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The correlation study on vitamin D receptor *ApaI* gene polymorphism and gestational diabetes mellitus among pregnant women.

Li-Ping Jin[1*], Lin-Jie Shi[2], Wen-Ping Zhang[3]

[1]Department of Maternity, Peace Hospital of Changzhi Medical College, Changzhi, PR China

[2]Department of Urology, Peace Hospital of Changzhi Medical College, Changzhi, PR China

[3]Department of Cardiac Surgery, Peace Hospital of Changzhi Medical College Changzhi, PR China

Abstract

Purpose: The aim of this study was to explore the correlation between vitamin D receptor *ApaI* gene polymorphism and Gestational Diabetes Mellitus (GDM).
Methods: 240 patients were divided them into GDM and non-GDM groups. DNA sampling was purified from peripheral blood and analysed by the PCR-RFLP for *ApaI* gene. BMI, Fasting Blood Glucose (FBG), and 1 h and 2 h Postprandial Glucose (PPG) were compared.
Results: The genotype frequency of vitamin D receptor *ApaI* gene polymorphism significantly differed between two groups. Compared with AA genotype patients, the risk of gestational diabetes mellitus was significantly increased in those with CC genotype. Logistic regression analysis showed that there was no significant correlation between *ApaI* C allele and GDM.
Conclusions: There was a significant correlation between vitamin D receptor *ApaI* gene polymorphism and GDM among pregnant women.

Keywords: Pregnant woman Vitamin D, *ApaI*, Gene polymorphism, Gestational diabetes mellitus.

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Introduction

Gestational Diabetes Mellitus (GDM) is defined as the normal glucose metabolism or potential impaired glucose tolerance before pregnancy, first discovery and diagnosis of diabetes in pregnancy. Epidemiologic study showed that its morbidity rate among pregnant woman is approximately 1%-14%, which is related to giant baby, birth injury, toxemia of pregnancy and even stillborn foetus. Typical clinical manifestations include insulin resistance, hyperglycaemia and adiposity [1-3]. The specific cause of GDM is still unclear, but inherent cause and life style play an important role in the pathogenesis and progress. It has been proposed that variants of the nuclear Vitamin D Receptor (VDR) gene are associated with a susceptibility to Type 2 Diabetes Mellitus (T2DM) and GDM. Partial genes possess polymorphism which could influence on the transcription and activity of protein. VDR is involved in the metabolism of insulin as one of the members [4]. A latest study showed that vitamin D could assist in regulating the growth, survive, inflammation of cells and the secretion of insulin. In addition, the related study also showed that there was a correlation between vitamin D deficiency, insulin resistance and the increased risk of GDM [5]. VDR gene encoding nuclear transcription factor contained four types of second gene polymorphisms with obvious characteristics: *BsmI* (A>G, rs1544410); *ApaI* (A>C, rs7975232); *FokI* (C N T, rs10735810) and *TaqI* (T>C, rs731236). The study showed that gene polymorphism of VDR was associated with the susceptibility of type I and II diabetes mellitus [6,7]. It is worth to note common features of glucose intolerance, insulin resistance and insulin secretion dysfunction between GDM and type II diabetes mellitus [8]. VDR principally mediates the anticancer activities of vitamin D. Many studies investigated the association between VDR gene *ApaI* polymorphism and multiple malignant tumors, but the relationship between VDR gene polymorphism and GDM susceptibility has been rarely investigated. So this study was designed to explore the correlation between vitamin D receptor *ApaI* gene polymorphism and GDM, aiming to provide reference and guidance for clinical diagnosis and treatment of GDM.

Materials and Methods

Baseline data

In this case-control study, all 240 patients admitted to our hospital from January, 2014 to June, 2015 were recruited. All participants divided into the GDM group (n=120) and non-GDM group (n=120). Baseline data of the patients in two groups could be found in Table 1, the diagnostic criterion of GDM was Oral Glucose Tolerance Test (OGTT) during 24-28 weeks gestation according to the standard of International

Association of Diabetes and Pregnancy Study Group (IADPSG) (0 minute 5.1 mmol/l, 60 minutes 10.0 mmol/l, 120 minutes 8.5 mmol/l). It would be diagnosed as GDM when more than one glucose level exceeded a threshold value. If all plasma glucose indexes were lower than threshold values, the subjects were diagnosed as Normal Glucose Tolerance (NGT) or non-GDM.

Exclusion criteria

Patients with a medical history of diabetes mellitus, liver disease, kidney failure or functional disorder, thyroid diseases, toxemia of pregnancy, hyperandrogenism or those receiving anti-seizure and anti-depressive drugs, etc. All surgical procedures were in accordance with the ethics committee of our hospital.

The separation and gene detection of DNA

According to manufacturer's instructions, DNA purification kit (Sinaclon) of gene group was utilized for extracting DNA samples from venous blood, and then restored at-20°C. The polymorphism of vitamin D receptor was detected by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). With regards to the detection of VDR *ApaI* (A>C) polymorphism, primers were first used to amplify 745-bp fragment (forward primer: 5'-CAGAGCATGGACAGGGAGCAAG-3'; reverse primer: 5'-GCAACTCCTCATGGCTGAGGTCTCA-3'). The total PCR reactant was 25 μl, which contained 12.5 μl 2 × PCR mixture (Sinaclon), 1 ul primer (10 pmol/μl) and 1 ul DNA masterplate (100 ng/l); 30 circles (94°C for 45 seconds, 67°C for 45 seconds and then 72°C for 45 seconds), extended 10 minutes at 72°C. After PCR reaction, added 2.5 μ *ApaI* restricted enzyme (Fermentas) to an equivalent quantity of reaction product (10 μl) and kept reaction for 4 hours at 37°C. The genotype was defined as AA (745 bp), CC (528 and 217 bp) or CA (528, 217 and 745 bp). Processed cataphoresis for digestion products in 8% acrylamide gel on silver nitrate stained gel. The detection of serum glucose level was based on the method of glucose oxidase.

Statistical analysis

We used chi-square test to detect and analyse the differences in VDR genotype and abnormal frequency between the experimental and control groups. After adjusting age factor, logistic regression analysis was adopted to analyse Odds Ratios (ORs) of VDR genotype and 95% Confidential Interval (CIs). Biochemical data differences between two groups were analysed and compared by t-test or ANOVA. If $p<0.05$, then the differences had statistical significance.

Results

Baseline data

As illustrated in Table 1, baseline data of the study subjects were included in the experimental and control groups. There was no significant difference in the average age between two groups (p=0.0001). Compared with the control group, Fasting Blood Glucose detection (FBG), 1 h and 2 h PPG in the experimental group were significantly increased (all $p<0.05$). There was a significant increase in the risk of GDM in patients with a family history of type II diabetes mellitus ($\chi^2=18.55$, OR: 3.44, 95% CI: 1.89-6.01; p=0.001).

Correlation analysis between genotype and allele frequency

As demonstrated in Figure 1, showed the differences in VDR *ApaI* polymorphic genotype and allele frequency of two groups. There were significant differences in the genotype frequencies of *ApaI* VDR polymorphism between the two groups ($\chi^2=8.2$, p=0.013, $D_f=2$). And CC genotype was more popular in the experimental group, the differences were obvious (18.3% *vs.* 2.7%, p=0.007). AA genotype was regarded as the reference, the GDM risk of the patients with CC genotype was higher (AA *vs.* CC, OR=2.876, 95% CI=1.245-8.117, P=0.006). Whereas there was no obvious correlation between other genotypes or combined genotypes (AA *vs.* CA, OR=0.933, 95% CI=0.538-1.663, P=0.6; AA *vs.* (CA+CC), OR=1.258, 95% CI=0.654-1.874, p=0.71). There was no significant difference in allele frequencies between two groups ($\chi^2=2.39$, p=0.16).

Logistic regression analysis showed no significant interaction between C *ApaI* allele and the risk of GDM. We further analysed the relationship between individual genotypes of VDR gene, population distribution and biochemical characteristics. The results showed there was no significant correlation (Table 2).

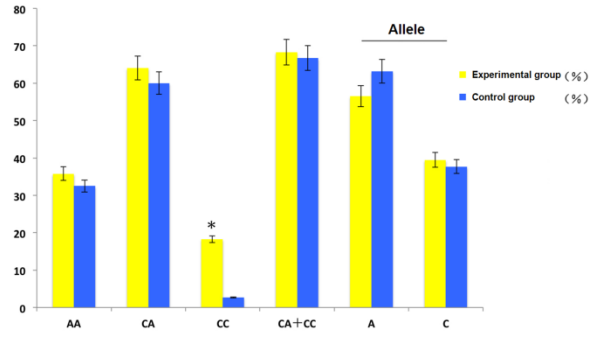

Figure 1. *Comparison of ^{ApaI} VDR genotype and allele frequency between experimental and control groups. *Represents statistical significance between two groups.*

Table 1. *Baseline data of study subjects between two groups.*

Variable	Experimental group (n=120)	Control group (n=120)	P value
Age (years)	29.54 ± 4.8	28.65 ± 3.89	0.072
History of abortion	0.35 ± 0.42	0.37 ± 0.35	0.661
Family history of type II diabetes mellitus	36.10%	15.10%	0.788

BMI (kg/m^2)	29.63 ± 5.87	24.87 ± 4.21	0.001
FBG (mg/dl)	111.3 ± 29.3	81.2 ± 8.3	0.001
1 h PPG (mg/dl)	170.5 ± 36.6	126.5 ± 29.7	0.001
2 h PPG (mg/dl)	143.2 ± 36.4	99.5 ± 25.2	0.001

Note: FBG: Fasting Blood Glucose Detection; PPG: Postprandial Glucose.

Table 2. *Logistic regression analysis of the correlation between Apal C allele genes and GDM risk between two groups.*

ApaI	Experimental group	Control group	OR (95% CI)	χ^2
C	n (%)	n (%)		
-	14 (11.7)	16 (7.3)	Reference	Reference
+	70 (58.3)	47 (39.2)	1.47 (0.5-3.9, P=0.28)	0.8 (d_f=1, P=0.42)
-	41 (34.1)	40 (33.3)	0.94 (0.7-1.6, P=0.87)	0.06 (d_f=1, P=0.77)
+	45 (37.5)	62 (28.2)	0.85 (0.6-1.4, P=0.39)	0.7 (d_f=1, P=0.42)

Discussion

More and more attention should be paid to the research on common genetic variants of vitamin D receptor gene in diabetes mellitus, GDM and other diseases [4,9-11]. In this study, we mainly explored the correlation between *ApaI* VDR gene polymorphism and GDM and found there was a significant difference in genotype distribution between GDM and non-GDM counterparts. The comparison between CC and AA genotypes was more common. It was found that VDR *ApaI* allele polymorphism was associated with the incidence of GDM in this population. There was no significant difference in allele genes in the susceptibility of GDM between the experimental and control groups.

In current study, the relationship between the polymorphism of VDR gene and GDM has been rarely investigated [12-14]. Ghamdi et al. reported that there was a certain correlation between VDR *Taql* polymorphism and GDM. Aslandi et al. found that there was a significant correlation between the *Fokl* VDR polymorphism and the risk of GDM among Iran population. Tawfeek et al. reported that there was a significant correlation between VDR *Bsml* polymorphism and the risk of GDM. The correlation between the *ApaI* gene polymorphism and GDM has not been reported.

VDR is a nuclear factor which can mediate physiological metabolic activity of a majority of vitamin D. Vitamin D plays a role by regulating the level of extracellular calcium in beta cells, and promotes the secretion of insulin. Similar to type II diabetes mellitus, typical features of GDM include insulin resistance and related functional activity damage in target organ tissues. The latest study showed that there was a certain correlation between vitamin D and cancer, disseminated sclerosis, asthma and diabetes mellitus, etc. [15-18]. Multiple epidemiologic studies detected serum vitamin D level in GDM and non-GDM population, and found that there was a certain correlation between vitamin D deficiency and the incidence of GDM [19].

Meantime, some evidences showed partial risk factors which coexist in both GDM and type II diabetes mellitus patients, such as genetic risk, etc. [20-22]. A meta-analysis study demonstrated that eight genes associated with type II diabetes mellitus are correlated with GDM susceptibility. Several scholars have detected a partial correlation between VDR gene polymorphism and the susceptibility of diabetes mellitus. Nosratabadi et al. reported that *Taql* polymorphism in Iran population was associated with the morbidity of type II diabetes mellitus. Oh et al. also found a significant correlation between *ApaI* polymorphism and type II diabetes mellitus in American population (p=0.058) [23]. Wang et al. conducted a meta-analysis study and reported that *Bsml* polymorphism was associated with type I diabetes mellitus in Asian population, whereas *Fokl* polymorphism was related to type II diabetes mellitus [24]. Bonakdaran et al. reported the effect of *BsmI, FokI*, VDR *ApaI* and *TaqI* gene polymorphisms on patients with type I diabetes mellitus and they found the genotype frequency of AA, FF and Bb was significantly increased. Mohammadnejad et al. found that VDR *Taql* gene polymorphism was significantly associated with type I diabetes mellitus [25]. Bilge et al. evaluated the insulin resistance and quality of life in obese subjects and non-obese counterparts and demonstrated that it would be a rational approach to identify routine serum 25 (OH) D levels of obese patients and administer a treatment to patients with a low level of vitamin D [26]. Obesity might be one of the risk factors of the incidence of GDM. In this study, we found the average BMI of GDM patients was obviously higher than that of the normal controls. Hence, further research was urgently needed to confirm the conclusion. In this study, we only detected VDR *ApaI* polymorphism and the sample size included in the study was relatively limited. In conclusion, there was a significant correlation between vitamin D receptor *ApaI* gene polymorphism and GDM among pregnant women.

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*Correspondence to

Li-Ping Jin

Department of Maternity

Peace Hospital of Changzhi Medical College

PR China