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The Role of ACTH and MC4R Gene in the Pathophysiology of Metabolic Syndrome

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Abstract

Background : The multifactorial metabolic syndrome is characterized by hormonal dysregulation and genetic susceptibility. The adrenal peel hormone (ACTH) has an vital part in the axis of the pituitary and adrenal gland and may contribute to metabolic defects. Additionally, genetic polymorphisms in the melanocortin 4 receptor (MC4R) gene, particularly rs17782313, have been linked to obesity and related metabolic conditions .**Objective** :To check the serum levels of ACTH and analyze the association of MC4R (rs17782313) C/T polymorphism with MetS in Iraqi patients, as well as explore the relationship between ACTH levels and MC4R genotypes. **Method**: The case study and evidence were conducted on 75 MetS patients and 75 health controls. Serum ACTH levels were measured using ELISA. Genotyping of MC4R (rs17782313) was performed using tetra-primer ARMS-PCR. Statistical analysis included t-tests, chi-square tests, and ROC curve analysis to assess diagnostic performance and genotype distribution .**Results** :ACTH levels in MetS sick were considerably greater than those in controls (26.44 2.88, P 0.001) (57.67 3.66). ROC analysis indicated a cutoff value of >37.51 pg/mL, with sensitivity and specificity of 98.7% and 97.3%, respectively (AUC = 0.980). With an odds ratio of 2.81 (P = 0.024), the TT genotype of MC4R was significantly more common in MS patients (24.0%) than in controls (12.0%). Patients with the TT genotype had higher ACTH levels, but the difference was not statistically significant (P = 0.175).

Key words : Metabolic Syndrome _ACTH_MCR4

دور هرمون ACTH وجين MC4R في الفيزيولوجيا المرضية لمتلازمة الأيض

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الخلاصة:

الخلفية: تُعد متلازمة الأيض (MetS) حالة متعددة العوامل تتميز بخلل في التنظيم الهرموني وقابلية وراثية للإصابة. يُعتبر هرمون الموجه لقشر الكظر (ACTH) من الهرمونات الأساسية في محور الغدة النخامية_ الكظر، وقد يلعب دورًا مهمًا في الاضطرابات الأيضية. كما أن التعددات الشكلية الجينية في جين مستقبل الميلانوكورتين 4(MC4R) ، وخصوصًا النمط الجينيrs17782313 ، قد ارتبطت بالسمنة واضطرابات الأيض المرتبطة بها. الهدف: هدفت هذه الدراسة إلى تقييم مستويات ACTH في الدم ودراسة العلاقة بين تعدد الأشكال C/T في الجين MC4R (rs17782313) ومتلازمة الأيض لدى المرضى العراقيين، بالإضافة إلى تحليل العلاقة بين مستويات ACTH والأنماط الجينية المختلفة لـ

الطرق: أهذه الدراسة شملت 75 مريضًا مصابًا بمتلازمة الأيض و75 شخصًا سليمًا كمجموعة ضابطة. تم قياس مستويات ACTH في مصل الدم باستخدام تقنية المقايسة المناعية المرتبطة بالإنزيم (ELISA). وتم تحديد النمط الجيني لتعدد الأشكال rs17782313 في جين MC4R باستخدام تقنية PCR بطريقة ARMS ذات التمهيد الرباعي. شملت التحليلات الإحصائية اختبار "T " للعينات المستقلة، واختار, chi-square ، وتحليل منحنى ROC لتقييم الكفاءة التشخيصية وتوزيع الأنماط الجينية. النتائج: كانت مستويات ACTH أعلى بشكل ملحوظ في مرضى متلازمة الأيض (57.67 ± 3.66 بيكوغرام/مل) مقارنة بالأصحاء (26.44 ± 2.88 بيكوغرام/مل، P < 0.001). أظهر تحليل منحنى ROC أن القيمة الحدية المثلى كانت >37.51 بيكوغرام/مل، بحساسية بلغت 98.7% ونوعية بلغت 97.3 (AUC = 0.980). كان النمط الجيني TT لجين MC4R أكثر شيوعًا بين مرضى متلازمة الأيض (24.0%) مقارنة بالأصحاء (12.0%)، بنسبة أرجحية بلغت 2.81 (P = 0.024). وعلى الرغم من أن حاملي النمط الجيني TT أظهروا مستويات ACTH أعلى، إلا أن هذا الفرق لم يكن ذا دلالة إحصائية.(P = 0.175)

الكلمات المفتاحية : متلازمة الأيض الغذائي ACTH_ _ مستقبل الميلانوكورتين 4

Conclusion:
Elevated ACTH levels and the presence of the MC4R rs17782313 TT genotype are related with increased danger of MS. ACTH may serve as a potential diagnostic biomarker, and MC4R polymorphism could contribute to the genetic predisposition to MS in the Iraqi population.

Introduction

Metabolic Syndrome (MetS) is a multifaceted disorder characterized by a cluster of conditions, including central obesity, insulin resistance, dyslipidemia, and hypertension. Cardiovascular diseases and kind 2 diabetes are significantly increased by these interrelated factors. The pathophysiology of MetS involves a complex interplay between hormonal regulation, genetic predispositions, and environmental influences (Loos & Yeo, 2022).

Adrenocorticotropic hormone (ACTH) plays a pivotal role in HPA axis, primarily stimulating cortisol production. Through its interaction with melanocortin receptors, particularly the melanocortin 4 receptor (MC4R), ACTH has been linked to energy homeostasis and appetite regulation in addition to its traditional endocrine functions (Mountjoy, 2015). MC4R receptor paired with G protein, mostly expressed in the central nervous method, is an integral part of food intake and energy expenditure regulation. Activation of MC4R by ACTH and other melanocortins has been shown to suppress appetite and influence metabolic processes (Ghamari-Langroudi et al., 2017).

One of the most prevalent monogenic causes of obesity is a genetic difference in MC4R gene. Both heterozygous and homozygous mutations can lead to hyperphagia, early-onset obesity, and metabolic disturbances. Recent studies have highlighted that pathogenic variants in MC4R are associated with increased visceral adiposity, hepatic fat accumulation, and insulin resistance, independent

of overall obesity levels (Clément & Meyre, 2021). Furthermore, the anorexigenic effects of ACTH have been demonstrated to be MC4R-dependent, as evidenced by studies in animal models lacking functional MC4R (Kühnen et al., 2019).

Understanding the synergistic roles of ACTH and MC4R in the development and progression of MetS could unveil novel therapeutic targets. The purpose of this review is to shed light on the molecular and clinical connections that exist between the metabolic syndrome, MC4R function, and ACTH signaling, in order to offer suggestions for potential treatments for this growing health problem.

Methods and Materials

A total of 150 participants were enrolled in the present study, including 75 patients diagnosed with metabolic syndrome (MetS) and 75 apparently healthy individuals who served as the control group. The Ethics Committee of the College of Biotechnology at Al-Qadisiyah University in Iraq approved the study protocol in 2024. All participants were adults from various regions in Iraq. The criteria set forth by the International Diabetes Federation (IDF) were used to make the diagnosis of MetS in the group. The inclusion criteria for both groups were based on age range (18–67 years), BMI classification, and absence of chronic diseases unrelated to metabolic syndrome. The mean age of the participants was 42.96 ± 8.61 years for the MetS group and 45.82 ± 9.76 years for the control group. BMI values were 28.79 ± 4.28 kg/m² and 22.10 ± 1.2 kg/m², respectively.

Pregnant and breastfeeding women were excluded. All participants provided written informed consent and concluded a questionnaire that involved medical history, lifestyle factors, and possible risk exposures. Anthropometric dimensions (height, weight, waist circumference) and blood pressure were found utilizing standard techniques height was measured to the close 0.5 cm, weight to the close 0.1 kg , and blood pressure utilizing a mercury sphygmomanometer . BMI was determined as weight separated by height in meters squared (kg/m).

For the biochemical analysis, venous blood samples were collected from each participant after an overnight fast. Plasma was seperated by centrifugation and stored at −20°C until analysis. The concentration of adrenocorticotropic hormone (ACTH) was determined using a commercially available ELISA kit (Elabscience, USA) according to the manufacturer's instructions. In order to guarantee validity of findings, each sample was examined twice. ACTH levels were statistically analyzed and compared between the MS and control groups. The data demonstrated that the mean ACTH levels of MetS sicks were considerably greater than those of healthy controls (26.44 2.88 pg/mL), with a greatly important difference (p 0.001). Additionally, ROC curves investigation

was done to evaluate the diagnostic potential of ACTH. An ACTH cutoff value of greater than 37.51 pg/mL was found to have an AUC of 0.980 (95% CI: 0.964–0.996) and a sensitivity of 98.7%, specificity of 97.3%, PPV of 97.4%, and NPV of 98.6%, respectively. This suggests that ACTH may be an effective biomarker for metabolic syndrome.

Genotyping

Genomic DNA was take out from blood specimens utilizing the gSYAN Frozen Blood Geneaid DNA Extraction Kit and examined using a nano-spectrometer at (260/280 nm).

There is a DNA concentration of 20 ng/µL and an average purity level of SPSS, or Statistical Package for the Social Sciences, was utilizing to perform statistical study. The proportions were utilized for description the obesity danger reasons. To summarize categorical data, we used the number and ratio for all sociodemographic, behavior, anthropometric, clinical, and biochemical elements of the research. For continuous variables, we used Mean and standard deviation . To inspect the change in covariates between high-danger and non-high-danger participants, an intergroup comparison was conducted using logistic regression, Pearson's chi-square test for definite variables, and independent-sample t-tests for continuous data. MC4R rs17782313 was inspected in connection to biochemical variables, body mass index , and demographics utilizing analysis of variance (ANOVA) .

Statistical Analysis

The data were analyzed utilizing SPSS program . Continuous variables were stated as mean ± standard deviation (SD), and comparisons between MetS patients and control subjects were performed using independent sample t-tests. Correlations between ACTH levels and metabolic parameters were measured utilizing Pearson's relationship factor. A p-value of <0.05 was considered statistically important.

Result

Adrenocorticotropic hormone (ACTH) levels in sicks with metabolic syndrome and healthy controls. The researchers compared ACTH levels in sicks MetS and healthy controls, and presented the results in Table (1) and Figure (1). The mean ACTH levels in patients with metabolic syndrome (MetS) and healthy controls were 57.67 ± 3.66 and 26.44 ± 2.88, respectively. The mean level was significantly higher in sicks with metabolic syndrome (MetS) than in healthy controls ($P < 0.001$).

Table (1): Adrenocorticotropic Hormone (ACTH) level in MetS patients and healthy control.

Groups		Adrenocorticotropic Hormone (ACTH) level
MetS patients	Mean ± SE	57.67 ± 3.66
	Range	36.61-83.95
Control	Mean ± SE	26.44 ± 2.88
	Range	2.74-44.73
p-value		< 0.001 † HS

n: number of cases; SD: standard deviation; †: independent specimens t-test; HS: highly significant at P ≤ 0.001.

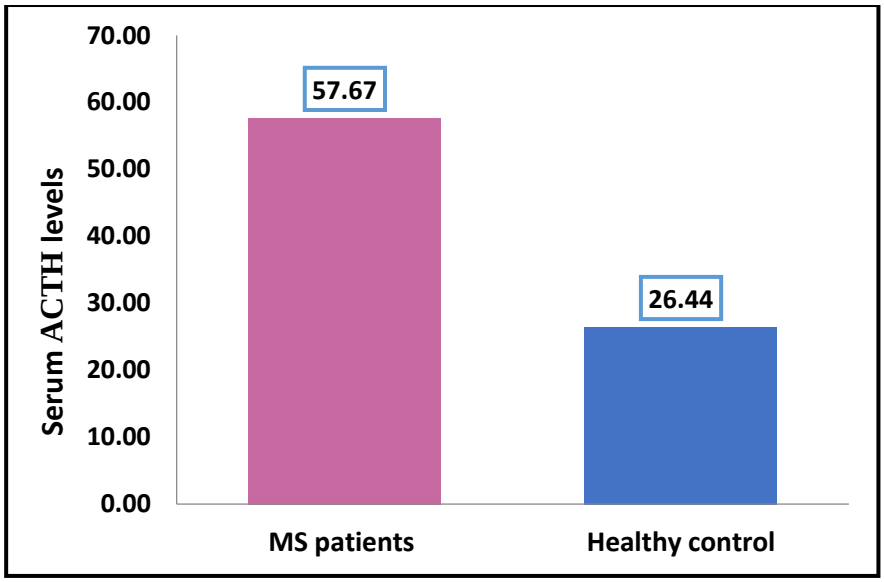

Figure (1): Mean ACTH levels of patients and healthy controls

Assessment of Adrenocorticotropic Hormone (ACTH) levels.

To evaluate the cut-off value of adrenocorticotropic hormone and its forecast of metabolic syndrome as a diagnostic or auxiliary diagnostic test, a receiver features(ROC) curve analysis was done, and results are revealed in Table (2) and Figure (2). The cut-off value of ACTH was greater than 37.51-fold, with sensitivity, specificity, positive predictive value (PPV), negative predictive value , and area below the curve of 98.7%, 97.3%, 97.4%, 98.6%, and 0.980 (0.964-0.996). According to the current results, ACTH is an excellent diagnostic indicator.

Table (2): Sensitivity and specificity of ACTH (> 37.51-fold) in MS disease

ACTH level	Patients $n = 75$	Healthy control $n = 75$
> 37.51	74	2
< 37.51	1	73
Sensitivity %	98.7 %	
Specificity %	97.3%	
PPV %	97.4 %	
NPV %	98.6%	
AUC (95%CI)	0.980 (0.964- 0.996)	

Cl: Confidence interval, AUC: Area under curve.

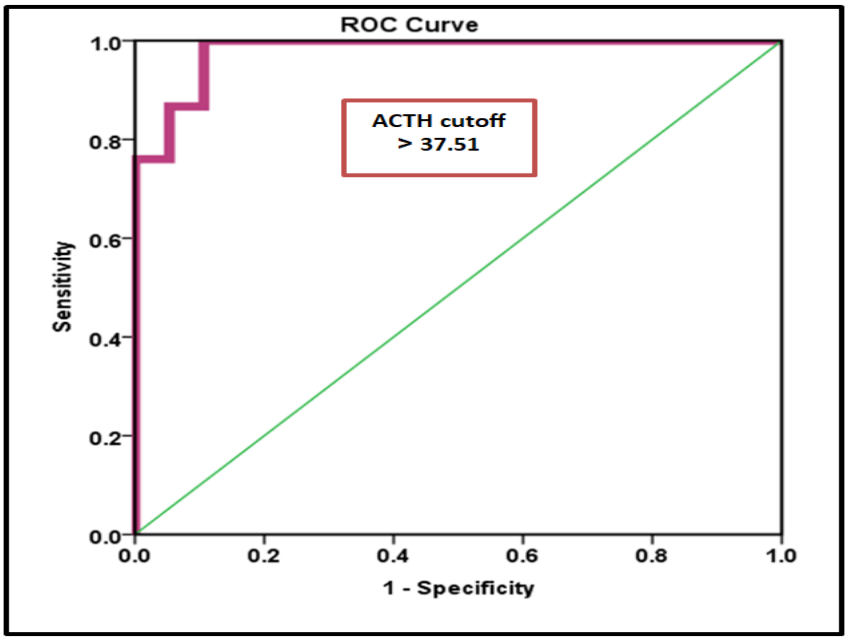

Figure (2): Receiver operator feature curve analysis of ACTH for the determination of potential diagnostic cutoff value.

Molecular research

Extraction of DNA

Genomic DNA was take out from blood specimens utlizing the gSYAN DNA extraction kit (Frozen Blood) Geneaid and analyzed by nanospectrometry at 260/280 nm. The purity range was 1.8–2.2, with a mean purity of 2, and the mean DNA concentration was 20 ng/µL. Melanocortin 4 receptor (MC4R) (rs17782313) C/T polymorphism was detected. The spreading of the MC4R (rs17782313) C/T polymorphism was detected by ARMS-PCR. There are three genotypes at this locus: CC, CT, and TT. Only the C allele at a product size of 218 bp was amplified in the homozygous wild-type genotype. The homozygous mutant genotype revealed amplification of only the T allele at a product size of 189 bp. Instead, the heterozygous genotype showed amplification of the C and T

alleles at product sizes of 218 and 189 base pairs, respectively (Figure 3). On the other hand, the heterozygous genotype revealed amplification of the C and T alleles.

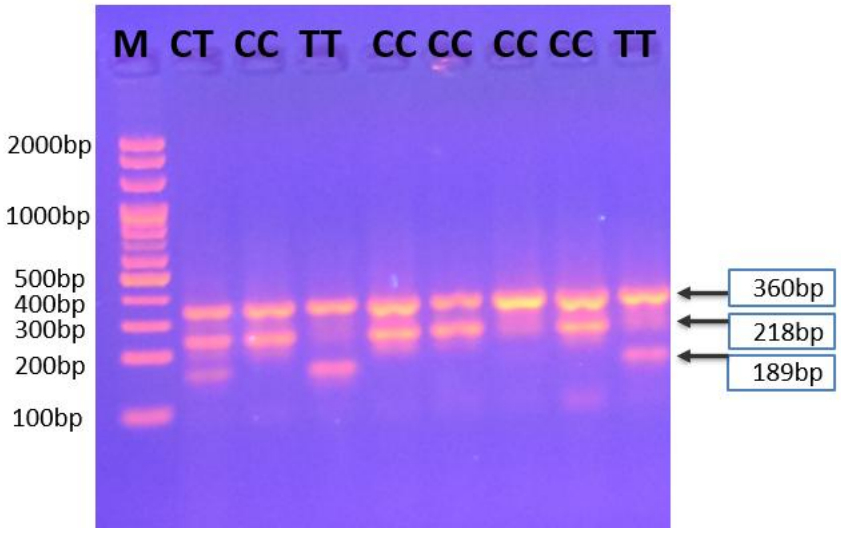

Figure (3): Agarose gel electrophoresis picture that revealed the T-ARMS-PCR product analysis of MC4R rs17782313 C/T gene polymorphism. Where M: marker (2000-100bp). The lane (CC) wild type homozygote was shown only C allele at 218bp T-ARMS-PCR product. The lane (TT) mutant type homozygote was shown only T allele at 189bp T-ARMS-PCR product, While heterozygotes (CT) were found as C and T alleles in T-ARMS PCR products of 218 and 189 bp in length. The intrinsic/extrinsic control collection was observed in T-ARMS PCR products of 360 bp in length. The Hardy-Weinberg equation was applied to the MC4R (rs17782313)C/T genotypes and their distribution in the controller collection, and the findings are revealed in Table 3.24. The homozygous wild-type CC genotype was found in 45 of 75 controls; heterozygous CT genotype was obtained in 21 of 75 controls, and homozygous mutant TT genotype was obtained in 9 of 75 controls (Table 3.24). The observed distribution of control group individuals according to the C/T genotypes of MC4R (rs17782313) was not statistically important changed from the probable distribution (P = 0.061).
Table (3): Hardy-Weinberg equation

Genotypes	Observed	Expected	χ^2	*P*
Homozygote reference CC	45	41.1	5.563	0.061 ¥ NS
Heterozygote CT	21	28.9		
Homozygote variant TT	9	5.1		

¥: Chi-square test; NS: Non-significant at *P* >0.05

Phenotype and allele analysis of the studied gene in patients and a healthy control group. Table 3-25 shows a association of the genotypes and allele frequencies associated with the SNP MC4R (rs17782313)C/T between patients with metabolic syndrome and healthy controls. Concerning the genotype shape, there was a significant difference in the distribution of genotype frequencies between patients with metabolic syndrome and healthy controls. The homozygous TT genotype was obtained to be a important danger cause (OR = 2.81), while the heterozygous C/T genotype was obtain to be an insignificant danger reason (OR = 1.67). This indicates that sicks with the homozygous TT genotype are about three times more likely to advance metabolic syndrome than those with other genotypes.

Table (4): *MC4R (rs17782313) C/T* POLY genotype frequency in MetS patients and healthy control

MC4R (rs17782313)	Patients N=75	Control N=75	*P*	OR	95% CI
Genotype frequency					
TT	18 (24.0%)	9 (12.0%)	0.024	2.81	1.12-7.05
C/T	25 (33.3%)	21 (28.0 %)	0.168	1.67	0.80-3.49
CC	32 (42.7 %)	45 (60.0 %)	Reference		
Allele frequency					
T	61 (40.7%)	39 (26.0 %)	0.007 ¥ S	1.95	1.20-3.18
C	89 (59.3 %)	111 (74.0%)		Reference	

¥: Chi-square test; NS: not significant at $P > 0.05$; S: significant at $P \leq 0.05$

The association between MC4R (rs17782313) C/T polymorphism and ACTH levels in MS patients

Table 3 shows the relationship between ACTH levels and the MC4R (rs17782313) C/T polymorphism in MetS patients. Patients with the CC, CT, and TT genotypes had mean serum ACTH levels of 56.5 3.24, 53.7 3.61, and 64.41 4.35, respectively. sick with the TT (mutant) genotype had higher ACTH levels than those in further groups, but this variance was not statistically important (P = 0.175). The relationship between ACTH levels and the MC4R (rs17782313) C/T polymorphism in MetS patients is shown in Table 5. †: one way ANOVA; NS: not important at P < 0.05.

ELISA parameters	ARMS-PCR finding			
	CC genotype	*CT genotype*	*TT genotype*	*P value*

	n =32	n = 25	n =18	
Adrenocorticotropic Hormone (ACTH) level				
Mean± SE	56.5 ± 3.24	53.7 ± 3.61	64.41 ± 4.35	0.175 † NS

n: number of cases; SE: standard error; †: one way ANOVA; NS: not significant at P 0.05.

Discussion

In the current study, ACTH levels were significantly greater in sicks with metabolic syndrome (MS) related to healthy controls ($P < 0.001$). This increase may reflect chronic activation of the hypothalamic-pituitary-adrenal axis, a common feature in MS and obesity, which is known to disturb endocrine and metabolic homeostasis (Ludescher et al., 2008). Hypersecretion of ACTH may result from stress-related stimulation of the HPA axis and/or impaired feedback regulation involving melanocortin pathways.

Simultaneously, the genotyping results for MC4R (rs17782313) revealed a significant association between the TT genotype and increased risk of MS (OR = 2.81, P = 0.024). The T allele was also more frequent in MS patients (40.7%) compared to controls (26.0%), indicating a possible functional role of this variant in the etiology of MS. These findings are consistent with studies suggesting that polymorphisms in *MC4R* are linked to altered energy balance, increased appetite, and susceptibility to obesity and metabolic disturbances (Loos et al., 2008; Xi et al., 2012).

The MC4R receptor is expressed in hypothalamus and plays a central part in regulating satiety, energy expenditure, and HPA axis through its interaction with ACTH and α-MSH (Mountjoy et al., 2001). Functional impairment due to rs17782313 polymorphism may diminish the receptor's response to ACTH or reduce downstream signaling, resulting in a disrupted feedback loop and persistent ACTH elevation, as observed in the TT genotype group. This disruption may also contribute to central adiposity and insulin resistance — hallmark features of MetS (Keller et al., 2012).

Comparison with previous studies supports our findings. In an Iraqi population, Al-Zubaidi et al. (2022) reported a higher frequency of the T allele among obese individuals with features of metabolic syndrome, suggesting a shared genetic predisposition. Internationally, Qi et al. (2008) reported that rs17782313 was strongly associated with BMI and insulin resistance in a large European cohort, supporting the link between MC4R variants and metabolic risk. Furthermore,

Rosmond et al. (2000) showed that individuals with impaired melanocortin signaling exhibited higher cortisol and ACTH levels, reinforcing the role of MC4R in HPA axis modulation.

In conclusion, the present findings suggest that elevated ACTH and MC4R gene polymorphism may interact in MetS patients. The TT genotype of MC4R rs17782313 may predispose individuals to ACTH dysregulation and associated metabolic disturbances. These findings suggest that endocrine dysfunction and genetic susceptibility play a role in the pathophysiology of MetS, which could be taken into account in future treatments and diagnostic procedures.

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