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Association of interleukin-4 polymorphism with diabetic retinopathy and neuropathy in a Sudanese population

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Abstract

Background: Interleukin-4 (IL-4) is a multifunctional cytokine; involved in the regulation of immune responses, as well as in the pathogenicity of many diseases, such as diabetes mellitus. Some researchers suggested that IL-4 protects the human pancreatic islet from cytotoxic damages, whereas others suggested some inhibitory actions of IL-4 on pancreatic islets. This study aimed to assess the role of IL-4 genotypes of intron 3 variable number of tandem repeats of the IL-4 gene in diabetic retinopathy and diabetic neuropathy in Sudanese patients with type 2 diabetes mellitus (T2DM). This case–control study was performed in a number of Khartoum state hospitals in Sudan. The study enrolled 181 Sudanese patients, 115 (57 females and 58 males) diagnosed with T2DM and 66 (29 females and 37 males) healthy persons who served as control subjects. Polymerase chain reaction was used for the analysis of IL-4, which was amplified using the following amplification sequence (forward primer: CACGACGTTGTAAAACGACTAGGC TGAAAGGGGGAAAGC; reverse primer: CTGTTCACCTCAACTGCTCC). Biochemical analyses for highly sensitive C- reactive protein (hs-CRP), glycated hemoglobin (HbA1c), fasting plasma glucose, total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein were performed using a chemical analyzer.

Results: The study showed that in the diabetic group, 49(42.6%) had diabetic retinopathy, whereas 7(6.1%) had diabetic neuropathy. The B1B1 genotype was found to be a higher risk factor for developing diabetic retinopathy than B2B2 [P = 0.028; Odds ratio (OR) = 1.381; 95% confidence interval (CI) 1.344–9.062], whereas the B1B2 genotype was found to be insignificantly associated with retinopathy (P = 0.357; OR = 1.570; 95% CI 0.654–3.887). Furthermore, hs-CRP and HbA1c were significantly increased in diabetic neuropathy with IL-4 B1B1 genotype.

Conclusions: IL-4 gene polymorphisms can be good markers for the early identification of risk for diabetic retinopathy and neuropathy in Sudanese people. The hs-CRP and HbA1c in diabetic patients with IL-4 B1B1 genotype may be predisposition predictors of diabetic neuropathy.

Keywords: Interleukin-4, Intron 3 VNTR, Type 2 diabetes mellitus, Retinopathy, Neuropathy, Sudan

Background

Interleukins are a type of cytokines produced by somatic cells as well as by leukocytes (Justiz and Qurie 2020). Interleukins play essential roles in the pathogenicity of many diseases (Balasubramanian et al. 2006) and have both autocrine and paracrine functions (Justiz and Qurie 2020). First identified in 1980, interleukin-4 (IL-4) is a multifunctional cytokine mainly produced by T cells, mast cells, eosinophils, and basophils (LaPorte et al. 2008). IL-4 is a potent prototypic member of the Th2-type cytokine subset that acts as a mediator for proinflammatory chemokines (Kuran et al. 2019; Al-Ayed et al. 2019). IL-4 suppresses adipocyte differentiation and thus

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enhances lipolysis (Tsao et al. 2014). It regulates the proliferation of macrophages, lymphocytes, and endothelial cells as well as apoptosis and the expression of many genes (LaPorte et al. 2008). IL-4 also inhibits tumor growth by exhibiting anti-angiogenic effects (Balasubramanian et al. 2006) and is involved in isotype switching from immunoglobulins (Kuran et al. 2019). Moreover, IL-4 regulates immune responses inducing helper T cell differentiation (Arababadi et al. 2012). Type 2 diabetes mellitus (T2DM) is a multifactorial genetic disease, characterized by different environmental and genetic causes (Cilensek et al. 2012). T2DM induces oxidative stress associated with various metabolic disturbances (Kundu el al. 2014; Pasnoor et al. 2013). Free radicals produced from T2DM damage cellular enzymes, increase lipid peroxidation, and promote insulin resistance, and lead to the development of diabetic complications (Maritim et al. 2003). The major complications of T2DM are due to the damage of small blood vessels including the eyes, nerves, and kidneys (Rosenberger et al. 2020). Diabetic retinopathy and diabetic neuropathy are the most important complications of T2DM (Pasnoor et al. 2013; Madsen-Bouterse and Kowluru 2008). Diabetic retinopathy is a severe microvascular complication of diabetes (Stamenkovic et al. 2018) and is also the most severe ocular disease (Madsen-Bouterse and Kowluru 2008). Like glaucoma, diabetic neuropathy is characterized by the quick degeneration of retinal ganglion cells and is a leading cause of acquired blindness (Stamenkovic et al. 2018).

Diabetic neuropathy is a multifactorial interaction of genetic, biological, and psychosocial factors (Hebert et al. 2017). Diabetic neuropathy affects 50% of patients with diabetes (Pasnoor et al. 2013; Prabodha et al. 2018). Diabetic neuropathy symptoms include tingling, numbness and altered pain sensation, which may damage the patient's skin (Rosenberger et al. 2020). However, proximal diabetic neuropathy may lead to painful muscle atrophy, weakness, and cognitive defects and disabilities (Oyenihi et al. 2015).

Aim of the study

The aim of this research was to assess the role of IL- 4 genotypes of intron 3 variable number of tandem repeats (VNTR) of the IL-4 gene in diabetic retinopathy and neuropathy in Sudanese patients with T2DM. VNTR was associated with IL-4 production (Nakashima et al. 2002).

Methods

Patient selection and data collection

This case–control study was conducted at Ribat University Hospital and Ahmed Gasim Fadul Hospital for Cardiac Surgery and Renal Transplantation in Khartoum, Sudan, in the period from 1 September 2015 to 30 May 2019. A total of 115 Sudanese patients suffering from T2DM (57 females and 58 males) and 66 healthy persons as control subjects (29 females and 37 males) were enrolled.

Ethical considerations

Ethical approval was obtained from the Ethics Research Committee of National Ribat University (Ph.D/medical–lab/2015/no:1). Written consent was obtained from all patients. Clinical and sociological data were collected through questionnaires conducted by a physician.

DNA extraction

Approximately 2.5 mL venous blood was collected from each patient and mixed with ethylenediaminetetraacetic acid (EDTA) anticoagulant. Genomic DNA was isolated from EDTA whole blood using Generation DNA purification capture column kit (Analytica Jena, Berlin Germany) according to manufacturer's instructions and then stored at − 20 °C.

Polymerase chain reaction for interleukin-4

IL-4 was amplified using the following amplification sequence (forward primer: CACGACGTTGTAAAA CGACTAGGCTGAAAGGGGGAAAGC; reverse primer: CTGTTCACCTCAACTGCTCC) (Tong et al. 2013).

The region that contains the VNTR of 70 bp within the IL-4 intron-3 was amplified by polymerase chain reaction (PCR). Primers were prepared using 10 µL primer added to 90 µL sterile de-ionized water. Forward and reverse primers were prepared in separate Eppendorf tubes. The PCR mixture was prepared by adding 0.5 µL forward primer, 0.5 µL reverse primer of each gene, and 17 µL sterile water to a PCR Premix tube before finally adding 2 µL DNA of patient sample for a total volume of 20 µL. The experiment consisted of DNA in 0.5 mL mixture (PCR Premix tube, Intron Biotechnology). Using PCR for all subjects, amplification was performed by initial denaturation at 95 °C for 10 min, followed by 30 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 7 min. The amplified products were further analyzed by electrophoresis in a 1.5% agarose gel and stained with 0.5 mg/mL (concentration) ethidium bromide. Approximately 2 µL of 100 bp ladder and 10 µL of PCR product were loaded on gel.

Biochemical methods

Biochemical analyses for fasting plasma glucose, total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were performed using a fully automated chemical analyzer (Mindray BS-380). But highly sensitive C-reactive protein (hs-CRP)

and glycated hemoglobin (HbA1c) were measured with a chemistry analyzer (COBAS Integra 400 plus).

Statistical methods

SPSS version 20 was used to calculate the means, standard deviations, probability, genotypes distribution and P-values. Odds ratio (OR) was assessed by Chi-squared test with 95% confidence intervals. One-way analysis of variance (ANOVA) was used for comparisons between groups. Statistical significance was considered when $P < 0.05$.

Results

In the diabetic group, the mean age was (53.60 ± 7.822 years), the mean duration period of the disease was (10.080 ± 7.044 years), and the mean body mass index was (27.76 ± 3.82) kg/m^2.

Intron 3 VNTR of IL-4 gene on the electrophoresis gel was identified as the following IL-4 genotypes: B1B1, B2B2, and B1B2, which were identified as the two repeats (183 bp allele, and three repeats (253 bp allele) of VNTR polymorphism and expressed as B1 and B2, respectively. Homozygote genotypes were expressed as (B1B2), in the electrophoresis gel. The biochemical parameters studied according to IL-4 genotype distribution included glucose, HbA1c, hs-CRP, total cholesterol, triglycerides, HDL, and LDL. ANOVA revealed only statistically insignificant differences among genotypes in glucose, total cholesterol, triglycerides, HDL, LDL, and body mass index (P-values = 0.513, 0.559, 0.458, 0.237, and 0.242, respectively). However, there were significant increases in HbA1c and hs-CRP levels in the B1B1 genotype (P = 0.003 and 0.001, respectively), as shown in Table 1.

The frequencies of IL-4 genotypes in T2DM patients and the healthy control group were 58 (50.4%) and 34 (51.5%), respectively, for B2B2; 27 (23.5%) and 5 (7.6%), respectively, for B1B1; and 30 (26.1%) and 27 (40.9%), respectively, for B1B2. The distributions of the IL-4 genotypes with risk analysis of T2DM showed that the B1B1 genotype was a high-risk factor for T2DM (P = 0.028; OR = 3.166; 95% confidence interval (CI) 1.114–8.991), whereas the B1B2 genotype was protective (P = 0.233: OR = 0.651; 95% CI 0.333–1.273) (Table 2).

The distributions of IL-4 genotypes with risk analysis of diabetic retinopathy showed that; the B1B1 genotype was a high-risk factor for diabetic retinopathy (P = 0.011; OR = 1.381; 95% CI 1.344–9.062), whereas the B1B2 genotype was insignificantly associated with retinopathy (P = 0.357; OR = 1.570; 95% CI 0.654–3.887) (Table 3). Furthermore, the prevalence in the diabetic group for diabetic retinopathy and diabetic neuropathy was 49 (42.6%) and 7(6.1%), respectively (Figs. 1, 2).

Comparisons of the mean values of the biochemical parameters and body mass index in Sudanese patients

Table 1 Comparison of biochemical parameters of interleukin-4 genotypes in type 2 diabetes mellitus (n = 115)

Genotypes	B1B1 (n = 27)	B2B2 (n = 58)	B1B2 (n = 30)	P value
Glucose (mg/dL)	189.11 ± 79.123	179.53 ± 86.665	165.53 ± 54.910	0.513
HbA1c (%)	9.637 ± 2.2937	8.097 ± 1.7053	8.170 ± 2.1552	0.003
hs-CRP (mg/L)	49.734 ± 9.593	9.852 ± 2.3632	2.073 ± 1.379	0.001
Total cholesterol (mg/dL)	218.48 ± 58.392	206.72 ± 60.971	202.13 ± 55.883	0.559
Triglycerides (mg/dL)	150.67 ± 72.568	141.76 ± 62.645	130.67 ± 41.207	0.458
HDL (mg/dL)	41.66 ± 8.777	44.41 ± 9.280	45.67 ± 8.872	0.237
LDL (mg/dL)	100.96 ± 37.892	114.72 ± 41.443	105.03 ± 29.417	0.242
BMI (kg/m2)	28.245 ± 4.4244	27.919 ± 3.6516	27.033 ± 3.6172	0.450

Comparisons were performed by one-way analysis of variance.

Significant differences between groups (P < 0.05). HbA1c, glycated hemoglobin; hs-CRP, highly sensitive C-reactive protein; HDL, high- density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index

Table 2 Distribution of interleukin-4 genotypes in type 2 diabetes mellitus and control groups and risk analysis

Genotype	T2DM, n (%)	Control, n (%)	X^2	OR (95% CI)	*P value*
B2B2	58 (50.4%)	34 (51.5%)		1.0 Reference	
B1B1	27 (23.5%)	5 (7.6%)	5.011	3.166 (1.114 –8.991)	0.028
B1B2	30 (26.1%)	27 (40.9%)	1.578	0.651 (0.333 –1.273)	0.233
Total	115 (100.0)	66 (100.0)			

Comparisons were performed by Chi-squared test

Significant differences between groups (P < 0.05). T2DM, type 2 diabetes mellitus; OR, odds ratio; CI, confidence interval

Table 3 Distribution of interleukin-4 genotypes in Sudanese patients with type 2 diabetes mellitus and risk analysis of developing diabetic retinopathy

Genotypes	Presence of retinopathy, n (%)	Absence of retinopathy, n (%)	X^2	OR (95% CI)	P value
B2B2	19 (38.8%)	39 (59.0%)		1 Reference	
B1B1	17 (34.7%)	10 (15.2%)	3.489	1.381 (1.344 –9.062)	0.011
B1B2	13 (26.5%)	17 (25.8%)	0.956	1.570 (0.654 –3.887)	0.357
Total	49	66	115		

Comparisons were performed by the Chi-squared test

Significant differences between groups ($P < 0.05$). OR, odds ratio; CI, confidence interval

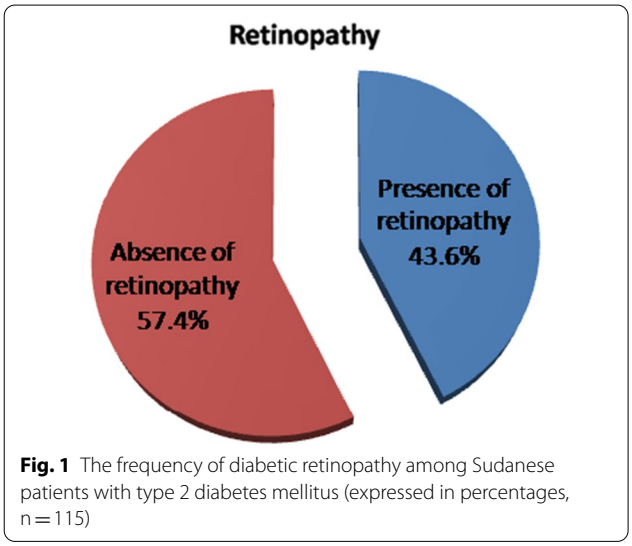

Fig. 1 The frequency of diabetic retinopathy among Sudanese patients with type 2 diabetes mellitus (expressed in percentages, n = 115)

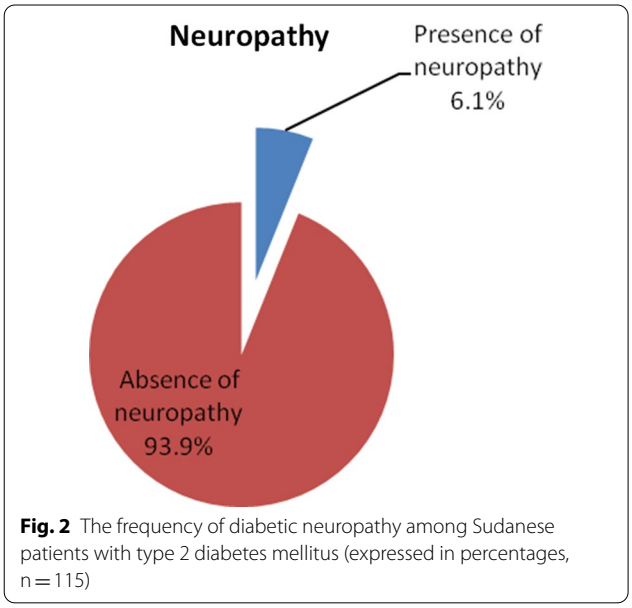

Fig. 2 The frequency of diabetic neuropathy among Sudanese patients with type 2 diabetes mellitus (expressed in percentages, n = 115)

with T2DM regarding the presence and absence of diabetic retinopathy and diabetic neuropathy are shown in Tables 4 and 5, respectively.

Discussion

Many studies (Maritim et al. 2003; Rosenberger et al. 2020) have investigated the role of immunological mechanisms in the pathogenicity of diabetic retinopathy and diabetic neuropathy. IL-4 is known as a type of chemokine and cytokine and acts as a mediator for inflammatory or proinflammatory functions. Inflammation plays a major role in increasing the production of free radicals and oxidative stress in the body. Poor glycemic control and oxidative stress most likely lead to diabetic complications, such as diabetic retinopathy and neuropathy**.** In the present study**,** the findings on the frequencies of IL-4 genotypes in T2DM (Table 2) suggest that the susceptibility to develop T2DM was three-folds higher in patients with IL-4 B1B1 genotype than those with IL-4 B2B2 genotype. Although genetic factors play a role in diabetic retinopathy, uncontrolled hyperglycemia is a critical risk factor in its pathogenesis (Pradhan et al. 2016). The findings of the present study further demonstrate that certain IL-4 genotypes particularly predispose toward diabetic retinopathy. These findings are contrary to a report by (Završnik et al. 2018) who did not find an association between IL-4 and diabetic complications in a Slovenian study of Caucasian patients with T2DM.

On the contrary, our study findings on the frequency of diabetic retinopathy (Figs. 1, 2) are consistent with a report by (Ciulla et al. 2003). Furthermore, Stamenkovic et al. 2018 reported that diabetic retinopathy was estimated at 50% in patients with diabetes. The prevalence of diabetic retinopathy in American people with T2DM, regardless of racial difference, is 28.5% (Yue Li et al. 2018), which is much lower than in Sudanese with T2DM.

Diabetic neuropathy is another common complication of T2DM. As reported by (Yeong et al. 2019), IL-4

Table 4 Comparisons of biochemical parameters and body mass index in Sudanese with type 2 diabetes mellitus with regards the presence or absence of retinopathy (n = 115)

Parameters	Presence of Retinopathy (n = 49)	Absence of Retinopathy (n = 66)	*P value*
Glucose (mg/dL)	164.35 ± 46.166	188.36 ± 93.474	0.073
HbA1c (%)	8.516 ± 2.2974	8.448 ± 1.8865	0.862
hs-CRP (mg/L)	21.186 ± 6.889	14.216 ± 3.520	0.481
Total cholesterol (mg/dL)	213.51 ± 63.143	204.41 ± 55.675	0.415
Triglycerides (mg/dL)	129.98 ± 56.556	149.11 ± 62.239	0.093
HDL (mg/dL)	44.92 ± 8.691	43.48 ± 9.405	0.403
LDL (mg/dL)	113.35 ± 41.474	105.71 ± 35.134	0.288
BMI (kg/m2)	27.298 ± 4.325	28.111 ± 3.409	0.262

Independent t-test was used

P < 0.05 was considered significant

HbA1c, glycated hemoglobin; hs-CRP, highly sensitive C-reactive protein; HDL, high- density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index

Table 5 Comparisons of biochemical parameters and body mass index in Sudanese with type 2 diabetes mellitus with regards the presence or absence of neuropathy (n = 115)

	Presence of Neuropathy (n = 7)	Absence of Neuropathy (n = 108)	*P value*
Glucose (mg/dL)	175.57 ± 61.722	178.30 ± 78.749	0.929
HbA1c (%)	11.900 ± 2.325	8.225 ± 1.874	0.000
hs-CRP (mg/L)	18.006 ± 5.369	4.543 ± 1.618	0.011
Total cholesterol (mg/dL)	227.86 ± 37.547	207.02 ± 59.897	0.366
Triglycerides (mg/dL)	129.71 ± 51.896	141.69 ± 61.28	0.613
HDL (mg/dL)	41.09 ± 1.577	44.29 ± 9.349	0.005
LDL (mg/dL)	98.71 ± 48.335	109.63 ± 37.397	0.464
BMI (kg/m2)	24.27 ± 5.338	27.995 ± 3.226	0.112

Independent t-test was used

P < 0.05 was considered significant. HbA1c, glycated hemoglobin; hs-CRP, highly sensitive C-reactive protein; HDL, high- density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index

plays a critical role in neurodegeneration and neuronal death by regulating oxidative stress in neurodegenerative diseases. While it is known that diabetes mellitus induces oxidative stress with various metabolic disturbances, the exact metabolic pathway is unclear, but the most commonly proposed one is enhanced cellular oxidative stress (Pang et al. 2020). In T2DM, hyperglycemia induces overproduction of reactive oxygen species, leading to damage to lipids, proteins, and DNA, which is key to the pathogenesis of diabetic neuropathy (Kasznicki et al. 2012). Genetic susceptibility and oxidative stress may have a role in the occurrence of T2DM and diabetic neuropathy (Stoian et al. 2015). In this study, the frequency of neuropathy in Sudanese with type 2DM is (6.1%). While the prevalence of diabetic neuropathy may reach as much as 67% according to a systemic review (Zhao et al. 2019), the present finding of 6.1% prevalence indicates that diabetic neuropathy is significantly lower in Sudanese with T2DM. While the mechanism of diabetic neuropathy is still not fully understood, the metabolic issues involving elevated HbA1c as revealed by this study indicates a significant association between diabetic neuropathy and increased HbA1c (Table 5), which is in agreement with previous reports (Kasznicki et al. 2012; Themistocleous et al. 2016).

Further findings in the present study include a significant increase in HbA1c and hs-CRP levels in patients with a B1B1 genotype, which affirm the report (Acharya et al. 2016), on the significant association of HbA1c with T2DM. The IL-4 B1B1 genotype may thus be a good predictor of the onset of diabetic retinopathy.

Conclusions

Many genetic causes are associated with the development of diabetic complications. The IL-4 gene polymorphism can be a good marker for early identification in Sudanese people at risk of diabetic retinopathy and neuropathy. The IL-4 B1B1 genotype may be an accurate predictor for the onset of diabetic retinopathy. This study is a conceptual investigation, and larger-scale future studies are needed to establish the exact role of IL-4 in diabetic retinopathy.

Abbreviations

IL-4: Interleukin-4; T2DM: Type 2 Diabetes Mellitus; hs-CRP: C- reactive Protein; HbA1c: Glycated Hemoglobin; VNTR: Variable Number of Tandem Repeats; EDTA: Ethylenediaminetetraacetic Acid; PCR: Polymerase Chain Reaction; SPSS: Statistical Package for Social Sciences; ANOVA: One-way Analysis Variance.

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Authors' contributions
ET prepared the research project, shared sample collection, did data analysis, wrote the paper, shared the consumables. He was the main supervisor of the research. GM prepared the project proposal, collected the samples, did the lab work, did data analysis, wrote the paper and shared the consumables. HA prepared the project proposal, did the clinical work, checked the patients and controls, did data analysis and wrote the paper, he was the co-supervisor of the project. SA prepared the project proposal, shared in data collection, did data analysis and wrote the paper, shared the consumables. AE did data analysis, wrote the paper, shared the consumables of the research. All authors have read and approved the final manuscript.

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Availability of data and materials
Data are available on request. Authors may be contacted at the email: carcar.2009@yahoo.com (Dr.Gaafar Mahmoud).

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the Ethics Research Committee of National Ribat University (Ph.D/medical–lab/2015/no:1). Written consents were obtained from all patients participated in this study.

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no financial relationships at present or had any in the previous years with any organizations that might have an interest in the submitted research.

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