RESEARCH ARTICLE

Impact of Smoking and Passive Smoking Habit in the XRCC1 Arg399Gln and RAD-18 Arg302Gln Gene Polymorphisms in Type 1 Diabetes Mellitus Patients

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ABSTRACT

Smoking is the most important habit problem in the world which contributed in disease development. The goal of the study is to detect the impact of the smoking habit in the *XRCC1* Arg399Gln and RAD-18 Arg302Gln gene polymorphisms in type 1diabetes mellitus patients, the aims implemented by using polymerase chain reaction with confronting two-pair primers (PCR-CTPP) method and allele-specific PCR for genes. The results included 16% were smokers, and 12% of them were passive smokers. The polymorphisms of the *XRCC1* gene show three genotyping (AG, GG, and AA) and two alleles (A and G), the GG was more frequent (60%) in patients than control (43.33%), and AG lower in patients (40%) than control (46.66%) (OR 1.312 P 0.619) and AA did not observe in patients, these differences were non-significant (OR 0.124, P 0.180). Another gene was RAD18 Arg302Gln (rs373572), three genotyping in this site of a gene (AA, AG, and GG) two of them were observed in the present study (AA and AG). GG did not observe non-significant differences (OR 0.22, P 0.34) of AA and AG and non-significant also with GG (OR 5, P 0.524). The effect of smoking habit included smoker, non-smoker, passive and non-passive smoker. The RAD18 gene did not show variation. The XRCC1 shows non-significant variations between non-smoker and smoker. The GG was more frequent (12%) than AG (4%) in smokers and also in non-smoker (44% and 40%) individuals. The passive smokers show non-significant differences also, 56% of non-passive smokers patients have GG (OR 0.3667, p = 0.4164) and 32% have AG, and 4%, 8% for passive smokers (OR 3.5000, p = 0.336). The present study concluded that the RAD18 and *XRCC1* gene nor relation with DM type 1 neither associated with the smoking habit in diabetes mellitus (DM) type 1 patients.

Keywords: Arg399Gln, Arg302Gln gene polymorphisms, Passive smoking, Smoking, *XRCC1* RAD-18, Type 1 diabetes mellitus

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by increased blood glucose by a deficiency in insulin secretion or glucose uptake by cells. It has become the most health problem in the world. Type 1 DM (T1DM) is an autoimmune disease caused by insulin deficiency, its incidence by complex interaction among environmental and genetic factors.[1] It represents about 10% of the DM cases and incidence increasing in much aerial of life.[2]

Smoking habits and passive smokers' effects on cellular functions have been proved. It is considered as one of the DM modifiable risk factors.[3] Investigations have clarified that smokers related with vascular damage dysfunction in the endothelial and activation clotting cascades.[4] In DM, the smoker companied with hyperglycemia accelerates vascular damage.[5,6] However, the other study pointed out that smokers are insulin resistant, which may lead to therapeutic failure in the patients and increase diabetic complications like nephropathy, neuropathy, and retinopathy, possibly, by the metabolic effects associated with an elevation in inflammation and endothelial dysfunction.[7]

The X-ray repair cross-complementing group 1 gene (*XRCC1* Arg399Gln) is found on chromosome 19, at 19q13.2, encoded to the enzyme has a major role in the base excision repair system via interaction with other proteins. About 50 variation regions have been reported the most frequently

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genotyping was studied is Arg399Gln (G/A transition) causes an amino acid substitution (Arg to Gln) at codon 399.19,20.[8,9] The *RAD18* Arg302Gln (rs373572) is gene encoded to E3 ubiquitin-protein ligase it is an enzyme interaction with other proteins contributed in the post replication DNA repair.[10]

Smoking also causes DNA damage and strand adduct, in addition to disturbance in oxidative stress,[11] all of these causes to mutation accumulation that lead to complications or another disease the present study investigated the *XRCC1* Arg399Gln and RAD18 Arg302Gln (rs373572) gene polymorphisms in the diabetes mellitus type one.

METHODOLOGY

A cross-sectional study was implemented on the T1DM, patients. All data and blood samples were collected from 25 patients who attended the Marjan hospital center of diabetic mellitus with written consents according to the ethical approval of the environment and health ministry in Iraq. DNA extracted from whole blood according to Alqaim *et al*..[12] Two DNA repair genes were investigated in present study, first the variant in the *XRCC1* Arg399Gln codon 399 by PCR-CTPP method (13). via four primers F1, TCC, CTG, CGC, CGC, TGC, AGT, TTC, T; R1 TGG, CGT, GTG, AGG, CCT, TAC, CTC, C; F2 TCG, GCG, GCT, GCC, CTC CCA ; and R2, AGC, CCT, CTG, TGA, CCT, CCC, AGG, C. the conditions included (94, 94,59,72 and 72)ºC for 1-minute to each step except final extension was 10 minutes, the products of amplification were 447bp for G allele (399Arg) and 222 bp for A allele (399 Gln) and 630 bp as a common band. The second gene was RAD18 Arg302Gln (rs373572) which genotyping using allele-specific PCR by the following nucleotides sequences F1 ATA, CCC, ATC, ACC, CAT, CTT, C. R1- GTC, TTC, TCT, ATA, TTT, TCG, ATT, TCT, T. F2- TTA, ACA, GCT, GCT, GAA, ATA, GTT, CG. R2- CTG, AAA, TAG, CCC, ATT, AAC, ATA, CA. The amplification products were 146-bp for (A) Arg allele, 106 bp of (G) Gln allele, the amplification condition were 94, 94, 58, 72, and 72ºC for the following times (5, 0.5, 0.5, 0.5 and 10) minutes respectively. All genotyping was observed using electrophoresis pattern of agaros gel 1%, 70 V, 20 mA, 0.5 X TBE for 45 minutes under ethedium bromide staining. Data were analyzed using odd ration and CI95% for patient groups.

RESULTS AND DISCUSSION

The aim of this study is implemented by detecting the impact of smoking habits in some DNA repair gene in DM1 patients. The age mean of study groups was 49.24 ± 12.65, 33.10 ± 11.38 years, the BMI was 31.07 ± 6.34, 28.06 ± 5.41 kg/m^2 for patients and control respectively, and the duration of disease was 11.70 ± 7.01 years.

The patient group included 16% smokers and 12% passive smokers, and the age, fasting blood glucose (FBG), body mass index (BMI), and duration were clarified in Table 1, where non-significant differences were observed between groups.

Smoking increased the FBG, but passive smoking did not affect also decreased in the BMI (Table 1). The smoking habit is one of the most harmful problems in society and although of the health awareness of the harmful effects and associated with disease development. Smokers still increase in the world.[14] Studies proved that smoking is considered as a risk factor of DM.[3,4] The associations between type 2 DM (T2DM) and smoking have been reported.[15-17] But the relation with DM1 was did not investigated. However, smoking can be led to insulin resistance, thus in diabetic muscle infarction (DMI) may be developed to failure in the glycemic control in the body.

Two DNA repair genes polymorphisms were studied in the present study *RAD-18* and *XCRR1*. Figure 1 shows the electrophoresis pattern of these genes

The polymorphisms of the *XRCC1* gene show three genotyping (AG, GG and AA) and two alleles (A and G), the GG was more frequent (60%) in patients than control (43.33%), and AG lower in patients (40%) than control (46.66%) (OR 1.312 p = 0.619) and AA did not observe in patients, these differences were non-significant (OR 0.124, p 0.180) and this clarified no

Table 1: Mean differences of age, FBG, BMI and duration in smoking habit of DM1 patients

Smoking habit	*AGE*	*FBG*	*Duration*	*BMI*
Non-smoker	51.04 ± 10.32	186.00 ± 66.82889	12.6429 ± 7.06955	32.1617 ± 5.84632
Smoker	39.75 ± 20.66	250.75 ± 174.320	6.7500 ± 4.64579	25.3784 ± 6.54217
P value	0.106	0.193	.126	0.048
Non-passive smoker	48.9545 ± 12.46510	199.7727 ± 95.07387	12.2727 ± 7.12595	31.1887 ± 6.62289
Passive smoker	51.3333 ± 16.77299	171.3333 ± 36.17089	7.5000 ± 5.22015	30.2531 ± 4.64576
p value	0.767	0.618	0.278	0.816

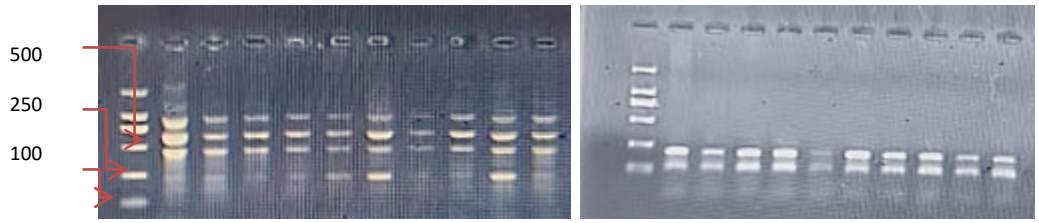

Figure 1: The agaros gel electrophoresis of *XRCC1* (left) and *RAD-18* (right) genes for patients and control groups

association of *XRCC1* genotyping with DM1 in the present study. Another gene was RAD18 Arg302Gln (rs373572), three genotyping in this site of gene (AA, AG and GG) two of them were observed in the present study (AA and AG), GG didn't observe in the present study, non-significant differences (OR 0.22, p = 0.34) of AA and AG and non-significant also with GG (OR 5, p = 0.524), the absence of AG and GG may be contributed in cell dysfunction in DM1 patients (Table 2).

The DNA repair genes were more important in cell functions because its role in mutations rapier and in the genome maintains and stability. The association of XRCC 1 with diabetes mellitus has been approved, a study implemented by Yesil-Devecioglu *et al.,*[18] but the association with type 2. They found a strong association with disease, in DM1 there was no studies referred to association *XRCC1*gene with DM1 disease. However, its relation with hyperglycemia, oxidative stress and DNA damage observed in T1DM have been investigated.[19-22] The *RAD18* ARG302GLN (rs373572) genotyping proved to be associated with different diseases like cancer,[23,24] diabetes, and hypertension.[25] The present study has shown no significant association with T1DM. The *RAD18* Arg302Gln (rs373572) genotyping in the T1DM needs more effort to prove its relation with hyperglycemia, oxidative stress, and damage in the DNA strands.

The effect of smoking habits included smokers, non-smokers, passive and non-passive smokers. The *RAD18* gene didn't show any variation because it does not relate to DM1 disease and all patients have AA genotyping. Thus no significant differences associated with a smoker or passive smoker were observed. The *XRCC1* shows significant non-variations between non-smoker and smoker. The GG was more frequent (12%) than AG (4%) in smokers and also in non-smokers (44% and 40%) individuals, the passive smokers showed non-significant differences also, 56% of non-passive smokers patients have GG (OR 0.3667, p 0.4164) and 32% have AG, and 4%, 8% for a passive smoker (OR 3.5000, p = 0.336).

Although of non-association the smoking with *RAD18* in the present study, other study show strong link some SNPs in RAD 18 gene with smoking in some cancer patients under chemotherapy.[26] The association between XRCC1, DNA adduct and smoking was shown a significant association with smoking in smokers for more than 40 years with 3-4 risk alleles in *XRCC1*.[27] The tobacco smoking was noticed that it related with elevation DNA adducts, especially in the diabetic patients that suffered from some cell dysfunction thus the studies deal with the smoking influence in DNA adducts in blood cells to identify a sensitive biomarker of effective intake of tobacco carcinogens.[28] Some studies show associated *XRCC1* with smoking in different types of cancers.[29,30] The present study needs more investigations about other SNPs in DNA repair genes in T1DM and relation with DNA damage caused by smoking. However, the present study concluded that neither the RAD18 and XRCC1 gene nor relation with T1DM is associated with smoking habit in T1DM patients.

Table 1: The genotyping of *RAD-18 and XRCC1* in DM1 patients and control

Genotyping RAD18 Arg302Gln (RS373572)	*Patients*	*Control (%)*	*Odd ratio*	*p-value*
AA	100%	92.85	0.2235	0.3409
AG	0%	7.14	0.010–4.8786	
GG	0	0	5.0000	0.5247
			0.035–711.86	
XRCC1				
AG	40%	46.66	1.3125	0.6199
			0.4481–3.8441	
GG	60%	43.33	0.1244	0.1807
AA	0	10	0.0059–2.6319	

Table 2: The genotyping of RAD-18 and XRCC1 in smoker, non-smoker, passive smoker and non-passive smokers DM1 patients and control

Categories	*Genotyping*			*Odd ratio*	*p-value*
RAD 18	AA	AG	GG	-	-
Non-smoker	84%	0	0	-	-
Smoker	16%	0	0	-	-
Non-passive smoker	88%	0	0	-	-
Passive smoker	12%	0	0	-	-
XRCC1 Arg399Gln					
Non-smoker	0	40%	44%	0.3667	0.4164
Smoker	0	4%	12%	0.0326–4.1227	
Non-passive smoker	0	32%	56%	3.5000	0.3361
Passive smoker	0	8%	4%	0.2725–44.9524	

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