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Evaluation of Serum Leptin Level with Genes *(LEPR and TMEM18)* Expiration in Obese Individuals

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Annotation: Background: obesity is linked to early death and poses a severe danger to public health. Non-communicable diseases, including type 2 diabetes, cardiovascular conditions, high blood pressure, and various cancers, significantly contribute to the global health burden. Studying the genes, hormones, and proteins that has correlation with obesity will help explain why some people, even in identical situations, are more likely to become obese than others.

Aim: Identify the effect of Leptin hormone, gene expiration of LEPR and TMEM18 and differences between obese and healthy individuals in obesity people.

Method: This study was carried out in laboratories of College of Science, Wasit University in Iraq form November 2023 to July 2024.This study were include 80 peoples the ages (18-50) they were measuring the BMI of them . Fifty obese people (25 male and 25 female) they are diagnosed clinically. The control group consists of 20 apparently healthy individuals (10 male and 10 female). The current study was in agreement with hospital ethics and

approvals from all participants. Measuring Leptin, LEPR and TMEM18 Genes expiration in obese and control groups by EIESA assay and Real Time PCR according to type of test.

Results. The Results was showed that mean concentrations of leptin hormone and LEPR were significantly lower in obese individuals than in the control group. Leptin hormone 1.47 ± 0.061 (obese) vs. 2.35 ± 0.411 (control) (P = 0.002). LEPR 0.52 ± 0.18 and 1(control) (P= 0.001).Also, the mean levels TMEM18 genes were significantly higher in obese individuals compared control group. TMEM18 3.43 ± 0.78 (obese) vs. 1.00 (control) (P = 0.001).

Conclusions: It is concluded from the present study that the gene expression of LEPR, TMEM18 and Leptin hormone important roles in the development and severity of obesity.

Keywords: Obesity, Leptin, LEPR and TMEM18.

Introduction

A typical definition of obesity is the accumulation of excessive body fat that poses a health concern. Body Mass Index (BMI), which assesses an individual's weight in relation to height, is the most effective and widely used method for identifying obesity (1).Numerous physiological functions, including as metabolism, proliferation, and cellular homeostasis, are impacted by obesity (2).

Several hormones, including leptin regulate hunger, satiety, and energy metabolism .Leptin, primarily produced by adipose tissue, acts as a satiety factor and regulates food intake and energy expenditure (3) (4).

Estimates of obesity's heritability range from 40% to 75%, highlighting the significant hereditary component. Genome-wide association studies (GWAS) have identified a variety of genes that influence energy regulation and appetite, contributing to both monogenic and polygenic forms of obesity (5). The leptin receptor gene (*LEPR*) is integral to the regulation of body weight and energy homeostasis. Mutations in *LEPR* have been associated with severe early onset obesity, hyperphagia, and metabolic disorders (6). Additionally, transmembrane protein 18 (*TMEM18*) was identified as a genetic locus associated with obesity-related traits. Its expression in different

tissues, such as the brain, indicates its potential involvement in controlling appetite and metabolism (7).

2. Methods:-

2. 1 Two main subjects were included in the study:

2.1 subjects were included in the study:

This study has included (80) person with the ages rang (18-50). Fifty people as obesity people they have more than 25 BMI and thirty healthy people as control group have BMI (19.5-24.5).

2.2. EIESA Kit Assay

2.2.1 Human Leptin ELISA Kit.

The procedure of this kit down According to the company China (BT LAB) the catalog number (E1559Hu).

Reagent Preparation:

Before use, all reagents were maintained at room temperature. To prepare a 6ng/ml standard stock solution, mix 120ul of the standard (12.8ng/ml) with 120ul of the standard diluent. Let the standard sit for 15 minutes with gentle stirring before proceeding with dilutions. Duplicate standard points were created by serially diluting the 6ng/ml standard stock solution 1:2 with a standard diluent to achieve concentrations of 3ng/ml, 1.5ng/ml, 0.75ng/ml, and 0.375ng/ml. Any leftover solution should be stored at -20°C and used within a month.

2.3 Molecular assay

2.3.1. Total RNA Extraction:

All samples had their total RNA extracted using the TRIzol LS Reagent, adhering to the manufacturer's instructions, as detailed below:

In an Eppendorf tube, 250 µL of blood from each sample was combined with 750 µL of Trizol LS Reagent. The mixture was homogenized using a vortex. Subsequently, 200 µL of chloroform was added, and the mixture was shaken vigorously for 15 seconds. It was then placed on ice for 5 minutes. The mixture underwent centrifugation at 12,000 rpm for 10 minutes at 4℃. Following centrifugation, the mixture separated into a lower organic phase, an interphase, and a clear upper aqueous phase, with RNA remaining solely in the aqueous phase. The aqueous phase was transferred to a new 1.5 mL tube, and an equal volume of isopropyl alcohol was added. The mixture was inverted for 10 seconds and incubated at -20℃ for 10 minutes. It was then centrifuged again at 12,000 rpm for 10 minutes at 4℃, and the supernatant was carefully removed. An 80% ethanol solution was added and mixed thoroughly by inverting or vortexing. The mixture was centrifuged at 12,000 rpm for 5 minutes at 4℃, and the supernatant was carefully removed. The pellet was dried using hot air. Finally, the RNA was dissolved in RNase-free water and incubated for 10 minutes at 60℃ before being stored in freezing conditions until needed.

2.3.2. Estimation of RNA Purity and Concentration:

The NanoVue Nanodrop spectrophotometer (England) was employed to assess the concentration and purity of extracted RNA, thereby determining the quality of samples for subsequent RT-qPCR analysis. The RNA concentrations of the samples varied from 73 to 147 ng/µl. Absorbance measurements were conducted at two specific wavelengths, 260 nm and 280 nm, to evaluate RNA purity. An A260/A280 ratio approximating 2.0 indicated that the RNA sample was pure.

2.3.3. Quantitative Real Time PCR:

A NanoVue Nanodrop spectrophotometer from England was employed to assess the concentration and purity of the extracted RNA, which was crucial for evaluating the quality of samples for further RT-qPCR analysis. RNA concentrations in the samples varied between 73 ng/µL and 147

ng/µl. To assess RNA purity, absorbance was measured at 260 and 280 nm. An A260/A280 ratio close to 2.0 indicated that the RNA sample was pure.

2.3.4. cDNA Synthesis of Mrna

Using a cDNA kit from Add bio Company in Korea, total RNA was converted into complementary DNA (cDNA) through reverse transcription. This process was carried out in a 25 µl reaction volume, following the guidelines provided by the manufacturer.

The conversion involved three key steps:

1. Reverse transcription reactions were conducted in an RNase-free environment.
2. It was advised to use clean pipettes and filter tips.
3. The RNA templates and all reagents were thawed on ice, with each solution being gently mixed.

2.3.5. Primers Design:

The primer design process was carried out using the primer3 web version 4.1.0, accessible online at http://primer3.ut.ee, for the genes *TMEM18*, *LEPR*, and *GAPDH*, and was verified using UCSC programs. Alpha DNA Ltd. (Canada) synthesized and lyophilized the primers.

2.3.5.1. Primer Preparation:

For each assay in this study, the necessary primers were prepared as follows:

The lyophilized sample was dissolved in nuclease-free water following the manufacturer's guidelines, creating a stock solution with a concentration of 100µM, which was stored at -20°C. By diluting 10µL of each primer stock solution in 90µL of nuclease-free water, a working solution with a concentration of 10µM was obtained and kept at -20°C until needed.

2.2.4 Statistical analysis:

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation after performance of Kolmogorov- Smirnov normality test and making decision about normally and non-normally distributed variables. **Independent sample t-test** was used to study difference in mean between any two groups provided that the variable is normally distributed. **One way anova test** was used to study difference in mean among more than two groups provided that the variable is normally distributed. **Chi-square test** was used to study association between any two categorical variables. In order to detect the cutoff value that predict a positive finding, **receiver operator characteristic (ROC)** curve analysis was used with its corresponding area under the curve (AUC), accuracy level, sensitivity, specificity and level of significance (P). **Pearson correlation** was used to evaluate the correlation between any 2 numeric variables and the results were expressed as correlation co-efficient (r) and the level of significance (P). The level of significance was considered at P-value of less 0.05 and highly significant level at 0.01 or less (8).

3. Results and Discussion:

3. 1 Leptin hormone parameters

The comparison of leptin hormone levels between obese people and healthy control subjects has been carried out, and the results are demonstrated in table (3-1) and figure (3-1). The mean leptin hormone level was 1.47± 0.061 in obese people and 2.35 ± 0.411 in the healthy control group. The mean level was significantly lower in obese people than in healthy controls (P= 0.002).

Table (3-1): Leptin hormone level in obesity and healthy controls.

Hormone parameters	Obesity people *n* = 50	Healthy control *n* = 20	*P value*
Leptin hormone level			
Mean± SD	**1.47 ± 0.061**	**2.35 ± 0.411**	**0.002** † **S**
Range	0.10 – 2.98	0.15- 6.72	

n: number of cases; **SD**: standard deviation;; †: independent samples t-test; S: significant at P ≤ 0.05.

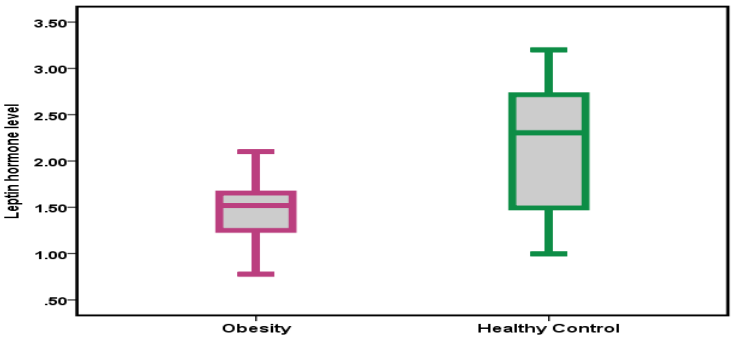

Figure (3-1): Mean of Leptin hormone level in obesity people and control groups.

Based on the findings, Leptin functions as the principal regulator of the brain-gut axis, conveying a satiety signal through its interaction with the central nervous system receptors located in the hypothalamus. Activation of hypothalamic leptin receptors results in the suppression of food intake and promotion of energy expenditure pathways. Notably, leptin levels decreased in response to weight reduction. Despite the capacity of leptin to suppress appetite, the majority of obese individuals exhibit hyperleptinemia and leptin resistance, which impedes the effectiveness of exogenous leptin therapy (9) (10). This paradoxical condition, wherein elevated circulating leptin levels fail to suppress appetite or induce weight loss, is a defining characteristic of obesity and is termed leptin resistance. Leptin resistance significantly contributes to obesity, thereby complicating efforts by most individuals to achieve and maintain weight loss (11).

3.1.1 Diagnostic accuracy of Leptin hormone.

To evaluate the Leptin hormone cutoff value as well as to predict the obesity as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in (3-2), and figure (3-2). The Leptin hormone cutoff value was > 1.63-fold with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under curve of 74.0%, 75.0%, 88.1%, 53.6% and 0.744 (0.591- 0.896). The current findings suggest that leptin is a valid diagnostic marker.

Table (3-2): Sensitivity and specificity of Leptin hormone (> 1.63-fold) in Obesity

Leptin hormone	Obesity people *n* = 50	Healthy control *n* = 20
> 1.63	37	5
< 1.63	13	15
Sensitivity %	74.0 %	
Specificity %	75.0 %	
PPV %	88.1 %	
NPV %	53.6%	
AUC (95% CI)	0.744 (0.591- 0.896)	

n: number of cases ,CI: Confidence interval, AUC: Area under curve.

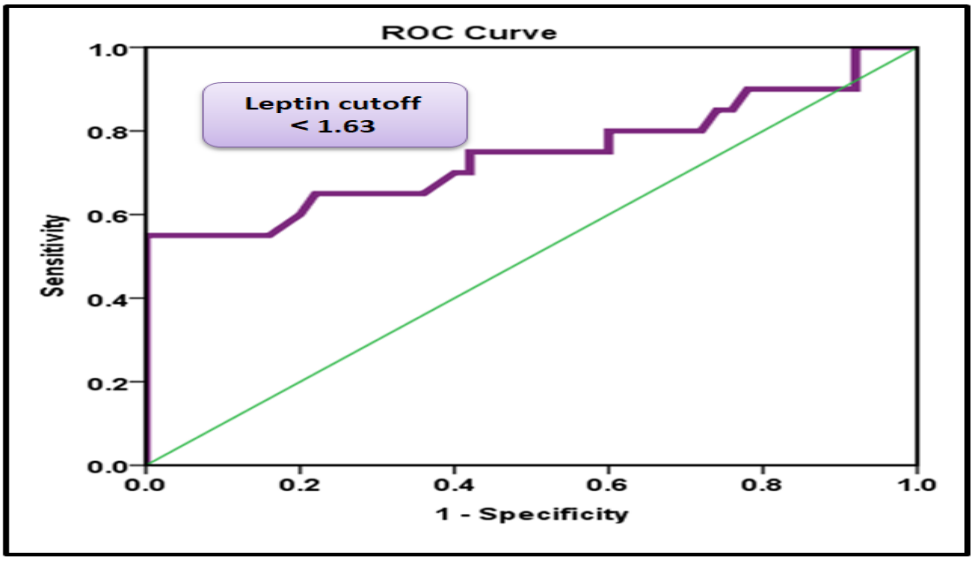

Figure (3-2): Receiver operating characteristic curve for Leptin hormone levels to distinguish obesity people from healthy control subjects.

Leptin levels in the blood were directly proportional to percentage body fat and hence measurement of leptin can be used to assess the severity of obesity. Individuals who are more prone to develop obesity-related comorbid conditions can be identified by the elevated levels of leptin.

These findings are in agreement with a report by Considine *et al.*, (1996) where they determined that the serum concentration of leptin is considerably higher in obese individuals compared to thin individuals. They demonstrated a strong correlation between body mass index (BMI) and plasma concentrations of leptin, and it was proposed that measurements of leptin concentration could serve as a valuable diagnostic test for obesity (12).

3.2 Leptin receptor (*LEPR*).

The comparison of Leptin receptor (*LEPR*) between obesity people and healthy control subjects has been carried out and the results were demonstrated in table (3-3) and figure (3-3).Mean of *LEPR* were 0.52 ± 0.18 and 1, in obesity people and healthy control group respectively, The average level was significantly lower in the obese group than in the healthy control group (P= 0.001).

Table (3-3): Leptin receptor level in obesity people and healthy controls.

Protein parameters	**Obesity people *n* = 50**	**Healthy control *n* = 20**	***P value***
Leptin receptor (*LEPR*) level			
Mean± SD	**0.52 ± 0.18**	**1.0**	**≤ 0.001 † HS**
Range	0.19 – 0.90	**1-1**	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; S: significant at P > 0.05.

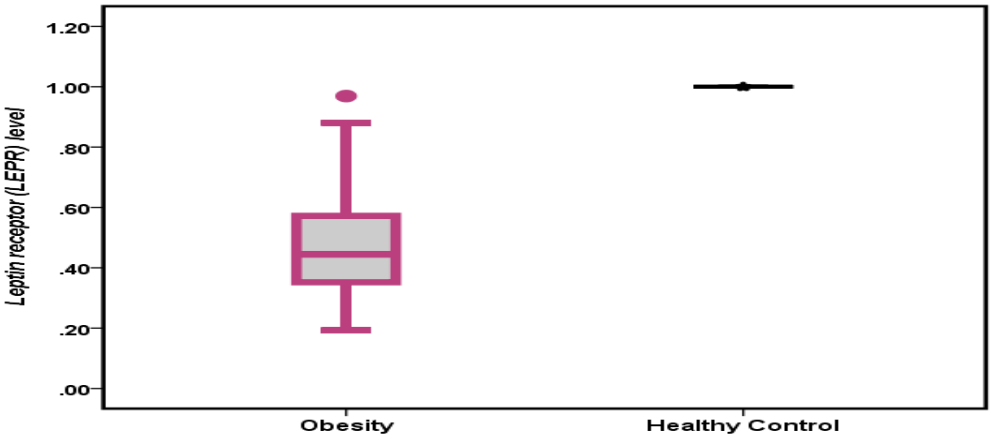

Figure (3-3): Mean of *LEPR* level in obesity people and control groups

These findings suggest that obesity may hinder leptin signaling due to a decrease in receptor availability. Individuals with obesity often show decreased expression of leptin receptor Ob-Rb mRNA compared to those with normal weight. Leptin resistance, in which the body becomes less sensitive to leptin signals, can result from genetic alterations in the *LEPR*, leading to excess food intake and lowered energy expenditure.The present research is in agreement with the report of Farooqi *et al.*, (2007) that established the occurrence of rare homozygous mutations in the LEPR gene leading to severe early onset obesity. *LEPR* mutations, including the Q223R polymorphism, have been associated with obesity in different populations. Mutations or alterations in gene expression of *LEPR* may influence receptor function, thus the variation in energy homeostasis and fat storage in individuals (13).

3.2.1 Diagnostic accuracy of *LEPR*.

To assess the LEPR cut-off value and its ability to predict obesity as either a diagnostic or supplementary diagnostic test, a receiver operator characteristic (ROC) curve analysis was performed, with the findings presented in (3-4) and figure (3-4). The LEPR cutoff value exceeded 0.98-fold, achieving a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and an area under the curve of 100.0%, 100.0%, 100.0%, 100.0%, and 1.000 (1.000-1.000), respectively. These results suggest that LEPR is an outstanding diagnostic marker.

Table (3-4): Sensitivity and specificity of *LEPR* (> 0.98-fold) in Obesity

LEPR	**Obesity people *n* = 50**	**Healthy control *n* = 20**
> 0.98	50	0
< 0.98	0	20
Sensitivity %	100.0 %	
Specificity %	100.0 %	
PPV %	100.0 %	
NPV %	100.0%	
AUC (95% CI)	1.000 (1.000- 1.000)	

n: number of cases ,CI: Confidence interval, AUC: Area under curve.

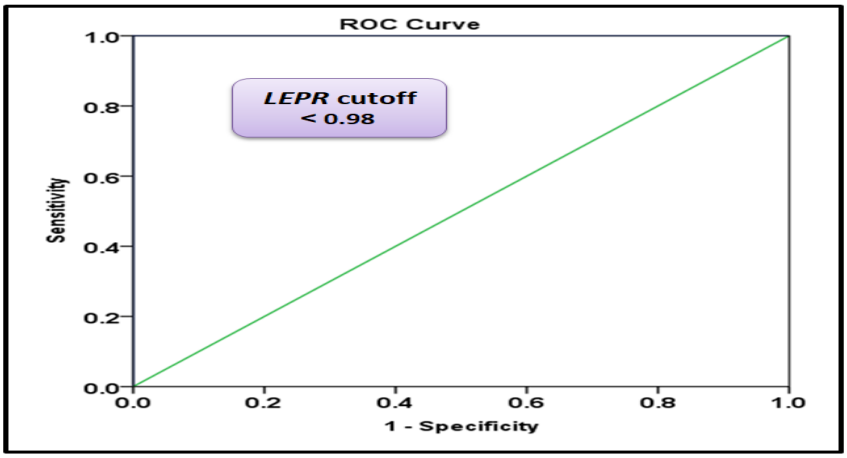

Figure (3-4): Receiver operating characteristic curve for *LEPR* levels to distinguish obesity people from healthy control subjects.

These study show that use of genetic sequence-based methods for *LEPR* mutation analysis is extremely sensitive and specific for diagnosing leptin receptor-based obesity syndromes. This enables the identification of affected individuals more accurately and to guide specific treatment options, such as weight control programs or novel drugs for the leptin pathway.

Genetic screening for *LEPR* has thus emerged as a valuable tool in the diagnostic assessment of obese individuals with severe obesity, facilitating more precise determination of genetic etiology and improving the prospect of early intervention. These results are in agreement with study by Clement *et al.,* (1998) provided early evidence of the link between *LEPR* mutations and obesity, showing that obesity people with *LEPR* deficiency exhibited severe obesity from a young age and did not respond effectively to leptin due to defective receptors (14).

3.3 Transmembrane protein-18 (*TMEM-18*).

The study compared transmembrane protein-18 (*TMEM-18*) levels between obese individuals and healthy control subjects, with the findings presented in table (3-5) and figure (3-5). The average *TMEM-18* levels were 3.43 ± 0.78 for those with obesity and 1 for the healthy control group. The mean level was significantly higher in obese individuals than in healthy controls (P= 0.001).

Table (3-5): *TMEM-18* level in obesity people and healthy controls.

Protein parameters	**Obesity people *n* = 50**	**Healthy control *n* = 20**	***P value***
Transmembrane protein-18 (*TMEM-18*) level			
Mean± SD	**3.43 ± 0.78**	**1.0**	**≤ 0.001 † HS**
Range	1.01 – 8.75	**1-1**	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; S: significant at P > 0.05.

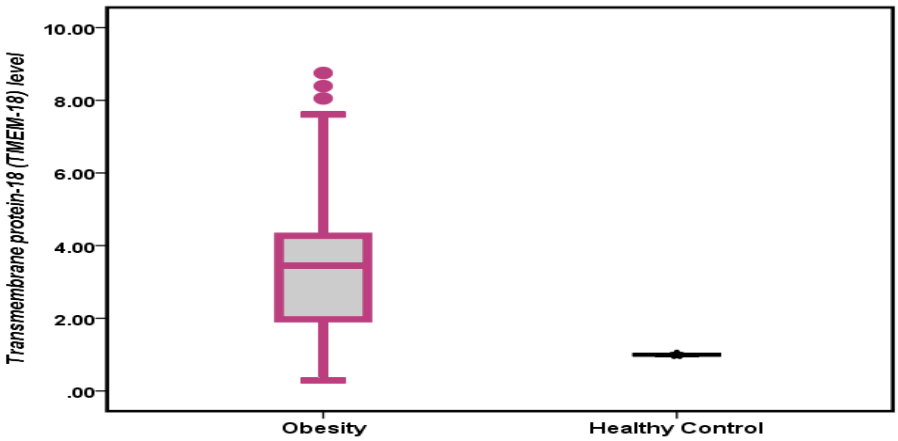

Figure (3-5): Mean of *TMEM-18* level in obesity people and control groups

These findings indicate that *TMEM18* is a significant genetic determinant of obesity risk. Research has demonstrated that specific variants of *TMEM18* are strongly correlated with increased body mass index (BMI) and heightened susceptibility to obesity. *TMEM18* is highly expressed in the hypothalamus, a brain region that is essential for regulating food intake and energy expenditure.

These results are consistent with the study by Chehadeh *et al.*, (2020), which identified a significant association between *TMEM18* gene polymorphism rs12463617 and overweight/obesity in female participants from a young UAE Arab population (p =0.044). Although *TMEM18* influences obesity risk, particularly in young females, its impact is likely part of a broader genetic framework involving multiple genes and their interactions. Sex-specific associations and gene-gene interactions highlight the importance of population-specific and sex-specific genetic profiling in understanding the heritability of obesity (15).

3.3.1 Diagnostic accuracy of *TMEM-18.*

To assess the TMEM-18 cutoff value and its potential to predict obesity as either a diagnostic or supplementary diagnostic test, receiver operator characteristic (ROC) curve analysis was performed. The findings are presented in table (3-6) and figure (3-6). The TMEM-18 cutoff value was determined to be less than 1.06-fold, with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area, the curve all, 100.0%, 100.0%, 100.0%, 100.0%, and 1.000 (1.000-1.000), respectively. These results suggested that TMEM-18 is an outstanding diagnostic marker.

Table (3-6): Sensitivity and specificity of *TMEM-18* (< 1.06-fold) in Obesity

TMEM-18	**Obesity people *n* = 50**	**Healthy control *n* = 20**
< 1.06	50	0
> 1.06	0	20
Sensitivity %	100.0 %	
Specificity %	100.0 %	
PPV %	100.0 %	
NPV %	100.0%	
AUC (95% CI)	1.000 (1.000- 1.000)	

n: number of cases ,CI: Confidence interval, AUC: Area under curve.

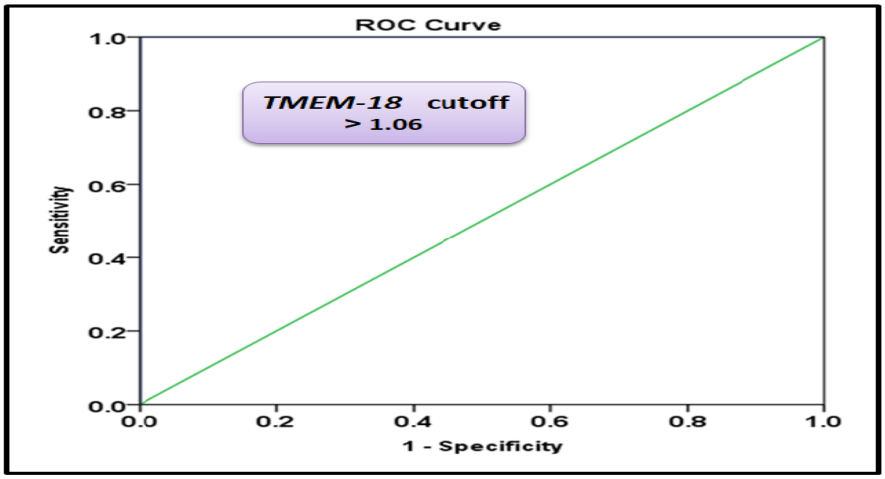

Figure (3-6): Receiver operating characteristic curve for*TMEM-18* levels to distinguish obesity people from healthy control subjects.

The diagnostic accuracy of *TMEM18* in predicting obesity has been substantiated by robust genetic associations and functional studies. As research continues to investigate the mechanisms by which *TMEM18* influences body weight regulation, it remains a promising target for elucidating the complex genetic landscape of obesity. This finding was recently corroborated by a study conducted by Landgraf *et al.*, (2020), which demonstrated that large-scale genome-wide association studies (GWAS) have consistently identified *TMEM18* as one of the top candidates for obesity risk. For instance, *TMEM18* is not only genetically associated with obesity but also plays a critical role in adipocyte differentiation. Research has indicated that downregulation of *TMEM18* impairs adipocyte formation and is clinically associated with obesity and insulin resistance (16).

Conclusions:

The gene expression of *LEPR*, *TMEM18 genes* and Leptin hormone important roles in the development and severity of obesity.

References

1. Carbone, S. (2013). Obesity and Diastolic Heart Failure: Is Inflammation the Link? Translational Medicine, 03(03).
2. Vick, L. V., Canter, R. J., Monjazeb, A. M., & Murphy, W. J. (2023). Multifaceted effects of obesity on cancer immunotherapies: Bridging preclinical models and clinical data. Seminars in Cancer Biology, 95, 88–102.
3. Ribiere, C., & Plut, C. (2005). Nutritional regulation of leptin signaling. Current Hypertension Reports, 7(1), 11–16.
4. Çakır, I., Bagchi, R. A., White, A., Lin, J. D., Cone, R. D., Wang, Q., Mckinsey, T. A., Hagen, S., Ghamari-Langroudi, M., Porter, D. T., Lee, P., Jana, S., Hadley, C. K., Pan, P. L., & Litt, M. J. (2022). Histone deacetylase 6 inhibition restores leptin sensitivity and reduces obesity. Nature Metabolism, 4(1), 44–59.
5. Mahmoud, R., Kimonis, V., & Butler, M. G. (2022). Genetics of obesity in humans: Aclinical review. International Journal of Molecular Sciences, 23(19), 11005.
6. Nunziata, A., Lennerz, B., Funcke, J.-B., Von Schnurbein, J., Fischer-Posovszky, P., Borck, G., Brandt, S., Wabitsch, M., Gierschik, P., & Moepps, B. (2018). Functional and Phenotypic Characteristics of Human Leptin Receptor Mutations. Journal of the Endocrine Society, 3(1), 27–41.
7. Golden, A., & Kessler, C. (2020). Obesity and genetics. Journal of the American Association of Nurse Practitioners, 32(7), 493–496.

8. Daniel W.W., (2018). Biostatistics: A Foundation for Analysis in the Health Sciences, John Wiley & Sons New York.
9. Bence, K. K., Hotamisligil, G. S., Xue, B., Delibegovic, M., Neel, B. G., Kahn, B. B., & Gorgun, C. Z. (2006). Neuronal PTP1B regulates body weight, adiposity and leptin action. Nature Medicine, 12(8), 917–924.
10. Münzberg, H., Bates, S. H., Björnholm, M., & Myers, M. G. (2005). Leptin receptor action and mechanisms of leptin resistance. Cellular and Molecular Life Sciences, 62(6).
11. Myers, M. G., Cowley, M. A., & Münzberg, H. (2008). Mechanisms of Leptin Action and Leptin Resistance. Annual Review of Physiology, 70(1), 537–556. Münzberg, H., Bates, S. H., Björnholm, M., & Myers, M. G. (2005). Leptin receptor action and mechanisms of leptin resistance. Cellular and Molecular Life Sciences, 62(6).
12. Considine, R. V., et al. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. New England Journal of Medicine, 334(5), 292-295.
13. Farooqi, I. S., Undlien, D. E., Lessan, N., Ercan, O., Myhre, A. G., Perna, F., Wangensteen, T., Bottomley, B., Keogh, J. M., Ghodsi, M., Barroso, I., Lank, E., Fontana, S., De Rosa, V., Matarese, G., Stanhope, R., Dattani, M., Collins, S., Retterstøl, L., … O'Rahilly, S. (2007). Clinical and Molecular Genetic Spectrum of Congenital Deficiency of the Leptin Receptor. New England Journal of Medicine, 356(3), 237–247.
14. Clément, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J. M., Basdevant, A., Bougneres, P., Lebouc, Y., Froguel, P., & Guy-Grand, B. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature, 392, 398–401.
15. Chehadeh, S. E. H., Osman, W., Nazar, S., Jerman, L., Alghafri, A., Sajwani, A., Alawlaqi, M., AlObeidli, M., Jelinek, H. F., AlAnouti, F., Khalaf, K., & Alsafar, H. (2020). Implication of genetic variants in overweight and obesity susceptibility among the young Arab population of the United Arab Emirates. *Gene*, *739*, 144509.
16. Landgraf, K., Klöting, N., Gericke, M., Maixner, N., Guiu-Jurado, E., Scholz, M., Witte, A. V., Beyer, F., Schwartze, J. T., Lacher, M., Villringer, A., Kovacs, P., Rudich, A., Blüher, M., Kiess, W., & Körner, A. (2020). The obesity-susceptibility gene TMEM18 promotes adipogenesis through activation of PPARG. Cell Reports, 33(3), 108295.