November 2017

Association of vitamin D binding protein polymorphism with risk of type 2 diabetes mellitus in a Pakistani urban population: A case control study

Khalida Iqbal
Aga Khan University

Najmul Islam
Aga Khan University, najmul.islam@aku.edu

Iqbal Azam
Aga Khan University, iqbal.azam@aku.edu

Ali Asghar
Aga Khan University

Naseema Mehboobali
Aga Khan University, naseema.mehboobali@aku.edu

See next page for additional authors

Authors
Khalida Iqbal, Najmul Islam, Iqbal Azam, Ali Asghar, Naseema Mehboobali, and Mohammad Perwaiz Iqbal

ORIGINAL ARTICLE

Association of Vitamin D binding protein polymorphism with risk of type 2 diabetes mellitus in a Pakistani urban population: A case control study

Khalida Iqbal,[1] Najmul Islam,[2] Iqbal Azam,[3] Ali Asghar,[4] Naseema Mehboobali,[5] Mohammad Perwaiz Iqbal[6]

Abstract

Objective: To assess if genotypes/diplotypes of vitamin D binding protein have any association with type 2 diabetes mellitus.

Methods: This case-control study was conducted from January 2013 to July 2015 at the endocrinology clinics of the Aga Khan University Hospital, Karachi, and comprised adult patients with type 2 diabetes and their age- and gender-matched healthy controls. Venous blood was obtained and assessed for serum/plasma 25 hydroxyvitamin D, parathyroid hormone, calcium, alkaline phosphatase and creatinine. Deoxyribonucleic acid was isolated and genotyping was done by polymerase chain reaction-restriction fragment length polymorphism procedures.

Results: Of the 330 participants, there were 165(50%) cases and as many controls. There were 116(70.3%) males and 49(29.7%) females in each group. The mean age of the patients was 48.82±9.23 years and that of the controls was 46.27±8.77 years (range: 22-70 years) (p=0.010) Mean serum concentration of 25 hydroxy vitamin D was significantly higher among the patients compared to the controls (p<0.001), but not significantly different by genotypes or diplotypes (p>0.05). Multiple conditional logistic regression revealed an association of group-specific 1-2 genotype with patients when adjusted for age, body mass index, and serum levels of 25 hydroxy vitamin D with matched adjusted odds ratio (95% confidence interval) being 3.1(1.22-7.88).

Conclusion: Group-specific 1-2 genotype of vitamin D binding protein gene was associated with the risk of type 2 diabetes.

Keywords: Vitamin D binding proteins, Gene polymorphism, Diabetes mellitus, Gc protein. (JPMA 67: 1658; 2017)

Introduction

Recent reports have shown that vitamin D deficiency is widespread in both developed and developing countries. Nearly 1 billion people around the world appear to be suffering from vitamin D deficiency or insufficiency.[1] The problem is more serious in Pakistan where 86% of the adults have been reported to be suffering from vitamin D deficiency with levels of 25 hydroxy(OH) vitamin D3 [25(OH)D] less than 20 ng/ml,[2] while 97% of females in a community based-population in Karachi were found to have insufficient vitamin D with levels of 25(OH)D less than 30 ng/ml.[3] There is some evidence to suggest that there is a relationship of plasma levels of 25(OH)D and phenotypes of vitamin D binding protein (VDBP) which is a major transporter of vitamin D3 and its metabolites and is also known as a group-specific (Gc) protein.[4] This is indicative of a role of VDBP gene polymorphism in determining serum concentrations of vitamin D3 in certain populations.[4,5]

Type 2 diabetes mellitus (T2DM) is one of the major threats to human health in Pakistan. The prevalence of T2DM in Pakistan has been reported to be 6% in men and 3.5% in women in urban areas,[6] and because of changing lifestyle, it has been estimated that by the year 2035 Pakistan will be ranked 8th in the world in terms of the number of diabetic patients.[7]

A few recent studies have demonstrated an association of serum levels of 25(OH)D and risk of T2DM,[8,9] suggesting that the VDBP polymorphism could be playing some role in the pathogenesis of T2DM.

Few reports show association between the VDBP polymorphism and T2DM. The association has been found mostly in non-Caucasians,[10] while no relationship has been reported in European White populations.[11,12]

To the best of our knowledge, no study has been carried out to investigate the association of the VDBP polymorphism with T2DM in a South Asian population. The current study was planned to find out whether genotypes/diplotypes of VDBP have any association with T2DM, and to investigate any relationship of vitamin D deficiency with T2DM.

Subjects and Methods

This case-control study was conducted from January 2013

[1,5,6]Department of Biological and Biomedical Sciences, [2,4]Department of Medicine, [3]Department of Community Health Sciences, Aga Khan University, Karachi, Pakistan.

Correspondence: Mohammad Perwaiz Iqbal. Email: perwaiz.iqbal@aku.edu

to July 2015 at the endocrinology clinics of the Aga Khan University Hospital (AKUH), Karachi, and comprised adult patients with T2DM. Similar number of gender and age-matched (within 5 years) healthy controls were also recruited from the personnel of AKUH and other 3 healthcare institutions in Karachi. The sample in each group (T2DM patients and healthy controls) would achieve a power of 80% and was based on the assumption that the frequency of any of the 6 diplotypes among healthy controls would range between 25% and 65%, anticipated matched odds ratio (OR) of 2.25 or more, correlation coefficient of exposure between matched case-control subjects = 0.2 and $\alpha = 0.05$.[10] Patients diagnosed with T2DM on the basis of guidelines set by the International Diabetes Federation[13] (fasting serum glucose > 126mg/dl; clinical history), who were not taking vitamin D supplements during the last 6 months, were not suffering from tuberculosis, or liver disease, or uraemia or cancer, and were not pregnant were included. Similarly, healthy controls with no history of T2DM and who were not having fasting serum glucose >110mg/dl were included. They had not been taking vitamin D supplements during the preceding 6 months and were not suffering from any of the above-mentioned diseases or conditions. Demographic characteristics of the two groups were collected using a pre-structured questionnaire after the study protocol was approved by the institutional ethics review committee.

Then, 10ml of venous blood (with at least 4 hours of fasting) was collected and transferred in equal volumes to a tube containing ethylenediaminetetraacetic acid (EDTA) and another with no anti-coagulant. Plasma/serum was obtained and analysed for 25(OH)D, parathyroid hormone (PTH), calcium, alkaline phosphatase (ALP), phosphate and creatinine using kits following manufacturer's instructions (Roche Diagnostics, Indianapolis, Indiana, United States). The minimum detection limits of 25(OH)D, PTH, calcium, ALP, phosphate and creatinine were 4 ng/ml, 1.2 pg/ml, 0.4 mg/dl, 3 U/l, 0.31 mg/dl and 0.2 mg/dl, respectively.

Genomic deoxyribonucleic acid (DNA) was extracted by salting out method. VDBP(Gc) gene was amplified by polymerase chain reaction (PCR) using the forward primer (5 'TAAT-GAGCAAATGAAAGAAG3') and reverse primer (5 'TGAGTAGATTGGAGTGCATAC3') as described by Ito et al.[14] The amplified region is 462 base-pairs (bp) and it covers point mutations rs7041and rs4588. A typical PCR mixture contained 100ng DNA, 1xPCR buffer, 200μM deoxyribonucleotides, 1.5mM magnesium chloride ($MgCl_2$), 1U Taq polymerase (Promega, Wisconsin, United States) and 10 pM each of the primers in a total volume of 10 μl. The amplification conditions were 94°C for 30sec, 56°C for 30sec, 72°C for 30sec for 35 cycles.

Genotypes were identified by restriction fragment length polymorphism (RFLP). The PCR product was digested separately by HaeIII and StyI-HFTM (New England Biolabs Inc., Ipswich, Massachusetts) at 37°C for 6 hours. Gc1F remained uncut by both enzymes. GcIS was cut by HaeIII only, resulting in 295bp and 167bp bands. Gc2 was digested by StyI-HFTM into 302bp and 156bp bands. The digested products were resolved on 3.0% agarose gel containing 0.2 μg/ml ethidium bromide.

SPSS 19 was used for data analysis. Cross tabulations of Gc gene (Gc genotypes, diplotypes and alleles) and the type of subjects (cases and controls) were carried out to observe the frequency distribution of Gc genotypes, diplotypes and alleles in the two groups. Chi-square goodness of fit test of deviation from Hardy-Weinberg Equilibrium (HWE) was employed using trinomial equation.[15] Mean difference of 25(OH)D with respect toGc genotypes and Gc diplotypes in the patients and controls were compared using two-way analysis of variance (ANOVA). Mean ± standard deviation (SD) values of other quantitative variables such as age, body mass index (BMI), 25(OH)D, PTH, calcium, alkaline phosphatase, phosphate, and creatinine in the two groups were compared using paired samples t-test. McNemar's test was used for assessing the significance of qualitative variables, such as smoking, use of smokeless tobacco, sunlight exposure, use of statins, hypertension, ischaemic heart disease (IHD), hypercholesterolaemia, use of cooking oil/fat, engagement in exercise, extent of physical activity and vitamin D status. The relationship between Gc genotypes and T2DM separately and when controlling for other variables were assessed through simple and multiple conditional logistic regression and reported as crude and adjusted matched odds ratios with 95% confidence interval (CI). A $p<0.05$ was considered significant.

Results

Of the 330 participants, there were 165(50%) in each of the two groups. There were 116(70.3%) males and 49(29.7%) females in each group. The mean age of the patients was 48.82±9.23 years and that of the controls was 46.27±8.77 years (range: 22-70 years) ($p=0.010$). Mean values of age and BMI and serum/plasma concentrations of 25(OH)D and alkaline phosphatase were significantly different in T2DM patients compared to the controls ($p<0.05$).

Proportions of statin users, hypertensives and those having IHD and hypercholesterolaemia were significantly higher in the patients compared to the controls ($p<0.05$). Diabetics were more regular in physical exercise

Table-1: Demographic and clinical characteristics of type 2 diabetes mellitus (DM) patients and healthy controls (Mean ± SD).

Variable	Type 2 DM (n=165)	Healthy controls(n=165)	p value*
Age (Years)	48.82±9.23	46.27±8.77	0.010
BMI	28.96±4.13	25.24±3.45	<0.001
25(OH)D (ng/ml)	21.67±10.7	16.70±11.3	<0.001
PTH (pg/ml)	38.96±16.77	37.35±24.9	0.395
Calcium (mg/dl)	9.37±1.66	9.40±1.30	0.850
Alkaline phosphatase (U/l)	65.26±24.67	76.56±37.39	0.002
Phosphate (mg/dl)	3.62±0.63	3.68±0.64	0.395
Creatinine (mg/dl)	0.87±0.21	0.85±0.18	0.256
	n(%)	n(%)	
Smoking			0.033
Never	116(70.3)	135(81.8)	
Current	25(15.2)	13(7.9)	
Ever	24(14.5)	17(10.3)	
Smokeless tobacco			0.763
User	23(13.9)	25(15.2)	
Non-user	142(86.1)	140(84.8)	
Sunlight exposure			0.216
Yes	61(37.0)	50(30.3)	
No	104(63.0)	115(69.7)	
On statin medication			<0.001
Yes	89(53.9)	13(7.9)	
No	76(48.1)	152(92.1)	
Hypertension			<0.001
Yes	68(41.5)	25(15.2)	
No	96(58.5)	140(84.8)	
Ischaemic heart disease			0.027
Yes	11(6.7)	2(1.2)	
No	154(93.3)	163(98.8)	
Hypercholesterolaemia (> 200 mg/dl)			<0.001
Yes	93(56.4)	13(7.9)	
No	72(43.6)	152(92.1)	
Use of cooking oil/fat			0.001
Vegetable oil	114(69.1)	139(84.2)	
Vanspati ghee (hydrogenated oil)	10(6.1)	0(0.00)	
Both	41(24.8)	26(15.8)	
Engagement in exercise			<0.001
No	44(26.7)	110(66.7)	
Once/week	32(19.4)	17(10.3)	
2-5 times/wk	26(15.7)	12(7.3)	
Daily	63(38.2)	26(15.7)	
Extent of physical activity			0.02
Less than 1 h/day	60(36.6)	40(24.2)	
1-4 h/day	62(37.8)	61(37.0)	
> 4h/day	42(25.6)	64(38.8)	
Vitamin D status			<0.001
Deficient (< 20 ng/ml)	87(52.7)	115(69.7)	
Insufficient (20-30 ng/ml)	40(24.2)	32(19.4)	
Sufficient (> 30 ng/ml)	38(23.1)	18(10.9	

*Proportions in the two groups are compared using McNemar's test and simple conditional logistic regression, while mean values in patient and control groups are compared using Paired samples t-test.
BMI: Body mass index
25(OH)D: 25 hydroxy vitamin D
PTH: Parathyroid hormone
SD: Standard deviation.

Table-2: Distribution of frequencies of Gc genotypes and Gc diplotypes and mean levels of 25(OH)D in type 2 diabetes mellitus (DM) patients and healthy controls in a Pakistani population.

Gc gene	Type 2 DM patients (n=165)		Healthy controls (n=165)		p value*		
	n(%)	Mean ± SD 25(OH)D	n(%)	Mean ± SD 25(OH)D	Groups	Genotypes/Diplotypes	Interaction between Groups & Genotypes/Diplotypes
Genotype					0.031(F=4.707;df 1, 324)	0.159(F=1.848,df 2, 324)	0.707(F=0.347,df 2,324)
Gc 1-1	73(44.2)	22.7±11.4	91(55.2)	17.5±12.6			
Gc 1-2	82(49.7)	21.4±10.3	64(38.8)	15.8±9.7			
Gc 2-2	10(6.1)	16.2±7.8	10(6.1)	14.9±9.0			
Diplotypes					0.004(F=8.215,df 1,318)	0.291(F=1.239,df 5,318)	0.963(F=0.198,df 5,318)
1F-1F	6(3.6)	23.16±13.2	3(1.8)	15.4±13.3			
1S-1S	46(27.9)	22.37±11.5	61(37.0)	17.3±11.5			
2-2	10(6.1)	16.2±7.8	10(6.1)	14.9±9.0			
1F-1S	21(12.7)	23.3±11.1	27(16.4)	18.1±15.1			
1F-2	24(14.5)	24.3±10.1	18(10.9)	17.4±8.2			
1S-2	58(35.2)	20.2±10.2	46(27.9)	15.2±10.2			

*p value compares mean levels of 25(OH)D by Two way ANOVA. Groups are type 2 DM patients and healthy controls
Gc: Group-specific
DM: Diabetes mellitus
25(OH)D: 25 hydroxy vitamin D.

Table-3: Association of Gc genotypes with type 2 diabetes mellitus in a Pakistani population.

Genotype	Crude MOR (95% CI)[a]	MAOR (95% CI)[b]
Gc 1-1[c]	1.0	1.0
Gc 1-2	1.64 (1.03-2.62)	3.10(1.22-7.88)
Gc 2-2	1.16 (0.48-2.83)	2.98(0.43-20.66)

[a]Matched odds ratio (95% confidence interval).
[b]Matched adjusted odds ratio when the model was adjusted for age, BMI and serum levels of vitamin D.
[c]Most common genotype of Gc gene was taken as reference
Gc: Group-specific
MOR: Matched odds ratios
MAOR: Matched adjusted odds ratio
CI: Confidence interval.

compared to the controls, but significantly more controls were engaged in daily routine physical activity (greater than 4 hours per day) compared to the patients ($p<0.05$).

Serum levels of 25(OH)D were significantly higher in the patients compared to the controls ($p<0.001$) (Table-1).

The frequency distribution of alleles and genotypes was in accordance with HWE (p=0.98 among cases and p=0.99 among controls). Mean 25(OH)D levels were significantly higher among the cases than the controls, but not significantly different by genotypes or diplotypes (Table-2).

However, when the genotypes were compared using conditional logistic regression model, Gc 1-2 was found to be associated with T2DM. Matched, adjusted OR with 95% CI was 3.10(1.22-7.88) when the model was adjusted for age, BMI and serum levels of vitamin D (Table-3).

Discussion

Regarding the association of Gc gene polymorphism with T2DM, there have been conflicting reports. Hirai et al. showed significantly decreased proportion of Gc 1F allele in Japanese T2DM patients compared to Gc 1S and Gc 2 alleles.[10] However, no association between genotypes and haplotypes and T2DM could be found in Polish and French Caucasian populations.[11,12]

In a recent meta-analysis, moderate association of Gc gene polymorphism with increased susceptibility to developing T2DM was found in three populations in Japan and China.[16] Apart from studies on these Asian populations, no reports are available on the relationship of Gc gene polymorphism and T2DM in South Asian populations. Our results, therefore, are unique in reporting this association with T2DM in a Pakistani population. As against a modest association in other Asian populations, the odds of having T2DM were more than 3-fold in individuals with genotype Gc 1-2 compared to genotype Gc 1-1 (the most common genotype).

The underlying mechanism of this association between Gc genotypes and pathogenesis of T2DM is not clear at this moment. Variations in Gc protein could be influencing the serum levels of vitamin D metabolites, which in turn could be affecting the insulin secretory activity of pancreatic β cells.[17]

Pittas et al. have reported an association between low serum levels of vitamin D and type 2 DM.[18] However, our results showed no such association between serum levels of 25(OH)D and T2DM. Similar findings have been reported in a study from Western India, showing no relationship between vitamin D deficiency and T2DM in Indian population.[19] It appears that in the presence of very high prevalence of hypovitaminosis D in both T2DM patients and healthy controls in South Asian populations, including Pakistanis, any relationship of vitamin D deficiency with pathogenesis of T2DM is likely to remain inconclusive. Lauridsen et al.[20] have shown that serum concentration of vitamin D metabolites is highest in Gc 1-1, intermediate in Gc 1-2 and lowest in Gc 2-2. The same trend has been observed in the present study with respect to concentrations of 25(OH)D in individuals with these 3 genotypes in both T2DM and healthy control groups, though the differences were not found to be statistically significant. A couple of studies during the past few years have reported that common variants of Gc are genetic determinants of serum levels of 25(OH)D in Chinese populations, and Gc 2-2 had the lowest serum concentration of this metabolite.[21,22] In our study, too, Gc 2-2 group had the lowest mean concentration of 25(OH)D. How the Gc variant allele could be related to T2DM is unclear. Lifelong low levels of vitamin D among carriers of variant allele may have an adverse effect on health, making them more vulnerable to external risk factors.[23]

T2DM is a multi-factorial disease and its aetiopathogenesis could include not only genetic factors (such as Gc genotypes) but a host of nutritional and environmental factors as well.[24] Genome-wide association studies have revealed more than 60 gene loci in which variants have been found to be associated with T2DM.[25] We suggest that Gc gene variants also appear to have a role in T2DM in Pakistani population along with 6 novel T2DM susceptibility loci found in South Asian population.[26] On the basis of our data, it is conjectured that individuals with Gc 1-2 genotype in Pakistani population are more susceptible to the action of classical risk factors for T2DM, such as malnutrition, obesity, dyslipidaemia, stress, smoking, inflammation, age, gender, impaired glucose tolerance and physical inactivity,[6,24] making them more prone to developing this disease.

There are quite a few limitations of this study. We measured 25(OH)D by an immunoassay-based method which is less sensitive than the high-performance liquid chromatography(HPLC) and liquid chromatography-mass spectrometry (LC-MS) or MS methods. Moreover, the participants included in this study were mostly from urban areas and therefore the results might not be generalised.

Despite these limitations, the study had a reasonable sample size to investigate the relationship of Gc genotypes and T2DM. Moreover, matching of cases with controls for gender and age (within 5 years) at the time of recruitment of study participants is a major strength of this study as it eliminated the influence of 2 confounders. However, more data involving cases and controls from both rural and urban areas in all the 4 provinces of the country would be required to obtain conclusive evidence regarding the role of Gc polymorphism and T2DM in Pakistani population.

Conclusion

An association was found between Gc 1-2 genotype of VDBP gene and risk of T2DM in a Pakistani population. Hypovitaminosis D appeared to have no relationship with T2DM.

Acknowledgements: We are grateful to Ms. Shaheena Anwar for assistance in technical aspects of the study.

Disclaimer: None.

Conflict of Interest: None

Source of Funding: The study was supported by a grant from the Pakistan Science Foundation [No. PSF/Res/s-AKU (336)].

References

1. Holick MF. Vitamin D deficiency. N Engl Med. 2007; 357:266-81.
2. Zuberi LM, Habib A, Haque N, Jabbar A. Vitamin D deficiency in ambulatory patients. J Pak Med Assoc.2008;58:482-84.
3. Mehboobali N, Iqbal SP, Iqbal MP. High prevalence of vitamin D deficiency and insufficiency in a low income peri-urban community in Karachi. J Pak Med Assoc. 2015: 65:946-49.
4. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heick-endorffL,Mosekilde L, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int.2005;77:15-22.
5. Gozdzik A, Zhu J, Wong BY, Fu L, Cole DE, Parra EJ. Association of vitamin D binding protein (VDBP) polymorphisms and serum 25(OH)D concentrations in a sample of young Canadian adults of different ancestry. J Steroid Biochem Mol Biol. 2011; 127: 405-12.
6. Shera AS, Jawad F, Maqsood A. Prevalence of diabetes in Pakistan. Diabetes Res Clin Pract. 2007; 76: 219-22.
7. International Diabetes Federation. IDF Diabetes Atlas, 6th ed. Brussels: International Diabetes Federation, 2013. [Online] [Cited 2015 June 10]. Available from: URL: http://www.idf.org/diabetesatlas
8. Song Y, Wang L, Pittas AG, Del Gobbo LC, Zhang C,Manson JE, et al. Blood 25-dydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. Diabetes Care. 2013; 36: 1422-8.
9. Forouhi NG, Ye Z, Rickard AP, Khaw KT, Luben R,Langenberg C, et

al. Circulating 25-hydroxyvitamin-D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. Diabetologia. 2012; 55: 2173-82.
10. Hirai M, Suzuki S, Hinokio Y, Hirai A, Chiba M. Group-specific component protein genotype is associated with NIDDM in Japan. Diabetologia. 1998; 41: 742-43.
11. Malecki MT, Klupa T, Wanic K, Cyganek K, Frey J. Vitamin D binding protein gene and genetic susceptibility to type 2 diabetes mellitus in a Polish population. Diabetes Res Clin Pract. 2002; 57: 99-104.
12. Ye WZ, Dubois-Laforgue D, Bellanne-Chantelot C, Timsit J, Velho G. Variations in the vitamin D-binding protein (Gc locus) and risk of type 2 diabetes mellitus in French Caucasians. Metabolism. 2001;50:366-69.
13. International Diabetes Federation. Screening and diagnosis. In:Global Guidelines for Type 2 Diabetes. International Diabetes Federation, 2012; pp 9-14.
14. Ito I, Nagai S, Hoshino Y, Muro S, Hirai T. Risk and severity of COPD is associated with the group-specific component of serum globulin 1F allele. Chest. 2004;125:63-70.
15. Allele Frequencies and HardyWeinberg Equilibrium. [Online] [cited 2017 May 29]. Available from: URL:http://faculty.washington.edu/tathornt/sisg2013/Kerr/2HWE_Kerr.pdf
16. Wang G, Li Y, Li L, Yu F, Cui L, Ba Y, et al. Association of the vitamin D binding protein polymorphisms with the risk of type 2 diabetes mellitus: a meta-analysis. BMJ Open. 2014; 4: e005617.
17. Hirai M, Suzuki S, Hinokio Y, Hirai A, Chiba M, Akai H, et al. Variation in vitamin D-binding protein (group-specific component protein) are associated with fasting plasma insulin levels in Japanese with normal glucose tolerance. J ClinEndocrinol Metab. 2000; 85: 1951-3.
18. Pittas A, Lau J, Hu F, Dawson-Hughes B. Review: the role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J ClinEndocrinol Metab. 2007; 92: 2017-29.
19. Sheth JJ, Shah A, Sheth FJ, Trivedi S, Lele M,Shah N, et al. Does vitamin D play a significant role in type 2 diabetes? BMC Endocr Disord. 2015; 15: 5.
20. Lauridsen AL, Vestergaard P, Nexo E. Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women. Clin Chem. 2001; 47: 753-6.
21. Li LH, Yin X, Wu X, Zhang L, Pan SY, Zheng ZJ, et al. Serum 25(OH)D and vitamin D status in relation to VDR, GC and CYP2R1 variants in Chinese. Endocrin J. 2014; 61: 133-41.
22. Robien K, Butler LM, Wang R, Beckman KB, Walek D,Koh WP, et al. Genetic and environmental predictors of serum 25-hydroxyvitamin D concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr. 2013; 109: 493-502.
23. Sinotte M, Diorio C, Berube S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr. 2009; 89: 634-40.
24. Jiang X, Ma H, Wang Y, Liu Y. Early life factors and type 2 diabetes mellitus. J Diabetes Res. 2013; 2013: 485082.
25. Rathman W, Scheidt-Nave C, Reden M, Herder C. Type 2 diabetes: Prevalence and relevance of genetic and acquired factors for its prediction. Dtsch ArztebllInt. 2013; 110: 331-7.
26. Kooner JS, Saleheen D, Sim X, Sehmi J, Zhang W,Frossard P, et al. Genome-wide association study in South Asian ancestry identifies six noval susceptibility loci for type 2 diabetes. Nat Genet. 2013; 43: 983-9.