

# High Serum High Mobility Group A1 (HMGA1) Levels are associated with presence of Metabolic Syndrome: Case-control study

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### Abstract:

**Background:** Metabolic syndrome is a complex series of metabolic defects, characterized by high levels of serum glucose, hypertension, abdominal obesity, and dyslipidemia. The high mobility group AT-hook1, an architectural transcript factor, affects the homeostasis of glucose. No previous studies have been performed to examine whether HMGA1 can be secreted into the extracellular milieu.

**Aim of the study:** this case-control study aimed to examine whether HMGA1 secretes into the extracellular milieu and compares its serum level in two groups of metabolic syndrome (with and without diabetes) and a control group composed of apparently healthy individuals of Iraqi population with different nationalities.

**Patients and Methods:** Sixty-one patients with metabolic syndrome and thirty healthy Iraqi participants included in this study. Serum HMGA1 concentrations were determined by enzyme linked immuno sorbent assay (ELISA). Lipid profile, serum (glucose and insulin), HbA1c, systolic/diastolic blood pressure, body mass index, and waist circumference were also measured. The statistical analysis was done using IBM SPSS software for Windows version 26.0.

**Results:** Significant difference in HMGA1 level was seen (P = 0.000), between metabolic syndrome with diabetes, metabolic syndrome without diabetes and control group. Higher concentrations were seen in metabolic syndrome patients with diabetes followed by metabolic syndrome patients without diabetes and then the control group, and no significant difference was seen in the serum level based on nationality. Significant positive correlation was found between HMGA1 and fasting blood glucose (p=0.001) as well as between HMGA1 and HbA1c (p= 0.015) in patients with metabolic syndrome. Moreover there was a significant association between HMGA1 levels and the risk of metabolic syndrome. The risk of metabolic syndrome was found to be increased by a high HMGA1 level, odds ratio (OR), 0.411 (95% CI, 0.208-0.813).

**Conclusions:** This case-control study found that circulating HMGA1 concentration was significantly higher in Mets mainly in those with T2DM. Also, the high concentration of HMGA1 was found to present a significant risk of metabolic syndrome regardless of whether diabetes is present or not. Besides HMGA1 serum level was positively correlated with parameters of diabetes including HbA1c and FBG.

Keywords: ELISA, HMGA1 protein, Iraqi population, metabolic syndrome, Type 2 DM.

# Introduction:

Metabolic syndrome (MetS) is a complex series of metabolic defects, characterized by high levels of serum glucose, high blood pressure, central obesity, and dyslipidemia (1). Several studies have shown that MetS is associated with the development of heart

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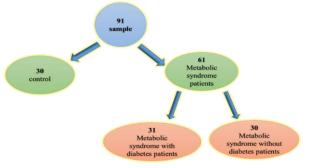
\*\*\*Ministry of Health and Environment, Kirkuk Health Dept. Kirkuk. <u>albustanyomar@gmail.com</u> disease, renal disease, and diabetes mellitus (2, 3). Diabetes mellitus (DM) is a group of metabolic disorders caused by a complex interaction of genetic and environmental factors (4), it is characterized by elevated blood sugar levels due to defects in insulin secretion, insulin action, or both (5, 6). There is an additive effect in the presence of MetS and insulin resistance, as these patients have a six- to seven times higher risk for Type 2 DM (7). High mobility group (HMG) proteins are a diverse group of basic proteins that are named for their ability to migrate quickly (hence high mobility) through the polyacrylamide gel because of its low molecular weight. HMG proteins have three groups: HMGN, HMGB and HMGA (8).

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HMGA proteins are small non-histone nuclear proteins which can bind DNA in the minor groove, and change the conformational state of chromatin and its accessibility through several regulatory factors involved in modulating gene expression (9). In humans, the HMGA1 gene resides on chromosome 6p21 (NC 000006.12) (10). HMGA1 positively regulates the activity of the INSR promoter, which is linked to the transcription start site of INSR leading to positive regulation of Insulin Receptor (INSR) expression and insulin signal transduction (11). A lot of nuclear proteins, in addition to their nuclear actions, can be released as well in the extracellular environment and participate in the pathologies of various diseases (12). No previous studies have been performed to examine whether HMGA1 can be secreted into the extracellular environment. So, this case-control study aimed to examine whether HMGA1 secretes into the extracellular milieu and then compares its serum level in two groups of metabolic syndrome (with and without diabetes) and a control group composed of apparently healthy individuals of Iraqi population with different nationalities.

# **Patients and Method**

Patients: Study population: This case-control study was carried out at Kirkuk city/ Iraq, internal medicine clinic under the supervision of an internal medicine specialist from February until Augusts 2022. One hundred individuals were selected to contribute in this study. Only (91) participants completed the courses of the study successfully. The participants were classified into the following groups: For Patients Group: 61 patients were divided into two main groups: First Group: contains 31 metabolic syndrome with type 2 diabetes, and second group: contain 30 metabolic syndrome without diabetes. For the control group: 30 individuals who are apparently healthy they have no components of metabolic syndrome criteria (such as diabetes mellitus, hypertension, dyslipidemia, and obesity) (Figure 1).



# Figure 1: study design.

**Inclusion criteria:** Patients and control groups who are ages over 30 years old of either sex who accepted to participate in this study. Metabolic syndrome was defined by means of "National Cholesterol Education

Program (NCEP) Adult Treatment Panel III (ATP III) guidelines" (13).

**Exclusion criteria:** Type 1 diabetes, any type of malignancy, pregnant and lactating women, autoimmune diseases, and patients with inborn errors of metabolism were excluded from this study.

Demographic, Anthropometric and Biochemical Evaluation: Data were collected using a researchermade questionnaire which consisted of individual. Such as age, gender, and smoking history. Body mass index (BMI) was calculated as weight divided by height (kg/m2). Blood pressure (systolic/diastolic) was measured with an MDF® desk mercury sphygmomanometer. Blood samples were collected following 12-h of overnight fast. Fasting serum glucose was measured by the enzymatic colorimetric method using a glucose oxidize test. HbA1c determined by latex enhanced immunoassay method. Serum total cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL- cholesterol), and lowdensity lipoprotein-cholesterol (LDL-cholesterol) were determined by enzymatic colorimetric methods using provided commercial kits by (GIESSES®DIAGNOSTICS, Italy). Very low-density lipoprotein-cholesterol (VLDL-cholesterol) is calculated as about one-fifth of triglyceride levels (14). electrochemiluminescence The sandwich immunoassay was method used to determine serum insulin by using the commercial kit provided by (Elecsys insulin, Cobas®, Germany).

Fasting blood glucose and serum insulin (FBG, FSI) levels were obtained in those with Mets patients without DM and the control group who were not using drugs that affected glucose tolerance (such as nonselective beta-blocker, thiazide diuretics, and glucocorticoids) to calculate Homeostasis Model Assessment-Insulin Resistance, beta cells function and insulin sensitivity (HOMA2-IR, HOMA2-B%, and HOMA2-S% respectively) which calculated with HOMA2

(http://www.dtu.ox.ac.uk/homacalculator/index.php).

Sample Collection: Blood was collected after at least 12 hours of fasting in patients and control groups, by vein puncture with plastic disposable syringes took up to 5mL of venous blood for biochemical analysis and after centrifuging at (2,000-3,000 rpm for 20 minutes), the leftover serum stored into aliquots  $(250\mu I)$  in an Eppendorf tube at (-20oC) until assayed for HMGA1 protein Measurement of HMGA1:Serum HMGA1 level was measured using the commercial Sandwich ELISA kits from SunLong Biotech Co., Ltd. (CHINA, Catalog no. SL3409Hu). Measurement was performed following manufacturer instructions, based on the principle of the ELISA technique (15).

# Statistical analysis

The statistical analysis was done using IBM "SPSS software for Windows version 26.0 (IBM Corp., Armonk, NY, U.S.)". Continuous variables were expressed in "mean  $\pm$  standard deviation (SD)" for normally distributed data and "median (IQR)" for skewed distributed data. The "Shapiro-Wilk test and Kolmogorov-Wilk test" were used to test the normality of the results. The "unpaired *t-test*" was used for normally distributed data and the "Mann-Whitney U test" was used for not normally distributed data to determine a significant difference in demographic characteristics and parameters between the groups. The "Kruskal-Wallis Test" was used to more than two

groups. Then, pairwise comparisons of groups were used whenever a significant difference between the three sample means has been revealed by (Kruskal-Wallis). The Spearman test was used to estimate the strength of the correlation between variables. Odds ratios and 95% confidence intervals for HMGA1 level and MetS were tested using binary logistic regression. A *P*-value of <0.05 was considered to be statistically significant.

# Results

Demographic, anthropometric and biochemical features of patients with metabolic syndrome and control group shown in Table 1.

 Table 1: Demographic, anthropometric and biochemical features of patients with metabolic syndrome and control group

Metabolic indices	Measurements	MetS N=61	Control N=30	P-value
Gender				
-Male	N (%)	26(42.6%)	17(56.7%)	-
-Female		35(57.4%)	13(43.3%)	
Age in years	Median(IQR)	53(42.5-63)	48(35-56)	-
Smoker	N (%)	6(9.8%)	12(40%)	-
WC (cm)	mean $\pm$ SD	$107.4 \pm 12.45$	$91.3 \pm 11.2$	0.000*
BMI (kg/m2)	mean $\pm$ SD	$30.48 \pm 4.6$	$27.25 \pm 3.9$	0.001*
TC (mg/dl)	mean $\pm$ SD	$182.2\pm46.9$	$133.1 \pm 60.4$	0.000*
TG (mg/dl)	Median(IQR)	160(118-205.5)	98(98-141.7)	0.000*
HDL (mg/dl)	mean $\pm$ SD	$56.2 \pm 14.02$	$48.3 \pm 8.4$	0.001*
LDL (mg/dl)	Median(IQR)	97(53-112.8)	55(53-110.5)	0.003*
VLDL (mg/dl)	Median(IQR)	32(23.6-41.1)	19(19-28.4)	0.000*
SBP (mmHg)	Median(IQR)	130(120-150)	120(120-130)	0.001*
DBP (mmHg)	Median(IQR)	90(80-97)	80(80-80)	0.000*
HbA1c (%)	Median(IQR)	6.5(5.9-7.3)	5.8(5-6)	0.000*
FBG (mg/dl)	Median(IQR)	113(100-132)	102(89.5-112.3)	0.013*

The "2-tailed standard t test" was used for comparisons of means. The "Mann-Whitney U Test" was used for comparisons of median. \* Statistically significant.

All samples were assessed for detection of HMGA1 level. Significant difference was seen between each group (P = 0.000). Higher concentrations were seen in metabolic syndrome patients with diabetes followed by metabolic syndrome patients without diabetes and

then the control group. Pairwise comparisons of groups displayed a significant difference between metabolic syndrome in each group and a control group .As shown in Table 2.

#### Table 2: Serum concentration of HMGA1 protein in all participant.

	Mets with T2D N=31	Mets without T2D N=30	Control N=30	P-value
Serum HMGA1(µg/L)	26.4(13.1-31.3)	10.8(10.5-11.2)	9.8(9.6-10.5)	0.000a 0.002b 0.000c 0.003d

a Independent sample Kruskal Wallis Test.

b Pairwise Comparisons between Mets with T2D & Mets without T2D.

c Pairwise Comparisons between Mets with T2D & control.

d Pairwise Comparisons between Mets without T2D & control .

Results are reported as median (IQR). HMGA1, High Mobility Group A1. Mets, metabolic syndrome. T2D, type 2 diabetes.

Based on their nationality, there was no significant difference seen in the HMGA1 serum level of metabolic syndrome patients. As shown in Table 3.

	Arab N=28	Kurd N=9	Turkmen N=24	P-value
Serum HMGA1(µg/L)	12.5(10.4-27.9)	11.9(10.3-25.2)	12.7(10.9-26.9)	0.637

Spearman correlation analysis of serum HMGA1 levels with anthropometric, clinical, and biochemical traits showed a significant positive correlation (weak) with HbA1c and FBG in metabolic syndrome patients. As shown in Table 4.

# Table 4: Spearman correlation analysis of serumHMGA1 levels with anthropometric, clinical andbiochemical traits.

	Mets N=61		Control N=30	
Variable	r	Р	r	Р
Gender (male	0.128	0.353	0.233	0.215
vs. female)				
Age	0.102	0.460	-0.275	0.141
WC	0.098	0.476	-0.074	0.697
BMI	0.225	0.098	-0.115	0.543
TC	0.193	0.157	0.105	0.581
TG	0.256	0.060	0.125	0.510
HDL	-0.125	0.364	0.052	0.919
LDL	0.253	0.062	0.093	0.624
VLDL	0.310	0.064	0.113	0.551
SBP	0.188	0.170	-0.193	0.307
DBP	0.117	0.396	-0.171	0.366
HbA1C	0.328	0.015*	0.105	0.582
FBG	0.423	0.001*	0.157	0.409
FSI	0.141	* 0.511	0.255	0.174
HOMA2-IR	0.116	0.590	0.286	0.125
HOMA2-B	0.038	0.859	0.063	0.740
HOMA2-S	-0.116	0.590	-0.279	0.135
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"Spearman correlation, \*\*Correlation is significant at the 0.01 level (2-tailed). \*Correlation is significant at the 0.05 level (2-tailed)".

Associations between Mets and serum HMGA1 levels were tested using logistic analysis. Among all study subjects, a significant association between HMGA1 levels and the risk of Mets was found. The risk of Mets was found to be increased by a high HMGA1 level, odds ratio (OR), 0.411 (95% CI, 0.208-0.813). As shown in Table 5.

Table 5: Association of serum HMGA1 withmetabolic syndrome.

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Variable	OR(95% CI)	P-value		
MetS (Total) vs. Control	0.411(0.208-0.813)	0.011*		
MetS with T2D vs. Control	0.510(0.275-0.947)	0.033*		
MetS without T2D vs.	0.406(0.189-0.872)	0.021*		
Control				
Binary logistic regression. OR, odd ratio.CI, confidence interval.				

Binary logistic regression. OR, odd ratio.CI, confidence interval. Mets, metabolic syndrome. T2D, type 2 diabetes.

# Discussion

High mobility group AT-hook1 (HMGA1, previously HMG-I/Y), an architectural transcription factor, is involved in several biological processes (16) and is a member of the "High Mobility Group (HMG)" family, that are identified as small non-histone nuclear binding proteins. An HMGA1 played a role in the transcription regulation of a number of genes involved in glucose homeostasis. Initially, HMGA1 was found to be essential for normal expression of the insulin receptor (INSR), a critical link in the action of insulin, and glucose homeostasis (9).

No previous studies have been performed to examine whether HMGA1 can be secreted into the extracellular milieu. Thus, the current study emerges as a novel finding for studying the serum level of HMGA1. Middle East population (Iraqi population) in Kirkuk province of different nationalities were subjects for this study. The main outcomes of this study are: (i) HMGA1 was detected in serum by using enzymelinked immune sorbent assays method according to the manufacturers' instructions of the kit; (ii) serum HMGA1 levels were significantly higher in MetS compared to healthy controls, and they showed an upward trend in metabolic syndrome associated with type 2 DM; (iii) serum HMGA1 levels was positively correlated with HbA1c and FBG(p=0.015 and 0.001 respectively) which suggested that HMGA1 might influence glucose homeostasis (9); (iv) high HMGA1 level was found to confer significant risk of metabolic syndrome regardless of whether diabetes is present or not. There is a study measuring plasma "High-Mobility Group Box-1(HMGB1)" which is another protein of the "High-Mobility Group (HMG) family" found that Plasma levels of HMGB1 increased in Chinese population with type 2 DM that could be caused by insulin resistance (11). A key feature of Kirkuk is its diversity - Kurds, Arabs, Turkmen, Shi'a, Sunnis, and Christians all coexist in Kirkuk. This study focuses on the three most common nationalities, Arabs, Kurds, and Turkmen. There is no significant difference seen in HMGA1 serum level of metabolic syndrome patients based on their nationalities (p=0.637). This study has some limitations: First, small sample size and one center focus, as this study enrolled only metabolic syndrome patients in Kirkuk city; therefore, caution is needed in generalizing the finding of this study with other populations. Second, this study only identified serum levels based on the ELISA method, and there could have been random measurement errors.

# **Conclusion:**

This case-control study found that circulating HMGA1 concentration was significantly higher in metabolic syndrome mainly in those with T2DM. Also, the high concentration of HMGA1 was found to present a significant risk of metabolic syndrome regardless of whether diabetes is present or not. Besides, HMGA1 serum level was positively correlated with parameters of diabetes including HbA1c and FBG.

### Authors' declaration:

### Conflicts of interest: None.

We hereby confirm that all the Figures and Tables in the manuscript are ours.

**Ethical considerations**: The study protocol was approved with the number (RECAUBCP4102021B) on the 4th of October 2021 by the Ethical Committee of the University of Baghdad-College of Pharmacy.

### Author contributions:

The work was designed, supervised, and performed by **Mirna, Eman and Omar**. The first draft of this manuscript was prepared by Mirna and Eman. Mirna analyzed the data and improved the overview of the manuscript. Collecting data was done by Mirna and Omar. All authors read and approved the final manuscript.

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# المستويات العالية للمجموعة عالية الحركة (HMGA11) A) في مصل الدم مّر تبطة بوجود متلازمة الأيض الغذائي : دراسة الحالات والشواهد

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الخلاصة:

المقدمة: متلازمة الأيض الغذائي هي مجموعة معقدة من الأضطر ابات الأيضية ، والتي تتميز بمستويات عالية من الكلوكوز في الدم ، وأرتفاع ضغط الدم، والسمنة في منطقة البطن ، وعسر دهون الدم. تؤثر المجموعة عالية الحركة ، وهي عامل نسخ معماري ، على توازن الكلوكوز. لم يتم إجراء دراسات سابقة لفحص ما أذا كان يُمكن إفراز HMGA1 في البيئة خارج الخلية.

**الهدف:** تهدف دراسة الحالة والشواهد هذه إلى فحص ما أذاً كان HMGA1 يُفرز في البيئة خارج الخلية ومقارنة مستواها في مصل الدم لمجموعتين من مرضى متلازمة الأيض الغذائي (مع أو بدون مرض السكري) ومجموعة ضابطة تتكون من أفراد يتمتعون بصحة جيدة على مايبدو من السكان العراقيين بمختلف القوميات.

**طرق الدراسة:** حوالي 61 مريض بمتلازمة الأيض الغذائي و 30 أصحاء عراقيين تم أخذهم لهذه الدراسة. تم تحديد تركيز HMGA1 بأستخدام مقايسة الامتصاصية المناعية للأنزيم المرتبط (الالبزا). كما تم قياس نسبة الدهون ووالسكر التراكمي وضغط الدم. ومؤشر كتلة الجسم ومحيط الخصر. التحليل الاحصائي تم بأستخدام برنامجspss النسخة السادسة والعشرون.

النتائج: شوهد فرق كبير في مستوى power (0.000) (HMGA1) بين مرضى متلازمة الأيض الغذائي المصابين بداء السكري , مرضى متلازمة الأيض الغذائي الغير المصابين بداء السكري والمجموعة الضابطة. حيث لوحظ تراكيز أعلى في مرضى متلازمة الأيض الغذائي المصابين بداء السكري يليهم مرضى متلازمة الأيض الغذائي الغير المصابين بداء السكري ثم المجموعة الضابطة، ولم يلاحظ أي فرق معنوي في مستوى المصل من حيث أختلاف القومية. يرتبط مستوى HMGA1 بشكل أيجابي مع مستوى السكر الصائم (0.001) وكذلك مع السكر التراكمي (50.00) في مرضى متلازمة الأيض الغذائي . وعلاوة على ذلك تم العثور على أرتباط واضح بين مستويات HMGA1 وخط الاصابة بمتلازمة الأيض الغذائي المصابين بداء السكري يليهم العور الغذائي . وعلاوة على ذلك تم العثور على أرتباط واضح بين مستويات HMGA1 وخطر الاصابة بمتلازمة الأيض الغذائي .

الاستُنتاجاتُ: وجدت دراسةُ الحالة والشواهد هذه أن تركيز HMGA1 كان أعلى بشكل ملحوظ لدى مرضى متلازمة الأيض الغذائي وبشكل اساسي لدى المصابين بداء السكري. أيضا وجد أن أرتفاع مستوىHMGA1 يمنح خطرا واضحاً للإصابة بمتلازمة الأيض الغذائي بغض النظر عن وجود أو عدم وجود داء السكري. إلى جانب ذلك كان مستوى HMGA1 في المصل مرتبطاً بشكل إيجابي بمعلمات مرض السكري والتي تشمل تحليل السكر التراكمي والسكر الصائم .

الكلمات المفتاحية: الاليزا، بروتين HMGA1، السكان العراقيون، متلازمة الأيض الغذائي، داء السكري النوع الثاني.