Association of *G22A* polymorphism of the adenosine deaminase (*ADA*) gene with biochemical characteristics in type 2 diabetic Palestinians

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ABSTRACT. The adenosine deaminase *G22A* polymorphism (20q.11.33) affects the level of adenosine deaminase (*ADA*) expression, which plays an important role in the regulation of intracellular and extracellular concentrations of adenosine. Recent studies reported greater ADA activity in diabetic patients and showed the role of ADA in the modulation of insulin activity and glucose homeostasis. We investigated whether the *G22A* polymorphism of the *ADA* gene is associated with type 2 diabetes mellitus (T2DM) in the Palestinian population and assessed the relationship between the *G22A* variant and fasting plasma glucose (FPG), glycated hemoglobin (HbA1c) and lipid profile among T2DM patients. A total of 231 individuals with T2DM and a control sample of 101 non-diabetic participants were randomly selected from those who were attending United Nations Relief and Works Agency (UNRWA) clinics for treatment and/or follow up. Genomic DNA was extracted from peripheral blood samples and PCR-RFLP was performed to identify the *TaqI* polymorphism *G22A* of the *ADA* gene. No significant differences were observed in the genotype and allele frequencies between T2DM patients and the control group. Yet,

among diabetic patients, the GG genotype was significantly associated with higher FPG and HbA1c when compared to the GA+AA genotype but had no influence on blood pressure, BMI or other metabolic parameters. In conclusion, we confirm that the GG genotype of the *ADA* gene is associated with poor glycemic control in T2DM Palestinians and points to the association of the *G22A* variant with decreased activity of the *ADA* enzyme, which is of paramount importance in the pathophysiology of T2DM.

Key words: Diabetes Mellitus Type 2; Fasting Plasma glucose; Adenosine Deaminase; Glycated Hemoglobin; Palestine

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a progressive condition resulting from insulin resistance and /or from a reduction in insulin production by the pancreas (Gow et al., 2016). Its prevalence is increasing rapidly in middle- and low-income countries including Palestine. The prevalence of diabetes in the Palestinian population was 15.3% in 2010, but estimates have predicted it to rise to 20.8% by 2020 (Abu-Rmeileh, 2012; Abu-Rmeileh et al., 2013). The number of new cases in Palestine reported annually, has ranged from 150 to 220 per 100,000 population, among which 95% of diabetic cases were diagnosed with T2DM (WHO, http://www.emro.who.int/pse/palestine-news). Diabetes mellitus is strongly associated with lifestyle, genetic and family related risk factors. Immunological disturbance in T2DM subjects has been attributed to cell-mediated immunity and inappropriate T-lymphocyte function (Prakash et al., 2006).

Adenosine deaminase (*ADA*), encoded by the *ADA* gene (gene map locus 20q13.11), is an enzyme that catalyzes irreversible deamination of adenosine to inosine and has a crucial role in regulating extracellular and intracellular adenosine concentration (Franco et al., 2007a; 2007b; Concetti et al., 2015). ADA is widely distributed in most mammalian tissues, but the highest activity is observed in lymphoid and fatty tissues (Niedzwicki and Abernethy, 1991; Husseini et al., 2009). High lymphocytic ADA activity is associated with alteration in the cell-mediated immune response; altered blood levels of this enzyme may predict immunological dysfunction associated with T2DM (Prakash et al., 2006). The action of adenosine on glucose and lipid metabolism in adipose tissue is similar to that of insulin (Johansson et al., 2008). Adenosine has antilipolysis activity in adipose tissue and acts directly to stimulate insulin activity by increasing accessibility of glucose transporter type 4 (GLUT4) to the cell surface for glucose transportation (Antonioli et al., 2014; Larijani et al., 2016), while it counteracts insulin action in the liver by activating A2B receptors (Yasuda et al., 2003; Antonioli et al., 2014). Han et al. (1998) demonstrated that blocking the action of adenosine with antagonists decreases transport of glucose to the skeletal muscles. Furthermore, it was reported that adenosine affects plasma levels of cholesterol and triglycerides by controlling liver cholesterol synthesis and thus the amount of fat tissue (Koupenova and Ravid, 2013). Several studies reported greater ADA activity in T2DM patients (Pinnelli, 2006; Khemka et al., 2013). High ADA levels result in an increase in serum TG levels in patients with T2DM by increasing lipolysis (Kaur et al., 2016). Prakash and Ananda Rao (2006) have demonstrated that elevated ADA activity is an important indicator of the immuno-pathogenesis of T2DM. Therefore, targeting adenosine

receptors has become promising in prophylaxis against diabetes and cardiovascular diseases.

Because the level of endogenous adenosine is affected by the enzyme ADA, polymorphisms within the *ADA* gene may have some effect on *ADA* activity. It is reported that the common *ADA* variant *G22A* polymorphism (c.22G>A, rs73598374) decreases the rate of conversion of adenosine to inosine in cells by approximately 20-35%, leading to a buildup of adenosine (Battistuzzi et al., 1981). Therefore, it is hypothesized that individuals with the A allele show reduced ADA enzymatic activity and higher levels of both circulating and intracellular adenosine compared to homozygous GG individuals (Riksen et al., 2008).

We investigated a possible association between *ADA* variant *G22A* with risk of T2DM. We also assessed the relationship between the *G22A* variant and fasting plasma glucose, glycated hemoglobin and lipid profile among type 2 diabetic Palestinians.

MATERIAL AND METHODS

Ethical considerations

This study was approved by the Research Ethics Committee of Al-Quds University (Rf no. 2/SRC/4). Each participant signed a consent form after providing information about the study objectives.

Study participants

A total of 332 participants were enrolled in this study. All participants were unrelated, aged >40 years and recruited within the period of 2016-2017 in collaboration with UNRWA (United Nations Relief and Works Agency) clinics (Hebron and Ramallah, Palestine). All cases (n=231) were diagnosed with diabetes mellitus type 2 and were on use of oral hypoglycemic agents. Subjects on insulin treatment were excluded from the study. The control group (n=101) was selected from healthy individuals who came to the same clinic for an annual heath check-up with no history of DM, and their fasting plasma glucose concentration was ≤100 mg/dl, fasting total cholesterol<220 mg/dl and BMI <30 kg/m.

Genomic DNA extraction and *ADA* genotyping

A 5-mL sample of blood was taken from each participant via venipuncture into vacuum tubes with ethylenediaminetetraacetic acid (EDTA) for genomic DNA extraction using a commercial kit (QIAGEN GmbH, Hilden, Germany). To characterize the *G22A* polymorphism of the *ADA* gene, a partial fragment (397 bp) was amplified using a published primer pair (Safranow et al., 2007). The amplified product was digested using *Taq I* restriction enzyme (Nunes et al., 2011) generating two bands (152, 245 bp) for the GG genotype, three bands (397, 245, 152 bp) for the GA genotype and one band of 397 bp for the AA genotype, indicating absence of a *Taq I* restriction site. All PCR-RFLP products were analyzed using 2% agarose gel electrophoresis in TAE (Tris-acetate EDTA) buffer.

Statistical Methods

All data were analyzed using SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.). The chi-square test was used to compare allelic and genotypic frequencies of the *ADA* gene. Comparison of biochemical and anthropometric parameters between groups was conducted using a one-way ANOVA. Statistical significance was defined as P<0.05.

RESULTS

The anthropometrical and biochemical parameters of the study participants (231 diabetic and 101 controls) are shown in Table 1. Significant differences in anthropometrical and biochemical parameters were observed between the two groups (P<0.05). Among the diabetic group, 14.3, 9.5, 6.9 and 5.6% had cardio vascular disease (CVD), nephropathy, diabetic foot and retinopathy, respectively. Only 5.6% had normal BMI; 32.9% were overweight and 61.5%, were obese.

Table 1. Anthropometrical and biochemical parameters of the diabetes study groups. Data are in means ± SD. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; TC: Total cholesterol.

Parameters	cases (n=231)	controls (n=101)	P-value
Sex (M/F)	119:112	53:48	-
Age at diagnosis (years)	50.9±9.9	NA	-
Age at sampling (years)	59.3 ±10.9	47.7±11.0	0.0001
BMI (Kg/m^2)	32.5±6.4	27.3±5.7	0.0001
SBP (mmHg)	136.1±16.2	121.3±10	0.0001
DBP (mmHg)	79.6±9.9.3	77.2±8.5	0.0001
FPG (mg/dl)	162.3±58.5	85.2±8.7	0.0001
TC (mg/dl)	184.3±45.6	168.4±33.8	0.0018

NA: Not applicable

Frequency distribution of *ADA* genotypes

The frequencies of *ADA* genotypes were in Hardy–Weinberg equilibrium for patients and controls (p>0.05). Table 2 shows the allelic and genotypic frequencies of the *G22A ADA* gene polymorphisms in T2DM cases and controls. The frequency of the GG genotype was higher in both groups; however, the difference was not significant (p=0.6).

Table 2. *ADA* genotype and allele frequencies in type-2 diabetes (T2DM) patients and controls

Genotype	T2DM (N=231) %	Controls (N=101) %
GG	66.7	69.3
GA	30.7	28.7
AA	2.6	2.0
MAF (%)	18	16

MAF: Minor allele frequency

Association of *ADA* genotypes with anthropometrical and biochemical measurements

As the frequency of the AA genotype in both groups was low, the GA and AA genotype carriers were grouped together in the analyses. The mean of biochemical

characteristics in carriers of GG genotype and carriers of at least one A allele (GA+AA genotypes) are shown in Table 3. In the diabetic group, GG carriers had significantly higher fasting plasma glucose (FPG) (P=0.026) and higher HbA1c (P=0.037) when compared with GA+AA genotypes. On the other hand, the mean serum levels of TG, TC, LDL and HDL showed no significant differences in GG carriers compared to GA+AA genotypes (P>0.05). Moreover, no association was found between the *ADA* genotype and cardio vascular disease or diabetes complications (P>0.05).

In the control group, there was no evidence of an association of *ADA* genotypes with FPG (85.5±8.9 for GG and 84.5±8.0 for GA+AA, P=0.64) or with serum cholesterol (TC) (165.8±33.3 for GG and 177.7±35.2 for GA+AA, P=0.14). As the measurements of TG, LDL, HDL and HBA1c were not available for the control group, we could not demonstrate the effect of *ADA* genotypes on these biochemical parameters. Both groups (cases and controls) showed no significant differences between the *ADA* genotypes and sex, age, BMI, as well as systolic and diastolic blood pressure.

Table 3. Demographic characteristics and biochemical measurements across *ADA* genotypes in type 2 diabetes mellitus (T2DM) patients in the Palestinian population. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; TC: Total cholesterol; TG: Triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

	T2DM (n=231)		
	GG	GA+AA	P-value*
Number	154	77	
Age(years)	59.8±10.5	58.4±11.9	NS
Age at diagnosis (years)	50.9±9.6	50.7±10.7	NS
BMI (kg/m^2)	32.5±6.3	32.6±6.7	NS
SBP (mmHg)	135.2±15.7	137.9±17.2	NS
DBP (mmHg)	81.3±8.7	78.9±9.6	NS
FPG (mg/dl)	168.4±63.8	150.3±44.2	0.026
HbA1c	8.3±1.97	7.76±1.80	0.037
TG (mg/dl)	177.6±127.9	181±127.8	NS
TC (mg/dl)	185.3±47.4	182.5±42	NS
HDL-C(mg/dl)	42.3±10.6	40.8±9.8	NS
LDL-C(mg/dl)	107.1±24.3	104.2±24.7	NS

Values are presented as mean ± standard deviation; P value was obtained by one way ANOVA, P<0.05 was considered significant. NS: Not significant.

DISCUSSION

To the best of our knowledge, this study is the first to evaluate *G22A ADA* (rs73598374) in T2DM patients in Palestine. The *G22A* polymorphism of the *ADA* gene is reported to be the only known polymorphism that affects the expression of ADA levels (Hirschhorn et al., 1994). In our study, the control group was restricted to those aged >40 years at the time of sampling to minimize misclassification given that they may develop diabetes later in their lives. Moreover, we excluded individuals with related traits of T2DM risk (obesity and hyperlipidemia) and/or had a family history of diabetes in first-degree relatives.

Our study revealed that the GG genotype is the most common genotype in both the control and the patient groups, which was also found in other studies (Domingos et al., 2014; Salehabadi et al., 2016).

However, the overall A allele frequency of the *ADA* gene in our study participants was much higher than reported in Brazilian, Polish, Chinese and Iranian populations (Liu et al., 2006; Safranow et al., 2007; Domingos et al., 2014). Studies enlightening the

importance of the *ADA* gene in the medical field and its clinical implications among Arab population are rare. A recent study, conducted on Egyptian women, evaluated the association of *ADA G22A* gene polymorphism with recurrent spontaneous abortion (RSA) (Farhan et al., 2017). In that study there was no significant difference in allelic frequency of *ADA G22A* gene polymorphism among RSA patients as regards the co-morbidities, including diabetes (Farhan et al., 2017).To our knowledge, no data on allele and genotype frequencies of this polymorphism among diabetic patients have been reported in the Arab population.

In agreement with earlier published studies (Domingos et al., 2014; Takhshid et al., 2015), our results revealed no significant difference in allelic and genotypic frequency between the study groups (P>0.05), thus indicating no association of *ADA G22A* polymorphism with risk of T2DM in Palestinians. Yet, our study demonstrated that GG carriers had a significantly higher FPG (P=0.026) and higher HbA1c (P=0.037) compared to those who had at least one minor allele (GA+AA).These findings support the hypothesis that *ADA G22A* gene polymorphism leads to a reduction in ADA enzyme activity in diabetic patients and consequently increases intracellular concentration of adenosine, thereby attenuating pathologic consequences of T2DM. Interestingly, similar results have been demonstrated in gestational diabetes among Iranian women (Takhshid et al., 2015). However, as T2DM is a multifactorial disease, the effect of a single nucleotide polymorphism on the pathogenesis of T2DM should be considered with other lifestyle, genetic and environmental risk factors. It should be noted that no association between FPG level and *ADA* genotypes in the control group. Therefore, further studies with larger sample size and greater statistical power are required to verify these findings.

Several studies reported that ADA activity is positively correlated with blood glucose levels and HbA1c, which is an important indicator of long-term glycemic control (Lee et al., 2011; Siddiq et al, 2011; Dasegowda, 2015; Sapkota et al., 2017). Further, it was shown that *ADA G22A* gene polymorphism leads to a 30% reduction in ADA enzyme activity and thus carriers of the GA and AA genotypes of the *ADA* gene had higher concentrations of adenosine, greater activity of its receptors and less tolerance to glucose compared to those with the GG genotype (Xu et al., 1998; Chen et al., 2013; Concetti et al., 2015).

On the other hand, the increase in ADA activity, and thus the decreased level of endogenous adenosine, resulted in an increase in lipolytic activity. Therefore, T2DM patients with elevated ADA levels may have hyperlipidemia due to increasing lipolysis (Kaur et al., 2016). Case control studies conducted by Kurtul et al. (2004; 2006) demonstrated that *ADA* activity was significantly increased in the serum of obese compared with non-obese subjects. In contrast, no significant difference was found in ADA activities between obese and non-obese Nepalese subjects with diabetes (Spakota et al., 2017). Another study showed a significant positive correlation between serum ADA level and FPG level among nonobese T2DM subjects, but no significant correlation in controls (Khemka et al., 2013). Moreover,   a previous study (Dasegowda et al., 2015) showed a significant positive correlation between ADA level and serum TG, TC and LDL cholesterol, whereas a negative correlation between ADA with HDL cholesterol levels was observed. In our study, we could not demonstrate an effect of the *ADA* variant on several traits that are associated with obesity and/or insulin resistant syndrome, such as BMI, blood pressure, and serum TG, TC, HDL and LDL cholesterol among the study subjects.

Dasegowda et al. 2015) reported that ADA levels can be used as an indicator to predict long term cardiovascular complications in T2DM. Reduced ADA activity has been found in chronic heart failure patients (Khodadadi et al., 2014). Safranow et al. (2007) showed that the A allele of the *ADA* gene can protect against coronary artery disease.

Another study conducted on Chinese Han participants showed that another polymorphism (rs452159) in the *ADA* gene is significantly associated with susceptibility to chronic heart failure (He et al., 2014).

In our study, 14.3% of the cases had CVD but no association was found across *ADA* genotypes. These findings are in accordance with a Brazilian study that reported no significant differences in allele frequencies between CVD risk in diabetic and control groups (Domingos et al., 2014).

In addition to the small sample size, there are a few limitations in our study, including non-estimation of serum insulin levels and homeostasis model of assessment (HOMA-IR), which are known to be related to ADA levels. A correlation study between serum ADA level and oral glucose tolerance test across *ADA* genotypes is also needed, which was, however, beyond the scope of this study.

In conclusion, our findings revealed an association of the *ADA G22A* variant with lower levels of FPG and HbA1c in diabetic patients and also point to its association with decreased activity of the ADA enzyme, which is of paramount importance in the pathophysiology of T2DM.

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CONFLICT OF INTEREST

None declared.

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