EUROPEAN ACADEMIC RESEARCH
Vol. VIII, Issue 10/ January 2021

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Association of PPAR-γ gene Pro12Ala Polymorphism with type 2 diabetes mellitus

MOHAMMAD RASEL MIAH
Senior Research Officer, TRIMAN Nutriceuticals Limited, Bangladesh
Md. ABDUL AWAL
Lab In-Charge, Ibn Sina Medical College Hospital, Dhaka, Bangladesh
ASOK CHANDRA DEBNATH
Senior Scientific Officer (SSO) & Lab In-charge
ZH Sikder Women's Medical College & Hospital, Bangladesh
BHABASINDHU TALUKDER
Executive, Microbiology, Medimet Pharmaceuticals Ltd., Bangladesh
Md. BADIUZZAMAN
Medical Technologist, MH Samorita Medical College & Hospital, Bangladesh
Md. JAMAN MIAH
Scientific Officer, ZH Sikder Women's Medical College & Hospital, Bangladesh
MOHAMMAD AL-AMIN
Senior Research Officer, TRIMAN Nutriceuticals Limited, Bangladesh
SUBAL CHANDRA ROY
Scientific Officer (RT-PCR Lab)
ZH Sikder Women's Medical College & Hospital, Bangladesh
Md. ABUL HOSSAIN[1]
Managing Director and Chief Researcher, TRIMAN Nutriceuticals Limited, Bangladesh

Abstract

Background: *Peroxisome proliferator activated receptor (PPAR) gene, a member of sub family of transcription factors, found to be associated with expression of number of genes involved in nutrient metabolism. PPAR gamma, one of the subtypes of the PPAR molecule suggested playing important role in adipocyte differentiation and gene expression. PPARG gene P12A polymorphism found to be associated with obesity, insulin resistance and T2DM in different populations.*

Objective: *To investigate the role of PPARG gene P12A polymorphism in pathogenesis of T2DM subjects.*

[1] Corresponding author: mahsujon71@gmail.com

Methods: *Blood samples were collected from 99 newly diagnosed T2DM and 91 healthy controls. Glucose-oxidase and enzyme linked immunosorbant assay (ELISA) was used. DNA was extracted using FAVORGEN spin column blood DNA kit. PPARG gene Pro12Ala polymorphism was analyzed by PCR-RFLP method using restriction enzyme HaeIII.*

Results: *Mean ± SD age of the T2DM subjects was significantly higher compared to the controls (p=0.001). BMI (Kg/m²) and insulin level in the two groups did not show statistical difference. Insulin-glucose ratio in the T2DM was significantly lower (p=0.007) compared to the controls. Genotype frequencies of the P12A polymorphic allele in control were 0.835, 0.165 and 0 for homozygous wild, heterozygous variant and homozygous variant respectively. In T2DM subjects, the frequencies were 0.778, 0.202 and 0.02 respectively. The genotype frequency distribution in the two groups did not show statistical significant association (p=0.331).*

Conclusion: *PPARG gene Pro12Ala polymorphism is not associated with the development of T2DM subjects of Bangladeshi origin.*

Keywords: PPAR-γ gene, Pro12Ala Polymorphism, IDDM, NIDDM, T2DM, MRDM

INTRODUCTION:

Diabetes is the heterogeneous group of disorders characterized by persistent hyperglycemia, polyuria and polydipsia. Basic defects in the pathogenesis of diabetes mellitus found to be defects in pancreatic ß-cell insulin secretion and / or insulin resistance (Reaven, 1998). National Diabetic Data Group (NDDG, 1979) first suggested a comprehensive classification of diabetes: Two main classes in this classification were insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), which has subsequently been endorsed by WHO sponsored Study Group with minor modification (WHO, 1980). Later in 1985 WHO proposed renewed classification which included malnutrition related diabetes

mellitus (MRDM) as a third major class of diabetes mellitus. In 1997 American Diabetes Association (ADA) proposed a revised classification of diabetes and subsequently WHO endorsed a classification that is widely regarded. More than 90% of all diabetics all over the world are of T2DM. However, in American Indian and South Pacific islanders, T2DM found to be the only form of the diabetes. Overall prevalence of diabetes varies between 15-20% in the world. The highest prevalence of T2DM (50%) was found among Pima Indians (41%; male 40% and female 42%) and a very low (0-1.4%; male 0% and female 1.4%) was observed among the Mapuches population in Chile and the prevalence was almost nil in rural and peri-rural population of Papua New Guinea (WHO 1994).

Diabetes is affecting the human health at an epidemic form. However, there is heterogeneity in its prevalence with regards to ethnicity. Earlier estimate has predicted that number of total diabetic would be 366 million in 2025 (King *et al.,* 1998). Later on Wild et al. (2004) have shown that in 2000 there were 250 million diabetics in the world and projected to be 500 million in 2030 which is much higher than the prior estimate. This surge in number of diabetes found to affect people mostly in the developing countries (IDF 2012). Over all prevalence of T2DM found to be about 8.3% which however varies in different race and regions (IDF 2012). In North American and Caribbean, the prevalence of T2DM is 10.5% whereas in Europe 6.7 %, Middle East and North Africa 4.3%, and South East Asia 8.7% (IDF 2012). The growth in total number of diabetic patients in the developing and developed countries which has clearly depicted the threat posed by fast increasing number of diabetic patients in world. It has been shown that in 2030 there will around 11.1 million diabetic patients in Bangladesh. Prevalence of diabetes in Bangladesh is also shown to be increasing like other countries. In rural Bangladeshi population it is found to be 4.3% (Sayeed *et al.,* 2003). However, impaired fasting glucose was 12.4% of the study subject. Prevalence of T2DM among Chakma population was reported to be 6.66% by the same group (Sayeed *et al* 2003). In another study T2DM prevalence was reported as 6.8% (Rahim *et al* 2007). These reports clearly suggest a rising trends of T2DM in the Bangladeshi population. Bhowmik et al (2013) has demonstrated that prevalence of T2DM in rural

population is 7.9% which is very close to that reported by Rahim et al (2008).

Three PPARs sub types are so far been well characterized; PPAR-alpha, PPAR- gamma, PPAR-delta. PPAR-gamma (PPARG) found to be involved in adipocyte differentiation and gene encoding the molecule located or chromosome 3p25 in human (Braissant *et al* 1996; Greene *et al* 1995; Beamer *et at*., 1997). PPARG found to have three isoforms – PPARG1, PPARG2, PPARG 3. Genes encoding PPARG isoforms are shown in the schematic diagram. These targets suggest that PPARγ influences lipid and glucose metabolism within adipocytes, as well as signaling from adipose tissue. PPARγ agonists also reduce 11ß-hydroxysteroid dehydrogenase 1 expression (11ß-HSD1) in adipose tissue. 11ß-HSD1 converts inactive cortisone to bioactive corticosterone within tissues, and over expression of this gene in mouse adipose tissue induces a 'Cushingoid state' in mice, with central Obesity, insulin resistance and hypertension (Savage, 2005). Hence, suppression of 11ß-HSD1 by PPARγ agonists might contribute to their insulin sensitizing properties.

METHODS:

A case-control study was conducted in the laboratory of the Department of Physiology & Molecular Biology, Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh. Sample size was 190 (blood specimen). 91 blood samples from healthy subjects as control and 99 from T2DM patients as case. The ethical permission was taken prior from the ethical review committee of Bangladesh Diabetic Somitee. The details explained to the subjects and written consent was taken before collection of samples. The biochemical parameters were analyzed. PPARG P12A genotype was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Statistical analysis was performed using Statistical Package for Social Science (SPSS) software for Windows version 17. Data were expressed as mean±SD, frequency (number) as appropriate. The statistical difference between two groups was assessed by unpaired Student's 't' test. A two-tailed p value of <0.05 was considered statistically significant.

RESULTS:

Table 1: Age, FG, AG, Insulin BMI and Insulin-Glucose ratio of the subjects (n=190)

Mean ± SD of age in years of the control and T2DM subjects were 40.2±11.0 and 44.2±9.6 respectively. Mean age of the T2DM group was significantly higher (p<0.001) compared to the controls. Mean ± SD BMI (Kg/m^2) of the control and T2DM subjects were 24.54±4.62 and 25.22±3.48 respectively. Mean BMI value in the two groups did not show statistically significant difference (p=0.281). Mean ± SD SBP of the control and T2DM subjects were 113±13 and 114±15 respectively. Mean SBP was normal in both two groups.

Variables	Control (n=91)	T2DM (n=99)	t/p value
Age (year)	40.2±11.0	44.2±9.6	-2600
BMI (Kg/m^2)	24.5±4.6	25.2±3.5	-3.84341637
SBP (mmHg)	113±13	114±15	-1.028419183
DBP (mmHg)	75±8	78±11	-28
FG (mmol/L)	5.1±0.5	11.0±3.8	-13300
2hAG (mmol/L)	6.5±1.1	18.6±5.7	-14200
Insulin (μIU/ml)	12.3±5.9	15.5±8.2	-21.25
Ins-Glu ratio	2.5±1.16	1.6±0.91	2.76/0.007

The results of two groups did not show statistically significant difference (p=0.563). The Mean ± SD of both control and case did not show statistically significant difference (p=0.066). Regarding the fasting glucose (mmol/L) level of the control and T2DM subjects, the mean with ± SD were 5.1±1.8 and 11.0±3.8 respectively. That showed the fasting glucose value in T2DM group was higher (p<0.001) than control. Mean ± SD of 2 hours blood glucose (mmol/L) in T2DM group was significantly higher (p<0.001) than control. Mean serum Insulin did not show statistically significant difference (p=0.080). Mean (±SD) Insulin-Glucose ratio of the control and T2DM subjects was 2.5±1.16 and 1.64±0.91 respectively. Mean Insulin-Glucose ratio was significantly higher in controls compared to the T2DM group controls (p=0.007).

Table 2: Distribution of frequency of PPARG gene Pro12Ala genotype and allele of control and T2DM subjects (n=190)

Genotype frequencies of the PPARG gene Pro12Ala polymorphic marker allele in control were 0.835, 0.165 and 0 for homozygous wild, heterozygous variant (Ht) and Homozygous variant (Hz) respectively. In T2DM subjects, the frequencies were 0.778, 0.202 and 0.02 respectively. The genotype frequency distribution in the two groups did not show statistically significant association (p=0.331).

Variable	Control (n=91)	T2DM (n=99)	Total (n=190)
Genotype			
Wild type (CC)	0.835 (76)	0.778 (77)	0.805 (153)
Ht variant (CG)	0.165 (15)	0.202 (20)	0.184 (35)
Hz variant (GG)	0 (0)	0.02 (2)	0.011 (2)
		χ^2=2.39	p=0.303
Allele frequency			
C	0.918	0.879	0.897
G	0.083	0.121	0.103
		χ^2=0.995	p=0.331

Chi squared (χ^2) test was performed to calculate statistical association. P value of <0.05 was considered as statistically significant.

Table 3: Hardy Weinberg Equilibrium of PPARG gene (n=190)

Hardy Weinberg Equilibrium for PPARG gene P12A variant allele among the total study subjects was tested. No significant association for equilibrium was observed (p=0.999).

N	Observed CC	CG	GG	χ^2/p
Total				
190	153	35	2	1.071/0.999
	P	0.897		
	Q	0.103		

Chi square (χ^2) test was performed to calculate statistical association. CC, Homozygous wild; CG, Heterozygous variant GG, Homozygous variant.

Table 4: Distribution of clinico-biochemical variables in the controls and T2DM with wild and variant genotype (n=190)

Mean (±SD) Age (years) in controls with candidate gene wild and variant allele was 39.8±10.7 and 42.6±12.5 respectively. The value did not show any statistically significant difference (p=0.431). In T2DM subjects the value was 43.9±9.9and 45.3 ±8.2 respectively which did not show any statistically significant difference (p=0.574). Mean (±SD) BMI in controls candidate gene with wild and variant allele was 24.5±4.6 and 24.5±5.1 respectively. The value did not show any statistically significant difference (p=0.955). In T2DM subjects the value was 25.1±3.4 and 25.7±3.8 respectively which did not show any statistically significant difference (p=0.472). Mean (±SD) SBP in controls with candidate gene wild and variant allele was 113±13 and 112±14 respectively. The value did not show any statistically significant difference (p=0.810). In T2DM subjects the value was 114±15and 114±15 respectively which did not show any statistically significant difference (p=0.918). Mean (±SD) DBP in controls with candidate gene wild and variant allele was 74.6±8.4 and 76.6±16.2 respectively. The value did not show any statistically significant difference (p=0.663). In T2DM subjects the value was 77.8±9.2 and 76.6±16.2 respectively which did not show any statistically significant difference (p=0.663). Mean (±SD) fasting glucose (mmol/L) level in controls with candidate gene wild and variant allele was 11.2±3.8 and 10.2±3.8 respectively. The value did not show any statistically significant difference (p=0.292). In T2DM subjects the value was 11.2±3.8 and 10.1±3.8 respectively which did not show any statistically significant difference. Mean (±SD) A.G. in controls with candidate gene wild and variant allele was 18.6±5.6 and 18.5±5.6 respectively. The value did not show any statistically significant difference (p=0.987). In T2DM subjects the value was 18.6±5.6 and 18.5±5.6 respectively which did not show any statistically significant difference.

Clinico-biochemical variables in the controls and T2DM subjects with wild and variant genotype			
Variables	Control		
	Wild (67)	Variant (11)	t/p
Age (yrs)	39.8±10.7	42.6±12.5	-1.837587007
BMI (Kg/m^2)	24.5±4.6	24.5±5.1	0.0570/.955
SBP (mHg)	113.1±13.3	112±14	0.241/0.810
DBP (mmHg)	74.6±8.4	75.6±8.1	0.437/0.663
Fasting (mmol/L)	11.2±3.8	10.2±3.8	1.059/0.292
2 h A G (mmol/L)	18.6±5.6	18.5±5.6	0.017/0.987
Ins-glu ratio	2.51±1.18	2.51±1.10	0.013/0.989
T2DM			
	Wild (77)	Variant (21)	
Age (yrs)	43.9±9.9	45.3±8.2	-0.984320557
BMI (Kg/m^2)	25.1±3.4	25.7±3.8	-1.531779661
SBP (mmHg)	114±15	114±15	0.103/0.918
DBP (mmHg)	77.8±9.2	76.6±16.2	0.437/0.663
Fasting glucose (mmol/L)	11.2±3.8	10.1±3.8	1.059/0.292
2 hours After (mmol/L)	18.6±5.6	18.5±5.6	0.017/0.987
Ins-Glu ratio	1.53±0.962	1.83±0.861	-1.209039548

DISCUSSION:

In the present study PPARG gene Pro12Ala variant was determined in 99 newly diagnosed type 2 diabetes mellitus subjects and 91 healthy volunteers. PPARG gene P12A has been found to modulate gene transcriptional activity (Deeb *et al.* 1998) and associated with altered insulin sensitivity (EK 2001). Among the reported PPARG gene various P12A has been widely studied**.** It has been reported that Ala12 variant is associated with decreased body mass index (Bouhaha *et al.,* 2008; Rosado *et al.,* 2007; Damcott *et al.,* 2004). The genotype frequency distribution in the two group did not showed significant association (p= 0.331). Allele frequencies of the polymorphic marker also did not significant association (p = 0.303). Unbiased collection of subjects in the present study was confirmed by lack of deviation of Hardy-Weinberg Equilibrium from the allele (P12A) in the study subjects. The variant 12Ala frequency was around 11% in the present study which is almost similar to that of European and European derived population also that (Mattevi *et al.,* 2007, Soskic *et al*., 2010 and Costa *et al*., 2009), which support the findings of the present study. Serum insulin level in T2DM group was higher but did not show statistically significant difference (p=0.080) but working of insulin-glucose ratio unmasked the significant level of secretory defect (p=0.007) in the T2DM group. The present data reconfirmed the previous findings that secretory defect present along

with insulin resistance present in T2DM of Bangladeshi population (Roy *et al.,* 2007).

CONCLUSIONS:

PPARG gene Pro12Ala polymorphism is not associated with the development of T2DM subjects of Bangladeshi origin.

REFERENCES:

1. Ahmed AM. History of diabetes mellitus. Saudi Med J 2002; **23(4):** 373378.
2. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C (2000). The common PPARgamma P12A polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet. **26**: 76-80.
3. American Diabetes Association (1997). Report of the Expert committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care **20**: 1183-1197.
4. American Diabetes Association (2013). Diagnosis and Classification of Diabetes Mellitus. Diabetes Care **36(1)**: 67-73.
5. American Diabetes Association (ADA) (1999). Diabetes care **22(1)**: 66-9.
6. Beamer BA, Negri C, Yen CJ, Gavrilova O, Rumberger JM, Durcan MJ, Yarnall DP, Hawkins AL, Griffin CA, Burns DK, Roth J, Reitman M, Shuldiner AR (1997). Chromosomal localization and partial genomic structure of the human peroxisome proliferator activated receptor-gamma (hPPAR gamma) gene. Biochem Biophys Res Commun **233**: 756–759.
7. Bouhaha R, Meyre D, Kamoun HA, Ennafaa H, Vaillant E, Sassi R (2008). Effect of ENPP1/PC-1-K121Q and PPARgamma-Pro12Ala polymorphisms on the genetic susceptibility to T2D in the Tunisian population. Diabetes Res Clin Pract **81**: 278-283.
8. Braissant O, Foufelle F, Scotto C, Dauça M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology 1996; **137**: 354-66
9. Damcott CM, Moffett SP, Feingold E, Barmada MM, Marshall JA, Hamman RF, Ferrell RE, (2004). Genetic variation in fatty acid-binding protein-4 and peroxisome proliferator-activated receptor γ interactively influence insulin sensitivity and body composition in males, Metabolism Clinical Experiment **53**: 303–309.
10. Defronzo RA (1997). Pathogenesis of type 2 diabetes: Metabolic and molecular implications for identifying diabetes genes. Diabetes Review **5**: 177-269.
11. DeFronzo RA (1998). Pathogenesis of type 2 diabetes: metabolic and molecular implication for identifying diabetes genes. In: American Diabetes Association, eds (Anmual Review Of Diabetes), Alexandria VA: American Diabetes, pp. 1-93.

12. Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K, Drivsholm T, Berglund L, Hansen T, Lithell H, Pedersen O (2001). Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 (PPAR-gamma2) gene in relation to insulin sensitivity among glucose tolerant Caucasians. Diabetologia **44**: 1170 –1176.
13. Fornage M, Jacobs DR, Steffes MW, Gross MD, Bray MS, Schreiner PJ (2005). Inverse effects of the PPAR(gamma)2 Pro12Ala polymorphism on measures of adiposity over 15 years in African Americans and whites. The CARDIA study. Metabolism: Clinical and Experimental **54**: 910-917.
14. Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K et al. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. Gene Expr1995; **4**: 281-299.
15. Group DECODA study Group: Comparison of the fasting and the 2-h glucose criteria for diabetes in different Asian cohort. Diabetologia. 2000;**43:**1470-1475
16. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Kimura S, Ito C, Kadowaki T (2000). The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. Biochem Biophys Res Commun **271***: 212 –216*.
17. Hara M, Alcoser SY, Qaadir A, Beiswenger KK, Cox NJ, Ehrmann DA. Insulin resistance is attenuated in women with polycystic ovary syndrome with the Pro12Ala polymorphism in the PPAR-gamma gene. J Clin Endocr Metab 2002; **87**: 772-775.
18. Hollenberg AN, Susulic VS, Madura JP, Zhang B, Moller DE, Tontonoz P, Sarraf P, Spiegelman BM, Lowell BB (1997). Functional antagonism between CCAAT/enhancer binding protein-a and peroxisome proliferator-activated receptor-G on the leptin promoter. J. Biol. Chem. **272**: 5283–5290.
19. IDF Diabetes Atlas. International Diabetes Federation. 5th Ed. 2012.
20. IDF Diabetes Atlas. International Diabetes Federation (IDF). 6th Edition 2013.
21. King H, Aubert RE, HermanWH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998; **21**: 1414-1431
22. Mahtani MM, Widen E, Lehto M, Thomas J, McCarthy M, Brayer J et al. Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nat Genet 1996; **14**:90–94.
23. Mattevi VS, Zembrzuski VM, and Hutz MH (2007). Effects of a PPARG gene variant on obesity characteristics in Brazil. Brazilian Journal of Medical and Biological Research **40**: 927–932.
24. Meirhaeghe A, Fajas L, Helbecque N, Cottel D, Lebel P, Dallongeville J, Deeb S, Auwerx J, Amouyel P (1998). A genetic polymorphism of the peroxisome proliferator-activated receptor gamma gene influences plasma leptin levels in obese humans. Hum Mol Genet **7**: 435–440.
25. Oh EY, Min KM, Chung JH, Min Y-K, Lee M-S, Kim KW et al Significance of pro12ala mutation in peroxisome proliferator-activated receptor-gamma2 in Korean diabetic and obese subjects. J ClinEndocrMetab 2000; **85**: 1801-1804.

26. Rahman M (2008). Associationof INS VNTR variants with type 2 diabetes mellitus in overweight to obese subjects in a Bangladeshi population. MS Thesis, BRAC University.
27. Rosado EL, Bressan J, Martins MF, Cecon PR, and Martinez JA (2007). Polymorphism in the PPARgamma2 and beta2- adrenergic genes and diet lipid effects on body composition, energy expenditure and eating behavior of obese women. Appetite **49**: 635–643.
28. Savage DB (2005). PPARG as a metabolic regulator: insights from genomics and Pharmacology **7**: 1-16.
29. Sayeed MA, Akter KP, Abdul LZ, Keramat ASM, Banu A (2003). Diabetes and impaired fasting glycemia in a rural population of Bangladesh. Diab Care 26: 1034-1039.
30. Sayeed MA, Mahtab H, Khanam PA, Latif ZA, Banu A, Khan AK (2007). Prevalence of diabetes and impaired fasting glucose in urban population of Bangladesh. Bangladesh Med Res Counc Bull **33**: 1-12.
31. Stefan N, Fritsche A, Haring H, Stumvoll M (2001). Effect of experimental elevation of free fatty acids on insulin secretion and insulin sensitivity in healthy carriers of the Pro12Ala polymorphism of the peroxisome proliferatoractivated receptor-γ2 gene. Diabetes **50**: 1143– 1148.
32. Tontonoz P, Hu E, Graves R, Budavari A, Spiegelman B (1994). mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev **8**:1224–1234.
33. Trinder P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. **6**: 24-25.
34. Turner NC, Clapham JC. Insulin resistance, impared glucose tolerance and non-insulin dependent diabetes, Pathogenic mechanism and treatment: current status and therapeutic possibilities. Prog Drug Res. 1998:**51**:33-94
35. Vidal-Puig AJ, Considine RV, Jimenez-Liñan M, Werman A, Pories WJ, Caro JF, Flier JS. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 1997; **99**: 2416–2422.
36. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes. Diabetes Care 2004; **27**: 1047-1053.
37. World Health Organization (1999). Definition, Diagnosis, and Classification of Diabetes Mellitus and it's complications: Report of a WHO Consultation. Part 1: Diagnosis, and Classification of Diabetes Mellitus. Geneva, World Health Organization.
38. World Health Organization, (2011). Global status report on noncommunicable diseases 2010. Geneva.
39. Yamamoto Y, Hirose H, Miyashita K, Nishikai K, Saito I, Taniyama M et al. PPAR (gamma) 2 gene Pro12Ala polymorphism may influence serum level of an adipocyte-derived protein, adiponectin, in the Japanese population. *Metabolism* 2002; **51**: 1407-9.
40. Zimmet P, Alberti KG, Shaw J (2001). Global and societal implications of the diabetes epidemic. Nature **414 (6865)**: 782-787.