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Association between CNR2 gene expression and the risk of diabetes mellitus disease

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ORIGINAL STUDY

Association Between CNR2 Gene Expression and the Risk of Diabetes Mellitus Disease

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Abstract

Endocannabinoid system (ECS) implicated in lipid and glucose metabolism, energy homeostasis expenditure and energy intake. In current study we focused on specific member of ECS which is CB2R. It is expressed in splenic tissue and immune cells. CB2R is one of the newest target of drug to treat chronic inflammatory diseases. This project aimed to assess the transcript level of CB2R in diabetic mellitus patients compare with its level in control group and evaluating some biochemical parameters including (glucose, cholesterol and triglyceride). As well as to characters the correlation type between above biochemical parameters and CB2R expression in patients group. mRNA of CN2R gene was extracted and cDNA was created to measure CB2R gene expression by using qRT-PCR assay. CB2R gene expression was significantly down regulated in blood samples of DM patients compared with its level in healthy group with fold change (0.563) fold. Biochemical parameters levels including (FBG, HbA1c, TC, TG, LDL, VLDL) were significantly increased in blood samples of DM patients compared with their levels in control group. However, HDL level was significantly reduced in DM patients (20.25 ± 0.93) compared with its level in control group (35.66 ± 9.34). There is no correlation coefficient was identified between CB2R gene expression and studied parameters while a non-significant negative correlation was demonstrated between cholesterol and CB2R gene expression (r = −0.205. p-value = 0.625). These finding refers to possible association role of CB2R with DM disease as well as its association with lipid regulation this assay may provide useful target for new research to imply DM treatment.

Keywords: Endocannabinoid, CB2 receptor, CNR2 gene, Diabetes mellitus

1. Introduction

The prevalence diabetes mellitus (DM) disease is continuing to increase and affect a numerous number of persons worldwide to reach epidemic proportions [1] genetic background and environmental factors are interacting and contributing to develop obesity and diabetes mellitus disease [2,3] molecular pathogenesis mechanisms of DM are not exactly recognized mainly [4], the pathogenesis includes insulin resistance, decreased in insulin secretion, beta cell disorder, inflammation, oxidative stress, mitochondrial dysfunction and apoptosis. However, the currently strategies of medications are effective to control diabetic disease but they have a side effects. Preventing or novel therapeutic that prevent insulin resistance IR and DM are required [5]. Therefore, an effective drugs are needed to produce or prevent the risk factors, which are associated with diabetic disease [6,7]. ECS receptors implicated in several physiological processes including pain reduction, apoptosis and DM regulation. CB2R is one subtype of endocannabinoid system (ECS) components [8]. CB2R activity could be changed directly by binding ligands or indirectly by alteration endocannabinoids levels [8]. Cannabinoids play a vital role in suppression oxidative stress, inflammation that a common of diabetes mellitus by activation CB2R [9−11] CB2R has been characterized as a peripheral receptor isoform which is expressed in the surface immune system cells like B lymphocytes, mast cells, killer cells, macrophages and on the spleen thymus, tonsils and lymphatic organs [12]. ECS has been identified to play a vital role in regulating glucose homeostasis and metabolism [13]. Agonists of CB2R contributed in antioxidant

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properties modulation inflammatory as well as its role in stimulation insulin secretion [14]. Insulin resistance, obesity and diabetes mellitus could be considered as a consequences of reducing immune system responses [15]. Recent study demonstrated that overexpression of CB2R in fatty liver diseases compared its vital role with related diseases including hypertriglyceridemia, type 2 diabetes and obesity [16]. The study of [17] identified that curcumin is agonist of CB2R and this is contributed in Cardioprotective responding of against the high fat diet and improve the nature membrane status that companied with diabetic diseases. According to suggesting role of CB2R in improving inflammation by normalizing glucose and fatty acid metabolism [18]. Thus this project is mainly aimed to assess the association between CB2R transcript and DM disease of Iraqi patients as well as to evaluate the relationship between CB2R and some chemical parameters such as glucose, cholesterol and triglycerides.

2. Materials and methods

2.1. Blood samples collections

One hundred blood samples were collected (fifty samples of diabetes mellitus patients and the other fifty samples of healthy group used as a control). Each sample was divided into two parts. One of them was input in tube containing EDTA to extract RNA sample and was stored at 4 °C until used. While the other part was saved in tube separator without added anti-coagulation for serum isolation. Then was centrifuged at 3500 rpm/1 min and separated serum was added to Eppendorf tube to be ready for biochemical analysis.

2.2. Biochemical analysis

(BioLAB kit, France) was used to measure glucose serum level after 10–14 fasting hours. Hemoglobin A1c (HbA1c) level was measured by utilizing Bodited kit (Korea). Cholesterol level was calculated according to Griffin and Lichtenstein [19] method. Triglyceride level was calculated fallowing [20] methods. However, enzymatic colorimetric method utilizing (BIOLABS kit, France) was used to measure HDL and LDL levels of blood samples.

2.3. Real time qPCR assay

RNA of whole blood samples were extracted, cDNA was created and treated from gDNA contamination. qPCR was performed according to previous described methods [21]. Forward TATGGGCATGTTCTCTGGAA and reverse GAGGAGCACAGCCAACACTA primer CB2R gene were used to amplify CB2R gene. Housekeeping gene GAPDH was amplified using forward primer GTCAAGGCTGAGAACGGGAA and revers primer TCGCCCCACTTGATTTTGGA. CT value of GAPDH was subtracted from CT value of CB2R gene (ΔCt), the conditions of reactions were utilized following: 95 °C (10 min) and (95 °C for 30 s per 1 min) for 40 cycle.

2.4. Statistical analysis

Computer program graph pad prism (version 8) was applied to analysis the obtained data. The mean of biochemical parameters of patients and healthy groups were compared using T-test (unpaired). ΔCt and fold change of CB2R gene was analyzed according to Livak and Schmittgen [22] methods. Correlation coefficient was performed to describe the association between CB2R gene expression and studied parameters.

3. Results

The level expression of CB2R gene was highly expressed in control group (ΔCt = 8.89 ± 1.02) compared with DM patients (ΔCt = 9.72 ± 0.61). Fig. 1(B) showed the down regulation of CB2R mRNA in blood samples of DM patients by 0.563 fold compared with control group (see Table 1).

FBG analysis showed a significant difference of its concentration in diabetic samples of patients (226.3 ± 26.90) compared with its low concentration

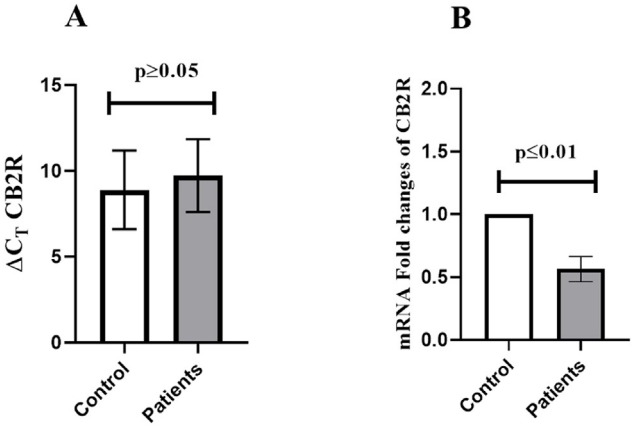

Fig. 1. Gene expression of CB2R in DM patients and healthy group. A compare ΔCt of DM patients with ΔCt of control group. B-fold change of CB2R expression in DM patients compared with healthy group expression level. Which optimized with the expression level of GAPDH gene. The lower value of ΔCt represents the highest expression level of the gene.

Table 1. CB2R expression level of DM patients and control group.

Individuals	C_T GAPDH	C_T CB2R	ΔC_T	$\Delta\Delta C_T$	Fold change
Control	28.67 ± 1.20	36.64 ± 1.36	8.89 ± 1.02	0.82 ± 0.61	0.563
Patients	25.01 ± 0.7193	34.74 ± 1.14	9.72 ± 0.61		

Mean ± standard error.

in the healthy group (109.6 ± 4.98). Glycated hemoglobin (HbA1c) analysis showed a highly increasing in DM group (8.16 ± 0.42) compared with healthy group (5.180 ± 0.29) at $p \leq 0.001$. Cholesterol level was decreased in control group (116.2 ± 6.33) compared with DM patients (160.1 ± 13.30). Triglyceride TG concentration was statically increased ($p \leq 0.05$) in DM samples (192.2 ± 24.69) compared with healthy group (91.76 ± 7.58). The level of HDL in blood samples of DM patients was significantly ($p \leq 0.05$) reduced (20.25 ± 0.93) compared with its level in healthy group (35.66 ± 9.34). While the level of LDL and VLDL were significantly raised in patients samples ($p \leq 0.01$) as shown Table 2 compared with their levels in healthy group.

Table 3 represented the correlation between the expression level of CB2R and studied biochemical parameters including (glucose, HbA1c, cholesterol and triglyceride). However, these results refers to non-significant correlation was identified between the gene expression and the above parameters ($p \geq 0.05$, $r = 0.515$, 0.369, 0.088 for glucose, HbA1c and triglyceride respectively. While anon significant negative relationship was identified between CB2R expression and cholesterol level ($p \geq 0.05$, $r = -0.2052$).

Table 2. Biochemical parameters levels in patients of DM and healthy group.

Biochemical parameters	Healthy subjects	Diabetes patients	p-value
FBG	109.6 ± 4.98	226.3 ± 26.90	*0.0152
HbA1c (%)	5.180 ± 0.29	8.16 ± 0.42	***0.0005
Cholesterol	116.2 ± 6.33	160.1 ± 13.30	**0.0100
Triglyceride	91.76 ± 7.58	192.2 ± 24.69	**0.0049
HDL	35.66 ± 9.345	20.25 ± 0.9372	*0.0202
LDL	81.68 ± 5.917	112.8 ± 6.549	**0.0059
VLDL	21.23 ± 3.230	37.49 ± 4.567	**0.0087

*$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$, mean ± standard error.

Table 3. Correlation association of CB2R expression with studied biochemical parameters of DM patients.

Biochemical parameters	*r*-value	95% confidence interval	p-value
Blood glucose	0.515	−0.082 to 0.840	0.0860
HbA1c (%)	0.369	−0.338 to 0.810	0.2930
Cholesterol	−0.205	−0.794 to 0.583	0.6259
Triglyceride	0.088	−0.573 to 0.680	0.8080

4. Discussion

Diabetic mellitus is identified as a metabolic disease resulting from reduction in insulin action, insulin secretion or both [23,24]. CB2R existed in all human body tissues and implicated in different physiological regulations [25,26]. CB2R is an essential element of endocannabinoid system encoded by CB2R gene is located on chromosome one and consisted of one translated exon and rounded by 3′ and 5′ un translated regions [27]. CB2R up regulated and enhanced fat inflammation, this is contributed in liver steatosis and insulin resistance [28]. Therapeutic are focused to improve alleviate of insulin resistance and insulin secretion thus CB2R represents a new target for several drugs to treat several inflammation diseases [29]. Therefore this study aimed to measure CB2R expression level in diabetic patients. CB2R expression was down regulated in blood samples of DM as shown Fig. 1B. This study consists with the study of [30] and it has been identified CB2R expression is decreased in podocytes under diabetic nephropathy condition. In contrast, CB2R mRNA level is unaltered in diabetic whole kidneys compared with healthy group [31]. It is believed that activation CB2R enhance the insulin secretion from beta-cells of pancreatic and promote the entering of calcium [14]. This is could be due to induce inflammatory cells in kidney tissus [30]. FBG level was significantly increased 226.3 ± 26.90 with DM patients compared with its level 109.6 ± 4.98 in control group Table 2. This is elevated could be resulted from reduction in the secretion of insulin or defects in insulin act or both [32]. Increasing FBG level consists with the study of [32]. HbA1c shows significant increasing with DM patients compared with control group. HbA1c was used to measure average blood glucose for 2–3 months. More than 8% of HbA1c was increased in DM patients [33]. Cholesterol, triglyceride, LDL and VLDL levels were increased significantly in blood serum of DM group compared with healthy group as shown in Table 2. While HDL was statistically reduced in DM group. These results agree with the results of [34] they demonstrated the reduction of HDL could be due to increase hepatic lipase activity that has a significant role in the metabolism of HDL. In contrast [35], did not identify significant increase in HDL level in DM patients. The reduction of LDL absorption in

fibroblasts may contribute in decreasing HDL and increasing LDL levels [34,36]. Increasing LDL and VLDL levels could be due to decrease VLDL removing from circulation system or increase it is production [37]. In contrast, triglyceride and cholesterol levels were down regulated in DM group compared to non-DM group, and low levels of HDL and LDL were identified in DM group compared with healthy group [38].

No correlation has been identified between CB2R expression and studied biochemical parameters as demonstrated in Table 3. According to CB2R expression was significantly low in DM group and this results agree with the study of [39] but as well as glucose and cholesterol level was identified at $r = -0.2052$ even though no significant correlation between CB2R mRNA level but still there is an inverse correlation [18] identified CB2R function by increasing the rate of oxidation of fatty acid and identified it is pathway in homeostasis of lipid as well as their data demonstrated that CB2R function can be modulated by using a specific agonist to regulate the oxidation rate of fatty acid. The association between DM and cannabinoids ligands have been characterized in numerous studies [29]. Number of experimental projects demonstrated that CB2R activation by agonists have a vital pharmacological impacts including immunoduatery cardioprotective, antioxidant, gastroprotcteive, nephroprotective, atheroprotective, cardio-protective [14,40]. However, the results of this study support previous studies and emphasis there is a relationship between CB2R expression and DM disease and these results highlight a new possible target to reduce or prevent DM but addition studies are required to explain the selective ligands of CB2R in DM diseases and evaluate the function ligands and its effect on DM.

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