LDL cholesterol; TC/HD total cholesterol and HDL cholesterol ratio; no. number of patients

* = significant p value

compared to females. The smokers had higher age, SBP, and TC/HD ratio but lower ABPI, BMI, duration, DBP and HD compared to nonsmokers. Patients of lower ABPI group had higher age, duration, systolic and diastolic blood pressure but lower TG and TC/HD ratio compared to the higher ABPI group.

On duration segregation (Table 3) of the genders—females of (A) group had higher BMI, LD, and ABPI compared to their males; none of the parameters differed in group B and group C females. Only females of C group had higher ABPI. On duration segregation of smokers and nonsmokers (Table 4) none of the parameters including ABPI differed between any groups. Duration segregation of low and high ABPI revealed only higher SBP in low ABPI group of intermediate duration (B).

Anova with post hoc paired T test and Bonferroni modification analysis: The mean differences between the three duration groups were significant for mean distribution of the parameters of age, SBP, duration of T2DM and ABPI. On post hoc analysis age did not have any significant difference amongst the 3 groups. SBP was significantly higher in both groups B and C compared to A but it did not differ between B and C. Duration was progressively higher in the 3 groups. ABPI was higher in group A compared to both B and C. The other parameters did not differ.

Pearson's correlation of ABPI with risk factors: (Table 6 and 7) Age predominantly had a variable correlation being significantly negative in intermediate duration smokers (group SmB), short duration nonsmokers (group NSmA) and short duration low ABPI (LAbA). Male gender had predominantly negative correlations being significantly (the reverse being true for female gender) so in total patients, duration groups A and C, both smokers and nonsmokers and high ABPI patients. BMI was predominantly positive in most groups but significant only in nonsmokers of duration group B. Duration of T2DM was mainly negative and was significantly so in total patients, high ABPI, group C of low ABPI but positive in longest duration of females (FC). Smoking had mostly negative correlations, but significant only in early duration (LAbA) of low ABPI. SBP had predominantly negative correlations significant in duration group B especially their males (MB). TC, HD, TC/HD and TG predominantly had positive correlations with ABPI; TC had significant positive association in low ABPI, and TG had positive correlation with total patients, males, and nonsmokers of group C; however, both HD and TCHD were never significant.

Linear regression analysis with ABPI as dependent variable: (1) In the total patients—analysis was done with age, gender, duration, BMI, smoking status, and TG (6 independent variables and 84 patients) and male gender (0.000) had negative impact while TG (0.017), had positive impact and Anova was significant (0.006). (2) In males, 5 independent variables were used for 49 patients—age,

Table 3 Clinicobiochemical parameters of the genders by duration segregation (mean ± standard deviation)

| Groups (no.) | Age (years) | BMI(kg/m$^2$) | SBP(mmHg) | DBP(mmHg) | ABPI |
|---|---|---|---|---|---|
| M-A(14) | 50.8±12.2 | 21.1±3.1 | 135.4±20.9 | 80±6.3 | 0.86±0.12 |
| F-A(14) | 44.8±8.9 | 24.9±3.4 | 131±14.2 | 84.2±7.4 | 0.96±0.11 |
| p-value | 0.16 | 0.005* | 0.53 | 0.13 | 0.04* |
| M-B(19) | 56.1±10.4 | 22.5±2.5 | 150.5±22.5 | 86±9.1 | 0.82±0.08 |
| F-B(9) | 49.3±9.6 | 21.8±4.2 | 146.4±15.7 | 87.5±10.1 | 0.88±0.08 |
| p-value | 0.12 | 0.58 | 0.64 | 0.70 | 0.09 |
| M-C(16) | 54.1±10.7 | 21.8±2.2 | 153.8±22.9 | 83.5±15.1 | 0.8±0.08 |
| F-C(12) | 55.1±9.7 | 24.1±4.2 | 143.7±22.8 | 86.7±12.4 | 0.88±0.09 |
| p-value | 0.8 | 0.08 | 0.28 | 0.54 | 0.04* |
| Groups(no.) | TC(mg/dl) | HDL(mg/dl) | LD(mg/dl) | TG(mg/dl) | TC/HD |
| M-A(14) | 160±40 | 37.4±10.8 | 86.1±25.9 | 171.4±93.6 | 4.4±1.1 |
| F-A(14) | 179.8±44 | 42.3±8.8 | 108.8±31.1 | 148.4±70.5 | 4.4±1.0 |
| p-value | 0.22 | 0.2 | 0.047* | 0.47 | 0.98 |
| M-B(19) | 192.3±44.1 | 42.2±10.1 | 121.7±37.5 | 144.6±63 | 4.7±1.4 |
| F-B (9) | 210±151 | 42±14.9 | 134±119.7 | 202±95.6 | 4.9±1.5 |
| p-value | 0.71 | 0.97 | 0.68 | 0.07 | 0.77 |
| M-C(16) | 195.4±112 | 39.1±18.6 | 125.4±97.3 | 155±86.1 | 6±1.5 |
| F-C (12) | 218.2±84 | 46.7±11.6 | 129.7±67.3 | 157±95.5 | 4.7±1.3 |
| p-value | 0.55 | 0.2 | 0.89 | 0.96 | 0.4 |

M male; F female; BMI body mass index; A, B, C duration groups (A = <1 year, B = 1–10 years, C= >10 years); SBP systolic blood pressure; DBP diastolic blood pressure; ABPI ankle brachial pressure index; TC total cholesterol; TG triglyceride; HD HDL cholesterol; LD LDL cholesterol; TC/HD total cholesterol and HDL cholesterol ratio; no. number of patients

* = significant p value

duration, SBP, TG and TC/HD; duration had negative (0.031) while triglyceride was found to have a positive impact (0.031). (3) For the females with 35 patients 4 variables were used—age, duration, TC and TC/HD; none had any significant impact and Anova was nonsignificant. (4) In the 3 duration segregated groups regression was done with 3 parameters as they each had 28 patients; in group A gender, BMI, and TG were used and gender had an negative impact (0.035) and Anova was significant (0.035); in group B—smoking, BMI, and SBP were used; BMI (0.027) had positive while SBP (0.014) had negative impact and Anova was significant (0.01); in group C independent variables were BMI, gender and TG and all had significant impacts on ABPI; male gender(0.002) and BMI(0.005) were negative and TG (0.012) was positive, Anova was significant (0.001). (5) In case of Smokers (N = 36) the independent variables were male gender, SBP, TG and age; male gender had significant negative impact (0.001) and TG was positive, Anova was significant (0.001). (6) In case of Nonsmokers (N = 48) regression was done with 5 variables (gender, duration, BMI, TG, HD); duration of T2DM (0.009) had a negative while TG (0.039) had a positive impact and Anova was significant (0.002). (7) Patients with low ABPI numbered 45 and 5 independent variables were used for regression—age, gender, TG, HD and TC; none had any significant impact and Anova was not significant (0.068). (8) For high ABPI patients numbering 39, duration (0.011) and male gender (0.038) had negative impact and Anova was significant (0.04), with gender, duration, Sm and TC as independent variables. In the model summary the adjusted R2 values were mostly between 0.21 to 0.29; value in females was 0 and in low ABPI group was 0.054; meaning the models could explain for 20 to 30% of ABPI status in most groups but failed to provide significant insight in the status in females and low ABPI group.

## Discussion

For the total number of patients, the prevalence of mild grades of PAD was fairly high in asymptomatic T2DM

Table 4 Clinicobiochemical parameters of Smoking status by duration segregation. (mean ± standard deviation)

| Groups(no.) | Age(years) | BMI(kg/m$^2$) | SBP(mmHg) | DBP(mmHg) | ABPI |
|---|---|---|---|---|---|
| Sm-A (11) | 50.5±10.9 | 22.4±3.8 | 137.8±22.4 | 83.3±4.5 | 0.87±0.13 |
| NSm-A (17) | 46±10.8 | 23.5±3.7 | 130.2±13.6 | 81.4±8.5 | 0.93±0.12 |
| p-value | 0.31 | 0.46 | 0.51 | 0.29 | 0.26 |
| | | | | | |
| Sm-B(13) | 56.7±11.4 | 22.5±2.4 | 155.5±22.1 | 86±7.2 | 0.81±0.06 |
| NSm-B(15) | 51.5±9.3 | 22.1±3.7 | 143.7±17.6 | 86.9±11.1 | 0.87±0.09 |
| p-value | 0.22 | 0.77 | 0.14 | 0.8 | 0.06 |
| Sm-C(12) | 54.3±11.1 | 22.1±2.3 | 151.3±24.5 | 82.5±11.1 | 0.84±0.09 |
| NSm-C(16) | 54.3±9.7 | 23.3±4 | 148±22.4 | 86.4±15.8 | 0.83±0.1 |
| p-value | 0.94 | 0.36 | 0.72 | 0.49 | 0.65 |
| | | | | | |
| Groups(no.) | TC(mg/dl) | HDL(mg/dl) | LD(mg/dl) | TG(mg/dl) | TC/HD |
| Sm-A (11) | 169.9±51.8 | 39.9±10 | 94.7±36.3 | 185.5±104 | 4.7±1.2 |
| NSm-A (17) | 169.7±36.9 | 41.7±9.8 | 99.2±26.9 | 143.4±61.8 | 4.2±0.93 |
| p-value | 0.99 | 0.22 | 0.71 | 0.19 | 0.27 |
| | | | | | |
| Sm-B(13) | 187.9±47.6 | 40.4±9.3 | 120.8±40.6 | 137.7±47.5 | 4.8±1.4 |
| NSm-B(15) | 206.7±111.6 | 43.6±13.4 | 130.1±92.8 | 185.3±93.3 | 4.8±1.4 |
| p-value | 0.59 | 0.47 | 0.74 | 0.098 | 0.95 |
| | | | | | |
| Sm-C (12) | 206±12.1 | 36.6±15.8 | 136.4±111.2 | 157.1±87.5 | 6.98±6.9 |
| NSm-C (16) | 204±79.9 | 46.6±15.6 | 120.3±60 | 154.7±92.2 | 4.5±1.2 |
| p-value | 0.97 | 0.11 | 0.63 | 0.94 | 0.16 |

Sm smoker; NSm non-smoker; BMI body mass index; A, B, C duration groups (A=<1 year, B=1–10 years, C= >10 years); SBP systolic blood pressure; DBP diastolic blood pressure; ABPI ankle brachial pressure index; TC total cholesterol; TG triglyceride; HD HDL cholesterol; LD LDL cholesterol; TC/HD total cholesterol and HDL cholesterol ratio; no. number of patients

* = significant p value

(53%) but more severe grades were not frequent (14%), this ratio was observable from the early stages [1]. This pattern is in contrast to other Indian reports [7, 8, 19]. Male gender, increasing duration of T2DM, had the most significant independent impact on ABPI lowering; age of the patient, SBP and smoking status were not so significant [19, 23]. These also differ with other Indian reports [24]. The SBP had better negative association than DBP. Probably higher BMI is protective [25]. The TC/HDL ratio was not more accurate a predictor than TC or HDL singly; role of TG is difficult to explain as it seems predominantly protective [11, 13, 15].

Male gender had significantly lower ABPI; they also had higher risks [26]. Duration, smoking, and SBP was better associated in males and higher BMI and age seemed protective. However it was only duration that had independent impact on ABPI lowering in males. Probably, gender as a risk factor had the most significant effect and other independent variables were dependent on it [27]. ABPI was significantly lower in smokers who also had higher percentage of males, age, SBP, and lower BMI and HD. The male gender had significant negative impact in smokers, but it was duration in nonsmokers. Smoking probably alters the usual impact of known risk markers in T2DM [28]. Low ABPI patients had longer duration of T2DM, higher age, blood pressure, proportion of males and smokers [16, 25]. Though most of the risk factors had negative correlation with ABPI except lipids, none had independent impact. In higher ABPI patients, duration and male gender had independent negative impacts. Low ABPI state could hardly be accounted by traditional risk markers, probably nontraditional markers or genetic influences are more important [19, 20]. Lipids correlated better in females, nonsmokers and higher ABPI but their individual impacts are questionable as most had a positive impact [25, 29].

On duration segregation of the total patients, mean ABPI was significantly higher in short duration patients; ABPI in intermediate and longest durations were similar. The gender did not have any significant impact in the intermediate duration [30]. Probably the role of gender became con-

Table 5 Clinicobiochemical parameters of ABPI levels - duration segregation (mean ± standard deviation)

| Groups(no.) | Age(years) | BMI(kg/m$^2$) | SBP(mmHg) | DBP(mmHg) | ABPI |
|---|---|---|---|---|---|
| L-Ab-A(12) | 45.6±11.8 | 21.5±3.1 | 132±24.5 | 81.1±6.7 | 0.78±0.05 |
| H-Ab-A(16) | 49.44±10.2 | 24.2±3.8 | 134±12.7 | 83±7.9 | 1.01±0.06 |
| p-value | 0.38 | 0.07 | 0.83 | 0.55 | 0.000* |
| L-Ab-B(17) | 56.4±10.7 | 21.6±3.0 | 156.5±22.2 | 87.8±11 | 0.78±0.04 |
| H-Ab-B(11) | 50.2±9.5 | 23.4±3.1 | 138±13.4 | 84±5.3 | 0.93±0.05 |
| p-value | 0.14 | 0.14 | 0.02* | 0.3 | 0.000* |
| L-Ab-C(16) | 54.1±9.5 | 23.3±3.8 | 151±24 | 84.5±15.4 | 0.76±0.05 |
| H-Ab-C(12) | 55.1±11.2 | 22.03±2.6 | 147±24.5 | 85±13 | 0.93±0.05 |
| p-value | 0.8 | 0.34 | 0.69 | 0.93 | 0.000* |
| Groups(no.) | TC(mg/dl) | HDL(mg/dl) | LD(mg/dl) | TG(mg/dl) | TC/HD |
| L-Ab-A(12) | 161.7±44.7 | 38.5±10.5 | 91.2±35 | 148.6±80.8 | 4.3±0.9 |
| H-Ab-A(16) | 175.9±41 | 40.8±9.8 | 102.2±26.5 | 168.3±88.5 | 4.5±1.1 |
| p-value | 0.39 | 0.55 | 0.35 | 0.55 | 0.71 |
| L-Ab-B(17) | 206.8±109 | 42.9±11.7 | 137.1±85.5 | 148.6±80.8 | 4.9±1.5 |
| H-Ab-B(11) | 184.5±53.1 | 40,9±11.9 | 108.3±42.9 | 185.8±71.5 | 4.7±1.3 |
| p-value | 0.53 | 0.67 | 0.31 | 0.23 | 0.66 |
| L-Ab-C(16) | 205±120 | 43.4±16.3 | 128±101.2 | 127.8±71.4 | 4.7±1.6 |
| H-Ab-C(12) | 205.3±70.4 | 40.9±16.7 | 126±56.8 | 192.9±98.2 | 6.6±6.9 |
| p-value | 0.99 | 0.69 | 0.95 | 0.052 | 0.29 |

ABPI ankle brachial pressure index; L-Ab low ABPI; H-Ab high ABPI; BMI body mass index; A, B, C duration groups (A=<1 year, B=1–10 years, C= >10 years); SBP systolic blood pressure; DBP diastolic blood pressure; TC total cholesterol; TG triglyceride; HD HDL cholesterol; LD LDL cholesterol; TC/HD total cholesterol and HDL cholesterol ratio; no. number of patients

* = significant p value

founded by the other risk factors which expressed better correlation in this duration segment, especially SBP. Duration, age, smoking and DBP had more pronounced inverse relations in the intermediate duration. Higher BMI had protective role in duration group B but had adverse impact in the longest duration. Higher age of onset associated with smoking may predispose to significant early ABPI lowering [21, 28]. However, impact of smoking and age is probably less in T2DM than in non diabetics [28].

Male gender and duration of T2DM had the most significant impact on ABPI decrement except in longest duration where higher BMI and age were major contributors; impact of duration was often more in males [12, 15, 25]. Higher BMI and female gender was probably protective in earlier durations; but the interplay of risk factors in females remain uncertain. Dyslipidemia being common association of diabetes, whether its impact is real or casual is difficult to comment. SBP was contributory in especially in males and intermediate duration. The impact of DBP was negligible [25, 28]. Higher age was marginally protective in earlier durations. Smoking seems to affect ABPI adversely after some duration but never significantly. Impact of smoking and age is less than in nondiabetic [28]. With increasing duration females probably become less predisposed, the post-menopausal state may be contributory. In the initial period it is probably the very diabetic state that is important for ABPI alterations; in intermediate duration multiple traditional risk factors play a role. In longer duration risk factor impacts are significantly altered; they are either masked by development of collaterals and vascular calcification or these patients are primarily less predisposed [31].

PAD in people with diabetes is different from others in its biology, clinical presentation and management [2, 22]. The very state of T2DM has been implicated as a major factor that independently predicts progression of PAD [18]. It is estimated that 8% of diabetics have PAD at the time of diagnosis of diabetes, 15% after 10 years, and 45% after 20 years [32]. Nontraditional risk factors, such as high

Table 6 ABPI correlations of risk factors of the broad groups- total patients, gender, smoking and ABPI segregation

| ABPI | | Age | Gen | Dur | Sm | SBP | DBP | BMI | TC | HD | TG | TCHD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T | PC | −.089 | −.402(**) | −.230(*) | −.167 | −.179 | −.001 | .178 | .025 | .039 | .273(*) | .037 |
| N=84 | Sig. | .423 | .000 | .035 | .129 | .104 | .995 | .104 | .818 | .724 | .012 | .741 |
| M | P C | −.029 | .(a) | −.239 | −.038 | −.237 | −.044 | .126 | .131 | .032 | .377(**) | .156 |
| N=49 | Sig. | .843 | . | .098 | .794 | .101 | .762 | .387 | .369 | .829 | .008 | .285 |
| F | P C | .007 | .(a) | −.168 | .227 | .051 | −.058 | .043 | −.158 | −.102 | .152 | −.169 |
| N=35 | Sig. | .970 | . | .334 | .190 | .771 | .740 | .806 | .364 | .561 | .383 | .333 |
| Sm | P C | −.209 | −.491(**) | −.028 | .(a) | −.298 | −.186 | .163 | .136 | .140 | .267 | .116 |
| N=36 | Sig. | .221 | .002 | .871 | . | .077 | .278 | .342 | .429 | .417 | .115 | .501 |
| NSm | PC | .038 | −.305(*) | −.366(*) | .(a) | −.038 | .072 | .168 | −.054 | −.082 | .281 | .005 |
| N=48 | Sig. | .800 | .035 | .011 | . | .800 | .626 | .254 | .716 | .579 | .053 | .975 |
| LAb | P C | −.254 | −.250 | −.243 | −.141 | −.010 | .112 | −.039 | .320(*) | .275 | .271 | .244 |
| N=45 | Sig. | .092 | .098 | .107 | .355 | .948 | .465 | .802 | .032 | .068 | .072 | .107 |
| HAb | P C | .073 | −.331(*) | −.398(*) | −.238 | −.012 | .040 | .179 | −.137 | .046 | .033 | −.178 |
| N=39 | Sig. | .657 | .040 | .012 | .144 | .943 | .807 | .275 | .404 | .780 | .840 | .279 |
| Dur A | PC | .107 | −.400(*) | .126 | −.221 | −.001 | .070 | .347 | .306 | .208 | .225 | .116 |
| N=28 | Sig. | .589 | .035 | .522 | .258 | .997 | .723 | .070 | .113 | .289 | .249 | .558 |
| Dur B | P C | −.157 | −.324 | −.315 | −.360 | −.397(*) | −.228 | .339 | −.027 | −.081 | .343 | .038 |
| N=28 | Sig. | .425 | .092 | .103 | .060 | .037 | .243 | .078 | .893 | .681 | .074 | .846 |
| Dur C | P C | −.021 | −.400(*) | .202 | .089 | .078 | .206 | −.238 | .088 | .065 | .331 | .126 |
| N=28 | Sig. | .917 | .035 | .304 | .653 | .694 | .292 | .223 | .658 | .743 | .085 | .522 |

T total patient; M male; F female; Sm smoker; NSm non-smoker; A, B, C duration groups; L low; H high; Ab ABPI; BMI body mass index; TC total cholesterol; TG triglyceride; HD HDL cholesterol; TCHD ratio of TC/HD; Dur duration of diabetes; SBP systolic blood pressure; DBP diastolic blood pressure; PC correlation value; Sig = p value of correlation; N: number of patients; * Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed);a: Cannot be computed because at least one of the variables is constant

levels of homocysteine, C-reactive protein (CRP), fibrinogen, interleukin IL-1 and IL-6, creatinine and cystatin C are also implicated for PAD. Relative impacts of traditional and nontraditional risk factors probably differ in PAD and CAD, site specific atherogenic mechanisms might be more important in PAD [20].

Strength of the study: 1) Reasonable patient number for broad group analysis; 2) Detailed dissection of patient group associations of risk factors of PAD.

Limitations of the study: 1) It being a cross sectional study, a single incidental correlation may not signify a true effect over longer periods; 2) for subgroup analysis the patient number was not reasonably adequate; 3) quantification of smoking is often difficult and was arbitrary.

In conclusion, PAD has to be identified early as the number of the modifiable risk factors become fewer or are altered with increasing duration [1, 6, 10, 12]. Calcified, poorly compressible vessels may artificially elevate ABPI values; it may be falsely negative with moderate aorto-iliac stenosis [31]. The different risk factors had differing impacts on ABPI at different durations on the gender types, smokers and non-smokers and high and low ABPI segments [10, 16, 23, 24, 31]. Indians are not less predisposed and asymptomatic PAD is not equal in genders as far as the severity is concerned [16, 24]. Intervention strategies have to be individualized and be aggressive in durations below 10 years.

Traditional risk factors fail to explain wholly the more adverse ABPI (≤0.8) state, ABPI status in females and it explains only about 20 to 30% in other groups. Probably local vascular events, sex hormones or genetic factors are more important [20, 33]. Relative impacts of traditional and nontraditional risk factors probably differ in PAD and CAD, site specific atherogenic mechanisms might be more important in PAD [20]. The previously reported PAD prevalence in India as well as the role of sex hormones in PAD needs further evaluation.

Table 7 ABPI correlations of risk factors—ABPI, gender and smoking status groups by Duration segregation

| ABPI | | Age | Gen | Dur | Sm | SBP | DBP | BMI | TC | HD | TG | TCHD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LAbA | PC | −.703(*) | −.408 | .262 | −.683(*) | −.156 | −.194 | .364 | .486 | .526 | .189 | −.034 |
| N=12 | Sig. | .011 | .188 | .411 | .014 | .629 | .546 | .245 | .109 | .079 | .557 | .916 |
| HAbA | P C | .260 | −.320 | .119 | −.320 | −.054 | −.042 | −.052 | .320 | .085 | .340 | .187 |
| N=16 | Sig. | .331 | .228 | .659 | .228 | .844 | .876 | .848 | .226 | .754 | .198 | .487 |
| LAbB | P C | −.057 | −.214 | −.156 | −.074 | −.219 | −.229 | .083 | .242 | .118 | .443 | .341 |
| N=17 | Sig. | .828 | .409 | .549 | .778 | .398 | .376 | .753 | .349 | .653 | .075 | .180 |
| HAbB | P C | .548 | −.261 | .137 | −.375 | .353 | .032 | .337 | −.014 | −.193 | .016 | −.144 |
| N=11 | Sig. | .081 | .438 | .688 | .256 | .287 | .926 | .310 | .968 | .569 | .964 | .673 |
| LAbC | P C | −.171 | −.244 | −.640(**) | .067 | .223 | .388 | −.241 | .405 | .300 | .128 | .287 |
| N=16 | Sig. | .527 | .363 | .008 | .806 | .407 | .137 | .370 | .120 | .259 | .635 | .281 |
| HAbC | P C | −.073 | −.488 | .419 | −.192 | .394 | .374 | .024 | −.408 | .220 | −.094 | −.288 |
| N=12 | Sig | .822 | .108 | .175 | .549 | .205 | .231 | .942 | .188 | .491 | .772 | .364 |
| MA | PC | .058 | .(a) | −.001 | −.145 | −.027 | −.097 | .332 | .281 | .414 | .185 | −.091 |
| N=14 | Sig. | .844 | . | .996 | .622 | .926 | .743 | .246 | .330 | .141 | .528 | .756 |
| FA | P C | .518 | .(a) | −.001 | .157 | .181 | −.020 | .038 | .196 | −.259 | .493 | .365 |
| N=14 | Sig. | .058 | . | .998 | .592 | .536 | .947 | .898 | .502 | .372 | .073 | .199 |
| MB | PC | −.233 | .(a) | −.329 | −.257 | −.525(*) | −.105 | .311 | .305 | −.025 | .662(**) | .325 |
| N=19 | Sig. | .336 | . | .169 | .289 | .021 | .668 | .195 | .204 | .918 | .002 | .174 |
| FB | P C | .305 | .(a) | .000 | .(a) | −.010 | −.572 | .533 | −.304 | −.171 | −.295 | −.551 |
| N=9 | Sig. | .425 | . | 1.000 | . | .980 | .107 | .139 | .426 | .660 | .440 | .124 |
| MC | PC | .185 | .(a) | −.035 | .327 | .028 | .095 | −.331 | .165 | −.167 | .391 | .377 |
| N=16 | Sig. | .494 | . | .897 | .217 | .919 | .728 | .211 | .541 | .537 | .134 | .149 |
| FC | P C | −.332 | .(a) | .751(**) | .363 | .361 | .289 | −.508 | −.135 | .210 | .322 | −.377 |
| N=12 | Sig. | .292 | . | .005 | .246 | .250 | .362 | .092 | .677 | .512 | .308 | .227 |
| SmA | P C | −.299 | −.467 | .150 | .(a) | −.166 | −.066 | .483 | .564 | .508 | .301 | .191 |
| N=11 | Sig. | .372 | .148 | .661 | . | .626 | .846 | .132 | .071 | .111 | .369 | .573 |
| NSmA | PC | .486(*) | −.271 | .070 | .(a) | .296 | .171 | .213 | .076 | −.082 | .311 | .156 |
| N=17 | Sig. | .048 | .293 | .788 | . | .249 | .511 | .412 | .773 | .755 | .224 | .551 |
| SmB | P C | −.720(**) | .(a) | −.117 | .(a) | −.440 | .140 | −.058 | .263 | −.089 | .526 | .329 |
| N=13 | Sig. | .005 | . | .703 | . | .133 | .648 | .851 | .386 | .772 | .065 | .272 |
| NSmB | P C | .401 | −.157 | −.267 | .(a) | −.267 | −.424 | .560(*) | −.155 | −.166 | .182 | −.129 |
| N=15 | Sig. | .138 | .577 | .336 | . | .336 | .115 | .030 | .582 | .554 | .515 | .646 |
| SmC | P C | .449 | −.562 | .348 | .(a) | −.224 | −.406 | −.318 | −.032 | .059 | −.047 | .144 |
| N=12 | Sig. | .144 | .057 | .268 | . | .485 | .191 | .313 | .920 | .855 | .885 | .656 |
| NSmC | P C | −.376 | −.467 | .044 | .(a) | .289 | .517(*) | −.197 | .219 | .123 | .568(*) | .130 |
| N=16 | Sig. | .151 | .068 | .870 | . | .278 | .040 | .464 | .415 | .651 | .022 | .633 |

T total patient; M male; F female; Sm smoker; NSm non-smoker; A, B, C duration groups; L low; H high; Ab ABPI; BMI body mass index; TC total cholesterol; TG triglyceride; HD HDL cholesterol; TCHD ratio of TC/HD; Dur duration of diabetes; SBP systolic blood pressure; DBP diastolic blood pressure; PC correlation value; Sig = p value of correlation; N: number of patients; * Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed);a: Cannot be computed because at least one of the variables is constant

## References


1. Norman PE, Davis WA, Bruce DG, Davis TME. Peripheral arterial disease and risk of cardiac death in type 2 diabetes: the Fremantle Diabetes Study. Diabetes Care. 2006;29:575–80.
2. Mohler ER. Therapy insight: peripheral arterial disease and diabetes—from pathogenesis to treatment guidelines. Nat Clin Pract Cardiovasc Med. 2007;4:151–62.
3. Sheehan P. Diabetes and PAD: Consensus Statement urges screening. Medscape Cardiol. 2004;8(1).
4. Younes NA, Ahmad AT. Diabetic foot disease. Endocr Pract. 2006;12:583–92.
5. American Diabetes Association. Peripheral arterial disease in people with diabetes. Diabetes Care. 2003;26:3333–41.
6. Marso P, Hiatt WR. Peripheral arterial disease in patients with diabetes. J Am Coll Cardiol. 2006;47:921–9.

7. Premlata G, Shanthirani S, Deepa R, Markovitz J, Mohan V. Prevalence and risk of peripheral vascular disease in a selected south Indian population. The Chennai Urban Population Study (CUPS). Diabetes Care. 2000;23:1295–300.
8. Mohan V, Vijayaprabha R, Rema M. Vascular complications in long-term South Indian NIDDMof over 25 years' duration. Diabetes Res Clin Pract. 1996;31:133–40.
9. Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, et al. Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA. 2001;286:1317–24.
10. Muhs BE, Gagne P, Sheehan P. Peripheral arterial disease: clinical assessment and indications for revascularization in patient with diabetes. Curr Diab Rep. 2005;5:24–9.
11. Agrawal RP, Sharma P, Pal M, Kochar A, Kochar DK. Magnitude of dyslipidemia and its association with micro and macro vascular complications in type 2 diabetes: a hospital based study from Bikaner (Northwest India). Diabetes Res Clin Pract. 2006;73:211–
4.
12. Wattanakit K, Folsom AR, Selvin E, Weatherly BD, Pankow JS, Brancati L, et al. Risk factors for peripheral arterial disease incidence in persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Atherosclerosis. 2005;180:389–
97.
13. Gimeno-Orna JA, Faure-Nogueras E, Sancho-Serano MA. Usefulness of total cholesterol/HDL cholesterol ratio in the management of diabetic dyslipidemia. Diabet Med. 2005;22:26–31.
14. Criqui MH. Peripheral arterial disease: epidemiologic aspects. Vasc Med. 2001;6(Suppl1):3–7.
15. Adler AI, Stevens RJ, Neil A, Stratton IM, Boulton AJ, Holman RR. UKPDS 59: hyperglycemia and other potentially modifiable risk factors for peripheral vascular disease in type 2 diabetes. Diabetes Care. 2002;25:894–9.
16. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002;287:2570–81.
17. Gilbey SG, Walters PJ, Edmonds ME, et al. Vascular calcification, autonomic neuropathy, and peripheral blood flow in patients with diabetic nephropathy. Diabet Med. 1989;6:37–42.
18. Aboyans V, Criqui MH, Denenberg JO, Knoke JD, Ridker PM,
Fronek A. Risk factors for progression of peripheral arterial disease in large and small vessels. Circulation. 2006;113:2623–9.
19. Morbach S, Lutale J, Viswanathan V, Mollenberq J, Ochs HR, Rajashekar S, et al. Regional variations of risk factors and clinical presentation of diabetic foot lesions. Diabet Med. 2004;21:91–5.
20. Pradhan AD, Shrivastava S, Cook NR, Rifai N, Creager MA, Ridker PM. Symptomatic peripheral arterial disease in women— nontraditional biomarkers of elevated risk. Circulation.
2008;117:823–31.
21. Adler A, Boyko E, Ahroni J, Smith D. Lower extremity amputation in diabetes mellitus: the independent effects of
peripheral vascular disease, sensory neuropathy and foot ulcers. Diabetes Care. 1999;22:1029–35.
22. American Diabetes Association. Preventive foot care in people with diabetes (Position Statement). Diabetes Care. 2003;26 Suppl
1:S78–9.
23. Contreras-Tellez EJ, Rodriguez-Moctezuma JR, Lopez-Carmona JM, Munguia-Miranda C, Aranda-Moreno C, Peralta-Pedrero ML. Differences of risk factors for peripheral arterial disease in type 2diabetes patients in urban and suburban populations. Rev Med
Inst Seguro Soc. 2007;45:117–22.
24. Mohan V, Premalatha G, Sastry NG. Peripheral vascular disease in non-insulin-dependent diabetes mellitus in South India. Diabetes Res Clin Pract. 1995;2:235–40.
25. Li J, Luo Y, Xu Y, Yang J, Zheng L, Hasima B, et al. Risk factors of peripheral arterial disease and relationship between low ankle-brachial index and mortality from all-cause and cardiovascular disease in Chinese patients with type 2 diabetes. Circ J.
2007;71:377–81.
26. Resnick HE, Carter EA, Sosenko JM, Henly SJ, Fabsitz RR, Nero FK, et al. Incidence of lower-extremity amputation in American Indians: The Strong Heart Study. Diabetes Care. 2004;27:1885–
91.
27. Nathera A, Beeb CS, Huak CY, Chew JL, Lin CB, Neo S, et al. Epidemiology of diabetic foot problems and predictive factors for limb loss. J Diabetes Complicat. 2008;22:77–82.
28. Walters D, Gatling W, Mullee MA, Hill RD. The prevalence, detection, and epidemiological correlates of peripheral vascular disease: a comparison of diabetic and non-diabetic subjects in an English community. Diabet Med. 1992;9:710–5.
29. Uusitupa M, Niskanen L, Siitonen O, Voutilainen E, Pyorala K. 5-year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level, and abnormalities in lipoprotein composition in non-insulin-dependent diabetic and nondiabetic subjects. Circulation. 1990;82:27–36.
30. Centers for Disease Control and Prevention (CDC): Lower extremity disease among persons aged 40 years and older, with and without diabetes- United States, 1999–2002. MMWR 2005;
54:1158–60.
31. Fiegelson HS, Criqui MH, Fronek A, Langer RD, Molgaard CA. Screening for peripheral arterial disease: the sensivity, specificity, and predictive value of noninvasive tests in a defined population. Am J Epidemiol. 1994;140:526–34.
32. National Diabetes Fact Sheet : National estimates and general information on diabetes in the United States. Atlanta. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 1997.
33. Daskalopoulou SS, Pathmarajah M, Kakkos SK, Daskapoulos ME, Holloway P, Milkailidis DP, et al. Association between ankle—brachial index and risk factor profile in patients newly diagnosed with intermittent claudication. Circ J. 2008;72:441–8.

ORIGINAL ARTICLE



# Glycemic and insulinemic responses to breakfast and succeeding second meal in type 2 diabetics

U. P. K. Hettiaratchi, Sagarika Ekanayake, Jayantha Welihinda, M. S. A. Perera

Int J Diab Dev Ctries.2011 ; 31 :199-207

Abstract The aim of the present study was to determine the parameters related to glycemic and insulinemic responses of type 2 diabetic patients to three low Glycemic Index (GI) breakfast meals and study the effects of each breakfast meal on a standard lunch meal. Breakfast meals were boiled chickpea, red rice (AT 353) meal and atta roti and the standard lunch was red rice (AT 353) with accompaniments. Study design was random cross over (n=11 age: 40–62 year). GI and Insulinemic Indices (II) of breakfast meals were calculated with white bread as the standard. Serum glucose peak concentration of chickpea was significantly lower than rice (p=0.0321), roti (p=0.0019) and bread (p=0.0001). GI of chickpea, rice and roti meals were 40±7, 64±11 and 88±9 respectively. GI of chickpea was significantly lower than rice (p=0.0466) and atta roti (p=0.0016) meals. Chickpea and rice breakfast meals had low GI and atta roti medium GI. GI values in diabetic patients were not significantly different (p>0.05) from that obtained previously in the same laboratory in healthy individuals. II of chickpea, rice and roti were 76±13, 90±20, 115±28. Glycemic and insulinemic responses showed a linear positive relationship (r=0.984) indicating that low GI was due to the macronutrients present in the meals. Breakfast meals given in the present study did not lower Glycemic nor insulinemic responses of the subsequent lunch meals. These data are useful especially in primary patient care for modulating diets of diabetic patients.

Keywords Glycemic index · insulinemic index · Type 2 diabetic patients · Second meal effect

U. P. K. Hettiaratchi : S. Ekanayake
e-mail: sagarikae@hotmail.com

## Introduction

Carbohydrate foods of diverse nature are digested at different rates releasing glucose into the blood stream. According to the postprandial glycemic responses these foods are categorized as low, medium and high using the respective Glycemic Index (GI) values [1]. The incorporation of foods with slow release carbohydrates (low/medium GI foods; lente carbohydrates) in the diets indicate a therapeutic potential in reducing insulin resistance [2], hyperglycemia between meals [3] and acute or chronic complications associated with type 2 diabetes [4, 5].

The increase in blood glucose following consumption of foods triggers the release of insulin which facilitates the uptake of glucose by extra-hepatic tissues. However, the amount of insulin released to counteract the effect of a carbohydrate load varies with food as well as health status of individuals, i.e., healthy or diabetic [6].

Certain foods ingested at breakfast have shown to improve glycemia and insulinemia of a subsequent lunch meal (4 h later) [7–9] which is recognized as the “second meal effect” [7–9]. A similar effect of dinner meals on breakfast (overnight second meal effects) had also been

demonstrated [10]. However, not all low GI foods elicit a second meal effect and improve the glycemic nor insulinemic responses of the subsequent meal [11].

Foods with slow release carbohydrates and meals that elicit second meal effects will be important in the long-term dietary management of impaired glucose tolerant (IGT) and type 2 diabetic patients [3]. This approach will lead towards a reduction in the degree of insulin resistance in diabetic patients and will be beneficial for healthy individuals as well. The prevalence of diabetes mellitus (DM) is rapidly increasing in Asian countries with India topping the list [12, 13]. Thus, the data on the dietary influences on above factors would help guide a clinician in primordial care, primary, secondary and even in tertiary prevention of diabetes. The underlying factors responsible for this problem have been recognized as life style changes that lead to reduced physical activity, consumption of foods with rapid release carbohydrates and excess calorie intake [14]. The correct guidance on diet is a cost effective and practical way to control or prevent diabetes mellitus (DM), which is a chronic disease with long term complications that affect the quality of life and productivity as well as the economy of a country.

Furthermore, an increase in the incidence of type 2 diabetes among children and teenagers in Asian countries extends this health issue to the next generation [13, 15]. Due to this epidemic nature of type 2 diabetes, early prevention strategies should be undertaken to combat this health issue before long. The objectives of this study therefore were to determine the glycemic and insulinemic responses of the Type 2 DM patients to three commonly consumed Sri Lankan breakfast meals with known GI [16] and further to determine the second meal effect of these breakfast meals on a rice lunch meal.

## Materials, subjects and methods

The details of breakfast and lunch meals are presented in Table 1. The breakfast meals analyzed were; meal 1-boiled and tempered chickpea with onions, meal 2—"atta" roti with onion salad (10 g), meal 3—boiled red rice (AT 353 obtained from Rice Research Institute, Sri Lanka) with meal accompaniments (lentil curry, coconut salad). The lunch served was boiled red rice (AT 353) with other meal accompaniments (lentil curry, boiled egg, green leafy salad, coconut gravy). White sliced bread without any meal accompaniments was used as the standard and given twice.

Type 2 Diabetes Mellitus (DM) patients (n = 11; age: 40–62 year, BMI: 25 ± 2 kg/m$^2$) with fasting blood glucose levels of 126–200 mg/dL and only on the oral drug, metformin participated in this study. Patients were requested to refrain from taking metformin for 36 h prior to a study day. The removal of metformin for the duration specified above was done to study the true physiological response to foods. Individuals were requested to refrain from vigorous physical activities, smoking and drinking alcohol the previous day. There was a 7–10 days interval between two study days and the patients resumed taking metformin during this period. Each patient completed the study (test—3 days, standard—2 days) within 4–6 weeks. The coefficient of variation (CV) of fasting serum glucose concentrations of the patients were 5–18%.

Ethical clearance for the study was obtained from Ethics Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura and Hospital Ethical Committee, Colombo South Teaching Hospital, Sri Lanka. Informed and written consents were obtained from the subjects before enrolling for the study.

The study was carried out as a randomised cross over design. Patients were requested to undergo 10–12 h overnight fast. Fasting venous blood samples were taken on arrival each morning by inserting a canula (20 G). Either a test meal or standard containing 25 g available carbohydrate portion was given with 250 mL of water on each day to be consumed within 15 min. Further blood samples (0.5–1.0 mL) were obtained at 30, 45, 60, 90, 120, 150 and 180 min intervals after the first bite [17] for analysis of serum glucose and insulin levels.

Serum glucose concentrations were determined by the enzymatic kit (GOD-PAP, Biolabo, France) and Glycemic Indices (GI) of the breakfast meals were calculated by taking the ratio of incremental area under curve (IAUC) of test and the standard [6].

Serum insulin concentrations were estimated with Elecsys insulin reagent kit (Roche Diagnostics GmbH, Germany) using Elecsys 1010 analyzer at Ceymed Laboratory (Sri Lanka). Insulinemic Indices (II) of breakfast meals were expressed as the ratio of incremental area under curve (IAUC) of test to that of the standard.

The effects of the three breakfast meals on a standard lunch meal were analyzed by serving the rice lunch meal containing 25 g available carbohydrate portion at 4 h (240 min) after taking the first bite of each of the breakfast meals or standard. Further venous blood samples were drawn at 30, 45, 60, 90, 120, 150 and 180 min intervals following ingestion of the lunch meal.

To determine the second meal effects of breakfast meals the IAUC of serum glucose and insulin response curves following ingestion of lunch meal were calculated.

The GI and incremental area under curve (IAUC) values are presented as mean±standard error of mean (SEM). The results were analyzed and correlations studied using Microsoft Excel (2003) and Minitab, USA (Version 14).

Table 1 Breakfast meals, lunch meals, preparation methods and portion sizes

| Food | Preparation method | Portion sizes |
|---|---|---|
| Breakfast meals: | | |
| 1. Chickpea | Chickpea was soaked overnight in excess water and boiled with sufficient amount of water. Chickpea (200 g) was tempered with coconut oil (10 mL) and onions (10 g). | 186 g |
| 2. "*Atta*" *roti* meal | | |
| *Atta roti* | *Roti* dough was prepared by mixing "*atta*" flour (whole wheat flour-50 g) with coconut scrapings (50 g). The dough was flattened on a circular plate (~ 15 cm) and cooked on a pan by turning the sides (~ 10 min). | 75 g |
| Onion salad | Chopped onions (50 g), chilli pieces (5), salt powder (5 g) were mixed and lime was added to prepare the onion salad. | 10 g |
| 3. Rice meal | | |
| Red rice | Red rice was boiled with water (1:2; w:v) in a rice cooker. | 86 g |
| Lentil curry | Lentils (200 g) were boiled with water (400 mL) and spices for 10 min. Lentil curry was prepared by adding coconut milk (63 mL), salt (20 mL), green chilli (10 g) and curry leaves (5 g). Curry was tempered with chopped onions (10 g) and garlic (5 g). | 38 g |
| Coconut salad | Coconut salad was prepared by grinding coconut scrapings (100 g) with chopped onions (20 g), garlic (5 g), dried chilli pieces (10 g), lime and salt powder (10 g). | 25 g |

Table 1 (continued)

| Food | Preparation method | Portion sizes |
|---|---|---|
| Lunch meal: | | |
| Red rice<br>Lentil curry | As in the rice meal (breakfast meal 3) | 86 g |
| Boiled egg | Eggs were boiled for 10 min. | 38 g |
| Green leafy salad (*Gotukola – Centella asiatica*) | Chopped *Centella asiatica* (100 g) was mixed with coconut scrapings (50 g), onions (20 g), garlic (10 g), green chilli (10 g), salt powder (10 g) and lime. | 25 g |
| Coconut gravy (*Kiri hodi)* | Coconut milk extracts (175 mL) were boiled with onions (10 g), garlic (5 g), green chilli (10 g), curry leaves (5 g) and turmeric powder (1 g) for ~ 15 min. | 30 mL |

## Results

The data related to glycemic and insulinemic responses following breakfast meals are stated in Table 2. The peak glucose concentrations of chickpea and rice meals were significantly low ($p=0.0001$, $p=0.0004$ respectively) when compared with the peak glucose concentration of the standard. Correspondingly a 27% and 14% reduction in peak glucose concentrations of chickpea and rice meals were observed compared with the standard. The peak glucose concentrations of chickpea were also significantly lower than that of rice meal ($p=0.0321$) and atta roti meal ($p=0.0019$). However, the serum glucose concentrations at 3 h from ingestion of test foods or standard were not significantly different ($p>0.05$; Fig 1).

The GI values of chickpea, red rice meal and atta roti (breakfast meals) were 40±7, 64±11 and 88±9 respectively (Table 2). Thus, the chickpea and rice meals can be categorized as low GI foods while the atta roti meal was medium GI when determined with type 2 diabetic patients. The GI of chickpea was significantly lower than that of rice ($p=0.0466$), and atta roti ($p=0.0016$).

When considering individual glycemic responses, all individuals ($n=11$) elicited low glycemic responses (low GI) with the chickpea meal. Low and medium GI were obtained for rice meal with 64% and 18% individuals

Table 2 Glycemic and insulinemic responses of breakfast meals and standard with type 2 diabetic patients

| Parameter | [a]Standard | [b]Chickpea | [b]Roti | [b]Rice meal |
|---|---|---|---|---|
| [1]Fasting glucose concentration | 8.8±3.1 | 8.3±3.2 | 8.7±3.1 | 8.6±3.2 |
| [1]Peak glucose concentration | *13.7±3.1 | *9.9±3.3 | *13.1±3.8 | *11.8±3.6 |
| Peak glucose time (min) | 90 | 120 | 90 | 60 |
| [1]Fasting insulin concentration | 11.4±5.4 | 10.4±9.3 | 9.7±6.6 | 13.5±6.7 |
| [1]Peak insulin concentration | 47.5±33.9 | 27.6±14.4 | 34.2±25.8 | 32.5±15.3 |
| Peak insulin time (min) | 90 | 150 | 90 | 120 |
| [2]IAUC (glucose) | *547±45 | *210±33 | *478±64 | *357±71 |
| [2]GI | 100 | *40±7 | *88±9 | *64±11 |
| [2]IAUC (insulin) | 4042±1125 | 2326±452 | 3142±755 | 2215±343 |
| [2]II | 100 | 76±13 | 115±28 | 90±20 |
| [3]GL | 17.9 | 7.3 | 15.7 | 11.6 |

[1]Values are given as mean±SD (Standard deviation); [2]Values are given as mean±SEM (Standard error of mean); [3]Glycemic Load (GL) values were calculated for the 25 g available carbohydrate portion; [a]n=12; [b]n=11; Glucose concentrations are expressed as mmol/L; Insulin concentrations are expressed as microIU/mL; *indicates the values that are significantly different

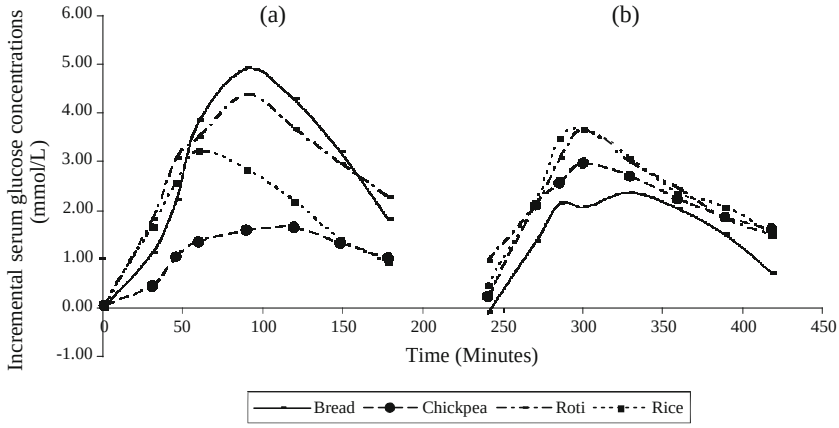



Fig. 1 Glycemic responses to (a) breakfast meals and (b) lunch meal following the breakfast meals and standard. Each test food represents an average of 11 (standard, n=12)

respectively. However, roti elicited each low and high GI with 45% subjects.

The peak insulin concentrations of chickpea meal were significantly lower ($p=0.0433$) compared with those of the standard (Fig. 2). The insulinemic indices (II) of chickpea, rice and roti meals were 76±13, 90±20, and 115±28 respectively (Table 2) and not significantly different from each other ($p>0.05$). The glycemic and insulinemic responses for the foods analyzed in the present study indicated a positive linear relationship ($r=0.984$; Fig. 3).

Glucose and insulin peak concentrations of lunch meals following consumption of standard and test breakfasts were not significantly different ($p > 0.05$). However, insulin concentra- tions at 3 h from ingestion of lunch meals were significantly lower in bread compared with rice meal ($p = 0.0344$) and atta roti ($p=0.0216$) but not with chickpea ($p= 0.07664$). IAUCs of glycemic nor insulinemic responses of lunch following ingestion of test breakfasts were not significantly different from that of the lunch following standard (Table 3, Fig. 1).

Serum glucose peak concentrations of lunch meals following the three breakfast meals and the standard were analyzed and all the glucose peak concentrations of lunch meals were observed to be similar or less (2.5–3.3 mmol/L) compared with the rice breakfast peak (3.2 mmol/L).

## Discussion

The present study was designed with the aim to study the suitability of some low GI breakfast meals in the diet regime of diabetic patients.

Chickpea and rice meal can be categorized as low GI while atta roti was medium GI (with respect to glucose as the standard). Inter individual variations to the breakfast meals were analyzed to assess the levels of variations of individuals to respective foods. Chickpea elicited minimum inter individual variations (100% low GI). However when atta roti and rice were consumed, the variations in glycemic responses of individuals were high.

Serum glucose peak concentrations of breakfast meals and standard were analyzed. The reduction of peak concentrations of chickpea, rice and roti meals by 27%, 14% and 3% compared to the standard respectively and also the peak concentrations of chickpea and rice being significantly lower than the standard indicates the potential of incorporating the two low GI plant based meals in South Asian diabetic meal plan (Table 2). Consumption of rice with a variety of meal accompaniments containing other macro and micronutrients should be promoted. A Glycemic response curve with a plateau was obtained for chickpea

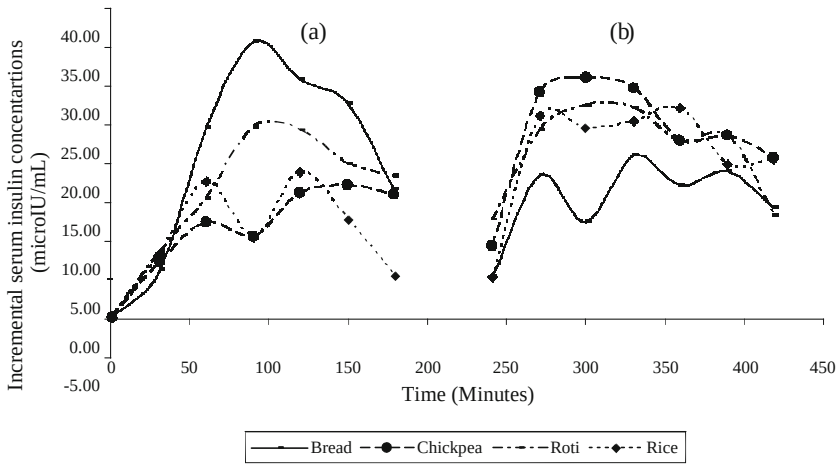



Fig. 2 Insulinemic responses to (a) breakfast meals and (b) lunch meal following the breakfast meals and standard. Each test food represents an average of 11 (standard, n=12)

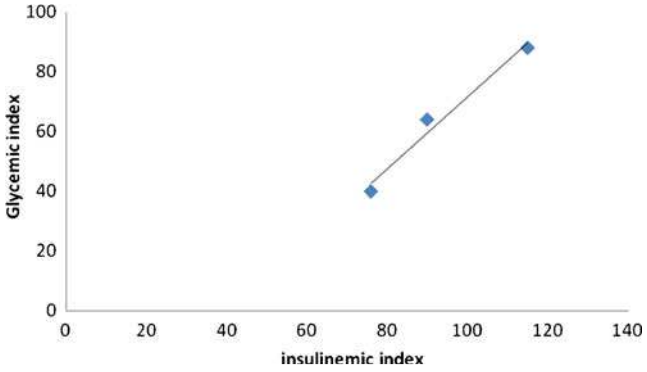



Fig. 3 Relationship between the glycemic indices and the insulinemic indices of the test foods. $R^2 = 0.974$

meal (Fig. 1) reinforcing the beneficial effects of legumes as the major carbohydrate portion in a meal. It would also be beneficial to include chickpea as a meal accompaniment with rice.

GI of chickpea containing similar load of available carbohydrate (25 g) and atta roti containing double the amount of available carbohydrates (50 g) determined previously in the same laboratory with healthy individuals [16] were observed to be low GI (29±5 and 67±9 respectively). GI of a rice meal containing all the components of the rice meal served for lunch with a 50 g available carbohydrate portion had been 60±5 with healthy individuals [18]. The data indicates the same categorization of foods with regard to GI in diabetic and healthy individuals except for roti. Although the GI values with diabetic patients were not significantly different ($p > 0.05$) from healthy individuals, [16] the GI values obtained with diabetics were always higher. The different degrees of insulin resistance and longer time to clear glucose from the circulation in the diabetic patients might be contributing to the increase (although not significant) in glycemic response when compared with healthy individuals. A similar observation had been reported previously for the two categories of individuals [19].

Roti elicited different categorizations in terms of GI with diabetic and healthy individuals, i.e., low GI with healthy individuals and medium GI with diabetic patients. This might be due to the evident trend of diabetic patients eliciting higher GI values compared with the healthy category. However, the GI values obtained with healthy individuals and diabetics were not significantly different ($p \geq 0.05$). These differences of GI values between the two categories could be due to the higher Glycemic responses elicited by the diabetics for the same foods compared to the healthy individual.

Delayed serum glucose peaking of test foods and standard were observed with the diabetic individuals compared with healthy individuals. This could be attributed to the differences in the digestion and absorption processes in diabetic patients, i.e., delayed gastric emptying [20], absorption of glucose into the blood stream and the diabetic patients being insulin resistant.

Insulin responses to three meals were also analyzed to observe the effect of meals on insulin secretion (Table 2). The glycemic and insulinemic responses of these foods indicated a positive linear relationship ($r = 0.984$) confirming the low GI foods elicit a lower insulin output and vice versa (Fig. 3). Thus, the low GI foods will be beneficial in reducing the blood glucose level as well as preserving the insulin output [21]. However, the linear positive relationship indicates that there were no insulin secretagogue properties of the meals tested as was reported for certain other foods i.e., dairy products [22]. Thus, the low GI of chickpea and rice meals were due mainly to the other factors such as proteins [chickpea −16 g [16], rice meal—10 g] and dietary fibre [chickpea—21 g [16], rice meal—13 g] present. As chickpea was given without any other accompaniments, the proteins and fibre content of the meal was only due to the legume itself. When considering the rice meal, the meal accompaniments included in the composite meal (Table 1) have also contributed to the total protein and fibre content. The accompaniments used in the rice meal are mainly vegetables, protein sources and other starchy sources with less carbohydrate contents compared to rice. Thus, the GI values of the meal accompaniments were not determined.

The “Glycemic load” concept (GL) introduced later in 1997 makes use of the Glycemic index values [23] in

Table 3 Glycemic and insulinemic responses of lunch meal following the breakfast meals and standard in type 2 diabetic patients

| Parameter | [a]Standard | [b]Chickpea | [b]Roti | [b]Rice meal |
|---|---|---|---|---|
| [1]Glucose concentration at 4 h (fasting) | 8.7±3.8 | 8.5±3.1 | 9.7±4.3 | 9.0±4.2 |
| [1]Peak glucose concentration | 11.2±4.1 | 11.3±3.5 | 12.4±4.8 | 12.3±4.6 |
| Glucose peaking time (min) | 90 | 60 | 60 | 60 |
| [1]Insulin concentration at 4 h (fasting) | 16.2±4.6 | 19.2±11.9 | 21.8±13.9 | 18.2±6.5 |
| [1]Peak insulin concentration | 32.1±18.4 | 41.2±31.5 | 36.9±18.2 | 40.3±17.7 |
| Insulin peaking time (min) | 90 | 60 | 60 | 120 |
| [2]IAUC (glucose) | 320±40 | 351±61 | 264±58 | 382±51 |
| [2]IAUC (insulin) | 2171±462 | 3098±668 | 2070±339 | 3196±648 |

Glucose concentrations are expressed as mmol/L; Insulin concentrations are expressed as microIU/mL; [1]Values are given as mean±SD (Standard deviation); [2]Values are given as mean ±SEM (Standard error of mean); [a]n=12; [b]n=11: h-hour

determining the actual Glycemic load of a normal serving size (NSS). According to GL values, foods are categorized as low (<10), medium [10–20], high (>20) against glucose as the standard [23]. When considering the portions of breakfast meals served in the present study only rice meal represented a normal edible portion while the portion of chickpea was more and roti less. According to the patients (100%), NSS of chickpea meal would be 2/3 of the portion given and roti, twice the amount. Thus, GL for the NSS of chickpea, rice and roti would be 5, 11, and 31 respectively indicating chickpea to be low GL meal, rice a medium and roti a high GL meal. This further stress the importance of including legumes, as the main carbohydrate source in a meal of diabetic food plan.

The second meal effects of breakfast meals (chickpea, rice meal and roti) were analyzed following the rice mixed meal as the lunch (Table 3). The IAUCs of neither glycemic responses nor insulinemic responses of lunch following ingestion of test breakfasts were significantly different ($p > 0.05$) from that of lunch following the standard. (Table 3, Fig. 1). The tested breakfast meals had no effect on the glycemic responses nor insulin responses following the subsequent lunch.

Serum glucose peak concentrations of the lunch meals following the three breakfast meals and the standard were analyzed to compare with the glucose peak concentration obtained with the rice breakfast meal. All the glucose peak concentrations of lunch meals were similar or less (2.5–3.3 mmol/L) compared with the rice breakfast peak concentration (3.2 mmol/L). The data of the present study indicate the suitability of the rice lunch meal also, with the diabetic patients despite not eliciting a second meal effect. Lunch meal served can also be consumed as a breakfast meal and according to the patients participated in the study (100%) had a high satiating factor.

The slow release (lente) carbohydrates and foods with high dietary fibre which can undergo colonic fermentation have been recognized as factors responsible in extending a second meal effect [10, 24]. However, in the present study we did not observe a second meal effect in spite of presence of lente carbohydrates in legumes (chickpea, lentils) and high dietary fibre (21 g) in chickpea [16]. The presence of 54% rapidly available glucose (RAG) content (unpublished data) and increased levels of low molecular weight carbohydrates in chickpea as observed with gel filtration chromatography (Unpublished data) might be responsible for not extending any second meal effect.

Although the second meal effect of foods had been determined mostly with healthy individuals [10, 11, 24] by serving a 50 g available carbohydrate portion, the present study was carried out with diabetic patients giving a lower carbohydrate load (25 g) as the data can be directly applied to the dietary therapy of the said category of individuals.

The data of this nature which focuses especially on diabetic patients can be used in primary patient care when modulating diets of diabetic patients. Effective dietary interventions with regard to Diabetes mellitus are both patient friendly and cost effective without any side effects. Hence, dietary strategies aimed at diabetes prevention should be more popularized.

Acknowledgements The patients participated in the study are gratefully acknowledged. Financial assistance by National Research Council, Sri Lanka (NRC-05-03), National Science Foundation, Sri Lanka (NSF/RG/2005/AG/10), and International Progamme in Chem- ical Sciences (IPICS -Sri 07), Uppsala University, Sweden are acknowledged. Authors wish to acknowledge the technical assistance given by Mrs. L.K.D.T. Dassanayake, Mrs. Amitha Jayawardena of Family Practice Centre, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka during the study.

Conflict of interest Authors declare no conflict of interest.

## References

1. Beals KA. The Glycemic index: Research meets reality. 2005. A special publication of the Unites states Potato Board.
2. Bornet FRJ, Billaux MS, Messing B. Glycemic index concept and metabolic diseases. Int J Biol Macromol. 1997;21:207–19.
3. Arvidsson-Lenner R, Asp NG, Axelsen M, Bryngelesson S, Haapa E, Jarvi A, et al. Glycemic index—relevance for health, dietary recommendations and food labelling. Scan J Nutr. 2004;48:84–94.
4. Barclay AW, Petocz P, McMilan-Price J, Flood V, Prvan T, Mitchell P, et al. Glycemic index, glycemic load, and chronic disease risk-a meta-analysis of observational studies. Am J Clin Nutr. 2008;87:627–37.
5. Zavaroni I, Bonora E, Pagliara M, Dall'Aglio E, Luchetti L, Buonanno G, et al. Risk factors for coronary artery disease in healthy persons with hyperinsulinaemia and normal glucose tolerance. N Engl J Med. 1989;320:702–6.
6. Brouns F, Bjorck K, Frayn N, Gibs AL, Lang V, Slama G, et al. Glycemic index methodology. Nutr Res Rev. 2005;18:145–71.
7. Jenkins DJA, Wolever TMS, Taylor RH, Griffiths C, Krzeminska K, Lawrie JA, et al. Slow release dietary carbohydrate improves second meal tolerance. Am J Clin Nutr. 1982;35:1339–46.
8. Wolever TMS, Bolognesi C. Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. J Nutr. 1996;126:2807–12.
9. Liljeberg H, Akerberg A, Bjorck I. Effect of the Glycemic index and content of indigestible carbohydrates of cereal-based breakfast meals on glucose tolerance at lunch in healthy subjects. Am J Clin Nutr. 1999;69:647–55.
10. Wolever TMS, Jenkins DJA, Ocana A, Rao V, Collier G. Second meal effect: low Glycemic index foods eaten at dinner improve subsequent breakfast Glycemic response. Am J Clin Nutr. 1988;48:1041–7.
11. Clark C, Gardiner J, McBurney M, Anderson S, Weatherspoon L, Henry D, et al. Effects of breakfast meal composition on second meal metabolic responses in adults with type 2 diabetes mellitus. Eur J Clin Nutr. 2006;60:1122–9.
12. King H, Aubert RE, Herman WH. Global burden of diabetes. Diabetes Care. 1998;21:1414–31.

13. Chowdhury TA, Grace C, Kopelman PG. Preventing diabetes in South Asians. Br Med J. 2003;327:1059–60.
14. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.
15. Hossain P, Kawar B, Nahas M. Obesity and diabetes in the developing world- a growing challenge. N Engl J Med.
2007;356:213–5.
16. Widanagamage RD, Ekanayake S, Welihinda J. Carbohydrate-rich foods: Glycemic indices and the effect of constituent macro-nutrients. Int J Food Sci Nutr. 2009;60:215–23.
17. FAO/WHO. Carbohydrates in human nutrition: report of a joint
FAO/WHO expert consultation. FAO Food and Nutrition paper 66; 1998. p. 1–140.
18. Hettiaratchi UPK, Ekanayake S, Welihinda J. Glycemic indices (GI) of three Sri Lankan wheat bread varieties and a bread-lentil meal. Int J Food Sci Nutr. 2009;60:21–30.
19. Atkinson F, Foster-Powell K, Brand-Miller J. International tables of glycemic index and glycemic load values: 2008. Diabetes Care. 2008;31:2281–3.
20. Kojecky V, Bernatek J, Horowitz M, Hep A. Prevalence and determinants of delayed gastric emptying in hospitalised type 2 diabetic patients. World J Gastroenterol. 2008;14:1564–9.
21. Hsu IR, Kim SP, Kabir M, Bergman N. Metabolic syndrome, hyperinsulinemia and cancer. Am J Clin Nutr. 2007;86:S867–71.
22. Nilsson M, Holst JJ, Bjorck I. Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose equivalent drinks. Am J Clin Nutr. 2007;85:996–1004.
23. Barclay AW, Brand-Miller JC, Wolever TMS. Glycemic index, glycemic load, and glycemic response are not the same. Diabetes Care. 2005;28:1839–40.
24. Nilsson A, Ostman E, Preston T, Bjorck I. Effects of GI vs content of cereal fibre of the evening meal on glucose tolerance at a subsequent standardized breakfast. Eur J Clin Nutr. 2008;62:712–20.

ORIGINAL ARTICLE



# Intensified insulin therapy during fasting of Ramadan in type 1 diabetic patients

Ali Abdel-Rahim, Nagwa Lachine, Mohamed Zeitoun, Wessam El-Gendi

Int J Diab Dev Ctries. 2011 ; 31 : 216-223

Abstract Evaluate safety of insulin glargine/aspart regimen in type 1 diabetics who are willing to fast Ramadan. Thirty-three patients with type 1 diabetes (mean age ± SD; 21.9 ± 8.7 years) were included. One week before the study, clinical and laboratory evaluations were undertaken. Patients on basal/bolus insulin had their doses adjusted. Those with another insulin regimen were changed to glargine/aspart regimen with adjustment of doses. At the beginning of Ramadan, patients were shifted to glargine once-daily and aspart before Iftar (sunset-meal), Suhur (sunrise-meal) and a meal in between. Total dose was reduced to 90% of pre-Ramadan dose. Patients were instructed to report any hypoglycemia, severe hyperglycemia or ketosis, and their self-monitored plasma glucose at five planned visits in Ramadan. All clinical and laboratory evaluations were repeated at the end-of-Ramadan. At the end of Ramadan, there was no report of severe hypoglycemia, hyperglycemia or diabetic ketoacidosis. Twenty patients suffered 70 hypoglycemia events; one discontinued fasting in 2 days. There was no significant change in HbA1c ($p = 0.373$) between pre-Ramadan (Mean ± SD; 6.5 ± 1.2%) and end-of-Ramadan level (Mean ± SD; 6.9 ± 1.2%). Moreover, there was no significant change ($p = 0.251$) in fructosamine level between pre-Ramadan (2.8 ± 0.9 mol/L) and end-of-Ramadan (3.2 ± 1.1 mol/L). Insulin dose had increased by 7% of the starting dose ($p = 0.0496$). There was no significant ($p > 0.05$) change in weight, BMI and lipid profile at the end–of-Ramadan. Patients with type 1 diabetes can fast Ramadan safely, using low-peak basal insulin and rapid-acting pre-meal insulin, under strict blood glucose-monitoring and close supervision.

Keywords Type 1 diabetes · Fasting · Ramadan · Insulin glargine · HbA1c

Introduction, Patients and Methods, Statistical analysis, Results, Discussion, Conclusions

A. Abdel-Rahim
aaarahim55@gmail.com

## Introduction

The study of epidemiology of diabetes and Ramadan (EPIDIAR study) reported that about 43% of patients with type 1 diabetes mellitus (T1DM) managed to fast a minimum of 15 days of Ramadan. [1] However, there are few studies addressing the management of those fasting patients as regards the most appropriate insulin regimen in terms of safety and maintaining euglycemia; thus fasting in Ramadan comes to be a challenge to both patients with diabetes and healthcare providers [2].

Furthermore, one of the Evidence-Based Medicine (EBM) pillars is to consider patients' values and culture. Hence, management of patients with T1DM in Ramadan is a step in the direction of EBM diabetes control.

Different suggested regimens are based upon clinical practice and not upon evidence-based studies. For that reason, there is no standard regimen for the situation of Ramadan fasting for patients with type 1 diabetes [3]. One of the recommended regimens for those patients is the intensified regimen using the long-acting low-peak insulin,

e.g. glargine once, combined with a rapid-acting insulin, e.g. aspart or lispro immediately before each meal [4].

Two studies examined the use of the intensified regimen in fasting patients with T1DM but with a few numbers of cases. One of them by Azar et al. (2005) examined this regimen in nine patients; however, only five patients actually continued fasting. One patient developed hyperglycemia, and two developed hypoglycemia events. In the other study Mucha et al. (2006) tested the hypothesis that insulin glargine would maintain euglycemia in patients with type 1 diabetes during an 18-h fast, such as would occur when a person with type 1 diabetes sleeps late or misses meals. The study was done upon 15 volunteers with no exercise and only for 2 days, one control day and one fasting day.

The purpose of this study was to examine the hypothesis that intensified regimen (insulin glargine once daily with three doses of insulin aspart as prandial insulin) in a total dose reduced by 10%, is safe; without exposing those patients to risk of severe hypoglycemia, diabetic ketoacidosis (DKA) or severe hyperglycemia and to study their glycemic profile.

## Patients and methods

This is an open-label, non-comparative, one-center interventional study. We do not have a standard regimen for management of fasting patients with T1DM, so we used non-comparative design type. We consider the current study as a step in the way of studying different regimens to develop appropriate guideline for management of those patients. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Alexandria University, Egypt.

### Patients

Seventy eight patients with T1DM were screened for the eligibility criteria. According to the inclusion and exclusion criteria, only 33 patients of this group were enrolled for the study. Patients were chosen from the out-patient diabetes clinic in the Main Alexandria University Hospital, Egypt. Inclusion criteria were Age ≥11 years, T1DM with HbA1c ≤8.5 or less, willing to fast Ramadan and signed an informed consent himself/herself or by parents in case of individuals less than 18 years. Exclusion criteria were pregnant or lactating females, patients who suffered DKA or severe hypoglycemia in the previous 3 months and patients with renal impairment, proliferative retinopathy or macrovascular diseases.

Table 1 Baseline characteristics

| | Range | Mean (SD) |
|---|---|---|
| Age (year) | 11–44 | 21.9 (8.7) |
| Duration of diabetes (year) | 1–17 | 6.3 (4.5) |
| Body weight (kg) | 37.5–97 | 74.1 (16.5) |
| BMI (kg/m$^2$) | 17.4–33.8 | 27.0 (4.6) |

The 33 patients consisted of 15 males and 18 females. Their baseline characteristics are shown in (Table 1).

### Methods

After the screening visit, all patients who enrolled for the study were evaluated on six visits (one visit before and 5 visits during Ramadan) as shown in (Fig. 1).

#### Phase I: before Ramadan

##### Visit (0): 1 week before Ramadan

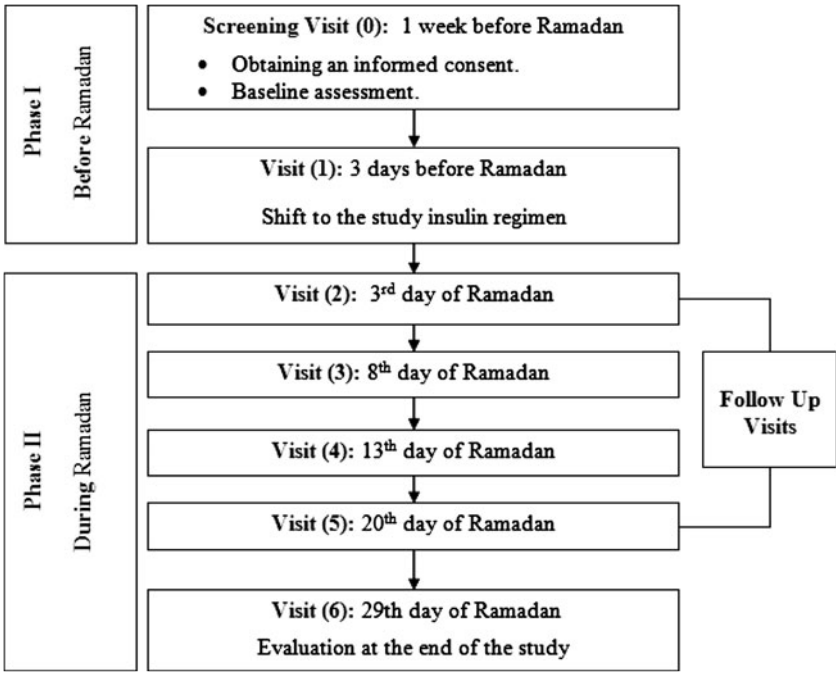
During this visit patients were screened for inclusion and exclusion criteria. The study protocol was explained to enrolled patients before signing the informed consent. Full history taking and physical examination were done. Laboratory assessment including HbA1c, fructosamine, lipid profile, ESR, blood urea, serum creatinine, urinary albumin/ creatinine ratio, CBP, ALT and Alkaline phosphatase levels were undertaken. Twenty of them were on basal bolus therapy (either analogues or conventional) insulin, 9 patients were on twice daily premixed insulin with short acting insulin before lunch and 4 patients were on twice daily premixed insulin without short acting insulin. All premixed insulins were conventional insulin.

##### Visit (1): 3 days before Ramadan

Patients without any of exclusion criteria were shifted from their current insulin regimen to a single dose of insulin glargine between 8 and 12 pm and insulin aspart immediately before each meal. The total daily insulin dose they were injecting was calculated and further divided into 50% glargine and 50% insulin aspart; insulin aspart was divided between three meals (25% before breakfast, 50% before lunch and 25% before dinner)

We provided patients with insulin preparations in the form of either re-filled or pre-filled pen devices for insulin glargine (Lantus® OptiSet pen or Solostar pen, Sanofi-Aventis, Frankfurt-Höchst, Germany) and pre-filled pen for insulin aspart; (NovoRapid® FlexPen®: Novo Nordisk, Bagsværd, Denmark:) and home blood glucose monitoring

Fig. 1 Study design




kit (Accu-Check® Go, Roche, Switzerland) and instructed them about their proper usage.

Patients were informed that once Ramadan started the insulin dose was to be reduced by 10% and was divided into 35% glargine and 65% aspart. The rationale for reducing the total dose and the basal insulin ratio is to keep the patient on the hyperglycemic side during the day-hours to avoid the undesired fasting hypoglycemia during the early days of Ramadan. On the other hand, the dose of the prandial insulin was increased to 65% to match the heavy meals and the intake of large amounts of juices and sweets during Ramadan.

Aspart dose was divided between three meals 50% immediately before the sunset-meal (Iftar), and 25% before the pre dawn-meal (Suhur) 25% before a meal in between the two meals. This was pre-calculated and clearly recorded in a written prescription for each patient and his family. Patients were encouraged to maintain a healthy balanced diet, increase fluid intake during the non-fasting hours, and to maintain their normal level of physical activity, but avoid severe exercise during hours of fasting.

Also patients were instructed to monitor their blood glucose level by daily capillary blood glucose testing, together with testing their urine for sugar and acetone, at least 5 times per day, on awakening from sleep, at 12–1 pm, 3–4 pm, just before end of fasting; Iftar (sun set), 2 h after Iftar and whenever they felt any symptoms. After the 1 week of Ramadan the patients were instructed to test the sugar profile three times: at 1 pm, 5 pm and 2 h after Iftar.

In case of emergency, including marked hypo- or hyperglycemia or DKA, patients were asked to report to the investigators by phone (available 24 h) by one of their relatives if they were unable to communicate. Furthermore, the patient was to be transferred to a hospital emergency. Whenever a patient developed severe hypoglycemia (requiring assistance of another person to administer carbohydrate, glucagon or other resuscitative actions according to the ADA criteria) [8] or developed DKA, he was requested to be admitted to emergency room, stop fasting and be excluded from the study.

Patients were advised to break their fasting for 1 day if they developed one attack of hypoglycemia in which the recorded blood glucose was less than 60 mg/dl

Phase II: during Ramadan

The average fasting period was about 12.5 h, approximately from 4:30 am to 5 pm.

Follow up visits

During visit (2), visit (3), visit (4) and visit (5), evaluation of the patient's general well-being during the preceding period was done. The patients' diaries for glucose profile were inspected, adjusting insulin doses individually according to each patient's metabolic profile and discussing patient's needs to change daily activities or meals. Also, reporting of any hypoglycemia, ketoacidosis, severe hyperglycemia or any other emergent adverse event was done. Clinical examination was made during each visit focusing on vital signs, any signs of dehydration, infection or metabolic disturbance.

Glargine dose was down-titrated if the blood glucose level in the day time (1:00 PM, 3:00 PM) or pre-Iftar (5:00

Table 2 Hypoglycemia events during the study

| Number of patients suffered hypoglycemia events | 20 patients |
|---|---|
| Number of hypoglycaemia events <70 mg/dl | 70 events |
| At awaken from sleep | 17 events |
| At 1 PM | 9 events |
| At 3 PM | 12 events |
| At 5 PM just before (Iftar) | 13 events |
| At 2 hours after (Iftar) | 19 events |
| Symptoms of hypoglycemia | |
| Documented symptomatic | 32 events |
| Asymptomatic | 38 events |
| Severe | 0 events |

PM) was less than 80 mg/dl, even if this was tolerated by the patient. On the other hand, glargine dose was up titrated by 1–2 units if the pre-Iftar blood glucose level was between 140 and 180 mg/dl and by 2 to 4 units if it was more than 180 mg/dl, based on patient's weight.

Insulin aspart dose was titrated up or down based on the blood glucose level in the morning for the pre-dawn meal (Suhur) dose and 2 h after Iftar for breakfast (Iftar) (5:00 PM) dose. The target blood glucose level was between 80 and 180 mg/dl. One unit of insulin aspart was added or reduced, for every 50 mg/dl blood glucose above or below target.

End visit: visit (6): 29th day of Ramadan

At the final visit the same parameters examined at visit (0) were repeated.

Statistical analysis

Inferential statistics and comparison within patients were undertaken using paired t-test. Data are presented as (means [SD]); a value for $P < 0.05$ was considered statistically significant. SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS Inc, Chicago, IL, USA) was used for the statistical analyses.

## Results

### Patients

All the included patients (n = 33) completed the study. Only one patient had to interrupt fasting on two different days because of asymptomatic hypoglycemia.

### Hypoglycemia events

No patient suffered severe hypoglycemia during Ramadan. 20 patients (61% of studied patients) suffered a total of 70 hypoglycemia events. Hypoglycemia is defined as blood-glucose level below 70 mg/dl according to the ADA criteria [7]. The calculated mean rate of hypoglycemia events was 2.1 events/ patient/ month. Maximum number of hypoglycemia events in one case was nine events & minimum was one. Details of such events are shown in (Table 2).

Nine patients (27% of the studied patients) suffered 11 hypoglycemia events during which the recorded blood glucose was less than 60 mg/dl; whenever these events

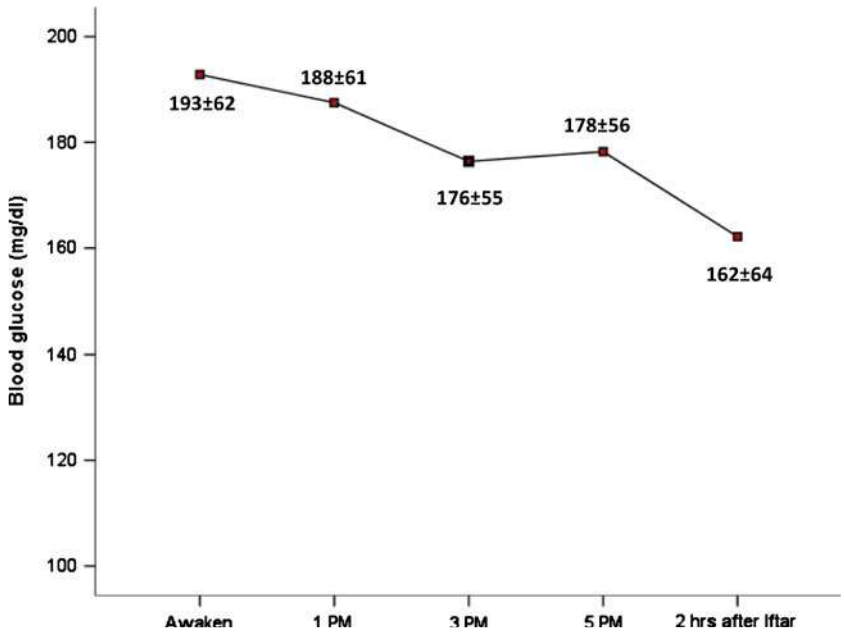



Fig. 2 Mean blood glucose levels at different times of the day

Table 3 Insulin dosage during the study

| | At start of Ramadan Mean (SD) | At end of Ramadan Mean (SD) | Percent change | p |
|---|---|---|---|---|
| Total Insulin (IU/day) | 57.8 (22.8) | 61.7 (24.7) | 6.75% | 0.0496* |
| Basal Insulin (IU/day) | 21.4 (9.0) | 23.1 (10.2) | 7.94% | 0.1094 |
| Prandial Insulin (IU/day) | 36.4 (15.9) | 38.6 (16.4) | 6.04% | 0.0727 |

*p is significiant

occurred during fasting (six events), patients were advised to interrupt fasting by protocol. However, only one patient followed this advice in two days. Other patients continued fasting with frequent measurements of their blood glucose without any deterioration.

## Hyperglycemia

No patient suffered from DKA or severe hyperglycemia requiring hospitalization.

## Glycemic profile

All subjects adhered to the protocol of self-monitoring of blood-glucose levels. The post-prandial glucose excursion was 18 mg/dl [=mean value of blood-glucose 2 h after the sunset meal (Iftar) – mean value of blood-glucose level at 5 pm]. Mean blood-glucose levels of patients at different times of the day are demonstrated in (Fig. 2).

We adjusted the total daily insulin doses individually for each subject at all visits; when required. The total insulin dosage was increased slightly by about 6.75% of the starting dose; this increase is statistically significant. Insulin glargine was increased by 7.94% while insulin aspart was increased by only 6.04% of the starting dose. (both values Not significant, Table 3).

HbA1c and fructosamine showed no significant difference ($p > 0.05$) between the pre- Ramadan and end-of-Ramadan (Table 4).

## Clinical assessment and laboratory investigations

Hemoglobin level, serum creatinine and alkaline phosphatase are the only variables that changed significantly at the end of Ramadan. Other variables did not change significantly (Table 4).

## Discussion

In our study, no patient suffered severe hypoglycemia. Furthermore, no patient was admitted to a hospital due to

Table 4 Clinical and laboratory parameters assessed before and at the end of Ramadan

| | Before Ramadan Mean (SD) | End of Ramadan Mean (SD) | p |
|---|---|---|---|
| Weight (kg) | 74.1 (16.5) | 74.6 (15.9) | 0.223 |
| Body mass index (kg/m$^2$) | 27.0 (4.6) | 27.3 (4.8) | 0.130 |
| HbA1c (%) | 6.5 (1.2) | 6.9 (1.2) | 0.373 |
| Fructosamine (mol/l) | 2.8 (0.9) | 3.2 (1.1) | 0.251 |
| Total cholesterol (mg/dl) | 203.7 (38.4) | 215.3 (42.7) | 0.107 |
| HDL-C (mg/dl) | 47.9 (17.6) | 44.4 (15.2) | 0.082 |
| LDL-C (mg/dl) | 138.0 (37.9) | 152.7 (38.6) | 0.056 |
| VLDL-C (mg/dl) | 17.8 (10.4) | 18.1 (9.9) | 0.867 |
| Triglycerides (mg/dl) | 79.6 (42.4) | 80.3 (33.4) | 0.936 |
| Blood urea (mg/dl) | 28.2 (7.0) | 26.8 (4.5) | 0.433 |
| Serum creatinine (mg/dl) | 0.75 (0.12) | 0.69 (0.14) | 0.014* |
| Urine albumin creatinine ratio (ug/mg) | 0.0509 (0.03) | 0.0513 (0.02) | 0.963 |
| ALT (U/L) | 26.1 (26.0) | 27.7 (25.3) | 0.584 |
| Alkaline phosphatase (U/L) | 269.7 (190.8) | 128.0 (83.8) | 0.000* |
| ESR (mm/hr) | 12.7 (10.6) | 12.3 (9.9) | 0.689 |
| Hemoglobin (g/dl) | 13 (1.7) | 14 (1.3) | 0.000* |
| WBCs (×10$^3$/cmm) | 7.6 (2.3) | 7.3 (2.6) | 0.397 |
| Platelets (×10$^3$/cmm) | 320.0 (80.2) | 299.7 (63.6) | 0.127 |

hypoglycemia. In addition, only one patient had to interrupt fasting on two different days because of hypoglycemia.

In accordance with our study finding, the absence of severe hypoglycemia during Ramadan fasting in patients with T1DM has been previously reported [8–11]. Azar et al. reported that two patients had to interrupt fasting because of daytime hypoglycemia; however, the severities of these attacks were not mentioned [5]. Bin-Abbas reported that three patients, maintained on twice-daily "split-mixed" insulin regimen, had to interrupt their fast during Ramadan, once or twice because of hypoglycemia. On the other hand, none of the patients on insulin pump therapy had to interrupt their fasting as a result of hypoglycemia. Nevertheless, both groups did not suffer severe hypoglycemia [12].

The calculated mean rate of hypoglycemia events in our study was 2.1 events/ patient/ month. Moreover, the maximum number of hypoglycemia events per patient/month was nine events. These results are in accordance with the results of the Diabetes Control and Complications Trial (DCCT) that an intensively treated individual with T1DM can experience up to 10 hypoglycemia events per week [13].

Asymptomatic hypoglycemia is expected in patients with T1DM, who are attempting tight glycemic control. The results of the present study showed that 46% of the total number of the hypoglycemia events was documented symptomatic hypoglycemia events, while 54% were asymptomatic. In the DCCT, about one-third of all episodes of hypoglycemia in intensively treated patients were not accompanied by sufficient signs or symptoms [13].

Another finding of our study is that, no patient developed DKA or was admitted to a hospital with hyperglycemia. This was previously reported in fasting patients with T1DM during Ramadan in other studies [5, 8–12, 14].

The EPIDIAR study, contrary to our study, reported a statistically significant increase in the rate of severe hypoglycemia and severe hyperglycemia in patients with T1DM during Ramadan [1]. Based on its results, the ADA workgroup report about "Management of diabetes during Ramadan" considered patients with T1DM at very high risk if they fasted during Ramadan [4]. However, the reliability of these results has been debated as it depended only upon a survey with patients after the end of Ramadan, which would be subjected to recall bias [15]. Another limitation of the EPIDIAR study is that there was no differentiation between the group of patients with micro / macro vascular complications, and the group without those complications [1].

In our study, we found that there is an increase in both body weight and BMI at the end of Ramadan. However, this increase is mild and statistically not significant. This is in accordance with the study of Kadiri et al., which reported no statically significant change in BMI after Ramadan fasting in patients with T1DM [9]. Al Nakhi et al., in disagreement with our study, reported a decrease in BMI after Ramadan fasting [8, 16]. Another finding of our study is that the lipid profile revealed no statistically significant changes either in total cholesterol, LDL-C, HDL-C, Triglycerides or VLDL-C. Al Nakhi et al., in agreement with this study, reported no statistically significant change in the levels of total cholesterol after Ramadan fasting in patients with T1DM [8]. The glycemic profile of the study subjects did not change significantly at the end of Ramadan as measured by HbA1c [16] and fructosamine. The mild increase in both HbA1c & fructosamine can be explained by the high caloric diet during Ramadan and the decrease in physical activities & working hours [17]. This finding is in accord with previous studies addressing the same question [5, 8, 10, 11].

The present study had some limitations, including the lack of a control group as we do not have a standard regimen to be used as control and a small sample size. Evidence based comprehensive guideline for management of patients with T1DM, who want to fast Ramadan are needed. This guideline should include a unified protocol for insulin dose adjustment, suitable insulin regimens and setting criteria to allow fasting in those patients. Our study, combined with other studies describing different regimens, is a step in that direction.

The results of our study demonstrate the possibility of fasting during Ramadan, for patients with T1DM using intensified insulin therapy, with minimal risks. However, there is a need to be compared with another insulin regimens, previously used in this group of patients during Ramadan, in a large scale randomized controlled studies.

In conclusion, the main outcome of this study was that patients with T1DM are able to fast successfully during Ramadan safely. The study showed that using the intensified insulin therapy (glargine as basal insulin with insulin aspart before meals) in this group of patients when appropriately selected is safe under strict blood-glucose self-monitoring and close supervision.

Conflicts of interest The authors have no financial disclosures to declare and no conflicts of interest to report.

## References

1. Salti I, Bénard E, Detournay B, et al. A population- based study of diabetes and its characteristics during the fasting month of Ramadan in 13 countries: results of the Epidemiology of Diabetes and Ramadan 1422/2001 (EPIDIAR) study. Diabetes Care. 2004;27:2306–11.
2. Al-Arouj M, Assaad-Khalil S, Buse J, et al. Recommendations for management of diabetes during Ramadan. Update 2010. Diabetes Care. 2010;33:1895–902.
3. Benaji B, Mounib N, Roky R, et al. Diabetes and Ramadan: review of the literature. Diabetes Res Clin Pract. 2006;73:117–25.

4. Al-Arouj M, Bouguerra R, Buse J, et al. Recommendations for management of diabetes during Ramadan. Diabetes Care. 2005;28:2305–11.
5. Azar ST, Khairallah WG, Merheb MT, et al. Insulin therapy during Ramadan fast for patients with type 1 diabetes mellitus. J Med Liban. 2008;56:46.
6. Mucha GT, Merkel S, Thomas W, et al. Fasting and insulin glargine in individuals with type 1 diabetes. Diabetes Care. 2004;27:1209–10.
7. Workgroup on Hypoglycemia, American Diabetes Association. Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia. Diabetes Care. 2005;28:1245–9.
8. Al Nakhi A, Al Arouj M, Kandari A, et al. Multiple insulin injection during fasting Ramadan in IDDM patients. Second International Congress on Health and Ramadan 1997, Dec 1–3, Istanbul, Turkey: 77.
9. Kadiri A, Al-Nakhi A, El-Ghazali S, et al. Treatment of type 1 diabetes with insulin lispro during Ramadan. Diabetes Metab. 2001;27:482–6.
10. Kassem H, Zantout M, Azar S. Insulin therapy during Ramadan fast for Type 1 diabetes patients. J Endocrinol Invest. 2005;28:802–5.
11. Hawli Y, Zantout M, Azar S. Adjusting the basal insulin regimen of patients with type 1 diabetes mellitus receiving insulin pump therapy during the Ramadan fast: a case series in adolescents and adults. Curr Ther Res. 2009;70:29–34.
12. Bin-Abbas BS. Insulin pump therapy during Ramadan fasting in type 1 diabetic adolescents. Ann Saudi Med. 2008;28:305–6.
13. Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977–86.
14. Merheb M, Chaiban J, Salti I, et al. Insulin pump therapy in type 1 diabetic patients: experience at the American University of Beirut Medical Center. Int J Diabetes Metabol. 2008;16:57–61.
15. Reiter J, Wexler ID, Shehadeh N, et al. Type 1 diabetes and prolonged fasting. Diabet Med. 2007;24:436–9.
16. Chandalia HB, Bhargav A, Kataria V. Dietary pattern during Ramadan fasting and its effect on the metabolic control of diabetes. Practical Diabetes International 1987;4:287–90.
17. Ewis SA, Hamed M, Afifi N. Ramadan fasting and obese type 2 diabetics: dietary habits, practice and attitude. Diabetes. 2001;50:195–7.

LETTER TO EDITOR



# Are low dose pioglitazone combinations justifiable?

Rakesh M. Parikh

Int J Diabetes Dev Ctries. 2011; 31:239

Dear Sir,

Recently number of pharmaceutical companies in India have started marketing various oral hypoglycemic agent (OHA) combinations with low dose (7.5 mg) pioglitazone. All these combinations are based on very small data from two studies conducted in the Japanese population. Based on these studies, low dose pioglitazone is promoted to be having efficacy comparable to that of 15 mg with significantly less side effects. The first study was conducted in Japanese women who were recently diagnosed to be diabetic [1]. The researchers concluded that though the HbA1C reduction was more in group receiving 15 mg pioglitazone, there was no statistically significant difference between standard dose (15 mg) and low dose group. The study has numerous limitations in addition to being representative of only Japanese females. All the participants were newly diagnosed diabetic with HbA1C of 7.57% and 7.69% in low dose and standard dose groups respectively. The study did not have any control arm on placebo though a similar reduction in HbA1C can be expected even by lifestyle modification in newly diagnosed diabetics. Pioglitazone being an insulin sensitizers, is not expected to cause hypoglycemia and it is expected that it will not lower HbA1C further after reaching normoglycemia irrespective of the dose. HbA1C of 6.96% was achieved in low dose group after 6 months while that in standard dose arm was 7.0%. Any further lowering in HbA1C after 6.96% would be at the cost of hypoglycemia which is not seen with pioglitazone and hence expecting any further lowering by increasing dose of pioglitazone will be unrealistic.

The second study was done in 14 male and 16 female diabetic subjects. All the participants were on sulphonylurea monotherapy at baseline and were additionally given pioglitazone 7.5 mg per day. The authors demonstrated a significant reduction in HbA1C and significant improvement in serum levels of adiponectin without any significant rise in side effects [2]. The study had very small number of participants and did not have any control arm.

Another study with 80 and 79 subjects being randomized to 7.5 mg and 15 mg of pioglitazone has shown that only standard dose of 15 mg pioglitazone could show significant improvement in HbA1C [3]. A separate study looking at dose response relationship with insulin sensitivity and secretion, has shown no effect of 7.5 mg and 15 mg of pioglitazone on fasting plasma glucose and fasting plasma insulin [4].

Dose calculation is a part of early phase II studies and is the foundation of designing phase III and phase IV studies. The scarce data available from poorly designed studies should not be used to promote use of low dose pioglitazone in the treatment of diabetic subjects.

R. M. Parikh
drrakeshparikh@gmail.com

## References

1. Majima T, Komatsu Y, Doi K, Shigemoto M, Takagi C, Fukao A, et al. Safety and efficacy of low-dose pioglitazone (7.5 mg/day) vs. standard-dose pioglitazone (15 mg/day) in Japanese women with type 2 diabetes mellitus. Endocr J. 2006;53:325–30.
2. Aso Y, Hara K, Ozeki N, Yatsuka C, Nakano T, Matsumoto S, et al. Low-dose pioglitazone increases serum high molecular weight adiponectin and improves glycemic control in Japanese patients with poorly controlled type 2 diabetes. Diabetes Res Clin Pract. 2009;85:147–52.
3. Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL. Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study. The Pioglitazone 001 Study Group. Diabetes Care. 2000;23:1605–11.
4. Miyazaki Y, Matsuda M, DeFronzo RA. Dose-response effect of pioglitazone on insulin sensitivity and insulin secretion in type 2 diabetes. Diabetes Care. 2002;25:517–23.

LETTER TO THE EDITOR



# Pseudoainhum of toes in type 2 diabetes

K. V. S. Hari Kumar, Sumit Bhasker, P. K. Banga

Int J Diab Dev Ctries. 2011 ; 31 : 241

A 43 year old woman known case of type 2 diabetes for 12 years presented with claudication pain and shortening of toes of left leg of 1 year duration. The claudication distance decreased gradually from 3 km to 1 km with no history to suggest rest pain. The pain involved the foot with sparing of thigh and calf muscles. She also noticed gradual shriveling and shortening of toes belonging to left foot. She denied similar complaint in other extremities, exposure to cold or consumption of ergot alkaloids. Past history revealed poor glycemic control (A1c: 9.2%) and microvascular disease in the form of retinopathy and nephropathy. Left foot examination revealed feeble pulse, diminished sensation to pinprick with absent ankle jerk. Toes of left foot were at varying stages of autoamputation with distal ulceration of 2nd toe (Fig. 1a). Radiograph showed resorption of terminal phalanges and osteopenia (Fig. 1b). Doppler study revealed normal major arteries with poor visualization of digital arteries (Fig. 1c). Based on the presentation and findings, she was diagnosed to have Pseudoainhum of toes. Pseudoainhum or Morior is defined as the autoamputation of toes or fingers with shriveling seen in chronic diseases like diabetes [1]. Our patient had features of both macro and microvascular disease with normal major arteries on doppler examination. She was treated with insulin, atorvastatin, ramipril, clopidogrel, aspirin, cilostazole and pentoxifylline. Marginal improvement was observed in the severity of pain and claudication distance with therapy.

Ainhum or spontaneous dactylolysis is a disease described in dark skinned individual of autoamputation of small toes [2]. The classical disease is usually preceded by a constricting band or groove encircling the toes. Pseudoainhum is similar process secondary to an identifiable disease independent of skin color. Though the pathogenesis is unclear, various theories like genetic predisposition, angiodysplasia, hyperkeratosis, trauma and environmental factors are proposed for the same [3]. The course of pseudoainhum is characterized by hyperkeratotic skin, fissuring, digital degeneration, skeletal erosion culminating into autoamputation. The differential diagnosis includes localized scleroderma, trauma, frost bite and ergot poisoning. Ainhum and Pseudoainhum are graded into 4 stages according to the stage of pathology [4]. Our patient had all the stages affecting different toes simultaneously. The therapy differs according to the stage of involvement. Oral vitamin A and etretinate are used in early stages and surgical procedures like release of constricting band, skin grafting and amputation are required in advanced stages.

K. V. S. H. Kumar
hariendo@rediffmail.com

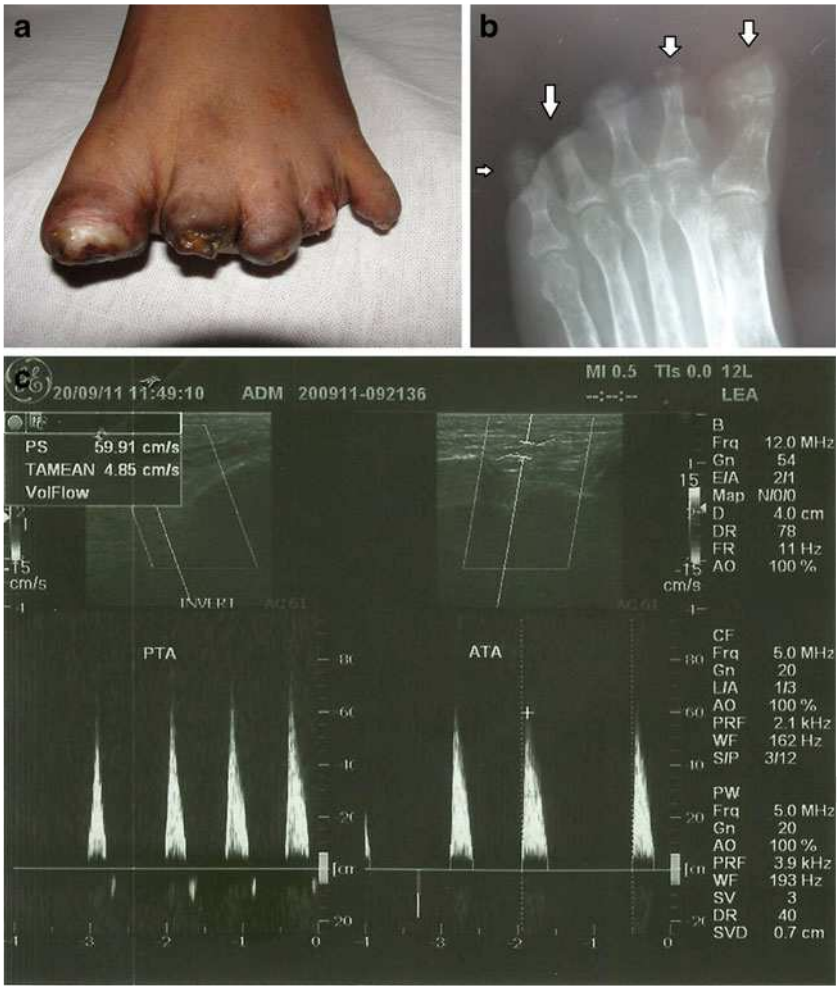



Fig. 1 a. Toes at varying stage of auto amputation with distal ulceration of second toe. b. Radiograph showing resorption of terminal phalanges and osteopenia. c. Doppler studies showing normal flow characteristic in major arteries with poor visualization of digital arteries

## References


1. Rashid RM, Cowan E, Abbasi SA, Brieva J, Alam M. Destructive deformation of the digits with auto-amputation: a review of pseudo-ainhum. J Eur Acad Derm Venereol. 2007;21:732–7.
2. Kerhisnik W, O'Donnell E, Wenig JA, McCarthy DJ. The surgical pathology of ainhum (dactylolysis spontanea). J Foot Surg. 1986;25:95–123.
3. Wollina U, Graefe T, Oelzner P, Hein G, Schreiber G. Pseudoainhum of all fingers associated with Reynolds' syndrome and breast cancer: report of a case and review of the literature. J Am Acad Dermatol. 2001;44:381–4.
4. Genakos JJ, Cocores JA, Terris A. Ainhum (dactylosis spontanea): report of a bilateral case and literature review. J Am Podiatr Med Assoc. 1986;76:676–80.

LETTER TO EDITOR



# Switching to three pre-meal injections of insulin glulisine from the basal-bolus insulin therapy improves glycemic control in a patient with type 2 diabetes who had anti-insulin antibody

Hidekatsu Yanai, Taro Yoshimi, Hidetaka Hamasaki

Int J Diab Dev Ctries. 2011; 31:240

Dear Sir,

Long-acting insulin analogs, the insulin glargine (glargine) and insulin detemir (detemir) induce a more prolonged, less peaked absorption profile compared with that of NPH insulin [1], and have been used in the basal-bolus insulin therapy for diabetic patients.

Here we report a type 2 diabetic patient who developed severe insulin resistance due to anti-insulin antibody after 2 years of the basal-bolus insulin therapy using insulin lispro (lispro) and detemir. Switching to three pre-meal injections of the newest rapid-acting insulin analog, insulin glulisine ameliorated hyperglycemia and decreased anti-insulin antibody level.

A 75-year-old man had been treated with the basal-bolus insulin therapy by five daily insulin injections: three injections of lispro before breakfast (10 units), lunch (6 units), and dinner (10 units) and two of detemir before breakfast (30 units) and at bedtime (50 units). His body weight was 73.4 kg and height 176.0 cm, BMI 23.7 kg/m$^2$. Plasma glucose (378 mg/dl), $HbA_{1c}$ [9.3% (NGSP value)],
and anti-insulin antibody ($^{125}$I-insulin binding rate, 35.9%; normal range, < 0.4%) levels was significantly elevated. Switching to the combination of glulisine and glargine brought down his blood glucose levels to 106–186 mg/dl by using 10, 8, and 8 units of glulisine before breakfast, lunch, and dinner, respectively, and 30 units of glargine at bedtime. Interestingly, further reduced dosing of glargine remarkably improved his glycemic control, and blood glucose levels were 88–180 mg/dl by using 8, 10, and 8 units of glulisine before breakfast, lunch, and dinner, respectively, and 10 units of glargine at bedtime. However, his anti-insulin antibody level was still high (37.5%). Three pre-meal injections of glulisine (14 units before each meal) and discontinuation of glargine use finally controlled blood glucose (151–195 mg/dl) and decreased anti-insulin antibody level ($^{125}$I-insulin binding rate, 25.1%).

Unlike other insulin analogs, glulisine allows for a viable drug product in the absence of hexamer-promoting zinc, which may provide immediate availability of glulisine at the injection site for absorption [2], while long-acting insulin analogs were designed to prolong absorption from subcutaneous tissue. The presence of insulin in a monomer form and the rapid absorption from the subcutaneous tissue may result in less anti-insulin antibody formation [3]. Therefore, switching to three pre-meal injections of glulisine from the basal-bolus insulin therapy may be effective in the treatment of patients with long-acting insulin analogs-mediated immunogenic insulin resistance.

H. Yanai
e-mail: dyanai@hospk.ncgm.go.jp

## References

1. Monami M, Marchionni N, Mannucci E. Long-acting insulin analogues vs. NPH human insulin in type 1 diabetes. A meta- analysis. Diabetes Obes Metab. 2009;11:372–8.
2. Becker RH, Frick AD. Clinical pharmacokinetics and pharmacodynamics of insulin glulisine. Clin Pharmacokinet. 2008;47:7–20.
3. Brange J, Owens DR, Kang S, Vølund A. Monomeric insulins and their experimental and clinical implications. Diabetes Care. 1990;13:923–54.

EDITORIAL



# Metabolic syndrome

K. M. Prasanna Kumar

Int J Diab Dev Ctries. 2011; 31:185–187

Metabolic syndrome (MS) is a constellation of individual risk factors of cardiovascular disease. The World Health Organization (WHO) was the first to publish an internationally accepted definition for metabolic syndrome in 1998, but the criteria that have received the most widespread acceptance and use in the United States are those established as guidelines in the ATP III (the third report of the National Cholesterol Education Program expert panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults).

The scientific evidence related to definition was reviewed and considered from several perspectives: (1) major clinical outcomes (2) metabolic components (3) pathogenesis (4) clinical criteria for diagnosis (5) risk for clinical outcomes and (6) therapeutic interventions [1]. Hence, there are many defini- tions and many criteria for diagnosis of metabolic syndrome like ATP III adult panel, WHO guidelines, IDF and AACE. There is no universal agreement on criteria for the diagnosis.

ATP III identified 6 components of the metabolic syndrome [1] that relate to CVD:

- Abdominal obesity
- Atherogenic dyslipidemia
- Raised blood pressure
- Insulin resistance and/or glucose intolerance
- Proinflammatory state
- Prothrombotic state

For the purposes of ATP III, metabolic syndrome is present when ≥3 of the following criteria are present: waist circumference >102 or >88 cm in men and women respectively; triglycerides ≥150 mg/dl; HDL cholesterol <40 or <50 mg/dl in men and women respectively; blood pressure ≥130/≥85 mmHg; and fasting blood glucose ≥110 mg/dl.

The WHO guidelines also viewed CVD as the primary outcome of the metabolic syndrome. In WHO guidelines, unlike the ATP III criteria, stipulate that insulin resistance is required for the diagnosis along with two other risk factors out of the following: high blood pressure, raised triglycerides, low HDL and increased BMI (or increased waist: hip ratio) and microalbuminuria. In addition, a higher blood pressure was required for the ATP III criteria. Like the ATP III criteria, the presence of type 2 diabetes does not exclude a diagnosis of metabolic syndrome. A potential disadvantage of the WHO criteria is that special testing of glucose status beyond routine clinical assessment may be required.

The AACE has proposed a third set of criteria for the insulin resistance syndrome [2]. These criteria appear to be a mixture of the ATP III and WHO criteria except that no defined number of risk factors is specified and the diagnosis is left to clinical judgment. When a person develops diabetes, the term insulin resistance syndrome no longer applies. Finding abnormal 2-hour glucose will improve prediction of type 2 diabetes.

International Diabetes Federation defines metabolic syndrome to include central obesity with 2 of the following 4 factors: raised triglycerides, low HDL cholesterol, and rised blood pressure raised fasting plasma glucose.

Thus the definition, criteria, pathogenesis and therapeutic intervention in the metabolic syndrome is controversial. By definition, syndrome is a group of symptoms that collectively indicate or characterize a disease, psychological disorder, or other abnormal condition. To recognize a syndrome a group of symptoms alone is not sufficient. The syndrome should have a common etiopathogenesis and a common pathway which leads to the syndrome. Do we have such a

K. M. Prasanna Kumar
dr.kmpk@gmail.com

unifying hypothesis in case of metabolic syndrome? To accept that metabolic syndrome is a risk for cardio vascular disease, the aggregate risk of metabolic syndrome should be more than the addition of individual risk factors. Each component of metabolic syndrome may have different cardiovascular risk, depending on the regional, ethnic, racial and geographical factors. In such a situation is it possible to have a universal definition and criteria for metabolic syndrome?

MS is a complex web of metabolic factors that are associated with a 2-fold risk of CVD and a 5-fold risk of diabetes. Individuals with MS have a 30%–40% probability of developing diabetes and/or CVD within 20 years, depending on the number of components present [2].

Metabolic syndrome is only a syndrome with multiple risk factors for future cardiovascular disease and diabetes mellitus and should not be misdiagnosed as a disease. How often do we dare to write in a case record of a person diagnosis of metabolic syndrome?

The association between MS and cancer mortality is yet to be proved. Research to focus on pre-cancer risk factors in metabolic syndrome is necessary. Studies have shown that MS is associated with an increased risk of all-cause cancer mortality in men. In a study by Jason R et al. [3] of 33,230 men aged 20–88 years, at baseline 28% of all the participants had MS. During an average of 14 years follow-up, there were a total of 685 deaths due to cancer. MS at baseline was associated with a 56% greater age-adjusted risk in cancer mortality. A relationship appears to exist between the number of MS components and the higher risk of all-cause cancer mortality and risk is escalated with the presence of three or more components. Thus MS and its implications extend beyond cardiovascular risk, like future risk of diabetes and may be cancer.

Metabolic syndrome and cardiovascular risk in South Asians is also heightened by their higher body fat, truncal subcutaneous fat, intra-abdominal fat, and ectopic fat deposition (e.g. liver fat). Further, cardiovascular risk cluster manifests at a lower level of adiposity and abdominal obesity. The cutoffs of body mass index and waist circumference for defining obesity and abdominal obesity, respectively, have been lowered and the definition of the metabolic syndrome has been revised for the Asian Indians in a recent consensus statement, so that physicians could intervene early with lifestyle management [4].

In recently held National Obesity and Metabolic Syndrome Summit in New Delhi, India, a consensus on diagnostic cutoffs of obesity (BMI, waist circumference) and optimal definition of the metabolic syndrome for Asian Indians was reached. Recommendations were as follows: BMI (kg/m2) cutoffs, normal, 18–22., overweight, 23–24., and obesity, ≥25; waist circumference cutoffs, ≥90 cm (male) and ≥80 cm (females) [5].

Epidemiologists in India and international agencies such as the World Health Organization (WHO) have been sounding an alarm on the rapidly rising burden of CVD for the past decade. The prevalence of MS among South Asians is higher than in other Asians and Europeans [6].In India, CVD is projected to be the largest cause of death and disability by 2020, with 2.6 million Indians predicted to die due to coronary heart disease, which constitutes 54.1% of all CVD deaths [7].

There is high prevalence of metabolic syndrome in the urban Indian population .In a study involving 19 973 subjects across India, prevalence of CVD risk factors among individuals aged 20–69 years,the overall prevalence of most risk factors was high, with 50.9% of men and 51.9% of women being overweight, central obesity was observed among 30.9% of men and 32.8% of women, and 40.2% of men and 14.9% of women reported current tobacco use. The study demonstrated very high levels of CVD risk factors among a relatively young population from 10 industrial settings across India [7].

There are many studies from Asia in general and India in particular. There are many studies on the urban prevalence of metabolic syndrome [8] and a few on the rural prevalence. Some studies [9, 10] compared Indian rural population with urban for prevalence of metabolic syndrome. Most of these studies have used one criteria for diagnosis (NCEP, ATP III, IDF, AACE or WHO criteria).

The study by Puneet Gowda et al. [11] is unique in that the same rural population of south India in Karnataka was studied both by modified National Cholesterol Education Programme-Adult Treatment Panel III definition and International Diabetes Federation criteria for the diagnosis of Metabolic Syndrome to evaluate the concordance or discordance of the diagnosis. There was significant discordance in the prevalence of metabolic syndrome using two criteria. The authors are of the opinion that the discrepancy could be the higher WC cut-off in the modified NCEP ATP III criteria, which excludes subjects considered obese as per IDF criteria. The differences in combinations of the individual parameters in the two criteria could also contribute to the discrepancy.

Nearly 70% of Indian population lives in the rural areas. The difference in health care, life style and economics are very wide between rural and urban India. The lower prevalence of obesity, hypertension, dysglycemia and dyslipidemia in rural areas is obviously due to dietary and lifestyle differences between rural and urban India. The psychological stress which is common in urban living cannot be quantified easily, though it is well accepted as one of the risk factors for cardiovascular disease. It is surprising that smoking which is a significant risk factor is not included in the metabolic syndrome. Increasing urbanization and internal migration of people from rural to urban areas contribute

to increasing prevalence of metabolic syndrome which is a pre-diabetic and cardiac risk factor.

Is the combined cardiovascular risk of each component of metabolic syndrome greater than the CV risk of metabolic syndrome? In fact, the individual components of the MS have been recognized and treated effectively by physicians for many decades. Life style modifications and pharmacotherapy wherever indicated will reduce the burden of metabolic syndrome.

Cardio-metabolic risk is high in South Asians, starting at an early age. Increasing awareness of the cluster of risk factors and how to prevent them should be emphasized in population-based prevention strategies in South Asian countries, primarily focusing on children and adolescent.

## References


1. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Final report. Circulation 2002;106:3143–420.
2. Einhorn D, Reaven GM, Cobin RH, et al. American College of Endocrinology position statement on the insulin resistance syn- drome. Endocr Pract. 2003;9:237–52.
3. Jaggers JR, Sui X, Hooker SP, et al. Metabolic syndrome and risk of cancer mortality in men. Eur J Cancer. 2009;45:1831–8.
4. Misra A, Khurana L. The metabolic syndrome in south Asians: epidemiology, determinants, and prevention. Metab Syndr Relat Disord. 2009;7:497–514.
5. Misra A, Chowbey P, Makkar BM, et al. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. JAPI. 2009;57:163–70.
6. Enas EA, Mohan V, Deepa M, Farooq S, Pazhoor S, Chennikkara H. The metabolic syndrome and dyslipidemia among Asian Indians: a population with high rates of diabetes and premature coronary artery disease. J Cardiometab Syndr. 2007;2:267–75.
7. Reddy KS, Prabhakaran D, Chaturvedi V, Jeemon P, Thankappan KR, Ramakrishnan L, Mohan BV, Pandav CS, Ahmed FU, Joshi PP, Meera R, Amin RB, Ahuja RC, Das MS, Jaison TM. Methods for establishing a surveillance system for cardiovascular diseases in Indian industrial populations. Bull World Health Organ. 2006;84:461–9.
8. Gupta R, Deedwania PC, Gupta A, Rastogi S, Panwar RB, Kothari K. Prevalence of metabolic syndrome in an Indian urban population. Int J Cardiol. 2004;97:257–61.
9. Misra R, Misra A, Kamalamma N, Naval K, Vikram NK, Gupta S, Sharma S, Patel T, Balagopal P. Difference in prevalence of diabetes, obesity, metabolic syndrome and associated cardiovascular risk factors in a rural area of Tamil Nadu and an urban area of Delhi. Int J Diab Dev Ctries. 2011;31:82–90.
10. Mahadik SR, Deo SS, Mehtalia SD. Increased prevalence of metabolic syndrome in non-obese Asian Indian-an urban–rural comparison. Metab Syndr Relat Disord. 2007;5:142–52.
11. Kempegowda P, et al. Prevalence of Metabolic Syndrome in Rural India - A disparity in definitions. Int J Diab Dev Ctries. doi:10.1007/s13410-011-0046-y.

ORIGINAL ARTICLE



# Prevalence of the metabolic syndrome in rural India—a disparity in definitions

Punith Kempegowda, Sara Rani Marcus, Priyank Solanki, Rashmi S. Reddy, Nandini D. R.,Mala Dharmalingam

Int J Diab Dev Ctries. 2011 ; 31 : 188-194

Abstract Although the metabolic syndrome is a known predictor of coronary heart disease and type-2 diabetes mellitus, it has no agreed definition. The concordance of its various definitions has been studied for a limited number of populations and there are few studies on the rural populations in India. The present study was done to determine the prevalence of metabolic syndrome in a rural population of South India and to evaluate the concordance of the modified National Cholesterol Education Programme-Adult Treatment Panel III definition and Interna- tional Diabetes Federation definition for the diagnosis of Metabolic Syndrome. Anthropometric and biochemical param- eters (fasting blood glucose and lipid profile) and blood pressure were measured using standard procedures. The prevalence of metabolic syndrome was calculated using the two sets of criteria and compared for their concordance. Descriptive statistics was used to analyze age and risk factors of metabolic syndrome. The chi-square test was applied to compare the prevalence of metabolic syndrome obtained from the two criteria (significant if $p<0.05$). An inter-rater reliability analysis using the kappa statistic was performed to determine consistency between the two sets of criteria in diagnosing the metabolic syndrome. The modified National Cholesterol Education Programme-Adult Treatment Panel III definition and International Diabetes Federation definition for metabolic syndrome identified overall age-adjusted prevalence of 17.8% and 20.5% respectively, which were not significantly different. Kappa statistics revealed only moderate agreement of 0.44 between the two sets of criteria. The impact of economic development and preponderance of genetic factors is increasing the prevalence of metabolic syndrome in rural India. It is important to determine which definition of the metabolic syndrome best predicts coronary heart disease and type 2 diabetes in this population in order to formulate effective public health policy.

Keywords Metabolic syndrome · Rural India · Prevalence · NCEP ATP III · IDF

P. Kempegowda
drpunith@gmail.com

## Introduction

The metabolic syndrome (MS) is a cluster of metabolic risk factors including central obesity, dyslipidemia, insulin resistance or glucose intolerance, hypertension and pro-inflammatory state. Insulin resistance, inflammation and central obesity have each been proposed as its primary cause, and indeed, this may vary between individuals. MS is associated with an increased risk of coronary heart disease (CHD) and type-2 diabetes mellitus (DM) [1]. Asian Indians are a population with high cardiometabolic risk which starts at an earlier age [2]. In India in the year 2000, 31.7% of all deaths reported were due to cardiovascular disease; 52% of these cardiovascular deaths occurred below the age of 70, compared with 23% in countries with established market economies [3]. Epidemiological data from rural South India revealed that in the year 2003–4,

32% of all deaths were due to circulatory system diseases [4]. Predictions of an increase in the prevalence of CHD in the rural and lower economic section on par with the other sections of the society irrespective of the age and gender have been reported [5]. The early diagnosis of MS may help in reducing the associated morbidity and mortality. While several studies on the prevalence of MS worldwide have been carried out, there are limited studies on its epidemiology especially in the rural population of India [6, 7].

Several organizations have attempted to develop a definition for MS. In 1998, the World Health Organization (WHO) proposed the first definition for metabolic syndrome. This was followed by European Group for the Study of Insulin Resistance (EGIR) and the National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) [8–10]. The NCEP ATP III was later modified in 2005 [11]. Recently, the International Diabetes Federation (IDF) proposed another definition which provides South Asian specific cut-off points for central obesity [12]. This diversity in definitions and the influence of environmental and ethnic variation amongst the different populations has led to different estimates of the prevalence of MS [6, 13].

Although numerous studies comparing the modified NCEP ATP III and IDF criteria have been carried out worldwide, similar studies on the Indian rural population are few [14–16]. This study was conducted to estimate the prevalence of MS and its risk factors in a rural lower socio-economic population of South India using IDF and modified ATP III criteria and to evaluate the concordance between the two criteria in diagnosing MS.

## Materials and methodology

This community-based, cross-sectional, randomized study was conducted in 2008 in Santhekallahalli sub center, Kaiwara Primary Health Center, Karnataka, India. Ethical clearance was obtained from the ethical review board of the institution. Informed consent was obtained from all the participants prior to their inclusion into the study.

The study population of 495 subjects comprised 304 men and 191 women, evenly spread over the age range 20–98, were from five villages around Santhekallahalli having a total population of about 8000. The number of subjects from each village was proportionate to the population of the village. The subjects from all the five villages belonged to the lower socio-economic group with similar life-styles and food habits, being agriculturalists by profession. Only subjects aged above 20 years that had lived in the study area for at least 6 months were included in the study.

Detailed information regarding demographic, socio-economic, behavioral and health status was collected from each study subject. Anthropometric measurements (waist circumference (WC), weight and height) and blood pressure were obtained using standard procedures. Blood samples were collected from each participant after an overnight fast. They were analyzed for fasting glucose levels, serum total cholesterol and serum triglycerides (enzymatic kit methods: Vital Diagnostics Pvt. Ltd, Mumbai); and serum High Density Lipoprotein (HDL-C) (Kit method: Bayer Diagnostics, Baroda) using a semi-auto-analyzer.

MS was defined based on the IDF [12] and modified NCEP ATP III criteria [11]. The components of these two definitions are given in Table 1.

## Statistical analysis

The statistical analysis was done using Statistical Package for Social Survey (SPSS) version 16. Descriptive statistics was used to analyze age and risk factors of metabolic syndrome. The age and gender-wise prevalence of MS was calculated

Table 1 Criteria for the definitions of metabolic syndrome used in the study

| Characteristic | Modified NCEP-ATP III criteria<br>Presence of any three of the following | IDF criteria<br>Presence of central obesity plus any two of the following |
|---|---|---|
| Adiposity | Waist circumference >102 cm for men and >88 cm for women | Waist circumference in Asians ≥90 cm for men and ≥80 cm for women |
| Impaired glucose regulation | Raised fasting plasma glucose (FPG) ≥110 mg/dL (6.1 mmol/L), or previously diagnosed type 2 diabetes | Raised fasting plasma glucose (FPG) ≥100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes |
| Raised blood pressure | Systolic BP ≥130 or diastolic BP ≥85 mm Hg, or treatment of previously diagnosed hypertension | Systolic BP ≥130 or diastolic BP ≥85 mm Hg, or treatment of previously diagnosed hypertension |
| Dyslipidemia | Raised TG level: ≥150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality; or Reduced HDL cholesterol: <40 mg/dL (1.03 mmol/L) in males and <50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality | Raised TG level: ≥150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality; or Reduced HDL cholesterol: <40 mg/dL (1.03 mmol/L) in males and <50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality |

using 10 year intervals. The chi-square test was applied to compare the prevalence of MS obtained from IDF criteria and modified ATP III criteria (significant, if $p<0.05$). An inter-rater reliability analysis using the Kappa (κ) statistic was performed to determine consistency among the two criteria in diagnosing MS. The level of agreement is considered poor with $\kappa \leq 0.20$, fair with $\kappa = 0.21$ to 0.40, moderate with $\kappa = 0.41$ to 0.60, substantial with $\kappa = 0.61$ to 0.80, and very good with $\kappa > 0.80$ [17]. To enable comparison with similar studies across the world, the prevalence of MS was age-adjusted by direct standardization method with 10-year bands using World standard population.

## Results

The ratio of males: females were 1.59:1. The mean ages of men and women were 47.12 ± 17.15 and 44.00 ± 15.19 years, respectively.

The BMI, WC, blood pressure and the various biochemical parameters assessed in the subjects are given in Table 2. The mean BMI of the study population was 21.36 ± 4.53 kg/m$^2$; there was no statistically significant gender difference. The mean WC was higher in the case of men when compared to women but within the cut-off points by any of the criteria, including the South Asian-specific definition. The prevalence of the risk factors of MS for each gender is given in Table 3. Central obesity was present in 9.21% of men and 19.37% of women by the modified NCEP ATP III criteria; this value increased to 36.84% in men and 34.55% in women by IDF criteria. This difference was mainly due to the IDF definition having South Asian specific criteria for central obesity. Hypertriglyceridemia was observed in 24.24% of the total population with a higher percent of men (26.97%) than women (19.89%) exhibiting hypertriglyceridemia, which was statistically insignificant. The HDL-C level was signif- icantly low in this population with women having lower HDL-C levels (93.2%) than men (76.97%) ($p<0.001$).

There was a significantly higher percentage of women than men with hypertension (39.8 v 34.1%; $p=0.036$). The prevalence of hyperglycemia (impaired fasting glucose and DM) was 29.29% in the total population with the prevalence in men being 29.93% and women 28.27%. However, the gender-specific difference was not observed in the prevalence of diabetes mellitus.

The age-adjusted prevalence of MS was 20.52% (crude prevalence 21.81%) as per the IDF criteria and 17.76% (crude prevalence 19.59%) according to the modified NCEP ATP III criteria. Interestingly, although the prevalence of MS in the total population using IDF criteria and modified NCEP ATP III criteria were similar, the affected population was different. While a higher percentage of men were found to have MS according to IDF criteria, the prevalence was higher in women using modified NCEP ATP III criteria, though not statistically significant. The kappa statistic revealed only a moderate agreement between the two criteria in diagnosing MS in the study population [total κ-0.44 (95% CI- 0.34–0.54), men κ-0.41 (95% CI- 0.28–0.53), women κ-0.49 (95% CI- 0.34–0.64)]. A Venn diagram showing the agreement and disparity between the two criteria among men and women is given in Fig. 1.

## Discussion

The prevalence of MS is frequently used in epidemiological studies to quantify cardiovascular risk in the population. However, depending on the definition of MS, there is marked variability in its prevalence among different ethnic groups [6, 13]. The socio-economic status, lifestyles and food habit variations also affect differences in urban and rural populations within an ethnic group leading to diversity in the prevalence of MS [6, 7, 18].

The distribution of the subjects in different age groups was uniform except in the higher ages, that is, above 70 years. A growing increase in the prevalence of MS was observed using both criteria; however, the maximum number was observed in the middle age group of 40–69 years. Similar observations have been reported both in India and globally [7, 18, 19].

Table 2 Anthropometric and Biochemical characteristics of the study population (N=495)

| Risk factors | Total (N=495) Mean± SD | Male (N=304) Mean± SD | Female (N=191) Mean± SD |
|---|---|---|---|
| Waist circumference (cm) | 80.72±13.41 | 84.38±12.38 | 74.91±12.95 |
| Body Mass Index (kg/m$^2$) | 21.36±4.53 | 21.17±4.25 | 21.66±4.94 |
| Systolic blood pressure (mm Hg) | 118.11±12.59 | 117.41±11.77 | 119.21±13.78 |
| Diastolic blood pressure (mm Hg) | 79.73±6.73 | 79.49±6.52 | 80.10±7.05 |
| Fasting blood glucose level (mg/dL) | 102.18±44.37 | 104.85±49.48 | 97.92±34.41 |
| Total cholesterol (mg/dL) | 172.66±35.35 | 169.28±34.88 | 178.05±35.52 |
| Triglycerides (mg/dL) | 139.15±46.01 | 141.17±48.23 | 135.94±42.17 |
| HDL-cholesterol (mg/dL) | 34.53±7.07 | 33.86±6.98 | 35.610±7.11 |

Table 3 Gender-wise comparison of the prevalence of risk factors of MS by the two criteria

| Risk factors | IDF | | | NCEP ATP III | | |
|---|---|---|---|---|---|---|
| | Total | Male | Female | Total | Male | Female |
| Central obesity | 178 (35.96) | 112 (36.84) | 66 (34.55) | 65 (13.13)* | 28 (9.21) | 37 (19.37) |
| Diabetes mellitus | 145 (29.29) | 91 (29.93) | 54 (28.27) | 121 (24.44) | 80 (26.32) | 41 (21.47) |
| Hypertriglyceridemia | 120 (24.24) | 82 (26.97) | 38 (19.89) | 120 (24.24) | 82 (26.97) | 38 (19.89) |
| Low HDL-cholesterol | 412 (83.23) | 234 (76.97)* | 178 (93.2) | 412 (83.23) | 234 (76.97) | 178 (93.2) |
| High blood pressure | 169 (34.14) | 93 (30.59)** | 76 (39.79) | 169 (34.14) | 93 (30.59) | 76 (39.79) |
| Metabolic syndrome[a] | 108 (21.81) | 67 (22.03) | 41 (21.46) | 97 (19.59) | 55 (18.09) | 42 (21.99) |

The numbers in parenthesis represent the %

*$p<0.001$, **$p<0.05$

[a] The overall age-adjusted prevalence of MS in the study population as per IDF criteria and modified NCEP-ATP III criteria is 20.52% and 17.76%, respectively

Prabhakaran et al. [7] reported a higher prevalence of MS in women compared to men in North India. However, the DECODE study reported a higher prevalence of MS among European men [20]. The present study did not find any statistically significant difference in the prevalence of MS between the men and women (Table 3). This is similar to the results seen in the Nigerian population in a study done by Adegoke et al., although the prevalence was much lower than in our study [21].

The prevalence of individual risk factors of MS was higher in the present study when compared to other similar studies across India [6, 18] and worldwide, [19] indicating the growing influence of modernization on the rural areas. Vaughan et al. reported an inverse association between the level of physical activity and MS with BMI as the mediating factor. They observed that physical activity in adults, particularly in women, could reduce the risk of MS and associated vascular diseases [22]. A recent multi-center study reports a measurable reduction in CHD risk factors with increased physical activity in overweight or obese, type 2 diabetic individuals [23]. Misra and Khurana reviewed the epidemiology of MS among South Asians and observed a high prevalence of MS and associated cardiovascular risk factors in all sectors of the society, including the rural community consequent to increasing mechanization [24].

The prevalence of central obesity according to modified NCEP ATP III criteria was significantly lower than IDF criteria ($p<0.0001$) in the present study. More subjects were considered normal when the modified NCEP ATP III criterion was applied. Since obesity is a major determinant of cardiovascular diseases, this may delay the interventions necessary to reduce the cardiovascular morbidity and mortality. Studies have shown that Asian Indians develop metabolic abnormalities at a lower BMI and WC than other groups [25]. Most of the cardiovascular risk factors manifest at a lower level of adiposity and abdominal obesity in South Asians as compared to Caucasians [24]. A major difference in the modified NCEP ATP III and IDF definitions for MS is the cut-off point for WC. Thus, the higher cut-off for WC in the modified NCEP ATP III criteria (>102 cm for men and >88 cm for women) in comparison to the IDF criteria for South Asians (>90 cm in men and >80 cm in women) decreases the predictive ability

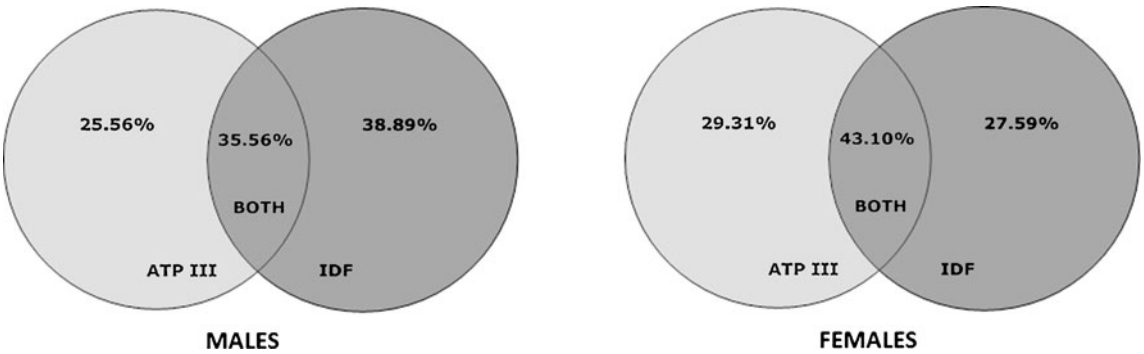



Fig. 1 Venn diagram showing the agreement and disparity in the diagnosis of metabolic syndrome using NCEP-ATP III and IDF criteria among the 90 males and 58 females who qualified for the diagnosis of metabolic syndrome by at least one of the definitions.

for MS by modified NCEP ATP III criteria among Asian Indians.

Reports from other Asian countries such as China and Japan report a low prevalence of dyslipidemia [26, 27]. Similar prevalence rates were reported from the African population by Ntandou et al. [28]. In our study, low HDL-C level (83.23%) was the most common abnormality causing dyslipidemia. Basit and Shera reported low HDL-C levels in 68–81% of the Pakistani population [29]. The predominance of atherogenic dyslipidemia, glucose intolerance, thrombotic tendency, subclinical inflammation, and endothelial dysfunction among Asians in comparison to Caucasians has been reported [24]. Low HDL-C levels could partly explain the disparity between various races. In our study, 93.2% of the women had low HDL-C levels compared to men, which is contrary to the present understanding. Most of the women in our study were in the reproductive age group wherein the protective effects of estrogen and progesterone are known to increase the HDL-C levels. This suggests that the percentage may be in fact higher or the protective effect is nullified in MS.

There was no statistically significant difference between the prevalence of MS according to the two definitions of MS in our study. Chow et al. reported a prevalence of 32.5% and 23.9% among men and women in rural Andhra Pradesh, India, using the modified NCEP ATP III criteria [30]. Mahadik et al. reported a prevalence of MS of 20.6% in their study of rural Indian population [18]. Rahim et al. studied the rural Bangladesh population for the prevalence of MS using modified NCEP ATP III and IDF criteria, and found a prevalence of 20.7% and 11.2% respectively [31]. Deepa et al. identified MS in 25.8% of the subjects by IDF criteria as against 18.3% applying NCEP ATP III criteria (2001) in the urban population of Chennai, India [6]. In an urban population of Pakistan, the prevalence of MS according to the IDF definition and modified NCEP ATP III criteria was 34.8% and 49% respectively [32]. In a recent study in Chandigarh (North India), Mangat et al. found a higher prevalence of MS (51.8% versus 41.2%) in the urban population in comparison to the rural counterparts (41.3% versus 34.9%) by both IDF and NCEP ATP III definitions [33]. The frequency of MS was higher among women than men by both criteria. The comparable prevalence of MS in the rural population with their urban counterparts may be due to the marked shift in lifestyle and nutrition of the rural population leading to the increased risk for CHD [24].

The concordance between the NCEP ATP III criteria and IDF criteria in diagnosing MS has been shown to vary from moderate to excellent agreement in various studies worldwide. While the Mexican study reported a high concordance (0.87) between the two criteria in diagnosing MS, Deepa et al. reported a concordance of 0.58 for the urban population of Chennai [6, 14]. Recent studies in Turkey and in the Republic of Seychelles reported a high concordance between the two criteria [15, 16]. In the present study, the concordance of subjects with MS according to modified NCEP ATP III criteria in comparison to IDF was moderate (0.44) with only 62 subjects diagnosed as having MS by both criteria. Individual consideration of the two criteria was unable to identify all the cases of MS from the study population. While IDF criteria recognized 108 subjects to have MS, 46 of these subjects were termed normal by the modified NCEP ATP III criteria. Similarly, of the 105 MS positive subjects according to modified NCEP ATP III criteria, 43 were normal according to IDF criteria. The rationale behind the discrepancy could be the higher WC cut-off in the modified NCEP ATP III criteria, which excludes subjects considered obese as per IDF criteria. The differences in combinations of the individual parameters in the two criteria could also contribute to the discrepancy. The superiority of one criterion over the other in the prediction of MS in the present study could not be established. Although the IDF criterion has been adjusted and adapted for the Asian population, it could not identify many of the cases in the community. The present definitions will overlook several cases of MS. In this study, although low HDL-cholesterol, hypertension and hypertriglyceridemia may be considered to identify MS; further investigations would be necessary to provide suitable criteria to define MS in this population. There is a need to develop more robust and applicable criteria which will increase the sensitivity and specificity of the parameters used to diagnose MS in different populations, especially Asian Indians.

## Conclusion

Comparison of the present findings with those reported by others suggests that the prevalence of MS in the rural population of India is increasing. This may be due to the impact of rapid lifestyle and nutritional transitions. These changes together with genetic factors in this population increase the risk for CHD and DM at an earlier age. In addition, the rural population lacks awareness of the risk factors for CHD and DM and have low accessibility to proper health care facilities. Early diagnosis of MS could help in timely intervention and reduction of the associated morbidity and mortality. To this end, it is important to use the definition of MS that best predicts the future incidence of CHD and DM. This study also highlights the inability of the current definitions to identify all the cases of MS in this rural population and hence there is a necessity for developing suitable region-based, specific cost-effective criteria to identify the affected population.

## References


1. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143–421.
2. Reddy KS, Yusuf S. Emerging epidemic of cardiovascular disease in developing countries. Circulation. 1998;97:596–601.
3. Ghaffar A, Reddy KS, Singhi M. Burden of non-communicable diseases in South Asia. BMJ. 2004;328:807–10.
4. Joshi R, Cardona M, Iyengar S, Sukumar A, Raju CR, Raju KR, et al. Chronic diseases now a leading cause of death in rural India— mortality data from Andhra Pradesh Health Initiative. Int J Epidemiol. 2006;35:1522–9.
5. World health Organization. NCMH Background papers-Disease burden in India Estimations and causal analysis. New Delhi (India), Ministry of Health and family Welfare, 2005. Available from http://www.whoindia.org/LinkFiles/Commision_on_Macroeconomic_and_Health_Bg_P2_Burden_of_Disease_Estimations_and_Casual_analysis.pdf.
6. Deepa M, Farooq S, Datta M, Deepa R, Mohan V. Prevalence of metabolic syndrome using WHO, ATPIII and IDF definitions in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-34). Diabetes Metab Res Rev. 2007;23:127–34.
7. Prabhakaran D, Chaturvedi V, Shah P, Manhapra A, Jeemon P, Shah B, et al. Differences in the prevalence of metabolic syndrome in urban and rural India: a problem of urbanization. Chronic Illn. 2007;3:8–19.
8. World Health Organization: Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva, World Health Organization, 1999. Available from http://www.staff.ncl.ac.uk/philip.home/who_dmg.pdf.
9. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med. 1999;16:442–3.
10. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001;285:2486–97.
11. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation. 2005;112:2735–52.
12. Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. Lancet. 2005;366:1059–62.
13. Lee CM, Huxley RR, Woodward M, Zimmet P, Shaw J, Cho NH, et al. Detect-2 collaboration: Comparisons of metabolic syndrome definitions in four populations of the Asia-Pacific region. Metab Syndr Relat Disord. 2008;6:37–46.
14. Guerrero-Romero F, Rodríguez-Morán M. Concordance between the 2005 International Diabetes Federation definition for diagnosing metabolic syndrome with the National Cholesterol Education Program Adult Treatment Panel III and the World Health Organization definitions. Diabetes Care. 2005;28:2588–9.
15. Can AS, Bersot TP. Analysis of agreement among definitions of metabolic syndrome in nondiabetic Turkish adults: a methodological study. BMC Publ Health. 2007;19:353.
16. Kelliny C, William J, Riesen W, Bovet P. Metabolic syndrome according to different definitions in a rapidly developing country of the African region. Cardiovasc Diabetol. 2008;7:27.
17. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–74.
18. Mahadik SR, Deo SS, Mehtalia SD. Increased prevalence of metabolic syndrome in non-obese Asian Indian—an urban-rural comparison. Metab Syndr Relat Disord. 2007;5:142–52.
19. Qiao Q, DECODE Study Group. Comparison of different definitions of the metabolic syndrome in relation to cardiovascular mortality in European men and women. Diabetologia. 2006;49:2837–46.
20. Balkau B. The DECODE study. Diabetes epidemiology: collab- orative analysis of diagnostic criteria in Europe. Diabetes Metab. 2000;26:282–6.
21. Adegoke OA, Adedoyin RA, Balogun MO, Adebayo RA, Bisiriyu LA, Salawu AA. Prevalence of metabolic syndrome in a rural community in Nigeria. Metab Syndr Relat Disord. 2010;8:59–62.
22. Vaughan C, Schoo A, Janus ED, Philpot B, Davis-Lameloise N, Lo SK, et al. The association of levels of physical activity with metabolic syndrome in rural Australian adults. BMC Publ Health. 2009;9:273.
23. Redmon JB, Bertoni AG, Connelly S, Fenney PA, Glasser S, Glick H, et al. Effect of the Look AHEAD Study intervention on medication use and related cost to treat cardiovascular disease risk factors in individuals with type 2 diabetes. Diabetes Care. 2010;33:1153–8.
24. Misra A, Khurana L. The metabolic syndrome in South Asians: epidemiology, determinants, and prevention. Metab Syndr Relat Disord. 2009;7:497–514.
25. Enas EA, Mohan V, Deepa M, Farooq S, Pazhoor S, Chennikkara H. The metabolic syndrome and dyslipidemia among Asian Indians: a population with high rates of diabetes and premature coronary artery disease. J Cardiometab Syndr. 2007;2:267–75.
26. Morimoto A, Nishimura R, Suzuki N, Matsudaira T, Taki K, Tsujino D, et al. Low prevalence of metabolic syndrome and its components in rural Japan. Tohoku J Exp Med. 2008;216:69–75.
27. Zuo H, Shi Z, Hu X, Wu M, Guo Z, Hussain A. Prevalence of metabolic syndrome and factors associated with its components in Chinese adults. Metabolism. 2009;58:1102–8.
28. Ntandou G, Delisle H, Agueh V, Fayomi B. Abdominal obesity explains the positive rural-urban gradient in the prevalence of the metabolic syndrome in Benin, West Africa. Nutr Res. 2009;29:180–9.
29. Basit A, Shera AS. Prevalence of metabolic syndrome in Pakistan. Metab Syndr Relat Disord. 2008;6:171–5.
30. Chow CK, Naidu S, Raju K, Raju R, Joshi R, Sullivan D, et al. Significant lipid, adiposity and metabolic abnormalities amongst 4535 Indians from a developing region of rural Andhra Pradesh. Atherosclerosis. 2008;196:943–52.
31. Rahim MA, Khan AK, Sayeed MA, Akhtar B, Nahar Q, Ali SMK, et al. Metabolic syndrome in rural Bangladesh: compar- ison of newly proposed IDF, modified ATP III and WHO criteria and their agreements. Diabetes Metab Syndr Clin Res Rev. 2007;1:251–7.
32. Hydrie MZ, Shera AS, Fawwad A, Basit A, Hussain A. Prevalence of metabolic syndrome in urban Pakistan (Karachi): comparison of newly proposed International Diabetes Federation and modified Adult Treatment Panel III criteria. Metab Syndr Relat Disord. 2009;7:119–24.
33. Mangat C, Goel NK, Walia DK, Agarwal N, Sharma MK, Kaur J, et al. Metabolic syndrome: a challenging health issue in highly urbanized Union territory of North India. Diabetol Metab Syndr. 2010;2:19.

ORIGINAL ARTICLE



# Relationship between limited joint mobility syndrome and duration, metabolic control, complications of diabetes as well as effects of the syndrome on quality of life

Ebru Umay, Alev Cevıkol, Ozlem Avluk, Ece Unlu, Aytul Cakcı

Int J Diab Dev Ctries. 2011 ; 31 : 207-216

Abstract Aim of this study was to examine relationship between Limited Joint Mobility syndrome (LJM) in patients with type-1 and type-2 diabetes and duration, metabolic control and other complications of diabetes, and to assess effects of the syndrome on hand functions and quality of life. Demographic characteristics, and micro-macrovascular and hand complications were recorded. Level of fasting blood sugar and HbA1c, superficial, pain, temperature, vibration and cortical senses, and deep tendon reflexes were measured. Hand dexterity was evaluated with nine-hole peg test. Grip and pinch strengths were recorded. Functionality of the hand with Hand Functional Index (HFI) and quality of life were evaluated with Short Form 36. Group 1 included 37.8% (n= 130) of 344 hands of 172 patients with LJM according to Rosenbloom classification, group 2 included 44.8% (n= 154) according to the same, and group 3 included 17.4% (n=60). In the comparison between groups, deterioration correlating with the increase in LJM stage was found in terms of duration of disease, level of fasting blood sugar and HbA1c, frequency of retinopathy and nephropathy, diabetic foot involvement, Dupuytren contracture, presence of CTS, superficial, pain, temperature and cortical senses, DTR, hand dexterity, grip strengths and function of the hand and quality of life except pain. In patients with long-term DM with poor metabolic control, presence of LJM can make contribution to prevent morbidity, mortality and functional disabilities of these patients.

Keywords Diabetes mellitus · Limited joint mobility syndrome · Complication · Hand function · Quality of life

This paper was accepted as a poster presentation in the Fifth International Physical Medicine and Rehabilitation Congress planned to be held between June 12 and 17, 2009.

E. Umay
e-mail: ebruumay@gmail.com

## Introduction

Diabetes Mellitus (DM) is a chronic, metabolic disease characterized with hyperglycemia developed as a result of absolute of relative insulin insufficiency or ineffectiveness that can cause increases in mortality and morbidity through its effects on various systems [1].

Increases in mortality and morbidity in DM is frequently related with micro and macrovascular complications occurring in the long term. Particularly microvascular complications cause functional disabilities with blindness, renal insufficiency, foot amputations and deterioration in the quality of life [2].

In addition to these complications, hands are more frequently involved in diabetics as compared to the normal population. Limited joint mobility syndrome (LJM) and sclerodactyly that is specific for DM and Dupuytren contracture, tenosynovitis of flexors and carpal tunnel syndrome (CTS), which are more frequently seen in DM although not specific, are complications related to hand involvement [3].

Limited Joint Mobility syndrome (LJM) is a complication with fibrosis in subcutaneous tissues and abnormal thickening of the dermis causing painless restriction in the extension movements of the hand joints. It was first described in 1971 by Jung et al. [4] as hand stiffness syndrome related to DM; however, with the demonstration

of its relation with juvenile type 1 DM by Rosenbloom [5], it was recognized as a complication.

LJM typically starts at the ulnar side of the hand in the fifth finger, and spreads to the radial side by involving metacarpophalangeal and interphalangeal joints, and starts to affect bigger joints in time. It has been reported that the extension restriction in the fingers causes disability in hand movements in time through the clumsiness in fine movements and weakness of fingers.

Relationship of LJM with micro- and macrovascular complications of DM has been shown in several studies [3, 6], and particularly, it has been reported that it can be used for the detection of microangiopathy in the early stages [5, 7].

The purpose of this study was to investigate the relation of limited joint mobility syndrome with the duration of diabetes, its metabolic control and other complications of diabetes in patients with types 1 and 2 DM together with this syndrome and to evaluate its effects on hand functions and the quality of life.

## Material and method

Total one hundred and seventy-two patients applying to our clinic between December 2005 and January 2009 with musculoskeletal system complaints, diagnosed with DM (directed from endocrinology clinic of our hospital) and limited joint mobility syndrome was found in their hands were included in the study.

Patients were informed about the study and their verbal approvals and written consent were obtained at the start of the study. Prayer sign was sought for in the hands of the patients for the diagnosis of LJM. Lack of contact of the palmar surfaces of the fingers when two hands are pressed together with elbows in flexion, wrists in extension and fingertips facing upwards in the vertical plane was considered as “positive prayer sign”.

Patients with trauma or injuries in the upper extremities in the history or cervical radiculopathy, and those with vitamin $B_{12}$ deficiency, thyroidal diseases, autoimmune and inflammatory rheumatoid diseases and patients with cere- brovascular diseases were excluded from the study.

Demographic data, disease characteristics and metabolic factors of the patients were recorded. Fasting blood sugar (FBS) and HbA1c levels were tested after fasting of 8 h as a minimum and 16 h as a maximum. Weights and heights of patients were taken, and basal mass index (BMI) was calculated according to the formula, (BMI) = (weight/(height)$^2$), in kg/m$^2$.

Classification of patients was made according to Rosenbloom classification [8]. Limited joint mobility classified by Rosenbloom as Stage 0: No limitation. Those with unilateral or doubtful findings, Stage 1: Mild limitation. Involvement of one or two interphalangeal joints or involvement of only the metacarpal joints (bilateral), Stage 2: Medium-level limitation. Involvement of three or more interphalangeal joints or involvement of only one finger or one large joint (bilateral), Stage 3: Severe limitation. Overt hand deformity at rest. According to this classification, 344 hands of 172 patients were grouped as Group 1 (stage 1), group 2 (stage 2) and group 3 (stage 3).

Presence of microvascular (retinopathy, nephropathy, neuropathy, diabetic foot involvement), macrovascular (atherosclerotic heart disease (ASHD)) and other hand involvements (Dupuyten contracture, flexor tenosynovitis, sclerodactyly, carpal tunnel syndrome) seen in patients related to DM were recorded.

In the patient, retinopathy was determined according to the results of the fundoscopic examination after consulting with the ophthalmology clinic, and nephropathy was determined by testing albuminuria, serum creatinine levels and creatinine clearance in the 24-hour urine, and patients with albuminuria of 30 g or more in the 24-hour urine and/or high levels of serum creatinine and/or proteinuria of mg or higher were found after consulting with the nephrology clinic.

Neuropathy was investigated by the same practitioner for all the patients using Medelec Synergy 10-channel (Oxford, U.K.) electroneuromyography (ENMG) device a with motor transmission study on upper and lower extremities by evaluating median, ulnar, tibial and peroneal nerves bilaterally, with sensory transmission study, bilateral median and ulnar evaluation and unilateral radial and sural nerve evaluation.

To determine ASHD, diagnoses of angina pectoris and cardiac diseases were questioned in the history of the patient.

Feet of the patients were evaluated with physical examination as regards neuropathic and vascular involvement with development of calluses, ulcers, joint motion ranges and presence of deformities and presence of diabetic foot was recorded.

Presence of CTS was investigated with provocative Phalen and Tinel tests and with the evaluation of sense in the innervation area of the median nerve in patients defining paresthesia in the thumb, and second and third fingers particularly t night. Evaluation with ENMG was performed for definitive diagnosis.

Consequences of micro- and macrovascular complications in the patients were evaluated as “present” or “absent”.

Evaluation of the superficial senses of the patients was performed with Semmes Weinstein Monofilament Test (SWMT). The lack of sense in 2.83 was evaluated as ‘deterioration of superficial sense’, lack of sense in 3.61 was evaluated as ‘diminished mild touch, lack of sense in 4.31 as ‘reduced protective sense’, lack of sense in 4.56 as

'loss of the protective sense', and lack of sense in 6.65 as 'could not be tested'. The smallest number of the monofilament sensed was recorded.

Pins were used to evaluate sense of pain. The patient was asked to differentiate between touches with blunt and sharp tips on each fingertip. Sense of pain was considered as 'normal' in case of eight correct answers out of ten trials.

To evaluate sense of heat, test tubes filled with cold water containing ice particles or hot water were used. These tubes were contacted to the fingertips for three seconds and the patient was asked to discriminate between hot and cold. Results were evaluated as 'normal' or 'deteriorated'.

For the evaluation of vibration sense, a diaposon of 256 Hz was used. Results were evaluated as 'normal' or 'reduced'.

Cortical sense was evaluated with static two-point discrimination test using pins. Ability to discriminate between two points 6 mm. apart and closer was considered as normal, discrimination between 7 and 10 mm. was considered as loss of sense in medium level, between 11 and 15 mm. was considered as reduced sense, and over 15 mm. was considered as 'loss of protective sense'.

Deep tendon reflexes (DTR) were tested in the upper extremities on biceps, brachioradialis and triceps muscles, and results were evaluated as 'normal' or 'reduced'.

Hand dexterities were evaluated with Nine-Hole Peg Test (NHPT). Periods were measured with a chronometer, and periods of 20 s and over were evaluated as 'loss of dexterity'.

Grip strength was measured with Jamar hand dynamometer. Measurements were repeated three times in the involved and intact hands each and mean values were taken in kilograms (kg). Pinch strengths were evaluated in three different positions (lateral, fingertip, palmar) with pinchmeter.

Functionality of the hand was evaluated with Hand Functional Index (HFI). Hands were evaluated one by one using the scale consisting of the first 9 items in the Keitel Functional Index that evaluates the movements of the wrist and fingers and were scored between 2 and 21.

Quality of life was evaluated with Short Form 36 related to health. Using the scale consisting of 8 subgroups, physical function, physical role limitation, emotional role limitation, general perception of health, vitality, bodily pain, mental health and social function were evaluated. Scoring was between 0 and 100. According to the subscales, 0 indicated the worst health status, and 100 indicated the best health status.

Data analyses were made using the Statistical Package for the Social Sciences 15.0 for Windows. Whether the distribution of normally distributed continuous variables, were assessed with the Kolmorov-Smirnov test. Descriptive statistics were shown using Chi-square tests as mean ± standard deviation for continuous variables and number of observations (%) for nominal variables. Because of all of continuous variables normally distributed, continuous variables were investigated with Anova test between groups. Significance of the differences between the groups for nominal variables was investigated with Spearman correlation analysis. Relation between sub-groups were shown using post hoc analysis (Tukey-HSD). The results were considered as significant for $p < 0.05$.

## Results

One hundred and seventy-two patients were included in the study. Of the patients, 129 (75%) were females and 43 (25%) were males, mean age was 62.58 ± 9.99, and 77 (44.8%) were type 1 while 95 (55.2%) were type 2 DM. The mean age at the onset of the disease was found as 51.45 ± 11.75 years and duration of the disease was found as 11.10 ± 7.48 years. Dominant hand was the right hand in 161 patients (93.6%) and left hand in 11 (6.4%). Involvement of both hands was present in all the patients (n = 344). While family history was positive in 93 patients (54.1%), it was negative in 79 (45.9%). While there were no diseases accompanying DM in 8 patients (4.7%), there was one accompanying disease in 95 patients (55.2%), two accompanying diseases in 47 (27.3%), three in 15 (%8.7) and four accompanying diseases in 7 (4.1%). Hypertension was determined as the most frequent disease accompanying DM with 142 patients (82.5%). Mean FBS levels was 164.76 ± 65.57 mg/dl, mean HbA1c was 8.87% ± 1.59, and mean BMI was 30.99 ± 5.28 kg/m².

Of the three hundred and forty-four hands having LJM according to Rosenbloom classification, group 1 constituted 37.8% (n = 130), group 2 constituted 44.8% (n = 154) and group 3 constituted 17.4% (n = 60). Complications seen related to DM were recorded as retinopathy in 142 (41.2%) of the 344 hands with limited joint mobility syndrome, as nephropathy in 161 (46.8%), as ASHD in 259 (75.2%), as neuropathy in 266 (77.3%), and as diabetic foot involvement in 89 (25.8%). Dupuytren contracture was found in 76 (22.1%), flexor tenosynovitis in 80 (23.2%), sclerodactyly in 22 (6.4%), and carpal tunnel syndrome was found in 154 (44.7%).

Distribution and comparison of demographic data, disease characteristics and metabolic factors are given in Table 1, distribution and comparison of complications are given in Table 2. Also, the results of correlation analysis that made to verify the correlation in terms of disease characteristics, metabolic factors and complications are presented in Table 3.

In comparison between groups in terms of disease characteristics, metabolic factors and complications, we found that, LJM stage increases with increasing duration of disease, high FBS levels and presence of retinopathy, nephropathy, diabetic foot involvement and sclerodactyly.

Table 1 Distribution and comparison of demographic data, disease characteristics and metabolic factors according to the groups

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| | n(%), mean ± SD | n(%), mean ± SD | n(%), mean ± SD |
| Gender | | | |
| Female | 96 (%73.8) | 120 (%77.9) | 42 (%70.0) |
| Male | 34 (%26.2) | 34 (%22.1) | 18 (%30.0) |
| Age | 63.72±8.57 | 63.16±10.04 | 58.63±12.03 |
| Type of Diabetes | | | |
| Type 1 | 50 (%38.5)[b] | 70 (%45.5) | 34 (%56.7)[b] |
| Type 2 | 80 (%61.5) | 84 (%54.5) | 26 (%43.3) |
| Age at the onset of the disease (years) | 52.81±10.52 | 52.48±11.39 | 46.00±13.89 |
| Duration of the disease (years) | 10.95±8.57[a] | 10.63±6.74[a] | 12.63±6.87[a] |
| Positive family history | 64 (%49.2) | 92 (%59.7) | 30 (%50) |
| Number of diseases accompanying DM | | | |
| No accompanying disease | 16 (%12.3) | 0 | 0 |
| 1 accompanying disease | 66 (%50.8) | 88 (%57.1) | 36 (%60.0) |
| 2 accompanying disease | 38 (%29.2) | 40 (%26.0) | 16 (%26.6) |
| 3 accompanying disease | 4 (%3.1) | 22 (%14.3) | 4 (%6.7) |
| 4 accompanying disease | 6 (%4.6) | 4 (%2.6) | 4 (%6.7) |
| BMI (kg/m²) | 31.99±6.76 | 30.67±3.66 | 29.63±4.85 |
| FBS level (mg/dl) | 153.33±47.82[a] | 158±59.20[a] | 175.64±77.30[a] |
| HbA1c level (%) | 7.70±1.63[b] | 7.95±1.44 | 9.46±1.75[b] |

mean ± SD: mean ± standard deviation, BMI body mass index, FBS Fasting blood sugar

[a]For the data compared, statistical differences were found between groups 1 and 3 and groups 2 and 3 ($p<0.05$)

[b]For the data compared, statistical differences were found between groups 1 and 3 ($p<0.05$)

Also, advanced stage (stage 3) more seen than early stage in patients with type 1 DM, high HbA1c level, Dupuytren contracture and CTS.

Distribution and comparison of physical examination, functional status of the hand, and the subgroup of quality of life assessment are given in Table 4 and the results of correlation analysis that made to verify the correlations are presented in Table 5.

In comparison between groups in terms of physical examination, functional status of the and quality of life assesment, was found that, with the increase of LJM stage decreased superficial, pain and heat senses, fingertip strengths, hand function, vitality level, mental health and social functionality and increases emotional role limitation. Also, advanced stage (stage 3) more seen than early stage in patients who have anormal cortical sense and DTR, loss of hand dexterity, reduced grip strength, decreased physical function and general health and increased physical role limitation.

## Discussion

In our study, relations of presence of LJM with the duration of diabetes, its metabolic control and complications, and the

Table 2 Distribution and comparison of complications according to groups

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| | n(%) | n(%) | n(%) |
| Retinopathy | 42 (%32.3)[a] | 64 (%41.5)[a] | 36 (%60.0)[a] |
| Nephropathy | 50 (%38.5)[a] | 73 (%47.4)[a] | 38 (%63.3)[a] |
| Atherosclerotic cardiac disease | 92 (%70.7) | 120 (%77.9) | 47 (%78.3) |
| Neuropathy | 98 (%75.4) | 120 (%77.9) | 48 (%80.0) |
| Diabetic foot involvement | 25 (%19.2)[a] | 32 (%20.8)[a] | 32 (%53.3)[a] |
| Dupuytren contracture | 22 (%16.9)[b] | 34 (%22.1) | 20 (%33.3)[b] |
| Flexor tenosynovitis | 24 (%18.5) | 42 (%27.3) | 14 (%23.3) |
| Sclerodactyly | 4 (%3.1)[a] | 8 (%5.2)[a] | 10 (%16.7)[a] |
| Carpal tunnel syndrome | 56 (%43.1)[b] | 66 (%43.5) | 32 (%53.3)[b] |

[a]For the data compared, statistical differences were found between groups 1 and 3 and groups 2 and 3 ($p<0.05$)

[b]For the data compared, statistical differences were found between groups 1 and 3 ($p<0.05$)

Table 3 The results of correlation analysis that made to verify the correlation in terms of disease characteristics, metabolic factors and complications

| Parameters | r | P |
|---|---|---|
| Type of Diabetes | −0.125 | 0.020 |
| Duration of the disease (years) | −0.174 | 0.001 |
| FBS level (mg/dl) | −0.163 | 0.012 |
| HbA1c level (%) | −0.081 | 0.013 |
| Retinopathy | −0.351 | 0.001 |
| Nephropathy | −0.181 | 0.013 |
| Atherosclerotic cardiac disease | 0.077 | 0.156 |
| Neuropathy | 0.158 | 0.282 |
| Diabetic foot involvement | −0.186 | 0.012 |
| Dupuytren contracture | −0.138 | 0.004 |
| Flexor tenosynovitis | 0.096 | 0.176 |
| Sclerodactyly | −0.113 | 0.016 |
| Carpal tunnel syndrome | −0.067 | 0.016 |

r rho coefficient, p correlation rate, BMI body mass index, FBS Fasting blood sugar

effects on the hand functionality and the quality of life were investigated. Between the groups, positive correlation was found between the stage of LJM and functional incapability of the hand, energy level, mental health and social functionality and negative correlation was found in superficial sense; between groups 1 and 3 and groups 2 and 3, positive correlation was found in FBS level, retinopathy, nephropathy, diabetic foot involvement, sclerodactyly, emotional role limitation and negative correlation was found in pain and heat senses and grip strength; between groups 1 and 3, positive correlation was found in HbA1c level, Dupuytren contracture, CTS, physical function, physical role limitation and general health and negative correlation was found in cortical senses, DTR, finger strengths and hand dexterity ($p<0.05$).

It has been reported in the literature that LJM is particularly related with the duration of DM, is generally seen in patients with long-duration disease and that duration of the disease can be an independent predictor for LJM [9]. It was reported in some studies that the incidence is 40–50% in patients with diabetes duration is longer than 12 to 16 years [10], and LJM was seen in 22.7% for the disease duration of 2–5 years, and in 89.3% for the disease duration of more than 10 years [11]. In our study also, mean duration of disease was found as 11 years, and positive correlation was found between the increase of duration and the increase of the stage of LJM, complying with the literature.

Articles reporting that continuous high levels of tissue glucose is effective on the development of LJM have been published [6, 12]. It was reported in the literature that increase in the stiffness of the joints correlated with the increased glycolization of collagen and that there is a relation between LJM and FBG [12]. The relationship between the level of HbA1c, which is used to define the changes in blood glucose levels in long periods, and LJM, and it was reported that level of HbA1c can be used as an independent risk factor for the development of LJM and a predictor, and that there was positive correlation between these two [13]. In our study also, FBS and HbA1c levels were found high, and a positive relation was determined with the stage of LJM.

It has been reported in the literature that there is a positive relation between the presence of LJM and microvascular complications of DM and although not very clear, the mechanisms for the development of these two groups of complications can be the same [9, 14]. It was reported in some other studies that LJM can be used as a risk factor when evaluating the presence of microvascular complications since it seen 4.5 years in the average before the occurrence of microvascular complications [15, 16].

The microvascular complication that relation with LJM is reported most frequently is the presence of retinopathy. Togetherness of LJM and retinopathy was found as 43–96.4% in the studies performed, and it was reported that this relationship became closer with the increasing severity of retinopathy and rate of development of severe retinopathy increased 2.5 to 2.8 folds [9, 17, 18–19].

In their study on patients with type 1 DM, Borch-Johnsen et al. [19] reported with a low sensitivity that LJM could be used as a potential predictor for the development of nephropathy. The rate of nephropathy in patients with LJM was reported as 40–90.9% in the literature and the relation with both micro and macroalbuminuria was shown [10]. In the study of Aoki et al. [20] it was reported that nephropathy stage increased with the increasing severity of LJM.

In our study also, retinopathy was found as 41.2% and nephropathy as 46.8% as compliant with the literature, and it was found that frequency of retinopathy and nephropathy increased with the increasing stage of LJM.

Diabetic neuropathy is reported as 10% at the time of DM diagnosis, and between 5–60% for all the patients with DM; this rate reaches 100% with the inclusion of subclinical transmission abnormalities. Neuropathy is present from the early stages of DM according to some authors, and can reach 80 to 90% when duration of diabetes reaches 10–12 years [21]. Big myelinated nerve fibers and non-myelinated nerves are affected in DM resulting in the reducing of superficial, pain, and heat senses, loss of two-point discrimination and DTRs, and reducing of gripping strength [22, 23]. It has been reported that sensory deterioration and strength losses prevent use of the joint and lead to decrease in joint movements and increase in stiffness [24]. Reducing of nerve transmission speed is

Table 4 Distribution and comparison of physical examination, functional status of the hand, and the subgroup of quality of life assessment

| Parameters | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| | n(%),mean ± SD | n(%),mean ± SD | n(%),mean ± SD |
| Superficial sense | | | |
| Normal | 68 (%52.3)[a] | 38 (%24.7)[a] | 2 (%3.3)[a] |
| Reduced slight touch | 40 (%30.8) | 66 (%42.9) | 10 (%16.7) |
| Reduced protective sense | 18 (%13.8)[b] | 32 (%20.8)[b] | 27 (%45.0)[b] |
| Protective sensory loss | 4 (%3.1) | 15 (%9.7) | 8 (%13.4) |
| Senses not tested | 0[b] | 3 (%1.9)[b] | 13 (%21.6)[b] |
| Sense of pain (normal) | 118 (%90.8)[b] | 124 (80.5)[b] | 35 (%58.3)[b] |
| Sense of heat (normal) | 120 (%92.3)[b] | 115 (%74.7)[b] | 32 (%53.3)[b] |
| Vibration (normal) | 6 (%4.6) | 4 (%2.6) | 1 (%1.7) |
| Two-point discrimination | | | |
| Under 6 mm | 34 (%26.2)[c] | 18 (%11.6) | 1 (%1.7)[c] |
| 7–10 mm | 50 (%38.5) | 40 (%26.0) | 13 (%21.6) |
| 11–15 mm | 28 (%21.5) | 58 (%37.7) | 21 (%35.0) |
| Over 15 mm. | 18 (%13.8)[c] | 38 (%24.7) | 25 (%41.7)[c] |
| DTR (normal) | 87 (%66.9)[c] | 88 (%57.1) | 25 (%41.7)[c] |
| NHPT (under 20 s) | 37 (%28.5)[c] | 34 (%22.1) | 3 (%5.0)[c] |
| Grip strength (kg.) | 15.05±6.40[c] | 14.34±8.86 | 11.99±6.22[c] |
| Lateral pinch strength (kg.) | 7.28±2.28[b] | 6.93±2.21[b] | 5.42±1.73[b] |
| Palmar pinch strength (kg.) | 5.62±1.91[b] | 5.23±1.78[b] | 4.59±1.46[b] |
| Fingertip pinch strength (kg.) | 4.73±1.73[b] | 4.10±1.65[b] | 2.65±1.43[b] |
| HFI (2–21) | 7.97±2.76[a] | 15.69±2.40[a] | 19.81±3.54[a] |
| Physical function (0–100) | 36.10±27.81[c] | 30.83±22.53 | 26.07±16.64[c] |
| Physical role limitation (0–100) | 10.58±23.02[c] | 8.20±12.55 | 3.33±8.64[c] |
| Emotional role limitation (0–100) | 35.61±35.37[b] | 29.58±31.75[b] | 19.99±35.66[b] |
| General perception of health (0–100) | 38.13±20.29[c] | 35.21±19.74 | 30.96±19.71[c] |
| Vitality level (0–100) | 42.69±21.52[a] | 31.00±18.63[a] | 13.90±8.23[a] |
| Pain evaluation (0–100) | 25.96±19.69 | 22.93±21.86 | 22.44±19.28 |
| Mental health status (0–100) | 63.90±19.82[a] | 53.23±13.48[a] | 41.68±21.10[a] |
| Social function (0–100) | 48.86±30.08[a] | 28.75±20.14[a] | 11.22±12.83[a] |

mean ± SD: mean ± standard deviation, DTR deep tendon reflexes, NHPT nine-hole peg test, HFI hand functional index

[a]For the data compared, statistical differences were found between group 1, group 2 and group 3 ($p<0.05$)

[b]For the data compared, statistical differences were found between groups 1 and 3 and groups 2 and 3 ($p<0.05$)

[c]For the data compared, statistical differences were found between groups 1 and 3 ($p<0.05$)

correlated with the reducing of senses of heat and vibration in diabetic neuropathy [25]. In the study of Rahman et al. [26], it was reported that evaluation of particularly the sense of vibration and with monofilaments can be used as the predictor of neuropathy. Togetherness of neuropathy and LJM was reported as 50–70.2% in the literature. Starkman et al. [9] found a relationship between the development of neuropathy and LJM in DM patients younger than 40 years of age with disease duration less than 20 years. In their study on 204 patients with type 1 DM, Beacom et al. [27] found significant decreases in vibration sense and ENMG transmission rates in patients with LJM as compared to those without LJM.

In our study, neuropathy was present in 77.3% or the patients, and deterioration in vibration sense was present in 97%. However, a statistically significant increase related to the stage of LJM could not be found. This can be explained with the presence of neuropathy in the majority of our patients and the decrease in vibration sense. Also, deterioration in the parameters of sense evaluation paralleling LJM stage suggests that neuropathy also increases with the LJM stage.

In the literature, it is reported that decrease in the mobility of foot joints also is found in cases where LJM is found in the hand [28]. Duffin et al. [29] reported in their study on 302 patients with DM that prayer sign was positive in 35% of the patients, and at the same time, they found the rate of restriction in subtalar joints in the feet was 35%, and reported that the prayer sign being positive could indicate the possibility of restriction in the mobility of other joints also. In our study also, positive correlation was found between the diabetic foot involvement and increase of the LJM stage.

There are too few studies in the literature on LJM and macrovascular complications. Together with this, coronary heart disease and hypertension is present in the majority of patients. In the study of Frost et al. [10], it is reported that

Table 5 The results of correlation analysis that made to verify the correlations in terms of physical examination, functional status of the hand, and the subgroup of quality of life assessment

| Parameters | r | P |
|---|---|---|
| Superficial sense (normal) | −0.151 | 0.005 |
| Sense of pain (normal) | −0.218 | 0.001 |
| Sense of heat (normal) | −0.128 | 0.017 |
| Vibration (normal) | 0.026 | 0.485 |
| Two-point discrimination (under 6 mm) | −0.112 | 0.038 |
| DTR (normal) | −0.090 | 0.019 |
| NHPT (under 20 s) | −0.049 | 0.016 |
| Grip strength (kg.) | −0.081 | 0.013 |
| Lateral pinch strength (kg.) | −0.377 | 0.004 |
| Palmar pinch strength (kg.) | −0.205 | 0.014 |
| Fingertip pinch strength (kg.) | −0.166 | 0.009 |
| HFI | −0.484 | 0.001 |
| Physical function | −0.153 | 0.005 |
| Physical role limitation | −0.016 | 0.003 |
| Emotional role limitation | −0.189 | 0.001 |
| General perception of health | −0.163 | 0.014 |
| Vitality level | −0.081 | 0.013 |
| Pain evaluation | 0.068 | 0.210 |
| Mental health status | −0.187 | 0.018 |

r rho coefficient, p correlation rate, DTR deep tendon reflexes, NHPT nine-hole peg test, HFI hand functional index

finding early subclinical macrovascular involvement signs is statistically significant particularly in women with LJM, and hypertension is seen more frequently as compared to patients without LJM. Arkkila et al. [30] reported that LJM constitutes a high risk for coronary heart disease. Slama et al. [31] found ASHD giving clinical findings by 11% in their study.

ASHD was found with a rate of 75.2% and hypertension 82.5% in our study. The reason may for lack of statistically significant changes with the stage of LJM is that majority of our patients has these complications.

It is reported in the literature that LJM and Dupuytren contracture, tenosynovitis and sclerodactyly develop with the same mechanisms [32]. Togetherness of LJM with Dupuytren contracture is reported in the literature as 12–95% [30, 31], with sclerodactyly as 6.4–85% [33], and with flexor tenosynovitis as 17.5–100% [34].

CTS is the most frequently seen mononeuropathy in DM; the risk increases in diabetics with duration of the disease is over 20 years and long-term high levels of blood glucose reportedly to reach 8–19% in asymptomatic cases and to 22–29% in symptomatic cases. This risk increases to reach some 75% in patients with LJM [35].

Although the frequency rates of Dupuytren contracture, sclerodactyly, flexor tenosynovitis and CTS in our study was harmonious with the literature, significant increases in LJM stage were found only regarding Dupuyten contracture, sclerodactyly and CTS. The reason for studied patients in the literature may have different features than our groups.

Hand grip strength and lateral pinch strength is reported to be reduced in patients with DM as compared to the normal population in the literature [36]. And it has been suggested that the reducing in the hand grip strength was related to peripheral neuropathy accompanying DM, cardiac diseases, vision problems and renal insufficiencies. Lundbaek et al. [37] reported that LJM could cause reducing in the muscular strength in the hand. In a study of Savaş et al. [14] evaluating the hand grip strength in patients with DM was found reduced, 23 kg in the average. In two studies in the literature performed on patients with DM using different hand function scales were reported relation with decreased hand muscular strengths between decreased in physical activity and deterioration in daily living activities. In our study, negative correlation was found for lateral, palmar and fingertip pinch and grip strengths with the stage of LJM. We think that lower values for hand grip strength as compared to the literature is the high incidence of micro- and macrovascular complications and neuropathy in our patients. In the literature in the study of Cederlund et al. [13], insufficiencies in daily living activities and hand functions were reported for LJM patients with findings of neuropathy. Also, in our study, inadequacy of hand functions and LJM stage were correlated with each other harmoniously with the literature.

There is no study same our study in the literature. It has been reported in the literature that in the evaluation of quality of life in patients with diabetes using SF-36, physical function played a role by 60–74%, physical role limitation by 56.5–64.5%, level of pain by 62.7–73%, general health perception by 48.9–63.9%, vitality level by 54.5–58.9%, social function by 74.8–81.4%, emotional role limitation by 63.2–80.9%, and mental health by 60.1–77.2% [38]. Hart et al. [39] have reported that macrovascular complications in patients with DM were related to both physical and mental functions; Papadopoulos et al. [40] however, reported that all but physical role limitation were affected in the presence of microvascular complications.

It was reported that occurrence of complications deteriorate the quality of life in the domains of physical function, physical role difficulties, and general health; quality of life was negatively affected with the increasing severity of the complications; domains that most badly affected were physical role limitation, vitality level and general health perception; and values for physical function and pain got worse in cases older than 10 years in duration [38].

Deterioration in all the quality of life parameters excluding pain level correlated with LJM stage was found

in our study. Lack of finding changes in pain levels correlated with LJM stage and finding the rates of other parameters lower as compared to DM patients without LJM suggest that quality of life in LJM is affected from hand involvement and microvascular complications that increase with the increasing stage in a correlated fashion contribute to this result.

As a secondary result, we found that, advanced stage LJM in patients with type 1 DM more seen than type 2 DM. Studies in the literature, were often evaluated patients with type 1 DM [18, 21, 28]. Therefore, we think that, especially in patients with type 1 DM should be more careful as regard development of complication, loss of hand function and reduce the quality of life.

## Conclusion

LJM, although seen rather frequently in diabetes, is a condition overlooked in general. Duration of diabetes is related with the metabolic control and microvascular complications and this relation gets stronger with the increasing stage. Serious inadequacy occurs in the functionality and quality of life of these patients with the increasing microvascular complications. We believe that detection of the presence of LJM particularly in DM of long duration with poor metabolic control will contribute to prevent morbidity, mortality and functional incapability in these patients.

## References


1. Smith LL, Burnet SP, McNeil JD. Musculoskeletal manifestations of diabetes mellitus. Br J Sports Med. 2003;37:30–5.
2. Bate KL, Jerums G. 3:Preventing complications of diabetes. Med J Australia. 2003;179:498–503.
3. Cagliero E, Apruzzese W, Perlmutter GS, Nathan DM. Musculoskeletal disorders of the hand and shoulders in patient with diabetes melitus. Am J Med. 2002;112:487–90.
4. Jung Y, Hohmann TC, Gerneth JA, et al. Diabetic hand syndrome. Metabolism. 1971;20:1008–15.
5. Rosenbloom AL, Frias JL. Diabetes Mellitus, short stature and joint stiffness. A new syndrome. Clin Rest. 1974;22:56.
6. Said G. Diabetic neuropathy: an up date. J Neurol. 1996;243: 431–40.
7. Kapoor A, Sibbitt Jr WL. Contractures in diabetes mellitus: The syndrome of limited joint mobility. Semin Arthritis Rheum. 1989;18:168–80.
8. Rosenbloom AL. Limitation of finger joint mobility in diabetes mellitus. J Diabet Complications Apr-Jun. 1989;3:77–87.
9. Starkman H, Gleason RE, Rand L, Miller DE, Soeldner JS. Limited joint mobility (LJM) of the hand in patients with diabetes mellitus: Relation to chronic complications. Ann Rheum Dis. 1986;45:130–5.
10. Frost D, Beischer W. Limited joint mobility in type 1 diabetic patients: associations with microangiopathy and subclinical macroangiopathy are different in men and women. Diabetes Care. 2001;24:95–9.
11. Ducić V, Babović-Vuksanović D, Grujić E, Arifhodzić N, Dinarević S. Changes in the small joints, skin and lungs in children and adolescents with insulin-dependent diabetes. Med Arh. 1989;43:3–7.
12. Isdale AH. The ABC of the diabetic hand–advanced glycosylation end products, browning and collagen. Br J Rheumatol. 1993;32:859–61.
13. Cederlund RI, Thomsen N, Thrainsdottir S, Eriksson KF, Sundkvist G, Dahlin LB. Hand disorders, hand function, and activities of daily living in elderly men with type 2 diabetes. J Diabetes Complications. 2009;23:32–9.
14. Savaş S, Bardak Y, Tunç ŞE, Çetin M, Akkuş S. Flexion contractures in a diabetic young man (Rosenbloom syndrome). Tr J Med Sci. 1999;29:711–4.
15. Esfahani MR, Asghari H, Piri N. Advanced proliferative diabetic retinopathy in four young patients with flexion contractures. Int Ophthalmol. 2008;28:247–316.
16. Amin R, Bahu T, Widmer B, Dalton R, Dunger D. Longitudinal relation between limited joint mobility, height, insulin-like growth factor 1 levels, and risk of developing microalbuminuria: the Oxford Regional Prospective Study. Arch Dis Child. 2005;90:1039–44.
17. Rosenbloom AL, Malone JI, Yucha J, Van Cader TC. Limited joint mobility and diabetic retinopathy demonstrated by fluores- cein angiography. Eur J Pediatr. 1984;141:163–4.
18. Arkkila PE, Kantola IM, Viikari JS. Limited joint mobility in type 1 diabetic patients: correlation to other diabetic complications. J Intern Med. 1994;236:215–23.
19. Borch-Johnsen K, Andersen PK, Deckert T. The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. Diabetologia. 1985;28:590–6.
20. Aoki Y, Yazaki K, Shirotori K, Yanagisawa Y, Oguchi H, Kiyosawa K, et al. Stiffening of connective tissue in elderly diabetic patients: relevance to diaetic nephropathy and oxidative stress. Diabetologia. 1993;36:79–83.
21. Rota E, Quadri R, Fanti E, Isoardo G, Poglio F, Tavella A, et al. Electrophysiological findings of peripheral neuropathy in newly diagnosed type II diabetes mellitus. J Peripher Nerv Syst. 2005;10:348–53.
22. Arıng A, Jones D, Falko J. Evaluation and Prevention of Diabetic Neuropathy. Am Fam Physician. 2005;7:2123–30.
23. England J, Gronseth G. Distal Symmetric polyneuropathy: A definition for clinical research. American Academy of Neurology. 2005;64:199–207.
24. Larkin JG, Frier BM. Limited joint mobility and dupuytren's contracture in diabetic, hypertensive and normal populations. Br Med J. 1986;292:1494.
25. Shy ME, Frohman EM, So YT, Arezzo JC, Cornblath DR, Giuliani MJ, et al. Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Quanti- tative sensory testing: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurol- ogy. Neurology. 2003;60:898–904.
26. Rahman M, Griffin SJ, Rathmann W, Wareham NJ. How should peripheral neuropathy be assessed in people with diabetes in primary care? A population-based comparison of four measures. Diabet Med. 2003;20:368–74.
27. Beacom R, Gillespie EL, Middleton D, Sawhney B, Kennedy L. Limited joint mobility in insulin-dependent diabetes: relationship to retinopathy, peripheral nerve function and HLA status. Q J Med. 1985;56:337–44.
28. Zimny S, Schatz H, Pfohl M. The role of limited joint mobility in diabetic patients with an at-risk foot. Diabetes Care. 2004;27: 942–6.

29. Duffin AC, Donaghue KC, Potter M, McInnes A, Chan AK, King J, et al. Limited joint mobility in the hands and feet of adolescents with Type 1 diabetes mellitus. Diabet Med. 1999;16:125–30.
30. Arkkila PE, Kantola IM, Viikari JS. Limited joint mobility in non-insulin-dependent diabetic (NIDDM) patients: correlation to control of diabetes, atherosclerotic vascular disease, and other diabetic complications. J Diabetes Complications. 1997;11:
208–17.
31. Slama G, Letanoux M, Thibult N, Goldgewicht C, Eschwege E, Tchobroutsky G. Quantification of early subclinical limited joint mobility in diabetes mellitus. Diabetes Care. 1985;8:329–32.
32. Ballantyne JA, Hooper G. The hand and diabetes. Curr Orthop.
2004;18:118–25.
33. Yosipovitch G, Hodak E, Vardi P, Shraga I, Karp M, Sprecher E, et al. The prevalence of cutaneous manifestations in IDDM patients and their association with diabetes risk factors and microvascular complications. Diabetes Care. 1998;21:506–9.
34. Sibbitt Jr WL, Eaton RP. Corticosteroid responsive tenosynovitis is a common pathway for limited joint mobility in the diabetic hand. J Rheumatol. 1997;24:931–6.
35. Cruz Martínez A. Diabetic neuropathy. Topography, general electrophysiologic features, effect of ischaemia on nerve evoked potential, and frequency of the entrapment neuropathy. Electromyogr Clin Neurophysiol. 1986;26:283–95.
36. Sayer AA, Dennison EM, Syddall HE, Gilbody HJ, Phillips DI, Cooper C. Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg? Diabetes Care.
2005;28:2541–2.
37. Lundbaek K. Stiff hands in long-term diabetes. Acta Med Scand. 1957;158:447–51.
38. Chittleborough CR, Baldock KL, Taylor AW, Phillips PJ, The North West Adelaide Health Study Team. Health status assessed by the SF-36 along the diabetes continuum in an Australian population. Quality of Life Research. 2006;15:687–94.
39. Hart HE, Bilo HJG, Redekop WK, Stolk RP, Assink JH, de Jong BM. Quality of life of patients with type I diabetes mellitus. Quality of Life Research. 2003;12:1089–97.
40. Papadopoulos AA, Kontodimopoulos N, Frydas A, Ikonomakis E, Niakas D. Predictors of health-related quality of life in type II diabetic patients in Greece. BMC Publ Health. 2007;2458:186.

ORIGINAL ARTICLE



# Salivary total antioxidant capacity in type 2 diabetes mellitus patients—a clinical and biochemical study amongst tobacco smokers

Roopashri Rajesh Kashyap, Gopakumar R. Nair, Subhas Babu Gogineni, Kumari N. Suchetha

Int J Diab Dev Ctries. 2011 ; 31 :194-199

Abstract This study was undertaken to determine the effect of smoking and diabetes mellitus individually and combined on the total antioxidant capacity (TAC) of saliva in type 2 diabetes mellitus. The study consisted of four groups, one control and three study groups, each with 20 subjects. Salivary and serum samples were collected from all the groups, which were further subjected to biochemical analysis. The observations of the study were subjected to statistical analysis using SPSS software version 12. The level of salivary TAC decreases secondary to diabetes mellitus and smoking. As the duration of diabetes mellitus increases, the level of total antioxidants decreases. Correlation of salivary TAC with Russell's index had a negative correlation. Smoking has a synergistic effect on diabetes mellitus in reducing the salivary total antioxidant level. Further research should be devoted to the possible benefits of supplementation with antioxidant supplements. Saliva is considered to be a representative of serum and salivary analysis is simple and noninvasive. From the clinical standpoint, it may be reasonable to conclude that salivary TAC can be a useful marker to assess the oxidative stress, as an indicator of progression of diabetes mellitus.

Keywords Diabetes mellitus · Total antioxidant capacity · Reactive oxygen species · Saliva · Free radicals · Smoking

R. R. Kashyap
e-mail: roopashri.r.k@gmail.com

## Introduction

Hyperglycemia in Diabetes Mellitus (DM) is associated with increased production of Reactive Oxygen Species (ROS), which in turn contribute to the development of diabetic complications. Numerous studies have shown that while different indices of free-radical damage increase, there is a decrease in the concentration of various individual antioxidant substances, indicating the presence of oxidative stress in DM. It has also been suggested that there is a link between the development of microvascular and macrovascular diabetic complications in the form of free oxygen radical damage[2]. The profound effect of DM on salivary antioxidant parameters may be of great importance with respect to the diagnosis and evaluation of the complications [3].

Cigarette smoke is a major exogenous source of free radicals. Obligatory use of the body reserve of antioxidants to detoxify the excess of free radicals in smokers therefore results in an alteration in the level of different antioxidants. Saliva is the first body fluid to encounter exogenous materials or gases that penetrate the human body. Smoking affects the antioxidants in the saliva, thereby affecting the protective mechanism of saliva [4, 5].

Oral health researches have been developing salivary diagnostic tools to monitor oral diseases including periodontal disease as well as caries. The major advantages for using saliva rather than blood in the diagnosis are easy access &

Table 1 Mean HbA1c, Russell's index and salivary TAC

| Groups | Mean HbA1c (%) | Russell's index | Saliva TAC (mM) | Duration of DM (yrs) | Duration of smoking (yrs) |
|---|---|---|---|---|---|
| Non diabetic, Non smokers | 5.9±0.2 | 2.65±1.05 | 3.65±1.53 | | |
| Non diabetic, Smokers | 6.0±0.4 | 4.68±1.88 | 1.60±0.77 | | 6.0±5.6 |
| Diabetic, Non smokers | 7.6±1.2 | 3.75±1.90 | 1.12±0.50 | 6.55±5.87 | |
| Diabetic, Smokers | 8.1±1 | 5.60±1.90 | 0.93±0.94 | 6.00±5.60 | 23.7±8.2 |
| P | <0.001* | <0.001* | <0.001* | 0.805** | 0.912** |

{* highly significant; ** non significant}

non-invasive collection. The correlation between depletion of total antioxidant capacity (TAC) and poor glycemic control suggests that measurement of TAC in diabetic patients can be used as an index of glycemic control and development of diabetic complications in both types of diabetes. Hence this study was undertaken to determine the effect of smoking and DM, singly and in combination on TAC of saliva.

## Subjects and methods

A total number of 80 subjects, above the age of 45 years were randomly selected for the study [6]. Study group comprised of 40 known diabetics either on diet and exercise or on oral hypoglycemic agents. Study groups were further divided into two groups of 20 each, according to the habit of smoking. Control group comprising of 40 adults without any systemic diseases, were further divided into two groups of 20 each according to the habit of smoking. Criteria for selection of smokers was, any form of tobacco smoking done every day as habit for at least 1-year duration. Subjects with other systemic diseases, those on medications other than hypoglycemics and those on deleterious habits other than smoking were excluded from the study.

The subjects were asked not to smoke or consume any food 2 hours prior to the collection of saliva. Following a thorough mouth rinse using distilled water, saliva was allowed to accumulate in the mouth for 5 min. Accumulated saliva was collected by spit method. Intra oral examination was done and findings were recorded. Periodontal attachment loss was assessed by Russell's periodontal index [7]. Blood sample was drawn and subjected for analysis of glycated hemoglobin (HbA1c) by Turbidimetric Inhibition Immunoassay and random glucose level by glucose oxidase-peroxidase method using Hitachi 902 automatic analyzer.

Estimation of total antioxidant capacity: 2 ml of collected saliva was stored at a temperature of +4°C in glass vials, which were then subjected to analysis by phosphomolybdenum method for the quantitative determination of total antioxidant capacity using Spectrophotometer. One milliliter of reagent solution was prepared using 0.6 M of sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate (30 ml sulfuric acid +218.4 mg sodium phosphate +247.2 mg ammonium molybdate made up to 50 ml). The assay is based on the reduction of molybdate to molybdenum and the subsequent estimation of a green phosphomlybdenum complex. An aliquot of 0.1 ml of sample solution containing a reducing species (ethanol) was combined in an Eppendrof tube with 1 ml of reagent solution. The tubes were capped and incubated in water

Table 2 Severity of periodontitis in different groups

| Severity | | | Groups | | | | Total |
|---|---|---|---|---|---|---|---|
| | | | Group 1 | Group 2 | Group 3 | Group 4 | |
| Russell's staging | Early destructive periodontal disease | N | 6 | 1 | 1 | | 8 |
| | | % within the group | 30.0% | 5.0% | 5.3% | | 10.4% |
| | Established destructive periodontal disease | N | 13 | 9 | 14 | 7 | 43 |
| | | % within the group | 65.0% | 45.0% | 73.7% | 38.9% | 55.8% |
| | Terminal disease | N | 1 | 10 | 4 | 10 | 26 |
| | | % within the group | 5.0% | 50.0% | 21.1% | 61.1% | 33.8% |
| Total | | N | 20 | 20 | 19 | 18 | 77 |
| | | % within the group | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |

Group 1: Non-diabetic, Non-smokers; Group 2: Non-diabetic, Smokers; Group 3: Diabetic, Non-smokers; Group 4: Diabetic, Smokers

bath at 37°c for 90 min. After 90 min. the samples were allowed to cool to the room temperature. The absorbance of the aqueous solution for each was measured at 695 nm against a blank using spectrophotometer. A typical blank solution containing 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample was incubated under the same conditions as the rest of the samples [8].

Data obtained were analyzed using chi-square test, Kruskal-Wallis H test, Spearman correlation coefficient ratio & Fisher's Exact Test using SPSS version 12 software. The P value $<0.01$ was considered as statistically significant.

## Results

The present study comprised of 80 male subjects above 45 year age, divided into four groups, $n=20\times4$, with 95% confidence level and 80% power, as follows; Group 1: 20 non-diabetic non-smokers; Group 2: 20 non-diabetic smokers; Group 3: 20 diabetic non-smokers; Group 4: 20 diabetic smokers.

*Salivary TAC*: Mean salivary TAC values were as given (Table 1). It was lower in group II, III& IV when compared to group I; it was significantly lower in group IV.

*Russell's periodontal index*: One subject in group 3 and three subjects in group 4 were completely edentulous. Hence they were not included for Russell's index. Amongst other 77 subjects, 10.4% had early destructive periodontal disease,
55.8% had established destructive periodontal disease and 33.8% had terminal disease. Terminal disease was seen with higher percentage in both the groups of smokers (Table 2).

## Correlations

Correlation of salivary TAC with Russell's index was highly significant with a negative correlation (Fig. 1).

On comparing group wise, in group 1, 2 and 3 salivary TAC showed negative correlation with Russell's index however, with group 4 the correlation was statistically significant (P = 0.046).

As the HbA1c level increased, there was a drop in the salivary TAC level (Fig. 2). However it was statistically not significant probably due to the smaller sample size.

Correlation of salivary TAC with type of smoking in groups 2 & 4 was not statistically significant. Correlation of salivary TAC with duration of smoking, revealed negative correlation in group 4. Correlation of salivary TAC with duration of diabetes showed negative correlation. However, it was not statistically significant.

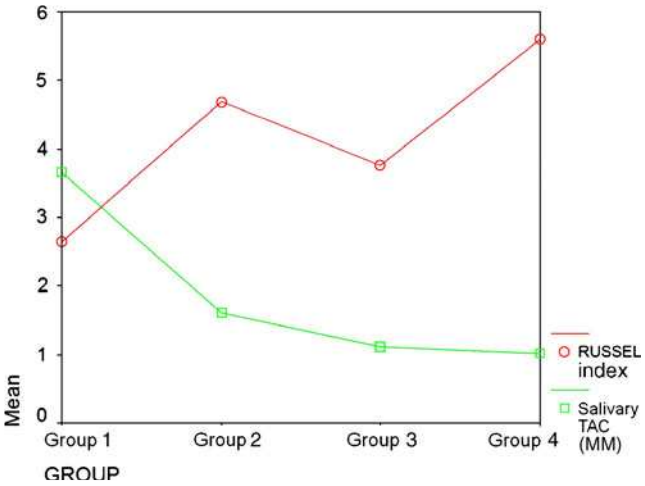



Fig. 1 Correlation of salivary TAC with Russell's index

## Discussion

Hyperglycemia in DM is associated with increased production of ROS, which in turn contributes to the development of diabetic complications. Saliva may be described as a heterogeneous fluid composed of proteins, glycoproteins, electrolytes and small organic molecules as well as compounds transported from blood [9]. The salivary antioxidant system includes various molecules and enzymes, of which the most important are the uric acid molecule and the peroxidase enzyme, both of which are water soluble [10].

The present study was carried out with the objective of estimating the TAC of saliva in diabetic smokers and nonsmokers and to correlate them with controls. The mean salivary TAC of smokers was significantly less than that of non-smokers, suggestive of the effect of smoking on salivary TAC. Saliva in smokers is constantly exposed to free radicals, which leads to a depletion in the TAC of saliva. A study by Zappacosta et al. has demonstrated that

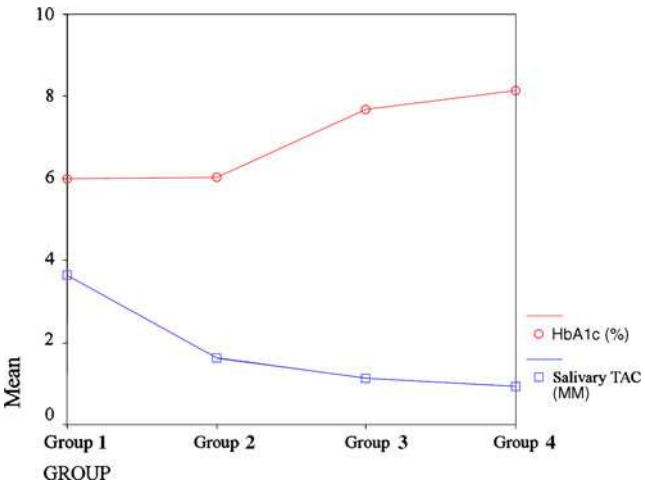



Fig. 2 Correlation of salivary TAC with HbA1c

even one cigarette reduces the concentration of glutathione in saliva, which, however, returns to the pre-smoking value after 1±2 h [11]. It can be considered that with chronicity, this mechanism may be hampered.

The present study showed negative correlation of salivary TAC with duration of diabetes. As the diabetic duration increases, there is an increase in the production of free radicals which would probably deplete the TAC as has been reported in previous literature [2]. However, the correlation between salivary TAC and HbA1c level was not statistically significant. A study conducted by Zloczower et al. has shown an overall increase in salivary antioxidant in DM patients. This study included estimation of selected individual antioxidants like peroxidase, superoxide dismutase and uric acid [12]. But, it has been suggested that antioxidant systems act in concert rather than alone and investigation of individual antioxidant may be misleading and measurement of individual antioxidant may be less representative of the whole antioxidant status [9].

Smoking as well as DM is implicated as the causative factors for periodontitis. Chronic generalized periodontitis is one of the main oral manifestations in diabetics. Mean Russell's index value for diabetic smokers was high amongst all the groups and was very highly significant. But when compared to diabetic nonsmokers, nondiabetic smokers had higher index value, suggestive of the major role played by smoking in the pathogenesis of periodontitis. In the present study, a negative correlation was observed between Russell's index and salivary TAC consistent with the cohort study conducted by Sculley et al. that periodontal disease is associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity [13].

In the present study, mean salivary TAC level is lowered in both groups of smokers, significantly more so in diabetic smokers. Salivary TAC revealed negative correlation with duration of smoking in both the groups, though it was statistically not significant probably because of smaller sample size. Compared to non-diabetics, the diabetic subjects had lower salivary TAC. Similar study conducted by Boemi et al. to analyze the association of smoking with the antioxidant enzyme paraoxonase in the serum of Type 2 diabetic patients, a deficiency of paraoxonase was consistently observed. Smoking was associated with a further, significant decrease in serum concentrations and activities of this enzyme [14]. DM as well as smoking is individually known to deplete the TAC. In diabetic smokers synergistic effect can be observed between DM and smoking in depleting the salivary TAC.

Salivary analysis is simple, noninvasive and can easily be obtained from the ambulatory patient. Several studies have been done to assess whether saliva can be used as a diagnostic tool. However, it harbors its own limitations. Collection of saliva must be performed in a meticulous manner and the saliva must be preserved in proper conditions for further analysis, in order to avoid various technique-related effects. Saliva, although considered to be a representative of serum, is influenced by other local factors affecting oral cavity. Serum glucose and HbAlc are still considered the proven gold standard for diagnosis and monitoring in diabetes. Though the objectives of the study were achieved, correlations were not statically significantly probably because of smaller sample size. The study has the confounding factor of self-reporting of smoking status and duration of diabetes and the medications taken for the same. However, this study adds to the existing literature as it is the first to examine salivary total antioxidant status in diabetic smoker patients. From the clinical standpoint, it may be reasonable to conclude that salivary TAC can be a useful marker to assess the oxidative stress, as an indicator of progression of DM and to monitor the efficacy of treatment. Further, it can be used to motivate diabetic smokers to abstain from the habit.

In conclusion, the findings of this study strengthen the need for extensive research to identify the potential relationship between depletion of TAC in saliva of diabetics and smokers. Further research should be devoted to the specific diabetes mellitus-related adverse effects and possible benefits after supplementation with antioxidant supplements. However this issue still remains open and needs to be further investigated and confirmed.

Conflicts of interest The authors declare that they have no conflict of interest.

## References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2011;34:S62–9.
2. Opara EC, Abdel-Rahman E, Soliman S, Kamel WA, Souka S, Lowe JE, et al. Depletion of total antioxidant capacity in type 2 diabetes. Metabolism. 1999;48:1414–7.
3. Reznick AZ, Shehadeh N, Shafir Y, Nagler RM. Free radicals related effects and antioxidants in saliva and serum of adolescents with type 1 diabetes mellitus. Arch Oral Biol. 2006;51:640–8.
4. Garg N, Singh R, Dixit J, Jain A, Tewari V. Levels of lipid peroxides and antioxidants in smokers and nonsmokers. J Periodontal Res. 2006;41:405–10.
5. Kanehira T, Shibata K, Kashiwazaki H, Inoue N, Morita M. Comparison of antioxidant enzymes in saliva of elderly smokers and non-smokers. Gerodontology. 2006;23:38–42.
6. Sherwin RS. Diabetes mellitus, Cecil textbook of medicine. 22nd ed. Philadelphia: Saunders; 1996. p. 1258–60.
7. Peter S. Essentials of preventive and community dentistry. 2nd ed. New Delhi: Arya (medi) Publishing House; 2003. p. 159–61.
8. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin e. Anal Biochem. 1999;269:337–41.

9. Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. J Clin Periodontol. 2002;29:189–94.
10. Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ. Characterization of the differentiated antioxidant profile of human saliva. Free Radic Biol Med. 2002;32:268–77.
11. Zappacosta B, Persichilli S, De Sole P, Mordente A, Giardina B. Effect of smoking one cigarette on antioxidant metabolites in the saliva of healthy smokers. Arch Oral Biol. 1999;44:485–8.
12. Zloczower M, Reznick AZ, Zouby RO, Nagler RM. Relationship of flow rate, uric acid, peroxidase, and superoxide dismutase activity levels with complications in diabetic patients: can saliva be used to diagnose diabetes? Antioxid Redox Signal. 2007;9:765–73.
13. Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. Clin Sci. 2003;105:167–72.
14. Boemi M, Sirolla C, Testa R, Cenerelli S, Fumelli P, James RW. Smoking is associated with reduced serum levels of the antioxidant enzyme, paraoxonase, in type 2 diabetic patients. Diabet Med. 2004;21:423–7.