Assessment of Vitamin D Receptor Gene Expression In Type 2 Diabetic and HypertensivePatients

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الخلاصة

تعتبر مستقبلات فيتامين د والمشفر عنها من جين VDR، احدى عوامل الأستنساخ المهمة التي ترتبط وظيفتها بتنظيم قدرة الجسم على مقاومة التعرض للأمراض المناعية والألتهابات ، ويمكن ان تلعب دورا مهما في امراضية مرضي داء السكري من النوع الثاني و ارتفاع ضغط الدم . شملت الدراسة الحالية (104) شخص لغرض تحديد مستويات التضخيم للحامض النووي منقوص الأوكسجين (DNA) منهم (40) مريض بداء السكري من النوع الثاني ، و(40) مريض مصاب بأرتفاع ضغط الدم للمقارنة مع (24) شخص من الأصحاء مظهريا كمجموعة سيطرة. تمت دراسة توزيع العينات حسب العمر و الجنس و مكان السكن و تاريخ العائلة المرضي ، وتم استخدام تفاعل البلمرة المتسلسل الكمي ذو الزمن الحقيقي لغرض تعيين مستويات التضخيم لجين ال VDR في العينات المدروسة .

أظهرت النتائج وجود ترابط معنوي مهم احصائيا بين مجموعتي المرضى و مؤشرات الجنس و تاريخ العائلة لكل من داء السكري وارتفاع ضغط الدم على التوالي في حين لم تظهر النتائج اي ترابط معنوي مع مكان السكن في مجموعة مرضى السكري بينما كان هناك ترابط معنوي في مجموعة مرضى ضغط الدم المرتفع . كما اظهرت النتائج بأن قيم نقطة الشروع بتضخيم جين ال VDR لمجاميع مرضى السكري و ارتفاع ضغط الدم و مجموعة السيطرة كانت (1.22±23.5) و (1.30±24.67) و (1.94±27.41) على التوالي ، اضهرت نتائج التحليل الأحصائي وجود اختلافات عالية المعنوية لمستويات التضخيم الجيني بين مجموعتي مرضى السكري والسيطرة في حين لم تكن هناك فروقات معنوية بين مرضى ارتفاع ضغط الدم و محموعة السيطرة ، كما اظهرت النتائج ايضا وجود فروقات احصائية معنوية بين مجموعتي مرضى السكري و ارتفاع ضغط الدم

الكلمات الفتاحية

جين ال VDR ، تفاعل البلمرة المتسلسل الكمي ذو الزمن الحقيقي، داء السكري، ارتفاع ضغط الدم.

Abstract

Vitamin D receptor (encoded by the VDR gene) is a transcription factor from the nuclear receptor subfamily. It has been reported that this gene has been participated in the regulation of susceptibility to autoimmune and inflammatory conditions, and could play a role in the pathogenesis of blood pressure and type (2) diabetes mellitus, so this study was conducted toevaluate the effect of some demographical parametersand to assess the gene expression levels of VDR gene in both type (2) diabetic and hypertensive patient groups in comparison with apparently healthy controls.A total of (104) individuals were enrolled in this study ,forty of diabetes mellitus type 2 patients, forty of blood hypertension patients and twenty four of apparently healthy individuals as a control group. The age, gender, living region and family historywere studied.Real time – quantitative polymerase chain reaction (RT-qPCR) technique was adopted to assess the amplification levels of VDR gene.

The results showed thatthere were significant associations between patients groups and gender and family history, respectively, and there was no significant association ($p \geq 0.05$) for the living region in diabetic patients.However, a significant positive association ($p \leq 0.05$) exists between living region and Diabetic condition.

RT-qPCR amplification results showed that the mean ± SD of threshold cycle (Ct) values of amplification cycles of diabetic, hypertension, and control groups were 23.5±1.22, 24.67±1.30 and27.41±1.94 respectively. The statistical analyses of quantitative amplifications revealed that there was highly significant difference ($p \leq 0.01$) between diabetic and control groups, and there were no statistically differences ($p \geq 0.05$) of hypertension patients in comparison with control group. The results also revealed significant difference ($p \leq 0.05$) between diabetic and hypertensive groups.

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Keywords

VDR gene, RT-PCR, Diabetes, Hypertension.

1. Introduction:

Type (2) diabetes mellitus (T2DM), formerly called non-insulin-dependent diabetes mellitus, obesity-related diabetes or adult-onset diabetes, is a metabolic disorder that is primarily associated with resistance to insulin hormoneand relative to deficiency of insulin and increasing the levels of blood sugars (hyperglycemia). Many genes such ashepatocyte nuclear factors,glucokinase, insulin promoter factors, and insulin receptor genes are consider a biomarker forT2DM gene abnormalities [1,2,3]. According to a global survey reported in (2004),the prevalence of diabetes has been estimated(2.8%) in (2000) and about (4.4%) in (2030), around the word.

So, the suspected number of people affected with diabetes ispredicted to increase from 171 million in (2000)to (360) million in (2030), most frequently will beT2DM. [2,4]

Vitamin D modulates the gene expression of insulin receptors,as well as insulin secretion, and exerts its activity on specific cellsby binding to the cytosolic/nuclear vitamin D receptor (VDR), which act as activation factors for transcription of many genes [3]. Vitamin D (1,25- dihydroxyvitamin D3) receptor gene is located on the long arm (q) of chromosome (12) at position (13.11), from base pairs (47,841,536) to (47,905,030), and consists of (14) exons, and has an extensive promoter region capable of generating multiple tissue-specific transcripts. [5,6,7,8]

This gene encodes the nuclear hormone receptor for vitamin D3, this receptor is important for the secondary bile lithocholic acid, and also plays critical roles in calcium homeostasis, bone development and mineralization, as well as control of cell growth and differentiation. [1,9]

The VDRgene is also serves as a good candidate gene for susceptibility to several diseases, due to regulating the renin-angiotensin system (RAS) that influencing the regulation of blood pressure. Hence molecular evaluation of VDR polymorphisms and its association with hypertension is suggested to help in the assessment of risk for the disease [10,11, 12] .Several studies have demonstrated that VDR are present in aortic endothelialand vascular smooth muscle cells [13], and also VDR polymorphisms predispose to the coronary artery diseases.[14].

The present study aimed to evaluate the effect of some demographical parameters include gender, family history andtype of living region, and to assess the gene expression levels through the quantification of amplification of VDR gene in both type (2) diabetic and hypertensive patient groups in comparison with apparently healthy controls using the accurate and reliable RT-qPCR methodology.

Materialsand Methods:

A total of (104)blood samples were studied, fortysamples from random clinically diagnosed diabetic type (2)patients(mean age 44±6.5 years) and forty samples are from hypertension patients(mean age 42±4.2 years) were involved in this study as a patients group

which were taken from Al-Hussain teaching hospital in Kerbala, Iraq. Beside the patient group, twenty foursamples from apparently healthy individuals with age matchingare served as a control group. The study conducted fromFebruary, (2015)to august, (2015). The gender, living region and family history for the disease were also studied.The official and ethical considerations were approved and provided for participant before sampling,

Blood Samples:

Two ml of peripheral blood samples were collected in EDTA anticoagulant tubes by vein puncture from all patients and controls. The collected blood samples were transmitted within (2)hours using cool box and subjected to DNA extraction and molecular analysis.

Genomic DNA purification:

Genomic DNA was purified from whole fresh blood using genomic DNA purification kit (Geneaid-South Korea), following the protocol provided by the manufacturer, DNA was quantified by the horizontal agarose gel electrophoresis and stored at (-20) Countil use.

The RT-qPCR assessment of VDR gene:

Real time- quantitative polymerase chain reaction (Rt-qPCR) was performed with the following primer sequences (forward primer: 5'-CAGAGCATGGACAGGGAGCAA-3' and reverse primer: -GCAACTCCTCATGGGCTGAGGTCTCA - 3') (1).

.Quantitative real-time PCR assays were performed in triplicate usingReal time PCR (Excecycler 96)®,BIONNER , South Korea. The Real time PCR system primer and SYBR Green master mix was used for quantitative assessment. The (25) µl of reaction volume containing(10)µl SYBR Green master mix,(1) µl of primer mixes ,(10) µl of RNase free water and (4) µl DNA template. Real-Time PCR protocol was as follows: initial denaturation by (1) cycle of (5) min at (95)°C, followed by 40 cycles of (30) sec. at (95)°C for denaturation, (40) sec. at (58)°C for annealing, (40) sec. at (72)°C for extension, and final hold at (8) °C.

Statistical analysis:

The obtained data were evaluated using Statistical Analysis System (SAS) (V 6.12, 2001) and chi- square were adopted for analysis of results, the appropriate p-values of less than (0.05) were considered as statistically significant, and value less than 0.01 was considered to be highly significant.

Results:

The study of demographical parameters (Sex, family history and living regions) revealed that (57.5%) of diabetic patients group were males and (42.5%)were females, and for hypertensive patients, (45%) of them were males and 55% were females,while for control group were (58.3%) males and (41.7%) females, (table 1), results also showed that there werestatistically significant differences($p \leq$

0.05) between males and females for both diabetic and hypertension groups.

Regarding to the family historyfor diabetic and hypertension patients, the results showed that (77.5%)of diabetic patients had positive family history in comparison with (22.5%) that had negative family history, while for hypertensive patients group the results showed that (67.5%)had positive family history and (32.5%)had negative family history, for control group , the results revealed that (25%) and (75%) had positive and negative family history respectively,and the statistical analysis revealed that there werehighly significant differences between positive and negative family history, ($p \leq 0.01$) for diabetes and ($p \leq 0.05$) for hypertension groups.

The distribution of the samples according to the living region revealed that (52.5%) of diabetic patients were live within urban regions while (47.5%)living in the rural regions, and regarding to the hypertension patients the percentages were (57.5%)and (42.5%) for urban and rural living regions respectively, while for control group , the distribution shows that (62.5%)of samples were living within urban regions in comparison with (37.5%) were live in rural regions, The results also showed no significant differences ($p \geq 0.05$) between two categories for diabetic patients while there was significant differences ($p \leq 0.05$) for living region of hypertension patients.

Table (1): The distribution of the studied groups according to the gender, family history for the diseases and living region.

Parameters*		Diabetes N(%)	HypertensionN(%)	Control
Gender	Male	23(57.5) A	18(45) B	14(58.3) A
	Female	17(42.5) B	22(55) A	10(41.7)A
Family history	Positive	31(77.5) A	27(67.5) A	6(25) B
	Negative	9(22.5) B	13(32.5) B	18(75)A
Living region	Urban	21(52.5) A	23(57.5) A	15(62.5)A
	Rural	19(47.5) A	17(42.5)B	9(37.5)B

*the similar vertical letters indicate for non- significant differences while the non- similar vertical letters indicate for significant differences within same parameter results.

The gene expression levels through the quantitative amplification of the target DNA region of VDR gene using RT-qPCRwere studied, and according to the threshold cycle (Ct) values of amplification cycles, the results revealed that the mean± SD of the Ct values for diabetic, hypertension, and control groups were (23.5±1.22, 24.67±1.30 and27.41±1.94) respectively.

The statistical analysis of the results regarding the gene expressions and threshold cycle (Ct) values of amplification revealed there were highly significant difference (P value=0.0024) between diabetic and control groups, and there were no statistically differences (P value = 0.2372) between hypertensive patients in comparison with control group. The results also revealed significant difference (P value = 0.0174) regarding Ct values between diabetes and hypertension groups Tables (2), Figs (1-3).

Table (2): The threshold cycle (Ct) values of VDR gene amplifications in diabetic, hypertensive and control groups.

Group	Threshold cycle (Ct)				
	Maximum Ct value	Minimum Ct value	mean± SD	P- value*	
Diabetes	25.37	19.42	22.5±1.22	0.0024 A	0.0174
Hypertension	27.30	23.10	25.67±1.30	0.2372 B	
Control	35.76	23.10	27.41±1.94	- - - - - B	

*the similar vertical letters indicate for non- significant differences while the non- similar vertical letters indicate for significant differences within same parameter results.

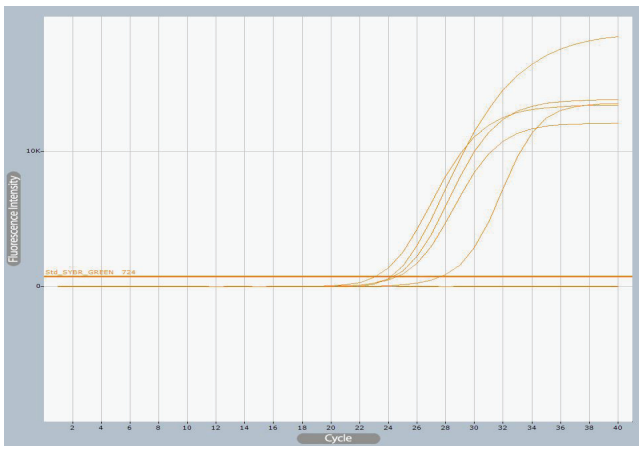

Fig. (1): RT-qPCR Amplification plot for synthesis of VDR gene in diabetic patients by Real time PCR (Excecycler 96)®,BIONNER , South Korea.

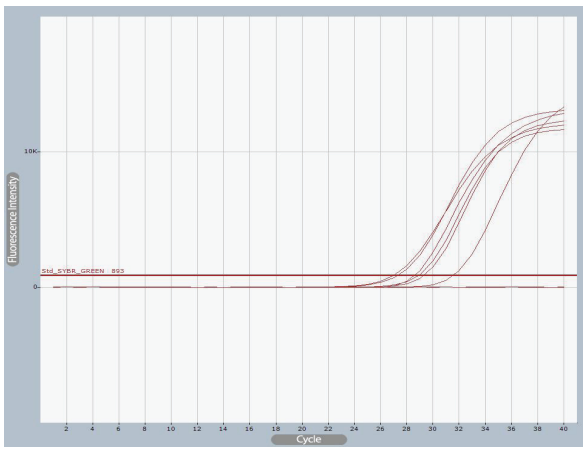

Fig. (2): RT-qPCR Amplification plot for synthesis of VDR gene in hypertensive patients by Real time PCR (Excecycler 96)®,BIONNER , South Korea.

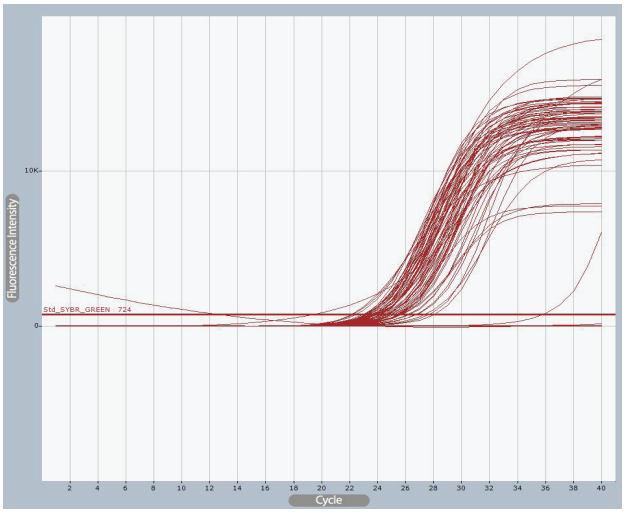

Fig. (3): RT-qPCR Amplification plot for synthesis of VDR gene in diabetic, hypertensive and control groups by Real time PCR (Excecycler 96)®, BIONNER, South Korea.

Discussion:

Diabetes and hypertension and its associated complications are part of a chronic disease global epidemic that presents a public health challenge, the study of some demographical parameters revealed(table 1) that the results of distribution of samples according to the gender were highest formale diabetic patients in comparison with female diabetic patients, and for hypertensive patients, the results revealed that the distribution for male were lower than female. While for control group the highest number of samples wasmale. Thesefindingsshowed significant differences between gender for both hypertensive and diabetic patients in comparison with control group. In diabetic, our results were agreed with the reports of Australian institute of health and welfare [15] that showed the incidence rate of diabetes regarding to the age groups which demonstrate that increasing of female gestational diabetic patients with age group range between(15-49) years old and the prevalence of diabetes type (2) will increase in males within increasing of age for (50 -75) years old. The results also compatible with other study findings that shows In the first half of the last century the prevalence of type (2) diabetes was higher among women than among men, but this trend has shifted, so more men than women are now diagnosed with type (2) diabetes. This change in the gender distribution of type (2) diabetes is mainly caused by a more sedentary lifestyle particularly among men, resulting in increased obesity [16].

The present study results were differ from Hariri et al. [17] findings on (4345) individuals, they showed (43.3%) of diabetic patients were male and (56.7%) of them were female. The present study findings revealed that the gender differences in the type (2) diabetes mellitus patients may be related to that women and men with diabetes face different challenges in the management of their condition during different hormonal and physiological condition that explain the effect of obesity and gestational period on diabetic prevalence in study samples for males in comparison with females that have mean age (44±6.5)years.

For hypertensive, the results agreed with previous study that revealed women have greater increases of cardiovascular risk than men [18].The present study were different from results reported by study used the tech-

nique of (24) hour ambulatory blood pressure monitoring shown that blood pressure is higher in men than in women at similar age groups due to presence of female hormones which may play a role in protecting females from developing hypertension [19].

Regarding the family history in diabetic patients, the present study showed that the high prevalence (77.5%) of samples have positive family history and the lowest (22.5%) were have negative ones, for hypertensive patients the results showed (67.5%)and (32.5%) were have positive and negative family history to the disease respectively in comparison with control group family history. Statistically, these results showed a significant association of family history with diabetes and hypertension. These findings support previous studies reported that family history to the disease is considered a powerful risk factor for diabetes [20, 21, 22,23,24] which suggested the potential benefit of use of family history for identification at-risk and undiagnosed individuals with diabetes and hypertensions.

In regard to living region, the present study revealed that the distribution of diabetic, hypertensive and control groups that live in urban regions were (52.5%), (57.5%) and (62.5%), in comparison with rural living regions were (47.5%), (42.5%) and (37.5%) respectively. The result of statistical analyses between patient and control groups regarding to the rural – urban living regions revealed non-significant differences for diabetic patients, these results are similar to the finding of Ogurtsovaet al. [25], both detected no significant differences of diabetes prevalence between rural and urban setting. Regarding to the hypertensive patient and control results the distribution revealed significant increasing of urban distribution of samples in comparison with rural, these results may be due to the trend of urbanization and lifestyle changes, including increasingly sedentary lifestyles, less physically demanding work and the style of nutrition.

Beside the role of vitamin D endocrine system in the calcium metabolism, it is involved in different aspects of cellular replication and differentiation in many target tissues, including the endocrine pancreas [26,27]. Now, it is clear that the VDRisimportant in the mechanism of insulin delivery and in the preservation of glucose tolerance. On the other hand, the vitamin D–VDR complex was reported as a potential controller of renin functions in human [28] and regulationof blood pressure through its effects on calcium homeostasis [29], vascular smooth muscle cell [30], and endothelial cell function [31]. Additionally, VDR may play a role in the mechanisms of inflammationand insulin sensitivity [32].

Regarding toVDR gene expression, in the present study, we examined the amplification level of VDR gene in patients suffering from the two mentioned diseases using qRT-PCR technique. We have shown that individuals with DMT2 have low threshold cycles comparing to the threshold cycles for hypertensive patients while for control group that have a

highthreshold cycle values. The statistical analyses revealed significantly VDR gene expression associated withDMT2, the current results might suggests that a certain level of VDR gene expression is the determining factor in controlling of DMT2.Previous studies have shown that certain mutations and single nucleotide polymorphisms (SNPs) in the VDR gene are associated with diabetes mellitus type (2) (DMT2) and hypertension (1,4,14).

In conclusion, this study provides evidence for the interaction between VDR gene expression and DMT2. To understanding the exact mechanism of how VDR gene expression affects the outcome of DMT2, it is recommended to study the expression levels of VDR gene in sufficiently large sample size to produce results with increased precision.And many parameters are important to study including epidemiological life course, social, behavioral, genetic and environmental factors.

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