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Lack of association of the HMGA1 gene variants with insulin resistance risk development in the Iraqi population: Case-control study

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ABSTRACT

Reduced sensitivity or responsiveness to the metabolic effects of insulin is the typical definition of insulin resistance. High mobility group A1 (HMGA1) can enhance the body's capacity to store fat and improve insulin sensitivity, so the abnormal decrease in HMGA1 or its variants may be related to type 2 diabetes and poor adipocyte differentiation, which results in insulin resistance. The goal of this study was to examine whether HMGA1 genetic variants in a sample of the Iraqi population can predict the risk of an increase in insulin resistance. This case-control study was carried out at a single center in Kirkuk, Iraq, from February to August 2022. This study included 30 healthy Iraqi participants and 30 patients without diabetes who had metabolic syndrome. Sanger sequencing of genomic DNA was used to identify HMGA1 polymorphisms and genotypes. The high prevalence of CC and GG genotypes of rs1023028442 and rs112081775 respectively was seen in the Iraqi population. Minor allele frequency of rs1023028442 was higher among metabolic patients without diabetes with (MAF=0.08) compared to the control group with (MAF= 0%). While (MAF=0.1) of rs112081775 was seen in metabolic patients without diabetes compared to (MAF=0.02) in the control group. After adjusting age, gender, and BMI as covariates, linear regression analysis showed no significant effect of these two variants (C>T, and G>A) on developing insulin resistance. Thus, the rs1023028442 with C>T genotype and rs112081775 with G>A genotype in the HMGA1 gene did not predict the risk of an increase in insulin resistance in the Iraqi populations.

Keywords: *HMGA1 gene; insulin resistance, Iraqi population; metabolic syndrome; diabetes mellitus.*

INTRODUCTION

A class of metabolic disorders known as diabetes mellitus (DM) is characterized by high blood glucose brought on by defects in insulin release, action, or both (1, 2). Insulin is an anabolic hormone that causes glycogen to build up in the liver and skeletal muscles (3). By preventing hepatic gluconeogenesis and lipolysis, insulin exerts its anabolic effects while raising the liver, muscles, and adipose tissues' glucose uptake (4). Insulin resistance (IR) is the term for decreased sensitivity or responsiveness to the metabolic effects of insulin (5). Insulin resistance, atherogenic dyslipidaemia, central obesity, and hypertension are all components of metabolic syndrome (MetS) (4).

A reliable tool for the surrogate assessment of IR is the "Homeostasis Model Assessment of IR (HOMA-IR)" (6). In comparison to other regions of the world, the Arab world will have the second-highest rise in the percentage of persons with DM in 2030 (7). Due to their low molecular weights, the diversified group of basic proteins known as the "high mobility group" (HMG) may move quickly through a polyacrylamide gel (8). The three families of HMG proteins are HMGB, HMGN, and HMGA. High mobility group A1 proteins (HMGA1) are small, non-histone nuclear proteins that are essential for binding DNA in the minor groove and modulating the expression of genes (9). Because the processes involved in maintaining glucose homeostasis depend on insulin release and activity, type 2 diabetes mellitus (T2DM) can develop as a result of problems in any of these mechanisms(10). The insulin receptor (INSR) promoter's activity is positively regulated by HMGA1, which binds to the INSR transcription start site and positively controls INSR expression (11). Insulin resistance and T2DM are caused by an abnormal decrease in intracellular expression of HMGA1 protein (12). This study was conducted to assess whether the HMGA1 genetic variants predict the risk of an increase in insulin resistance in a sample of the Iraqi population.

MATERIALS AND METHODS

Study population

From February to August 2022, this case-control study was conducted at a single center in Kirkuk, Iraq. About (60) subjects were involved in the study. These subjects were recruited into two groups: Group 1: about 30 patients with metabolic syndrome without T2DM. Group 2: Control Group (Looks healthy), contains 30 people who had no components of MetS criteria.

Inclusion criteria

The participants (Patients with metabolic syndrome and apparently healthy group) included both gender subjects over the age of 30. Metabolic syndrome was defined by "National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines" (13)

Exclusion criteria

Patients with diabetes, any type of malignancy, pregnant and lactating women, and subjects with polycystic ovarian syndrome were excluded from this study.

Demographical, Anthropometric, and Biochemical Evaluation

Collected data included age, gender, smoking history, waist circumference, body mass index (BMI), blood pressure, lipid profile, and glucose indices. After an overnight fast of 12 hours, blood samples were taken. Fasting serum glucose was measured by the enzymatic colorimetric method. HbA1c determined by latex enhanced immunoassay method. Serum total cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL), and low-density lipoprotein-cholesterol (LDL) were determined by enzymatic colorimetric methods. About one-fifth of triglyceride levels are regarded to be very low-density lipoprotein cholesterol (VLDL) (14).

Fasting serum (glucose and insulin) were measured in those with MetS without diabetes and in the control group who did not take medications that affected glucose tolerance, to calculate "Homeostasis Model Assessment-Insulin Resistance, beta cells function and insulin sensitivity" which was calculated with "HOMA2 calculator (http://www.dtu.ox.ac.uk/homacalculator/index.php)".

Sample Collection

Blood collected after at least 12 hours of fasting from patients and control group, by vein puncture with plastic disposable syringes took up to 5mL of venous blood, and 2 ml was added to an EDTA tube to detect the HMGA1 SNP.

DNA extraction

DNA from blood samples can be efficiently purified using the "Promega ReliaPrepTM Blood gDNA Miniprep System" for Genomic DNA (Promega Corp., WI, USA). Enzymatic amplification was accomplished using a hybrid thermal cycler and the Master Taq polymerase

enzyme through the polymerase chain reaction (PCR).

Primers

The NCBI GenBank database was used to retrieve the DNA sequences for the HMGA1 gene. The PCR primers were created using Primer Premier 3 software, as shown in (Table 1).

TABLE 1: The sequences of the primers, annealing temperature, product size (bp).

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)
HMGA-F	TGTAAAACGACGGCCAGTGTTTGTGGTTCTTG GTTCTTG	58	972
HMGA-R	CAGGAAACAGCTATGACGCTTCTTCACCTACC AGTTT		
"HMGA-F: forward primer. HMGA-R: reverse primer".			

Sequencing analysis

Products of PCR were sent to "Macrogen Corporation – Korea" for Sanger sequencing using the automated DNA sequencer ABI3730XL. The results were obtained via email, and geneious software was used to analyze them.

Ethical considerations

The Declarations of Helsinki's standards and guidelines for Good Clinical Practice were followed in the conduct of this study (15). Furthermore, the protocol was approved by the Ethical Committee of the Baghdad University - College of Pharmacy on October 4th, 2021, and assigned the number (RECAUBCP4102021B), with verbal informed consent, explain the study's methodology to the participants and let them know that the data will be collected anonymously.

Statistical analysis

The IBM SPSS software for Windows version 26.0 was used. Normally distributed data, were expressed as "mean ± standard deviation (SD)", and skewed data, as "median (interquartile range)". Genotypes and alleles were displayed in terms of frequency and number. The results normality was examined using the "Shapiro-Wilk test and the Kolmogorov-Wilk test". To find a significant difference in demographic traits and parameters between the groups, the "unpaired t-test" was used for data that were normally distributed, and the "Mann-Whitney U test" for data that were skewed distributed. After adjusting for covariates, "univariate linear regression analysis" was used to compare quantitative biochemical traits between wild and carriers. "Statistical significance was defined as a p-value of 0.05".

RESULTS

Metabolic syndrome indices including obesity indices, lipid profile, and blood pressure was significant differences between each group, while no significant difference was seen in their glucose indices including (HbA1c, FBG, FSI, and HOMA2-IR). As shown in (Table 2).

TABLE 2: Demographic, anthropometric, clinical and biochemical features of all participant.

Iraqi population	MetS without T2DM N=30	Control N=30	P-Value
Gender -Male -Female	 14(46.7%) 16(53.3%)	 17(56.7%) 13(43.3%)	 0.605
Age in years	53(40-62)	48(35-56)	0.000*
Smoker	1(3.3%)	12(40%)	0.001*
WC (cm)	107.6 ± 13.5	91.3 ± 11.2	0.000*
BMI (kg/m2)	31.5 ± 4.4	27.25 ± 3.9	0.000*
TC (mg/dl)	203 ± 42.7	133.1 ± 60.4	0.000*
TG (mg/dl)	188(142.6-223)	98(98-141.7)	0.000*
HDL (mg/dl)	56.9 ± 15.9	48.3 ± 8.4	0.011*
LDL (mg/dl)	102(97-136)	55(53-110.5)	0.000*
VLDL (mg/dl)	37.6(28.5-44.7)	19(19-28.4)	0.000*
SBP (mmHg)	130(120-142)	120(120-130)	0.000*
DBP (mmHg)	90(80-95)	80(80-80)	0.000*
HbA1c (%)	6(5-6.3)	5.8(5-6)	0.207
FBG (mg/dl)	103(88.8-114.8)	102(89.5-112.3)	0.790
FSI (µIU/mL)	9.67(9.70-16.56)	10.38(6.82-12.04)	0.584
HOMA2-IR	1.28(0.93-2.11)	1.34(0.96-1.62)	0.712

"Normally distributed data expressed as mean ± SD. While not normally distributed data expressed as median (interquartile range). The 2-tailed standard t test was used for comparisons of means. The Mann-Whitney U Test was used for comparisons of median. Fisher exact test was used for categorical variables. MetS, metabolic syndrome. T2DM, type 2 diabetes. WC, waist circumference. TC, total cholesterol.TG, triglyceride. HDL, high density lipoprotein. LDL, low density lipoprotein. VLDL, very low density lipoprotein. SBP, systolic blood pressure. DBP, diastolic blood pressure. HbA1c, Hemoglobin A1c. FBG, Fasting blood glucose, FSI, Fasting serum insulin, HOMA2-IR, Homeostatic model assessment method 2 for insulin resistance". * Statistically significant.

A "homozygous C allele" is indicated by a single "C" peak. A "homozygous T allele" is indicated by a single "T" peak. The "C/T heterozygous allele" is indicated by the presence of the "C" and "T" peaks (Figure 1A). A "homozygous G allele" is indicated by a single "G" peak. A "homozygous A allele" is indicated by a single "A" peak. The "G/A heterozygous allele" is indicated by the presence of the "G" and "A" peaks. (Figure 1B).

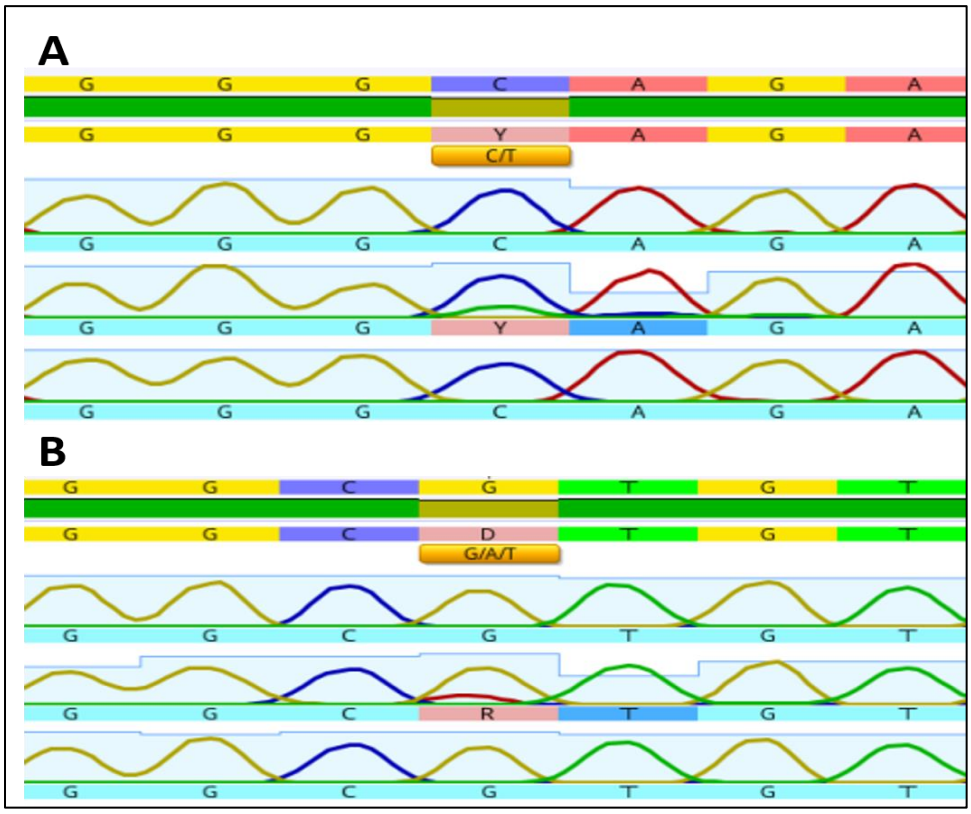

FIGURE 1: Analysis of A: rs1023028442 SNP. B: rs112081775 SNP of HMGA1 gene.

The high prevalence of CC and GG genotypes of rs1023028442 and rs112081775 respectively was seen. Minor allele frequency of rs1023028442 was higher among metabolic patients without diabetes with (MAF=0.08) compared to the control group with (MAF= 0%). While (MAF=0.1) of rs112081775 was seen in metabolic patients without diabetes compared to (MAF=0.02) in the control group. As shown in (Table 3).

TABLE 3: Distribution of HMGA1 genetic polymorphism in Iraqi populations

SNP	rs1023028442			rs112081775		
Genotype	CC N. (%)	C>T N. (%)	MAF	GG	G>A	MAF
MetS without T2DM	25(83.3%)	5(16.7%)	0.08	24(80%)	6(20%)	0.1
Controls	30(100%)	0(0%)	0	29(96.7%)	1(3.3%)	0.02
"SNP, single nucleotide polymorphism. rs: reference SNP. MAF, minor allele frequency. CC, and GG are the wild genotype. C>T, and G>A are the carrier (variant genotypes). MetS, metabolic syndrome. T2DM, type 2 diabetes".						

After adjusting "age, gender, and BMI" as covariates, linear regression analysis revealed no significant effect of these two variants (C>T, and G>A) on developing insulin resistance. As shown in (Table 4, 5).

TABLE 4: Effects of the HMGA1 rs1023028442 variant on insulin resistance.

Iraqi population	Measurements	Wild type CC N=55	Carrier C>T N=5	P-value
FBG	mean ±SD	104.09 ± 27.72	96 ± 43.2	0.207
FSI	mean ±SD	10.94 ± 5.96	11.2 ± 6.8	0.875
HOMA2-B %	mean ±SD	103.07 ± 68.56	167.86 ± 129.03	0.271
HOMA2-S%	mean ±SD	92.30 ± 55.90	87.44 ± 37.34	0.974
HOMA2-IR	mean ±SD	1.45 ± 0.80	1.4 ± 0.84	0.983
"Linear regression analysis, adding age, gender, and BMI as covariates, all values have been log-transformed to better approximate a normal distribution. FBG, fasting blood glucose. FSI, Fasting serum insulin". "HOMA2-B%, Homeostatic model assessment method 2 for beta cells function". "HOMA2-S%, Homeostatic model assessment method 2 for insulin sensitivity". "HOMA2-IR, Homeostatic model assessment method 2 for insulin resistance".				

TABLE 5: Effects of the HMGA1 rs112081775 variant on insulin resistance.

Iraqi population	Measurements	Wild type GG N=53	Carrier G>A N=7	P-value
FBG	mean ±SD	105.3 ± 29.36	88.71 ± 12.28	0.071
FSI	mean ±SD	10.9 ± 6.08	11.09 ± 5.57	0.660
HOMA2-B %	mean ±SD	102.77 ± 70.52	151.5 ± 105.16	0.079
HOMA2-S%	mean ±SD	93.13 ± 56.92	82.5 ± 29.6	0.832
HOMA2-IR	mean ±SD	1.45 ± 0.82	1.4 ± 0.69	0.826
"Linear regression analysis, adding age, gender, and BMI as covariates, all values have been log-transformed to better approximate a normal distribution. FBG, fasting blood glucose. FSI, Fasting serum insulin". "HOMA2-B%, Homeostatic model assessment method 2 for beta cells function". "HOMA2-S%, Homeostatic model assessment method 2 for insulin sensitivity". "HOMA2-IR, Homeostatic model assessment method 2 for insulin resistance".				

DISCUSSION

Hyperglycemia caused by T2DM is described by decreased tissue response to insulin as a result of insulin resistance (16). Several studies suggest that metabolic syndrome patients are five times more likely to develop type 2 diabetes (17). Both muscle and fat are important for preserving euglycemia. In this regard, a previous study showed that both in HMGA1-knockout mice and in people with HMGA1 variants, INSR expression is diminished in the muscle and adipose tissues (18). Poor adipocyte differentiation results in IR and T2DM, which may be related to the abnormal decrease in or variants of HMGA1. High mobility group A1 can promote the differentiation of adipocytes, enhance the ability for fat storage, and insulin sensitivity improvement (12).

HMGA1 at exon 6 (position 13) contains the low-frequency insertion polymorphism rs139876191 (also called IVS5-13insC), which has been linked to type 2 diabetes and insulin resistance in people of white European ancestry (19) and Chinese descent (20).

One of the most challenging aspects of case-control studies on IR and T2DM is choosing an appropriate control group, which is of great importance. The main issue is that the presence of a personal or family history of insulin resistance may eventually lead to developing T2DM. Therefore, without thorough testing, it is challenging to exclude participants with insulin resistance from a control group; as a result,

control groups may include a significant proportion of insulin-resistant individuals who will develop type 2 diabetes in the future. So, personal interviews were carried out with 30 healthy individuals to confirm there was no personal or family history (at least the first relative degree) of type 2 diabetes and associated conditions, such as hypertension, and dyslipidemia. This was done to decrease the number of IR individuals in the control group.

Variant types of rs1023028442 and rs112081775 called single nucleotide variants (SNVs) based on NCBI (https://www.ncbi.nlm.nih.gov/snp/), the mutations in introns do not affect protein sequences, and they are usually ignored when looking for normal or pathogenic genomic variation. However, introns comprise about half of the human non-coding genome and they can have important regulatory roles (21). According to another study, SNVs in intronic regions have not yet been examined for their potential to cause disease (22). The data of this study suggest that (rs1023028442 and rs112081775) SNVs in the intron region, are not associated with an increased risk of insulin resistance development. Another study showed no significant effect of another mutant variant called rs139876191 on developing IR in the French population (23) while another study shows that the rs139876191 mutant variant play role in the pathogenesis of disorders that are related to IR (24). Another study showed that when a mutation in HMGA1 occurred in peripheral blood lymphocytes of subjects with IR and T2DM, HMGA1 protein expressed at low levels. Restoration of HMGA1 protein expression improved INSR gene transcription and restored cell-surface INSR protein expression as well as insulin-binding capacity (12).

Given the small sample size, single-center focus, and the fact that this study only included patients with metabolic syndrome in Kirkuk City, caution should be applied when extrapolating its findings to other populations.

CONCLUSIONS

In conclusion, the rs1023028442 with C>T genotype, and rs112081775 with G>A genotype in the HMGA1 gene did not predict the risk of an increase in insulin resistance in the Iraqi populations.

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Declaration of conflicting interests

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