Arab American University

Faculty of Graduate Studies

Department of Health Sciences

Master Program in Cellular and Molecular Biosciences

The Correlation of genetic polymorphisms for leptin and leptin receptor, and serum level of leptin with hypertensive patients in Jenin.

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202020405

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This Thesis Was Submitted in Partial Fulfilment of the Requirements for the Master Degree in

Cellular and Molecular Biosciences

Palestine, Feb/ 2025

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Thesis Approval

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Declaration

I declare that, except where explicit reference is made to the contribution of others, this thesis is substantially my own work and has not been submitted for any other degree at the Arab American University or any other institution.

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Dedication

I would like to dedicate this thesis to my beloved family: my loving parents Ayman and Wijdan, caring husband Mohammad, and my two beautiful girls Yaqeen and Sarah. Also included are my supportive siblings (especially Hayat for many nights of babysitting). Without these pillars in my life, granting me constant courage and inspiration, this journey would not have been possible.

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Abstract

Background

An endocrine hormone that functions as a significant regulator of food intake, neuroendocrine output, metabolism, and fat accumulation is the human homologue of the LEPTIN gene (LEP), which has been identified as an obesity gene. Through sympathetic activation in the circulation or at the renal level, leptin may also influence blood pressure and contribute to the development of hypertension, as demonstrated by certain experimental studies. Microinjections of leptin have also suggested that higher levels of leptin have greater effects on sympathetic activation.

Objectives

This study aimed to investigate the association between gene polymorphisms (LEP, *LEPR*) among hypertensive patients from Jenin, West Bank.

Methodology

This study was carried out on forty-five HBP patients with BMI>30 Kg/m^2 and forty-five HBP patients with BMI≤25 Kg/m^2. Blood samples were collected in EDTA and plain tubes for all patients. DNA was extracted from all EDTA blood samples. Polymorphisms of *LEP*, and *LEPR* genes were genotyped by using the restriction fragment length polymorphism (RFLP)-PCR. (The LEP gene SNP rs7799039 (2548G > A), while LEPR SNPs rs1137101 (þ668A > G).

Results

Regarding the results of PCR-RFLP for LEP gene SNP rs7799039 (2548G > A), 0% of the cases were homozygous for the wild type genotype, compared to 20% in the normal BMI group. The heterozygous genotype was reported in 27% in cases, compared to 23% in the normal BMI group. The homozygous genotype was reported 18% in cases, compare to 13% in the normal BMI group. These results were statistically significant, with a higher frequency among patients (P=0.006). With no significance for *LEP* R SNPs rs1137101 (þ668A > G) polymorphism with P value (0.626456).

The mean for leptin concentrations were 13.7 ± 12.5 (mean ± SD) for 45 patients with BMI>30 and 41.3 ± 17.7 for 45 patient with BMI<25. These results were highly statistically significant, with a higher frequency among patients (P<0.001).

Conclusion

In conclusion, our current findings provide conclusive proof that the *LEP* gene polymorphism is associated with susceptibility to HBP.

This study suggests that having a *LEP* AA genotype is an independent risk factor for developing hypertension in obese individuals. To demonstrate a concrete link between these polymorphisms and HBP risk, further research should be conducted.

Keywords: Leptin, Hypertension, Body Mass Index.

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List of Definitions of Abbreviations

Abbreviations	Title
HBP	high blood pressure
GWAS	genome-wide association studies
BMI	body mass index
SNPs	single-nucleotide polymorphisms
LEP	Leptin
LEPR	Leptin receptor
WHO	World Health Organization
DNA	deoxyribonucleic acid
T	Thymine
C	Cytosine
CHD	coronary heart disease
BP	blood pressure
CVD	cardiovascular disease
CKD	chronic kidney disease
AHA	American Heart Association
ACC	American College of Cardiology
TOS	The Obesity Society
GBD	Global Burden of Disease
NS	nerve systems
LIF	Leukemia inhibitory factor
EDTA	Ethylenediaminetetraacetic acid
ELIZA	enzyme-linked immunosorbent assay
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism

OD	optical density
Cho	Cholesterol
TG	Triglyceride
HDL-c	high density lipoprotein cholesterol
LDL-c	low density lipoprotein cholesterol
P value	probability value
L	Ladder
US	United State

Chapter 1: Introduction

1.1 Introduction

Hypertension remains a critical public health issue along with the aging of the population worldwide. In recent decades, the prevalence of high blood pressure has largely increased in Europe and the United States. It has been widely accepted that the occurrence of hypertension arises from interactions between environmental and genetic factors. To date, more than 120 loci have been proven to be linked with hypertension susceptibility through genome-wide association studies (GWAS) (Huang et al., 2021).

In developed and developing countries, hypertension is identified as an expanding health crisis that causes major diseases, and it is highly affected by obesity and increased body mass index (BMI). It is the result of different changes caused by excessive fat accumulation in the body. Besides energy storage, the adipose tissue is also an endocrine organ that secrets adipokines. Adipokines play diverse roles in the pathophysiology of cardiovascular disease (Obradovic et al., 2021). Leptin is one of the most important adipokines will be investigated and evaluated in overweight and obese hypertensive patients compared to normal body-mass index (BMI) patients in this study.

Leptin has been affirmed to regulate various cardiac and vascular effects, including thrombosis, angiogenesis, and cardiac hypertrophy. Furthermore, it also has an impact on the control of metabolism, immunity, and reproduction (Wu & Sun, 2017) . The occurrence of single-nucleotide polymorphisms (SNPs) that may alter the circulation concentration of leptin adipokine has been connected to the genes *LEP* and *LEPR*, which encode leptin and leptin receptor on chromosomes 7 and 1, respectively. Both genes have been linked to sympathetic hyperactivity, obesity, and blood pressure dysregulation (De Faria et al., 2017).

The *leptin* (LEP) gene promoter region has one of the most researched SNPs, rs7799039 (2548G > A), while the exon 6 region of leptin receptor (*LEPR*) contains rs1137101 (þ668A > G) (De Faria et al., 2017). To investigate the role of *LEP* single nucleotide polymorphism (SNP) in the occurrence of hypertension, we will conduct a cross

sectional study to evaluate the association between *LEP* SNP and the risk of hypertension in a small Palestinian sample population.

In addition to the influence of LEP polymorphisms on serum, leptin levels, and the variability of metabolic phenotypes (leptin and lipid profiles) will be examined concurrently. This research will help increase understanding of the disease, its diagnosis, and effectively aid in its control and prevention. Identification of population at risk based on genetic polymorphism help deeper insight into the mechanisms underlying the disease. The resulting knowledge will help in the designing of selected preventive strategies, confirming that interventions are both effective and tailored to those most at risk.

1.2 Significance of the study

Current medical practices basically focus on the "treating the disease after its onset" while molecular medicine promises "preventing the disease before it occurs". Our study aims to reveal the association between hypertension and *LEP ,LEPR* genes variations. We intend to pinpoint the alleles linked to hypertension in the Palestinian population. This will finally lead to earlier detection, better prevention and personalized measures to control hypertension, particularly in the Palestinian population that are at higher risk.

1.3 Study Problem

The association of genetic polymorphisms in leptin and leptin receptor genes with hypertension has not previously been studied in Palestine. Therefore, the *LEP* and *LEPR* polymorphism (The LEP gene SNP rs7799039 (2548G > A), while LEPR SNPs rs1137101 (þ668A > G) is displayed in this study since there is no available data for the Palestinian population. Moreover. the concentration of the leptin hormone in the Palestinian population has not been investigated before.

1.4 Specific Objective

The overall aim of the study is to investigate the association between *LEP and LEPR* SNPs (The LEP gene SNP rs7799039 (2548G > A), while LEPR SNPs rs1137101 (þ668A > G) gene polymorphism and the occurrence of hypertension among patients from Jenin governorate, West Bank.

The specific objectives of the study are:

1. To find out the frequency of the common *LEP* and *LEPR* genotype and allele frequencies in the Palestinian hypertension patients based on age and sex.
2. To correlate the *LEP* and *LEPR* genotype with obesity BMI.
3. To investigate the association between *LEP* and *LEPR* genotype and the occurrence of hypertension among Palestinian patients referring to ministry of health in Jenin.

1.5 Research questions

Is hypertension related to *LEP* gene or *LEPR* gene variants? Do leptin and leptin receptor genes variants correlate serum leptin hormone levels? Do leptin and leptin receptor genes variants underly obesity or increase BMI?

1.6 Research Hypothesis

We hypothesize that leptin gene and leptin receptor gene polymorphisms are associated with leptin serum levels and some of these gene's variants are considered risk factors for hypertension.

1.7 Study limitations and determinants

There are some real limitations in the present study, especially the small sample size. The limited time frame to conduct this research made it unlikely to recruit a sufficient sample size. Also, the lack of study control group was due to limited financial resources and this was the second major limitation. The sample size and the limited number of hypertensive patients having normal or low BMI poses a challenge to make a reasonable statistical inference and therefore draw solid conclusions. Furthermore, the participants inhabit various

scattered villages, which make it difficult to track and follow-up. A major limitation was recruiting patients, due to data collection obstacles, it was hard-to-reach patients due to the poor filing system of hypertensive patients in the public care centers. Besides that, a considerable number of patients sought treatment in the private sector which made them unapproachable.

The main objective of our research was to see if there was an association between the frequency of *LEP* and *LEPR* gene polymorphisms with the development of hypertension in a cross-sectional study to better understand the current state of hypertension illness in our region.

1.8 Conceptual and operational definitions

1.8.1 Body mass index (BMI)

Estimating body fat is calculated by dividing body weight in kilograms by squared height in meters (kg/m2). The World Health Organization (WHO) defines overweight (25≤ BMI <30) and obesity (BMI ≥30) based on this straightforward and affordable procedure. However, the body fat percentage varies with age for a particular BMI, and the rate of change varies by sex, ethnicity, and personal characteristics. Furthermore, although BMI is insensitive to the true distribution of body fat, it does correlate with fat storage and metabolic health in large populations (Borga et al., 2018).

1.8.2 Single nucleotide polymorphisms (SNPs)

Every SNP indicates a variation in a single nucleotide, which is a building unit of DNA. For instance, in a specific DNA segment, an SNP may substitute the nucleotide thymine (T) for the nucleotide cytosine (C). SNPs are found naturally in DNA of all individuals. On average, they appear about every 1,000 nucleotides, meaning that an individual's genome has between 4 and 5 million SNPs. Many people have these variations; a variant must be present in at least 1% of the population to qualify as an SNP. More than

600 million SNPs have been discovered by researchers in populations worldwide (MedlinePlus Genetics, 2020).

1.8.3 Coronary heart disease

Coronary heart disease (CHD) is a constriction of the coronary arteries caused by the buildup of atherosclerotic plaques in the epicardial blood vessels, which stops blood flow, and decreases the oxygen supply to the heart muscle. Globally, CHD cases continue to be the main cause of death from cardiovascular disease worldwide (Sitinjak et al., 2023).

Chapter Two: Literature Review

2.1 High blood pressure (HBP) or hypertension

High blood pressure (HBP) or hypertension is the leading cause of cardiovascular disease and premature death worldwide since it is a highly prevalent chronic disorder. It is believed to be more responsible for the global illness burden and early mortality than other disease risk factors (Manuscript, 2017). Elevated systolic and/or diastolic blood pressure (BP) increases the risk of several cardiovascular disorders including stroke, coronary heart disease (CHD), heart failure, peripheral arterial disease and abdominal aortic aneurysms (MICHA, 2017). In 2010, hypertension, which is defined as systolic blood pressure of 140 mmHg and/or diastolic blood pressure of 90 mmHg, affected 31.1% of the world's adult population, or 1.39 billion people.

Due to population aging and increased exposure to lifestyle risk factors such as bad diets (i.e. high salt and low potassium intake) and a lack of physical activity, the prevalence of hypertension is increasing internationally. Nonetheless, variations in the prevalence of hypertension are not consistent across the globe. The prevalence of hypertension has significantly increased in poor and middle-income countries (LMICs) over the last 20 years, while it has modestly decreased in high-income countries (Mills et al., 2017).

Classification of hypertension

Systemic arterial hypertension is characterized by a persistent increase in blood pressure in the systemic arteries. BP is usually expressed as the ratio of the systolic BP (which is the pressure that the blood wields on the walls of the artery when the heart contracts) and the diastolic BP (the pressure when the heart relaxes). The BP thresholds that define hypertension depend on the measurement method (Table 1). A lot of causes can underlie hypertension.

Most patients (90–95%) have a highly heterogeneous or primary hypertension with a multifactorial gene and environment etiology. The hypertension family history is a frequent occurrence in patients with hypertension, with heritability (measure the variation in a trait is due to variation in genetic factors) estimated between 35% and 50% in most studies (Luft, 2001).

Genome-wide association studies (GWAS) have identified that 120 loci are associated with BP regulation and together explain 3.5% of the trait variance (Manuscript, 2017). These findings are becoming more important as we search for new pathways and new biomarkers to develop more-modern diagnostic and therapeutic methods for hypertension in the era of precision medicine (Dominiczak et al., 2017).

Table 2.1: Definitions of hypertension based on the 2013 ESH/ESC guidelines

Category	**Subtype**	**Systolic BP (mmHg)**	**Diastolic BP (mmHg)**
Office BP	NA	≥ 140	≥ 90
Ambulatory BP	Day time (awake)	≥ 135	≥ 85
	Night time (asleep)	≥ 120	≥ 70
	24hr	≥ 130	≥ 80
Home BP	NA	≥ 135	≥ 85

Several unique, monogenic forms of hypertension like Liddle syndrome, glucocorticoid-remediable aldosteronism and mutations in PDE3A have been described in which a single gene mutation fully explains the pathogenesis of hypertension and indicates the best treatment modality. If hypertension is caused by another condition for example, primary aldosteronism, pheochromocytoma or renal artery stenosis, it is referred to as secondary hypertension (Dexter, 1948).

Hypertension is the most common risk factor that cause cardiovascular disease (CVD). This includes heart failure, stroke, myocardial infarction, coronary heart disease, atrial fibrillation, peripheral artery disease, chronic kidney disease (CKD) and cognitive impairment. It is also the leading single contributor to all-cause death and disability in the world. The relationship between BP and the increased risk of CVD is continuous, starting as

low as 115/75 mmHg. Successful prevention and treatment of hypertension are the key to reducing disease burden and promoting longevity in the world's population (Forouzanfar et al., 2016).

The prevention of ensuing renal and cardiovascular disorders depends heavily on the treatment and management of hypertension. Numerous countries have reported varying percentages of hypertension awareness, treatment, and control. Nevertheless, these data have not been thoroughly examined to produce imprecise estimates in global locations with varying levels of economic growth. With a complicated etiology involving biological, psychological, socioeconomic, environmental, and other elements, obesity is a multifactorial disease. The processes and mechanisms by which it results in poor health outcomes vary (Jastreboff et al., 2019).

2.2 Obesity

The "2013 AHA [American Heart Association]/ACC [American College of Cardiology]/TOS [The Obesity Society] Guideline for the Management of Overweight and Obesity in Adults" (Jensen et al., 2014) uses the World Health Organization criteria to define overweight as a body mass index (BMI) ≥25 and <30 kg/m2 and obesity as a BMI ≥30 kg/m2 (Jensen et al., 2014). With significant variance by sex, age, and race/ethnicity, BMI has limitations in its predictive capacity to estimate body fat for any one individual, although having a great correlation with body fat percentage across populations (Rao et al., 2015).

According to the GBD (Global Burden of Disease) Obesity Collaborators, 603 million adults worldwide were obese, with the prevalence of obesity tripling in 73 countries between 1980 and 2015 and steadily rising in the majority of the other nations. Between 39% and 49% of the world's population, or 2.8 to 3.5 billion individuals, are thought to be overweight or obese (Maffetone et al., 2017). Furthermore, 4.0 million deaths in 2015 were attributed to excessive BMI, with cardiovascular disease (CVD) accounting for more than two-thirds of these deaths, according to the GBD investigators, even after accounting for smoking and ill health (Maffetone et al., 2017) (Bickel & Schöning, 2009).

2.3 The correlation between hypertension and obesity with BMI

Among obese people, CVD and hypertension were responsible for a significant percentage of BMI-related deaths (41%) and BMI-related disability-adjusted life-years (34%) (Bickel & Schöning, 2009). The crude prevalence of obesity in the United State(US) increased from 37.9% in 2013 - 2014 to 39.8% in 2015 - 2016, according to the most recent nationally representative estimates based on the National Health and Nutrition Examination Survey (Flegal et al., 2016).

At an uncorrected prevalence of 7.7% in the entire sample, class 3 obesity (BMI ≥40 kg/m2) is comparatively common. Racial/ethnic and sex variations in class 3 obesity prevalence range from 5.5% in non-Hispanic White males to 16.9% in non-Hispanic Black women (Flegal et al., 2016).

Adolescent obesity is a global health epidemic in pediatric populations; significant increases in the prevalence of obesity among teenagers over the last 35 years globally ultimately raise the risk of CVD in adulthood (Cardel et al., 2020). Furthermore, the current trend of obesity patterns in the US and other countries highlight the substantial influence that obesity will continue to have on global incidence and prevalence of cardiovascular disease (Poirier et al., 2006). Also obesity is linked to many diseases of the cardiovascular system, including stroke, venous thromboembolic disease, and pulmonary hypertension (Poirier et al., 2006).

The incredibly high frequency of hypertension in the obese population is a result of numerous diseases linked to obesity. In the US, around 50% of people with hypertension are obese .Thus, compared to less than one-fifth of people of normal weight, more than one-third of the obese population in the US has been diagnosed with hypertension (Saydah et al., 2015).

In addition to noting that fat would raise the risk for various diseases, the ancient Greeks appreciated the idea that obesity resulted from an imbalance between energy intake and expenditure. However, the prevalence of obesity has rapidly increased globally, nearly doubling between 1980 and 2016, due to modern lifestyles that provide easy access to high-energy-dense foods and beverages (Hanley et al., 2010).

The relationship between obesity and hypertension is not specific, multifaceted and closely connected with other diseases present in obesity. The diagnosis and monitoring of

hypertension in obesity is complicated because of the difficulty in measuring blood pressure in these patients. In addition, highly complex underlying factors contribute to several challenges to treat hypertension patient suffering of obesity, extending the greater morbidity and mortality observed in this population. Historically, excess weight with obesity and the control of body size have always been aim of general consideration and speculation (Haslam, 2016).

Because not everyone who consumes a lot of calories and does not exercise ends up overweight, scientists think that hereditary variables are just as significant in the etiology of obesity as environmental factors (McNiven et al., 2011). According to family and twin studies, genetic variables account for 10–30% of the risk for metabolic syndrome, 50% of the risk for type 2 diabetes, and 80% of the variation in body mass index (BMI) (Phillips, 2013).

2.4.1 Adipokine

Adipose tissue secretes bioactive molecules called adipokines, which have a range of impacts on both health and illness. They are crucial in controlling cancer, inflammation, immunological response, metabolism, and cardiovascular health. Nonetheless, problems linked to obesity may be exacerbated by adipokine dysregulation (Clemente-Suárez et al., 2023).

Accordingly, adipose tissue functions as both an active endocrine organ, that secretes a variety of chemicals known as adipokines, and a passive energy storage organ. Adipokines are cytokines that control a number of physiological functions, including hunger, inflammation, metabolism, immunology, and cardiovascular health. Leptin, adiponectin, resistin, and numerous more substances are examples of adipokines (Clemente-Suárez et al., 2023). White or brown adipocytes, as well as their size, quantity, location, and interactions with other cells, determine the kind and quantity of adipokines that adipose tissue produces (Clemente-Suárez et al., 2023).

Ghrelin, neuromedin-beta, leptin, appetite-regulating peptides, the endocannabinoid system (a system made up of cannabinoid receptors and enzymes involved in the synthesis and degradation of endocannabinoids), and uncoupling proteins (related to the effectiveness

of energy metabolism in the human body) are some of the biological factors contributing to the etiology of obesity (Rudkowska & Pérusse, 2012). The two most important peptides that control hunger and energy are leptin and ghrelin.

2.4.2 Leptin

Leptin was discovered in 1994, its name was derived from the Greek word *leptus,* that means thin. Leptin is a polypeptide encoded by obese (ob) gene, which is expressed mainly in the white adipose tissue (Loktionov, 2003). Leptin production is proportionately associated with obesity. Several of which reactions may not always correspond with changes in fat mass, even though leptin levels do decrease during fasting and its absence is linked to hyperphagia in both humans and animals. This finding implies that leptin has a more important function in maintaining body energy balance than in meal-to-meal intake (Loktionov, 2003).

2.4.3 Leptin's hormone synthesis and secretion

Adipose tissue is the primary source of the peptide hormone leptin's synthesis and secretion. Despite being identified as a hormone that controls energy homeostasis, leptin has been shown to have a variety of physiological roles, including that of a proangiogenic factor and metabolic regulator (Biesiada et al., 2016). It has recently been demonstrated that leptin and its receptor play a role in the processes that cause and advance breast cancer (BC). Findings from a few BC epidemiology research have demonstrated a correlation with levels of circulating leptin (Petridou et al., 2000).

With a molecular weight of 16 kD and a 167 amino acid composition, leptin is a protein that belongs to the adipokine family and is currently understood to be a hormone. It is mostly produced in adipocytes, and its amount depends on the abundance of accumulation of body fat (Zhang Y et al., 1994). To carry out its central function and engage with hypothalamic receptors found in neurons in a variety of nuclei, including the arcuate, paraventricular, and Centro medial ones, leptin crosses the blood–brain barrier (BBB) (Zhang et al., 1997).

The main site of action is the hypothalamic arcuate nucleus, which is made up of two different neuronal populations. One releases proopiomelanocortin, which forms the anorexigenic route (satiety inducer), while the other releases neuropeptide Y, which forms the orexigenic pathway (appetite inducer). Leptin , as evidenced by numerous studies that show it inhibits the orexigenic pathway and activates the anorexigenic pathway (White & Tartaglia, 1996).

2.4.4 Leptin hormone

An endocrine hormone that functions as a significant regulator of food intake, neuroendocrine output, metabolism, and fat accumulation is the human homologue of the LEPTIN gene (*LEP*), which has been identified as an obesity gene (Obradovic et al., 2021). Through sympathetic activation in the circulation or at the renal level, leptin may also influence blood pressure and contribute to the development of hypertension, as demonstrated by certain experimental studies. Microinjections of leptin have also suggested that higher levels of leptin have greater effects on sympathetic activation (Sánchez-Margalet et al., 2003).

Since women's leptin levels are known to be several times higher than men's, it is possible that women's nerve systems (NS) may be more affected. While a lot of research has looked at the relationship between leptin levels and its hypothalamic receptor with blood pressure, the gender-specific effects of genetic variants in *LEP* as well as leptin levels in the regulation of blood pressure are still anxious (Ma et al., 2009).

2.4.5 Leptin hormone resistance

As stated previously, leptin is a hormone that may successfully lower body weight and food consumption, which initially suggests that it may be used to treat obesity (Obradovic et al., 2021). Nonetheless, it has been noted that obese people have high circulating leptin levels and are insensitive to the injection of exogenous leptin. Leptin resistance is the incapacity of leptin to produce its anorexigenic effects in obese people and, as a result, its

lack of clinical value in obesity (Carter et al., 2013). For leptin to be used effectively in the treatment of obesity, it is essential to comprehend the molecular mechanisms generating leptin resistance (Carter et al., 2013).

One important factor is that leptin must pass through the blood-brain barrier in order to enter the hypothalamus and start its anorexigenic effects. There are a number of mechanisms in this environment that are not entirely known, and new methods for reestablishing the leptin response in obese patients have emerged recently (Obradovic et al., 2021) . Therefore, leptin, an anorexigenic hormone that is predominantly produced in adipose tissue, may be a better risk marker for CVD than current BMI (Figure 2.1: Obradovic et al., 2021).

Figure 2.1:

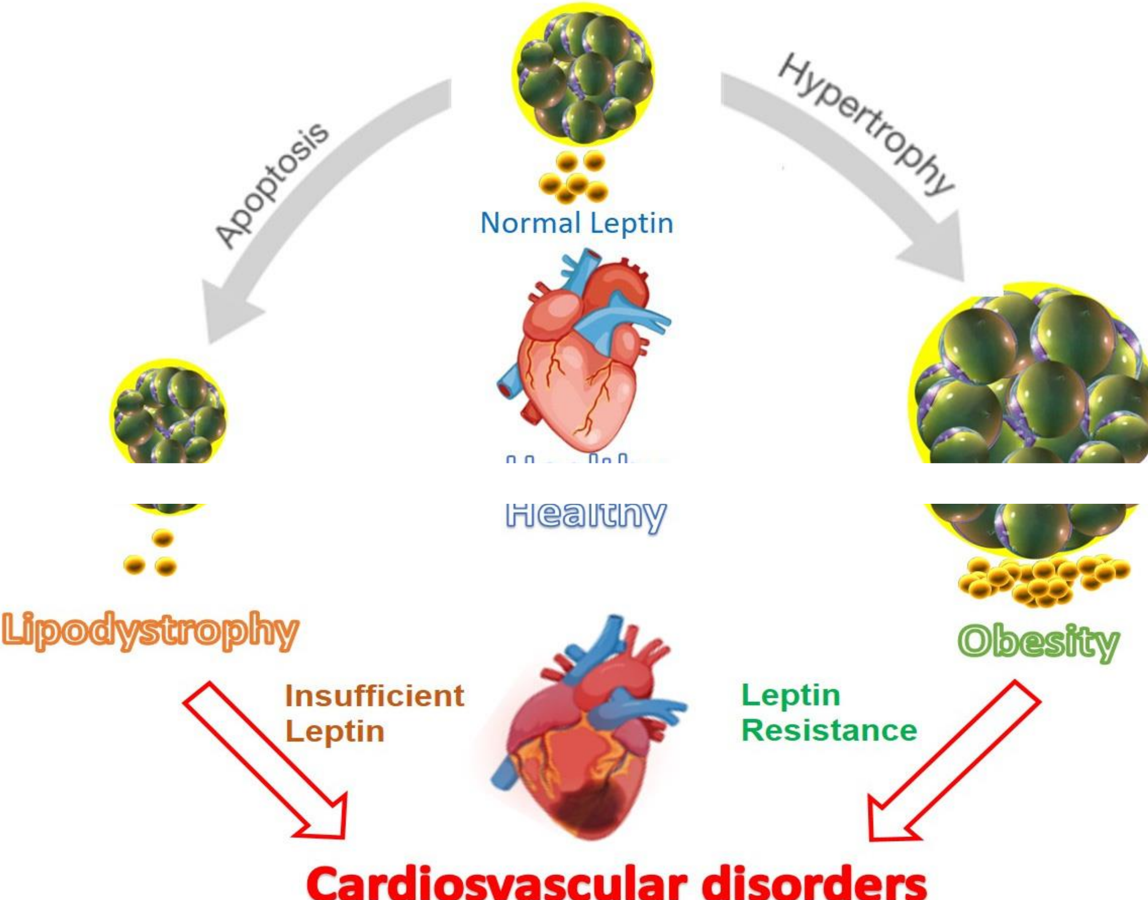


Figure 2.1 Paradoxical effect of leptin: relationship between circulating leptin levels and cardiovascular dysfunction. For proper cardiovascular function, it is essential to maintain circulating leptin levels within a narrow normal range. In conditions of lipodystrophy, resulting from widespread adipose tissue apoptosis or the inability to develop adipose tissue correctly, extremely low levels of circulating leptin promote cardiovascular disorder. Conversely, in diet-induced obesity, hyperleptinemia acts as an important factor in developing cardiovascular dysfunction due to leptin resistance (modified from reference (Passos et al., 2007).

Figure 2.2:

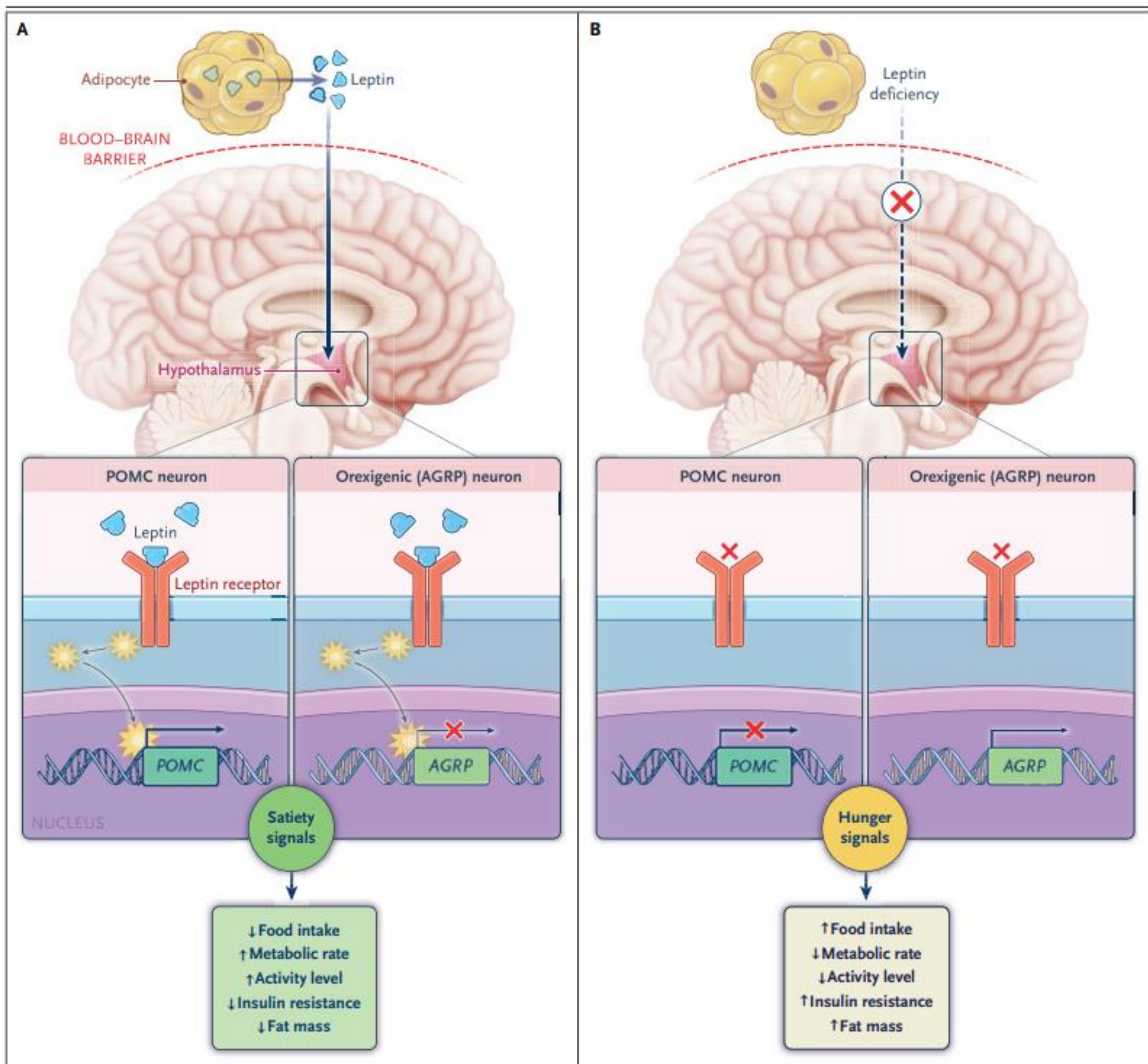


Figure 2.2 Obesity, Metabolism, and Leptin Signaling. Adipocytes produce leptin, which is then released into the bloodstream (Panel A). It penetrates the blood-brain barrier and attaches itself to the hypothalamic leptin receptor, which promotes proopiomelanocortin (POMC) and suppresses orexigenic neurons, hence preventing the synthesis of agouti-related protein (AGRP). These alterations lead to increased energy expenditure and appetite suppression. Obesity, increased food intake, decreased energy metabolism, and eventually left ventricular hypertrophy are caused by a deficiency in leptin (Panel B) (Rosen, 2023).

2.4.6 Leptin receptor

LEPR is located on chromosome 1p31. *LEPR* is a molecule distributed in various tissues, and it can mediate the important impact of leptin as a hormone for whole-body energy homeostasis (L et al., 1995).

Similar to the signal-transducing subunits of IL-6, G-CSF, and leukemia inhibitory factor (LIF) receptors, the leptin receptor is a single membrane-spanning receptor that is a member of the class I cytokine receptor superfamily (Zhang et al., 1997). Because of alternative gene splicing, the leptin receptor has at least six isoforms: OBRa, OBRb, OBRc, OBRd, OBRe, and OBRf. Only OBRb, the receptor isoform with the largest cytoplasmic domain length, can fully transduce signals (Lee G H et al., 1996). The JAK/STAT, MAPK/ERK1/2, and PI3K signaling pathways are among the signaling pathways that are triggered when leptin binds to the leptin receptor (Sánchez-Margalet et al., 2003). Obesity, reproductive problems, thymic atrophy, immune system dysfunction, and other notable abnormalities are caused by the loss of leptin/leptin receptor signal in leptin-deficient ob/ob and leptin receptor-deficient db/db mice (Han & Wang, 2015) . Blocking leptin signaling pathway could become potential therapy for certain disorders.

2.4.7 Single nucleotide polymorphisms (SNPs) of *LEP* and *LEPR*

Single nucleotide polymorphisms (SNPs) are among the variants influencing genetic differences. SNPs can identify a person's susceptibility to specific metabolic disorders and, consequently, to diseases, even though they are typically not the only factors that cause disease (Costa et al., 2010). Leptin G-2548A (rs7799039), leptin receptor Q223R/Gln223Arg 668A>G (rs1137101), and K109R/Lys109Arg 326A>G (rs1137100) are among the gene polymorphisms found in human leptin and leptin receptors (Hao et al., 2019). In previous studies, several SNPs of *LEPR*, such as rs1137101,rs1137100, rs6700896, and rs8179183, have been proven to be associated with a variety of chronic diseases (diabetes mellitus, hypertension, and some cancers) (Li et al., 2017).

The occurrence of single-nucleotide polymorphisms (SNPs) that may alter the circulation concentration of leptin adipokine has been connected to the genes *LEP* and *LEPR*, which encode leptin and leptin receptor on chromosomes 7 and 1, respectively. Both genes have been linked to sympathetic hyperactivity, obesity, and blood pressure dysregulation. The *LEP* gene promoter region has one of the most researched SNPs, rs7799039 (2548G > A), while the exon 6 region of *LEPR* contains rs1137101 (þ668A > G) (De Faria et al., 2017).

2.5.1 Other literature review

It is reported that patients with essential hypertension were reported to be hyperleptinemic and plasma immunoreactive leptin was reported to correlate with blood pressure (Takizawa et al 2001). Galletti (2008) also reported that hypertensive patients may be leptin resistant with high circulating leptin levels independent of body mass. Transgenic skinny mice overexpressing leptin were reported to exhibit elevated blood pressure, suggesting a direct effect of leptin on the pathogenesis of hypertension independent of obesity, and the machine seemed to be mediated by sympathetic activation (Dunbar JC et al., 1997).

These results indicated leptin as a strong candidate factor for hypertension, either through its direct effect on hypertension or through its effect on obesity. However, the role of leptin in the development of hypertension and CAD in the general population is far from clear (Gu P et al 2012).

The purpose of the current study is to examine the function of plasma leptin in hypertension patients who are overweight or obese to those with normal BMI. Additionally, to find out how the *LEP* SNP contributes to the development of hypertension in a Palestinian population.

Chapter Three: Materials and Methods

3.1.1 Study design

A cross-sectional study, quantitative and qualitative will be used. Quantitative for leptin concentration in serum, and qualitative for genetic determination (LEP gene SNP rs7799039 (2548G > A), while LEPR SNPs rs1137101 (þ668A > G).

3.1.2 Study area

A cross-sectional study conducted among Palestinian hypertension patients in the period between May 2024 and November 2024. Hypertension patients were recruited from health centers in Jenin.

3.1.3 Sampling method and sampling size

The study was approved by the Palestinian Ministry of Health and committed to the Helsinki declaration. A total of ninety (90) hypertensive patients under therapy were enrolled in the study. Participants signed an informed consent before data collection or sample provision**.** Participating patients were (47) males and (42) females aged 40 to 74 years. Based on their BMI patients were divided into two groups; BMI > 25 kg/m2 and with BMI < 30 kg/m2.

For genotyping Leptin and Leptin Receptor genes, Five milliliters of blood were withdrawn from the patients included in the study divided into 3 ml in a sterile ethylenediaminetetraacetic acid (EDTA) vacutainer and 2 ml in a sterile PLAN vacutainer. DNA was extracted from the whole blood of the EDTA tube by using a DNA extraction kit (genomic DNA was extracted using QIAamp (QIAGEN, Heldin/Germany) Blood DNA extraction mini kit protocol according to manufacturer’s recommendations and kept at −20

°C until analyzed. DNA concentration and quality (260/280) was analyzed by a Nano-Drop analyzer (Implen).

For measurement of biochemical markers, Leptin concentration was determined by the ELISA kit. Genotyping for detection of *LEP*, *LEPR* polymorphisms was performed for all patients using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

3.1.4 Inclusion and exclusion criteria

The following inclusion criteria apply:

- 40 to 74 years of age.
- Diagnosis of hypertension at least 6 months before the study.
- Takes medications of hypertension.
- Return for required follow-up in Ministry of Health in Jenin.

For individuals with hypertension, the following exclusion criteria apply:

- The patient age less than 40 years and more than 74 years.
- The patient with BMI between 25-30.
- The patient who refuses to give informed consent.

3.2 Molecular and Serology testing

3.2.1 Blood collection and DNA extraction

About five milliliters of blood were collected from each subject 3 ml in EDTA tube and 2ml in plain tube. Genomic DNA was extracted from buffy coat using the QIAamp mini kit (QIAGEN, Heldin/Germany) according to manufacturer's guidelines. In a sterile 1.5ml-microtube, 200µL blood buffy coat sample was mixed with 25µL proteinase K. An exact volume of 200µL of AL lysis buffer was added to the microtube. The mixture was incubated at 56°C for 15 minutes. After this, 250µL of absolute ethanol was added to the mixture and vortexed well. The lysate was then incubated at room temperature (15–25°C) for 5 minutes.

The lysate was transferred to a QIAamp Min Elute column and centrifuged at 8000 rpm for 1 minute. The Min Elute column was then washed twice with the washing buffers AW1 and AW2, respectively. Then, 500µL of ethanol (96–100%) was added to Min Elute column and centrifuged at 8000 rpm for 1 minutes. Finally, using 200µL of Buffer from the kit, DNA was eluted into a sterile microtube. The extracted DNA was kept at -20°C until use. For serological tests blood was collected in plane tube and the collected serum was stored at -20 °C until use.

3.2.2 DNA Quantification

A Nano Drop 2000/2000c (IMPLEN, Germany) was used to determine the concentrations and quality of DNA (OD260/OD280). One µL of buffer AVE was used as a blank, followed by 1µL of DNA. The ratio OD260/OD280 was computed to represent the quantity of DNA to protein, which should be > 1.8 to be considered appropriate for analysis.

3.2.3 Genotype Analysis

DNA samples of patients and controls were genotyped for *LEP* and *LEPR* polymorphisms (LEP gene SNP rs7799039 (2548G > A), while LEPR SNP rs1137101 (þ668A > G). Genotype analyses of *LEP* and *LEPR* were performed by using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

3.2.3.1 *LEP* (NC_000007.14) polymorphism (rs7799039 (2548G > A)

For the *LEP* gene, DNA samples were amplified with the following primer pair: forward primer (5' TTTCCTGTAATTTTCCCGTGAG 3'); reverse primer (5'AGAGATTAAAGCAAAGACAGGC 3'), using polymerase chain reaction (PCR) (Table 3.1). PCR was performed in a final volume of 25µL, consisting of 12.5µL Master mix

(2X) (Promega Corp., USA), 1µL forward primer (10pmole), 1µL reverse primer (10pmole), 5.5µL nuclease-free water and 5µL genomic DNA (85ng/µL).

The PCR thermocycling protocol (Biometrat advanced) was as follows: an initial denaturation at 95°C for 10 minutes, followed by 35 cycles of amplification at 94°C for 60 sec, 52°C for 90 sec, and 72°C for 60 sec. A final extension step of 72°C for 10 min is preformed to complete the elongation processes. Reaction products were analyzed on a 2% agarose gel stained with 1mg/mL ethidium bromide prepare with 1X TEP buffer. Was carried out in 1X TBE buffer, at 100V, for approximately 40-60 minutes.

3.2.3.2 *LEPR* (NC_000001.11) polymorphism (rs1137101 (þ668A > G)

For the *LEPR* gene, DNA samples were amplified with the following primer pair: forward primer (5 AAACTCAACGACACTCTCCTT' 3'); reverse primer (5' TGAACTGACATTAGAGGTGA 3'), using polymerase chain reaction (PCR). PCR was performed in a final volume of 25µL, consisting of 12.5µL Master mix (1X) (Promega Corp., USA), 1µL forward primer (10pmole), 1µL reverse primer (10pmole), 5.5µL nuclease-free water and 5µL genomic DNA (85ng/ reaction).

The PCR thermocycling protocol was as follows: an initial denaturation at 95°C for 7 minutes, followed by 35 cycles of amplification at 94°C for 60 sec, 52°C for 90 sec, and 72°C for 60 sec. A final extension step of 72°C for 5 min is preformed to complete the elongation processes. Reaction products were analyzed on a 2% agarose gel stained with 1mg/mL ethidium bromide.

Table 3.1: Primer sequences used in PCR

Primer	**Sequence**	**Product size**	**Method used**
LEP F *LEP* R	5' TTTCCTGTAATTTTCCCGTGAG 3' 5' AGAGATTAAAGCAAAGACAGGC 3'	**249**	**RFLP PCR**

LEPR F *LEPR* R	5’ AAACTCAACGACACTCTCCTT 3’ 5’ TGAACTGACATTAGAGGTGA 3’	**80**	**RFLP PCR**

The PCR products were then digested with Hhal for *LEP* gene and Msp1 for *LEPR* gene (New England Biolabs, Hertfordshire, United Kingdom) restriction enzyme, according to manufacturer’s instructions. The RFLP conditions were performed for 15 min 37℃ according to the protocol shown in Table 3.2, using a thermo-cycler for incubation.

Table 3.2: Component and volumes used in RFLP PCR

Component	**Volume**
Buffer, 10X	2.5 µL
Restriction enzyme (New England Biolabs, Hertfordshire, United Kingdom)	0.5 µL
Nuclease free water.	2 µL
PCR product (amplicon)	10 µL
Total volume	15 µL

The restriction DNA amplification products were loaded into wells of 4% agarose gel (Lorenzo, Italy) stained with 1mg/mL ethidium bromide. A horizontal standard electrophoresis system (BIO-RAD, China) was carried out in 1X TBE buffer, at 100V, for approximately 4 hours.

The LEP rs7799039(-2548G>A) polymorphism was identified with the PCR-restriction fragment length polymorphism (PCR-RFLP). HhaI restriction enzyme used to digested the PCR products, confirmed by the presence of three bands.

The normal homozygous Ob/Ob was confirmed by the presence of two DNA fragment (188 and 61bp), a single DNA fragments for mutant homozygote ob/ob (249bp), whereas the heterozygous Ob/ob genotype was confirmed by the presence of three fragments (249, 188, and 61bp).

The *LEPR* rs1137101 (+668A>G) polymorphism was identified with the PCR-restriction fragment length polymorphism (PCR-RFLP). MspI restriction enzyme used to digested the PCR products, confirmed by the presence of three bands. The normal homozygous Db/Db was confirmed by the presence of a single DNA fragment (80bp), two DNA fragments for mutant homozygote db/db (57 and 23bp), whereas the heterozygous Db/db genotype was confirmed by the presence of three fragments (80, 57, and 23bp).

DNA sequencing analysis

To confirm the RFLP results, two randomly LEP PCR products and one LEPR PCR product were sent for sequencing. The PCR products were initially cleaned up by adding 5µL of PCR product with 1µL of clean up reagent Eppic FAST (A&A biotechnology) and incubating for 15 minutes at 37°C. The BigDyeTM Direct Cycle Sequencing Kit (Thermo Fisher Scientific, USA) was used to perform Sanger sequencing on the Biosystem 3500 Genetic Analyzer. The Finch TV chromatogram viewer was used to look at nucleotide variations.

3.2.4 Biochemical and serology Tests

3.2.4.1 Leptin concentration

Leptin concentration was determined by ELISA kit from DRG company by the following steps: Secure the desired number of Microtiter wells in the holder, then dispense 15 µL of each Standard, controls and samples with new disposable tips into appropriate wells. After that dispense, 100 µL Assay Buffer into each well and thoroughly mix for 10 seconds. It is important to ensure good mixing in this step. Incubate for 120 minutes at room temperature. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (300 µL per well). Add 100 µL Antiserum to each well. Incubate for 30

minutes at room temperature. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (300 µL per well) and dispense 100 µL Enzyme Complex into each well. Incubate for 30 minutes at room temperature. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets. Add 100 µL of Substrate Solution to each well. Incubate for 15 minutes at room temperature. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.

Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution with microtiter plate reader. The results have been calculated automatically by a micro plate reader using a curve fit.

3.2.4.2 Lipid profile

Lipid profile, cholesterol (Cho), triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) concentrations were taken from patients` files.

3.3.1 Statistical analysis

For data input and analysis, a predesigned Statistical Package for Social Science Version 30 (IBM SPSS Statistics) software was used. Unpaired t-test was used to assess the risk given by the serology test results, with P value of less than 0.05 considered as significant. Chi square ($\chi 2$) test was used to study genotype frequency differences between cases and controls were employed. Statistical significance was defined as a P value of less than 0.05

Chapter Four: Results

4.1.1 Demographic and clinical features of the studied subjects

Ninety (90) Palestinian participants were recruited for this study during the years 2024-2025. All participants were residents of the Jenin governorate. Forty-five (45) participants were suffering from high blood pressure (HBP) and increased BMI (>30), while other forty-five (45) participants were suffering from HBP but with normal BMI (<25).

As shown in table 1 the high BMI group comprised 20 males (44.4%) and 25 females (55.6%), this group participants age range was 40 to 72 years (60.18 ± 8.37) (mean ± SD). The normal BMI control group involved also 45 participants, 28 males (62.2%) and 17 females (37.8%) with age range 40 to 74 with a mean ± SD (61.69 ± 8.39 years). The difference between the mean age of the two groups was statistically not significant (P value = 0.09). There was also no significant difference between males and females ages.

Five (5) (11.1%) of the increased BMI group were smokers, and fourteen (14) (31.1%) of the normal BMI group participants were smokers, showing a significant difference in the number of smokers between two groups probability value (P value = 0.02). Chi square test was used to compare the differences in age, gender and smoking.

Table 4.1: Age, sex and smoking distribution among HBP patients

Variable	**HBP Patients** **N=45 (%)**	**HBP Controls** **N=45 (%)**	**P value**
Age(year) **Mean ± SD**	60.18±8.37 yr	61.69 ± 8.39 yr	0.951
Sex n (%)			
Male	20 (44.4%)	28 (62.2%)	0.09
Female			

	25 (55.6%)	17 (37.8%)	
Smoking			
Smoker	5(11.1%)	14(31.1%)	0.02*
Non smoker	40(88.9%)	31(68.9%)	

4.1.2 Medical history and clinical tests results

The clinical tests, as well as smoking data, were retrieved from the patient's files. The clinical tests result of the serum levels of triglyceride (TG), high density lipoproteins (HDL-c), low density lipoproteins (LDL-c) and cholesterol (Cho) were shown in Table 4.2. The concentration for lipid profile tests TG, LDL-c, HDL-c and Cho were very close for both groups, P-value for TG, HDL-c, LDL-c and cholesterol between the two groups were (0.889, 0.382, 0.277, 0.592) respectively, without significance in P-value. P-values were calculated by the T-test.

Table 4.2: Concentration for lipid profile serology tests (TG, LDL-c, HDL-c. Cho)

	BMICAT	N	Mean	Std. Deviation	Std. Error Mean	P-value
TG	BMI>30	45	130.76	70.019	10.438	0.889
	BMI<25	45	129.04	42.629	6.355	
LDL	BMI>30	45	98.22	34.419	6.131	0.382
	BMI<25	45	91.18	40.434	5.028	
HDL	BMI>30	45	47.44	14.525	2.165	0.277
	BMI<25	45	44.04	14.983	2.234	

Cho.	BMI>30	45	170.11	43.879	6.541	0.592
	BMI<25	45	165.07	45.108	6.724	

4.2 Genetic analysis of the studied subjects

4.2.1 Molecular analysis

4.2.1.1 *LEP* polymorphism

The *LEP* rs7799039(-2548G>A) polymorphism was identified with the PCR-restriction fragment length polymorphism (PCR-RFLP). HhaI restriction enzyme used to digested the PCR products, confirmed by the presence of three bands.

The normal homozygous Ob/Ob was confirmed by the presence of two DNA fragment (188 and 61bp), a single DNA fragments for mutant homozygote ob/ob (249bp), whereas the heterozygous Ob/ob genotype was confirmed by the presence of three fragments (249, 188, and 61bp) as shown in Figure 4.3(A+B).

Figure 4.1(A)

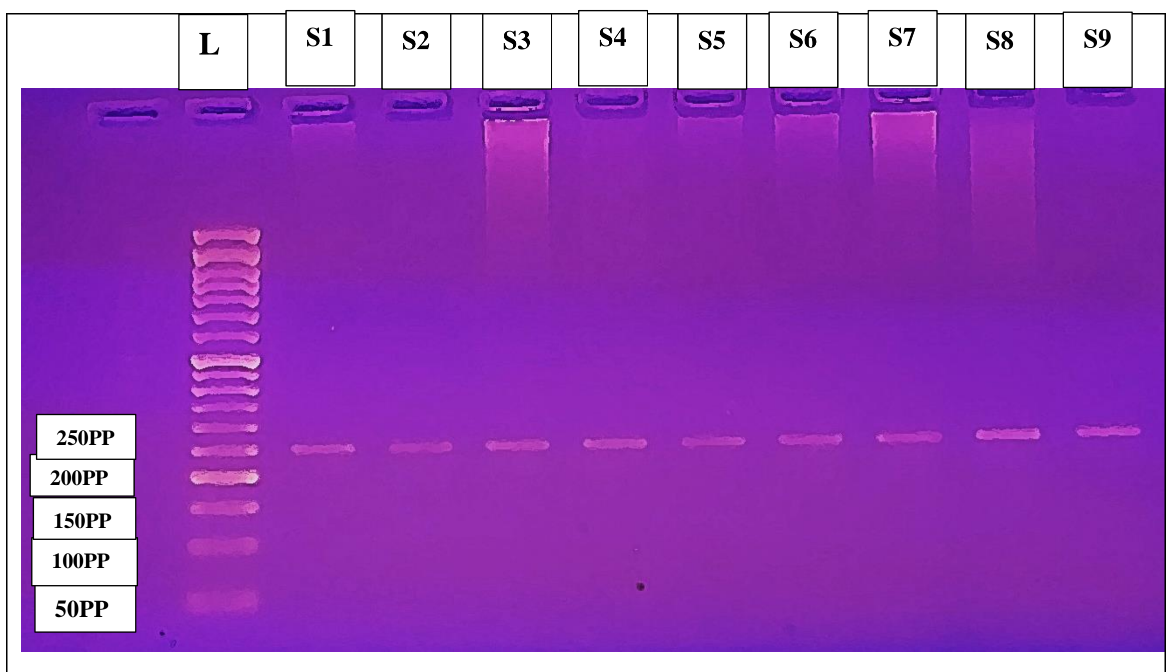
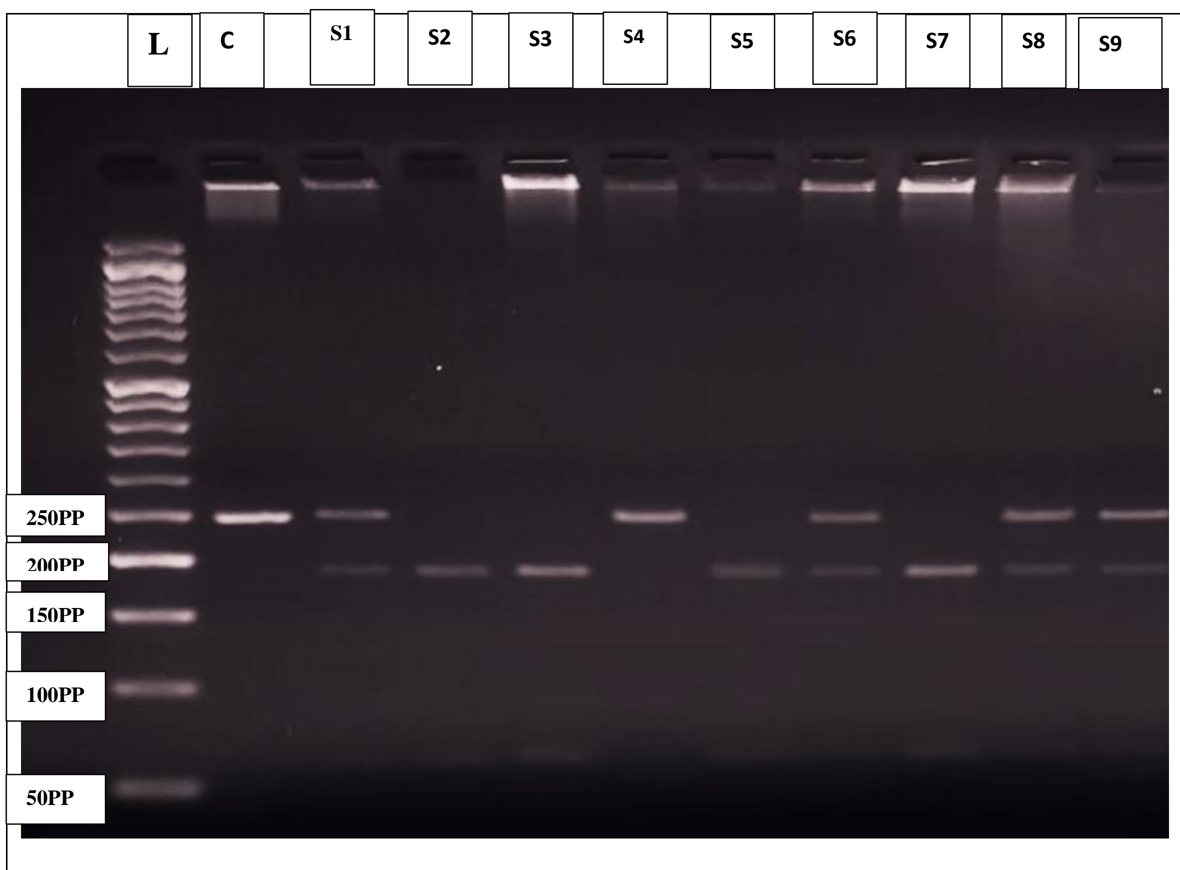


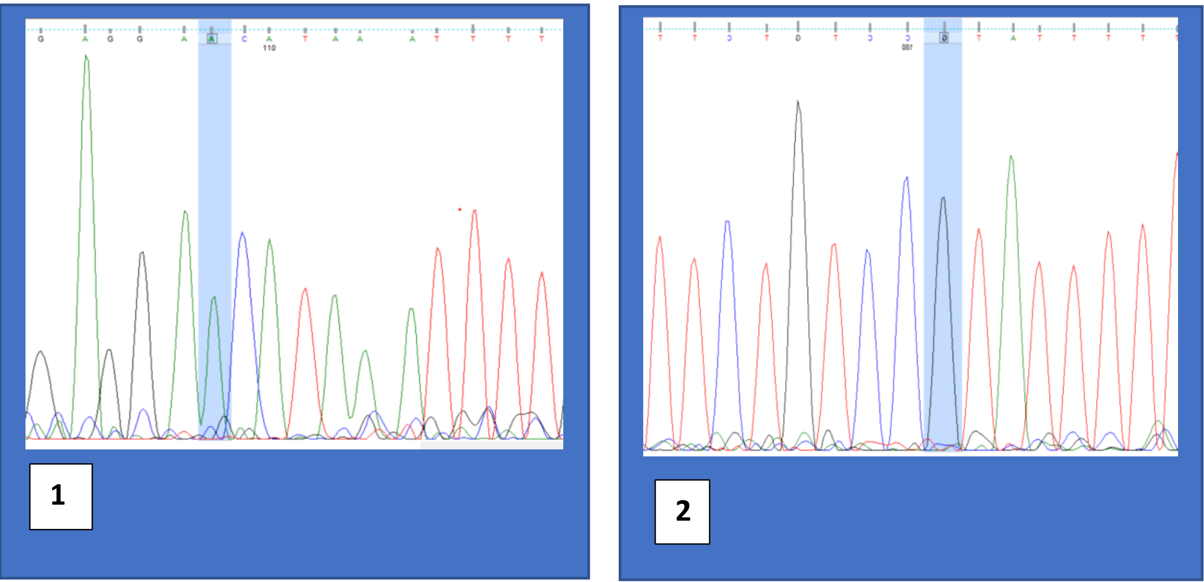
Figure (4.1) A+B: Genotyping of the *LEP* polymorphism. A: agarose gel electrophoresis (2.5%) for PCR product(249pp) L: DNA ladder (50bp)

Figure 4.1 B:



B. Agarose gel electrophoresis (4%) pattern of selected RFLP products of the *LEP* polymorphism. L: DNA ladder (50bp). Lane C is positive control (PCR product without restriction enzyme). S 4 represent (249pp) fragment of the homozygous (AA genotype). S S1,6,8 and 9 represent are heterozygous (AG genotype) Hhal digested products confirmed by the presence of three bands (249pp, 188pp, and 61 bp). 2,3,5 and 7 correspond to the wildtype homozygous (GG genotype) resulting in tow band (188bp and 61pp).

Figure 4.1 C:



Sanger sequencing chromatograms showing the sequence for two random samples, sample 1 with AA genotype, and sample 2 with GG genotype.

4.2.1.2 *LEPR* polymorphism

The *LEPR* rs1137101 (+668A>G) polymorphism was identified with the PCR-restriction fragment length polymorphism (PCR-RFLP). MspI restriction enzyme used to digested the PCR products, confirmed by the presence of three bands. The normal homozygous Db/Db was confirmed by the presence of a single DNA fragment (80bp), two DNA fragments for mutant homozygote db/db (57 and 23bp), whereas the heterozygous Db/db genotype was confirmed by the presence of three fragments (80, 57, and 23bp) as shown in figure 4.2(A+B).

Figure 4.2(A):

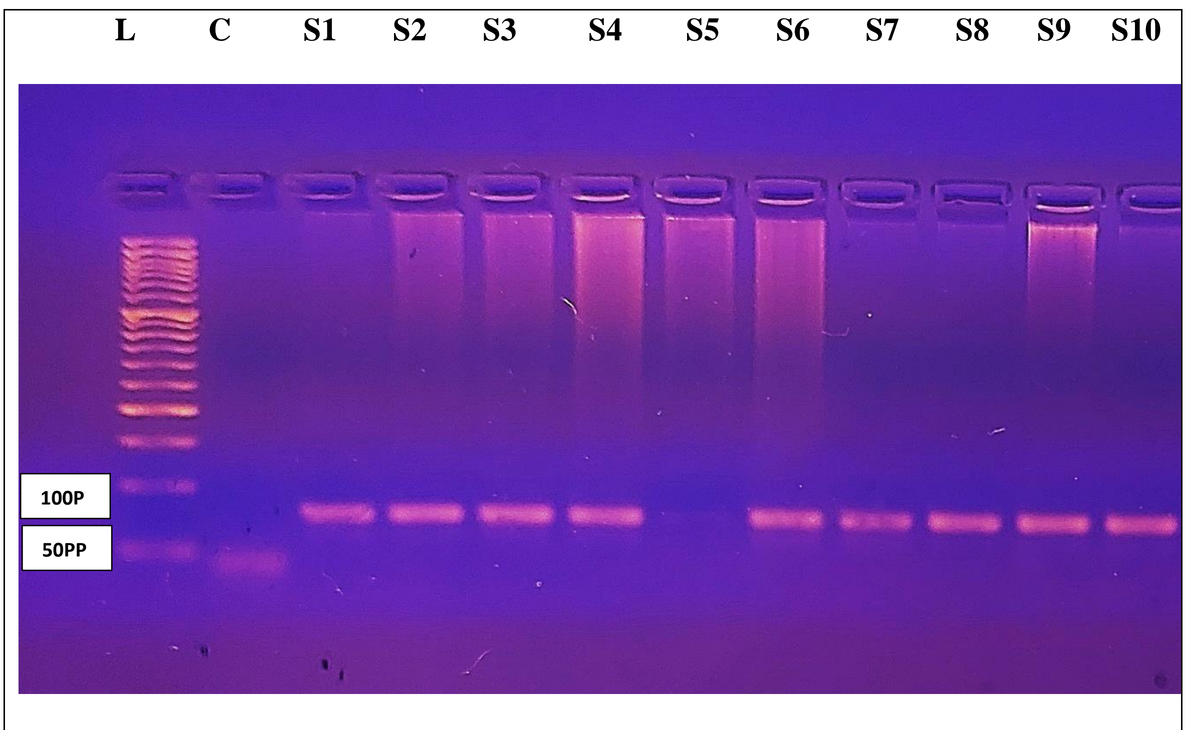
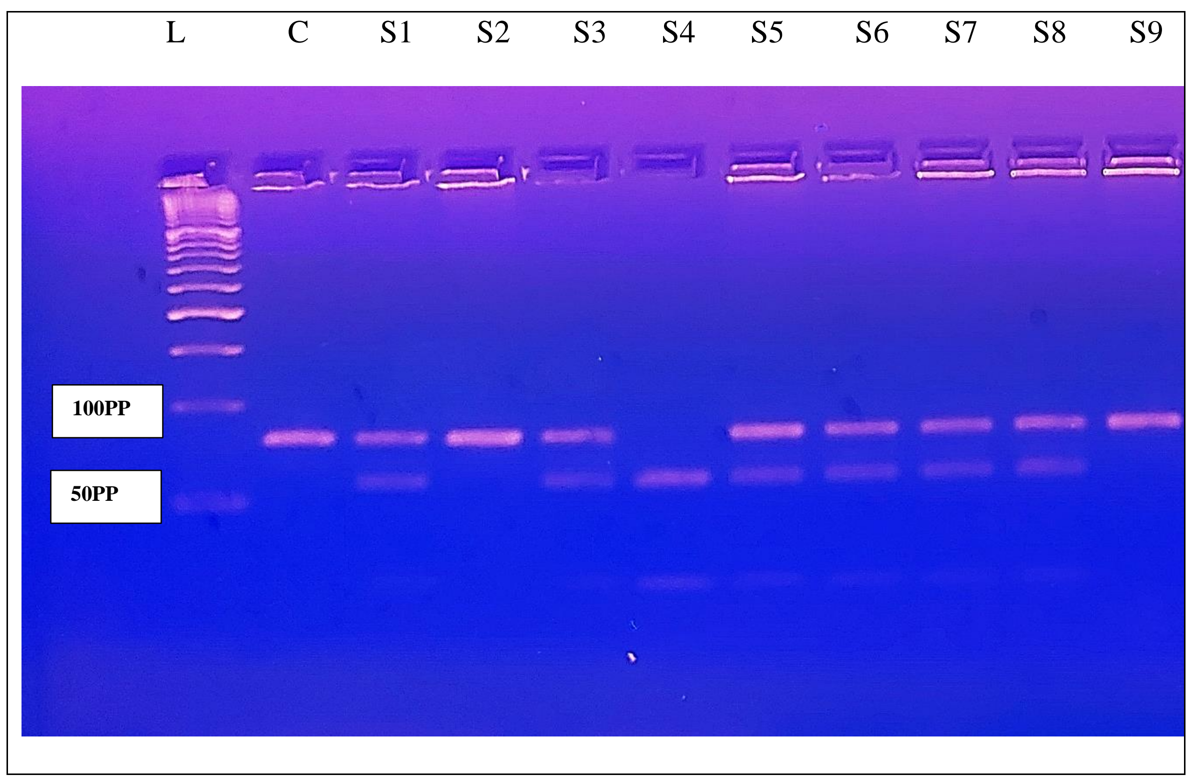


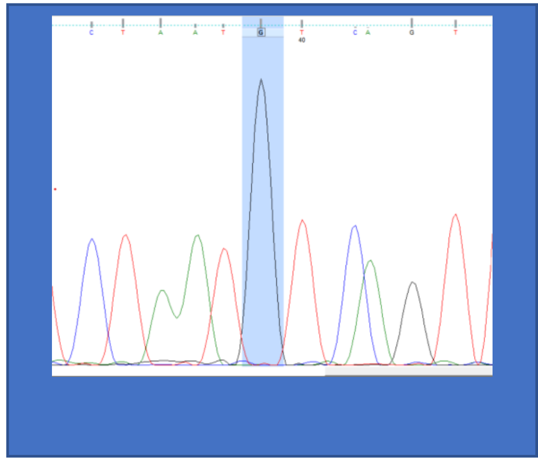
Figure 4.1 (A): Genotyping of the *LEP* R polymorphism (rs1137101 (+668A>G). Agarose gel electrophoresis (2.5%) for PCR product(80pp). L: DNA ladder (50bp)

Figure 4.2(B):



B. Agarose gel electrophoresis (4%) pattern of selected RFLP products of the *LEP* R polymorphism. L: DNA ladder (50bp). Lane C is positive control. S 2 and 9 represent (80bp) of the wildtype homozygous (AA genotype). S 1,3,5,6,7 and 8 represent are heterozygous (AG genotype) MspI digested products confirmed by the presence of three bands (80, 57, and 23 bp). S 4 correspond to the homozygous (GG genotype) resulting in tow band (57pp and 23bp).

Figure 4.1 (C):



Sanger sequencing chromatograms showing the sequence for one random sample, with GG genotype.

4.2.2 Genotypic analysis

The Chi-square ($\chi 2$) was used to test the association between the genotypes and alleles in the cases and controls. Regarding the results of PCR-RFLP for LEP, 0% of the cases were homozygous for the Ob/Ob genotype, compared to 20% in the controls. The Ob/ob heterozygous genotype was reported in 27% in cases, compared to 23% in the controls. The ob/ob homozygous genotype was reported 18% in cases, compare to 13% in the control. These results are statistically significant, with a higher frequency among patients (P=0.006).

For *LEPR* 35.5% of the cases were homozygous for the Db/Db genotype, compared to 44.4% in the controls. The Db/db heterozygous genotype was reported in 51.1% in cases, compared to 46% in the controls. The db/db homozygous genotype was reported 13.3% in cases, compare to 9% in the control. It was found that there was no significant association between the frequency of Db and db alleles in patients compared to controls (P=0.626) The results of genotype frequency are summarized in Table 4.3.

Table 4.3: Association analysis between *LEP, LEPR* polymorphisms with BMI.

Polymorphism	Genotypes/Alleles	Patients N=45 (%)	Controls N=45 (%)	P-value
LEP	Ob/Ob (GG)	0(0%)	9(20%)	0.006325*
	Ob/ob (GA)	27(60%)	23(51%)	
	ob/ob (AA)	18(40)	13(29%)	
LEPR	Db/Db (AA)	16(35.5%)	20(44.4%)	0.626456
	Db/db (AG)	23(51.1%)	21(46%)	
	db/db (GG)	6(13.3%)	4(9%)	

SPSS was used for the statistical analysis. Output summarizes the results of a Chi-Square Test conducted to evaluate the relationship between two polymorphisms: *LEPR* and *LEP* polymorphism. Results can be interpreted as: Pearson Chi-Square value = 7.051, Degrees of Freedom (df) = 4, and P-value = 0.133. The P-value (0.133) is greater than the typical significance threshold (0.05), indicating that there is no statistically significance association between *LEPR* and *LEP* polymorphism.

4.3 Clinical and Serology analysis

The mean for leptin concentrations were 13.7 ± 12.5(mean ± SD) for 45 patients with BMI <25 and 41.3 ± 17.7 for 45 patients with BMI >30, These results were statistically significant, with a higher frequency among patients (P < 0.001) as shown in Table 4.4

Table 4.4: Levene's Test for Equality of Variances

Leptin concentration	F	Sig.	df	Significance One-Sided p	Two-Sided p
Equal variances assumed	3.193	.077	88	**<.001**	**<.001**

Table 4.4: Leptin concertation between high BMI and normal BMI

	BMICAT	N	Mean	Std. Deviation	Std. Error Mean
Leptin Con.	Normal W	45	13.6889	12.44410	1.85506
	Obese	45	41.2889	17.73238	2.64339

These results were statistically significant, with a higher concentration among patients' group (P=0.001).

Chapter Five: Discussion

To the best of our knowledge, this is the first genetic study among the Palestinian population to examine the genotypic and allelic distributions of *LEP* and *LEPR* genes polymorphisms in hypertension patients. Accordingly, the aim of this study was to investigate the association of (*LEP* and *LEPR*) gene polymorphisms with BMI in Palestinian population. We hypothesize that *LEP* and *LEPR* polymorphisms act as risk factors for high blood pressure. Therefore, we genotyped forty-five (45) patients suffering from HBP with increased BMI and same number of patients suffering from HBP but having normal BMI.

Our findings are in agreement with previous studies have suggested that obesity can occur at any age, but it becomes more common with older age. The process of aging is accompanied with a reduction in muscle mass, bone mass, and strength levels, in addition to an increase in entire body fat mass, especially visceral fat mass. As a result, decreasing lean mass and percent lean mass with age is a significant indicator of aging (Jiang et al., 2015).

Concerning gender distribution, about 55.6% of high BMI patients (25/45) who participated were females. But about 37.8% of normal BMI patients were females. Khalid M Salih & Luma Qasim Ali, 2022 showed that there is a significant difference ($P < 0.05$) in obesity status between females and males (5.7% and 2.1% respectively) (Khalid M Salih & Luma Qasim Ali, 2022). The world is experiencing a pandemic of obesity. Overall, more women than men are obese, while there are significant differences in the prevalence of overweight and obesity among men and women within and between nations. Women in developing nations, especially those in the Middle East and North Africa, are more likely to be overweight or obese than men (Kanter & Caballero, 2012).

Other study showed that in the study group mean BMI was not different in males and females (29.24 vs. 29.32) kg/m2 (Hussain et al., 2019) . Other BMI researches results were higher than the previously mean BMI of participants from an urban community in Yemen (23.9 ± 5.1) kg/m2 and (21.8 ±8.9) kg/m2 in females and males, respectively (Gunaid, 2012). Also, other work showed that the overall mean BMI was considerably higher in females than in males (28.0 vs. 25.4)(Al-Sharafi & Gunaid, 2014).

The high prevalence among females in this study might be due to physiologic and genetic differences between men and women, women may consume more sugar-laden foods than males, but they also frequently report eating healthier foods.

Acculturation influences weight gain in both men and women through various sociocultural processes. Both men and women have experienced excess weight gain as a result of the nutrition shift occurring in many developing nations, although women's levels of physical activity have been more significantly impacted. Additionally, larger body sizes are viewed by some cultures as a sign of fertility, health, or prosperity in both men and women.

Further research on gender differences in overweight and obesity can help us better comprehend the worldwide obesity pandemic as it progresses.

In our study patients comprised 45 individuals with high BMI, 5 patient (11.1%) were current smokers, and the control group included 14 smokers (31.1%). Literature confirms that current smokers were less likely to be obese than never smokers or former smokers (Dare et al., 2015).

Obesity, associated with aberrant lipids and poor cardiovascular outcomes, is becoming increasingly common condition in Pakistan (Tanzil, et al 2016). Research literature support a relationship between BMI and lipids. Lipid profiles are associated with

lifestyle, age, intraabdominal adiposity, obesity, and BMI (Esther Omotoye & Fadupin, 2016).

In the current study we evaluate the correlation between BMI and lipids in patients from both groups. Metabolic disorders such as cardiovascular diseases (CVD), diabetes, dyslipidemia, hypertension, hyperinsulinemia, and high serum uric acid have been found to be strongly influenced by body fat and blood lipids (Dixit et al., 2014).

In this study HBP patient with normal BMI when compared to overweight and obese. BMI did not show significant differences in the mean values of TC, TGs, LDL and HDL. A study by Yadav NK et al., reported that obese type2 Diabetes mellitus patients, in comparison with diabetic obese control patients revealed statistically significant increase in the levels of TC, TGs, LDL except HDL levels in the both groups did not show statistically significant difference (Yadav et al., 2012).

Another study on Iraqi women found that high BMI is consistently associated with an aberrant lipid profile characterized by increased TGs and LDL -C, and low HDL-C (Ali & Al-Zaidi, 2011).

Our results are consistent with a study by Hussain et al. which reported slightly increased TC, LDL-C, triglyceride and HDL between groups. The mean differences of TC, TGs, LDL and HDL-C showed no statistically significant correlation with BMI (Hussain et al., 2019).

Literatures includes hypertension patients were performed according to age and the BMI, in both groups < 60 and ≥ 60 years, TC, LDL-C, and non-HDL-c levels were higher in the hypertensive population, whereas HDL-C and TG differed slightly between the hypertensive and non-hypertensive populations with no statistical significance. Also, in

the BMI < 23 or ≥ 23 kg/m2 groups TC, LDL-C, and non-HDL-C levels were higher in the hypertensive population, but HDL-C levels were higher in the hypertensive population.

In the lipid profiles, TC, LDL-C, and non-HDL-C were higher in the hypertensive population (P < 0.001) (Chen & Cheng, 2022).

One of the most common illnesses connected with obesity is hypertension, which is considered a major risk factor for stroke, myocardial infarction, and heart failure. According to epidemiological research, overweight or obesity causes 65-75% of primary (essential) hypertension (Garrison et al., 1987).

The literature showed that linear relationship exists between BP and indices of obesity, including BMI, in all population examined, including White, Black and Asian populations. There are studies in primary care settings report that 60–76% of patients with high BMI have hypertension, increase in BMI more than a 5-year led to increase the risk of hypertension 30% more when compared with people with normal weight did not change (Hall et al., 2019).

The present study revealed the frequency of the *LEP* polymorphism with Ob/Ob, Ob/ob and ob/ob genotype in both group of HBP patients. Regarding the results of *LEP*, 0% of the cases were homozygous for the Ob/Ob genotype, compared to 20% in the controls. The Ob/ob heterozygous genotype was reported in 27% in cases, compared to 23% in the controls. The ob/ob homozygous genotype was reported 18% in cases, compared to 13% in the control. These results were statistically significant, with a higher frequency among patients (P = 0.006). A literature interventional study showed that individuals without the A allele (GG) of SNP rs7799039 in *LEP* polymorphism displayed a substantially higher improvement of LDL-cholesterol and triglycerides than A allele carriers (GA,AA) after a diet (Primo et al., 2020) .

Adults with the AA genotype consumed considerably more calories each day [GG: 2853 (1215) kcal versus GA + AA: 3431 (1609) kcal], according to a case-control study including Arab patients (Boumaiza et al., 2012).

Additionally, allele A of rs7799039 was found to be significantly associated with the change in energy intake from pre-pregnancy to pregnancy in a cohort study of pregnant Brazilian women; bearers of the A allele had a lower total mean adjusted energy intake (Sugawara & Nikaido,1972). However, the A allele of the LEP-rs7799039 polymorphism had no influence on appetite, according to a randomized intervention trial conducted in high-BMI White men (Dougkas et al., 2013). In another study with a Caucasian population lack of association was also demonstrated (Bienertova-Vasku et al., 2008), furthermore in a prospective cohort of Brazilian children (Zandoná et al., 2013). A guanine to adenine substitution in the promoter region is the rs7799039 polymorphism. This mutation may alter gene expression, most likely at the transcriptional level, altering the level of leptin generated by adipose tissue and, as a result, affecting BMI (Shabana & Hasnain, 2016).

The significance of leptin in obesity hypertension is supported by the finding that *LEPR* antagonism reduced blood pressure in obese rabbits. Furthermore, compared to lean control mice, leptin-deficient (ob/ob) mice have lower blood pressure and SNA but significant obesity, dyslipidemia, insulin resistance, and hyperinsulinemia. In ob/ob mice, leptin infusion resulted in considerable weight loss, but it also increased blood pressure. All of these findings point to leptin raising blood pressure and sympathetic activation in obese animals (Hall et al., 2019).

For *LEPR* 35.5% of the cases were homozygous for the Db/Db genotype, compared to 44.4% in the controls. The Db/db heterozygous genotype was reported in 51.1% in cases, compared to 46% in the controls. The db/db homozygous genotype was reported 13.3% in

cases, compared to 9% in the control. It was found that there was no significant association between the frequency of Db and db alleles in patients compared to controls (P = 0.626). One of the first findings of our study is the lack of relationship of *LEPR* polymorphism with BMI between HBP group.

In the literature, *LEPR* gene has been widely studied in obesity-related hypertension. This study compared AA vs. GG and indicated that this SNP may modulate blood pressure levels and hart rate in a treatment-resistant hypertension subject with no statistically significant (De Faria et al., 2017) . Although conflicting results in accordance with our findings a study showed between the overall case and the control population it was significant between the wild type and Homozygote (P = 0.001), and between wild type and heterozygous (P = 0.001),

Also AA subjects had higher body fat percentage (Etemad et al., 2013). The rs1137101 polymorphism leads to an amino acid change and change from neutral to positive charge in the extracellular domain of the receptor that may impair the ability of leptin to bind to its receptor. This provides of leptin resistance status (Szapary, et al 2020).

According to another study, if BP control is directly dependent on *LEPR*, persons with the most common genotype of rs1137101 may be prone to high blood pressure, but the rare genotype may provide protection due to a dysfunctional leptin receptor. Previous studies reported no difference in leptin levels between homozygous genotypes for rs1137101. It has been proposed that inadequate leptin receptors in humans have insufficient feedback regulation in leptin synthesis (Alexander et al., 2003).

In our study, the mean for leptin concentrations were statistically significant, with a higher frequency among patients (P < 0.001). In other study, there was a statistically significant difference (P < 0.05) in leptin blood levels among older obese people. Similarly,

20% of non-obese old people and 45% of obese elderly people had hypertension, with a systolic BP increase predominating (Sanchez-Rodriguez et al., 2000). Older adults who are obese had greater serum leptin levels, which may indicate that leptin resistance and/or a decline in leptin receptors are linked to aging. Associated obesity is the cause of the high prevalence of hypertension as people age (Jura & Kozak, 2016).

Like insulin resistance, leptin resistance results from excessive production, but its absence causes obesity. Evidence that elderly people frequently exhibit leptin resistance suggests a potential connection between leptin and aging (Jura & Kozak, 2016). Leptin sensitivity is linked to youth, but a lack of leptin activity with aging is linked to both obesity and the distribution of body fat (Carter et al., 2013).

According to research on leptin administration resistance, elderly rats with high amounts of body fat had endogenous serum leptin levels that were three times higher than those of young rats (Scarpace et al., 2000). According to research by Scarpace et al., leptin treatment subcutaneously for seven days raised serum levels and significantly reduced food consumption in young rats, suggesting that older people are more resistant to the effects of leptin (Scarpace et al., 2000).

In conclusion, our current findings provide conclusive proof that the *LEP* gene polymorphism is associated with susceptibility to HBP. This study suggests that having a *LEP* AA genotype is an independent risk factor for developing hypertension in obese individuals. To demonstrate a concrete link between these polymorphisms and HBP risk, more studies with large cohorts and different study designs such as cohort study are needed. A cohort study design will allow the follow up of patients for at least 18 months which will enable researchers to clearly investigate the differences in the outcome of patients.

Finally, if we can identify an association between SNPs and therapy response and disease complications, we may be able to incorporate this test into the standard blood testing protocols before the initiation of treatment.

References

Al-Sharafi, B. A., & Gunaid, A. A. (2014). Prevalence of obesity in patients with type 2 diabetes mellitus in Yemen. *International Journal of Endocrinology and Metabolism*, *12*(2), 1–5. https://doi.org/10.5812/ijem.13633

Alexander, C. M., Landsman, P. B., Teutsch, S. M., & Haffner, S. M. (2003). NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes*, *52*(5), 1210–1214. https://doi.org/10.2337/diabetes.52.5.1210

Ali, Z. A. U. A., & Al-Zaidi, M. S. (2011). The association between body mass index, lipid profile and serum estradiol levels in a sample of Iraqi diabetic premenopausal women. *Oman Medical Journal*, *26*(4), 263–266. https://doi.org/10.5001/omj.2011.63

Bickel, M., & Schöning, A. (2009). Bill & Melinda gates foundation. In *Handbook of Transnational Economic Governance Regimes* (Vol. 377, Issue 1). https://doi.org/10.1163/ej.9789004163300.i-1081.23

Bienertova-Vasku, J. A., Bienert, P., Tomandl, J., Forejt, M., Vavrina, M., Kudelkova, J., & Vasku, A. (2008). No association of defined variability in leptin, leptin receptor, adiponectin, proopiomelanocortin and ghrelin gene with food preferences in the Czech population. *Nutritional Neuroscience*, *11*(1), 2–8. https://doi.org/10.1179/147683008X301379

Biesiada, L. A., Glowacka, E., Krekora, M., Sobantka, S., Krokocka, A., & Krasomski, G. (2016). The impact of excessive maternal weight on the nutritional status of the fetus-the role of leptin. *Archives of Medical Science*, *12*(2), 394–401. https://doi.org/10.5114/aoms.2016.59267

Borga, M., West, J., Bell, J. D., Harvey, N. C., Romu, T., Heymsfield, S. B., & Leinhard, O. D. (2018). Advanced body composition assessment: From body mass index to body composition profiling. *Journal of Investigative Medicine*, *66*(5), 887–895. https://doi.org/10.1136/jim-2018-000722

Boumaiza, I., Omezzine, A., Rejeb, J., Rebhi, L., Ouedrani, A., Ben Rejeb, N., Nabli, N., Ben Abdelaziz, A., & Bouslama, A. (2012). Relationship between leptin G2548A and leptin receptor Q223R gene polymorphisms and obesity and metabolic syndrome risk in Tunisian volunteers. *Genetic Testing and Molecular Biomarkers*, *16*(7), 726–733. https://doi.org/10.1089/gtmb.2011.0324

Cardel, M. I., Atkinson, M. A., Taveras, E. M., Holm, J.-C., & Kelly, A. S. (2020). Obesity Treatment Among Adolescents. *JAMA Pediatrics*, *174*(6), 609. https://doi.org/10.1001/jamapediatrics.2020.0085

Carter, S., Caron, A., Richard, D., & Picard, F. (2013). Role of leptin resistance in the development of obesity in older patients. *Clinical Interventions in Aging*, *8*, 829–844.

Chen, S., & Cheng, W. (2022). Relationship Between Lipid Profiles and Hypertension: A Cross-Sectional Study of 62,957 Chinese Adult Males. *Frontiers in Public Health*,

10(May), 1–11. https://doi.org/10.3389/fpubh.2022.895499

Clemente-Suárez, V. J., Redondo-Flórez, L., Beltrán-Velasco, A. I., Martín-Rodríguez, A., Martínez-Guardado, I., Navarro-Jiménez, E., Laborde-Cárdenas, C. C., & Tornero-Aguilera, J. F. (2023). The Role of Adipokines in Health and Disease. *Biomedicines*, *11*(5). https://doi.org/10.3390/biomedicines11051290

Costa, V., Casamassimi, A., & Ciccodicola, A. (2010). Nutritional genomics era: Opportunities toward a genome-tailored nutritional regimen. *Journal of Nutritional Biochemistry*, *21*(6), 457–467. https://doi.org/10.1016/j.jnutbio.2009.10.012

Dare, S., Mackay, D. F., & Pell, J. P. (2015). Relationship between smoking and obesity: A cross-sectional study of 499,504 middle-aged adults in the UK general population. *PLoS ONE*, *10*(4), 1–12. https://doi.org/10.1371/journal.pone.0123579

de Faria, A. P., Ritter, A. M. V., Sabbatini, A. R., Modolo, R., & Moreno, H. (2017). Effects of leptin and leptin receptor SNPs on clinical- and metabolic-related traits in apparent treatment-resistant hypertension. *Blood Pressure*, *26*(2), 74–80. https://doi.org/10.1080/08037051.2016.1192945

Dexter, L. (1948). Mechanisms of human hypertension. *The American Journal of Medicine*, *4*(2), 279–284. https://doi.org/10.1016/0002-9343(48)90243-5

Dixit, A. K., Dey, R., Suresh, A., Chaudhuri, S., Panda, A. K., Mitra, A., & Hazra, J. (2014). The prevalence of dyslipidemia in patients with diabetes mellitus of ayurveda Hospital. *Journal of Diabetes and Metabolic Disorders*, *13*(1), 2–7. https://doi.org/10.1186/2251-6581-13-58

Dunbar JC, Hu Y, and Lu H. Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. Diabetes 1997, 46: 2040-3.

Dominiczak, A., Delles, C., & Padmanabhan, S. (2017). Genomics and Precision Medicine for Clinicians and Scientists in Hypertension. *Hypertension*, *69*(4), e10–e13. https://doi.org/10.1161/HYPERTENSIONAHA.116.08252

Dougkas, A., Yaqoob, P., Givens, D. I., Reynolds, C. K., & Minihane, A. M. (2013). The impact of obesity-related SNP on appetite and energy intake. *British Journal of Nutrition*, *110*(6), 1151–1156. https://doi.org/10.1017/S0007114513000147

Esther Omotoye, F., & Fadupin, G. T. (2016). Effect of Body Mass Index on Lipid Profile of Type 2 Diabetic Patients at An Urban Tertiary Hospital In Nigeria. *IOSR Journal of Dental and Medical Sciences*, *15*(09), 65–70. https://doi.org/10.9790/0853-1509086570

Etemad, A., Ramachandran, V., Pishva, S. R., Heidari, F., Aziz, A. F. A., Yusof, A. K. M., Pei, C. P., & Ismail, P. (2013). Analysis of Gln223Agr polymorphism of Leptin Receptor gene in type II diabetic mellitus subjects among Malaysians. *International Journal of Molecular Sciences*, *14*(9), 19230–19244. https://doi.org/10.3390/ijms140919230

Flegal, K. M., Kruszon-Moran, D., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2016). Trends in obesity among adults in the United States, 2005 to 2014. *JAMA - Journal of*

the American Medical Association, *315*(21), 2284–2291. https://doi.org/10.1001/jama.2016.6458

Forouzanfar, M. H., Afshin, A., Alexander, L. T., Biryukov, S., Brauer, M., Cercy, K., Charlson, F. J., Cohen, A. J., Dandona, L., Estep, K., Ferrari, A. J., Frostad, J. J., Fullman, N., Godwin, W. W., Griswold, M., Hay, S. I., Kyu, H. H., Larson, H. J., Lim, S. S., … Zhu, J. (2016). Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet*, *388*(10053), 1659–1724. https://doi.org/10.1016/S0140-6736(16)31679-8

Garrison, R. J., Kannel, W. B., Stokes, J., & Castelli, W. P. (1987). Incidence and precursors of hypertension in young adults: The Framingham offspring study. *Preventive Medicine*, *16*(2), 235–251. https://doi.org/10.1016/0091-7435(87)90087-9

Galletti F, D'Elia L, and Barba G. High-circulating leptin levels are associated with greater risk of hypertension in men independently of body mass and insulin resistance: results of an eight-year follow-up study. J Clin Endocrinol Metab 2008, 93: 3922-6.

Gunaid, A. A. (2012). *العدد Obesity , الثاين عرش املجلد الثامن عرش املجلة الصحية لرشق املتوسط* *overweight and underweight among adults in an urban community in Yemen اليمن يف* *حرضي جمتمع يف البالغني بني الوزن ونقص الوزن وفرط البدانة. 18*(12), 1187–1193.

Hall, J. E., do Carmo, J. M., da Silva, A. A., Wang, Z., & Hall, M. E. (2019). Obesity, kidney dysfunction and hypertension: mechanistic links. *Nature Reviews Nephrology*, *15*(6), 367–385. https://doi.org/10.1038/s41581-019-0145-4

Han, T. J., & Wang, X. (2015). Leptin and its receptor in hematologic malignancies. *International Journal of Clinical and Experimental Medicine*, *8*(11), 19840–19849.

Hanley, M. J., Abernethy, D. R., & Greenblatt, D. J. (2010). Effect of obesity on the pharmacokinetics of drugs in humans. *Clinical Pharmacokinetics*, *49*(2), 71–87. https://doi.org/10.2165/11318100-000000000-00000

Hao, J. Q., Zhang, Q. K., Zhou, Y. X., Chen, L. H., & Wu, P. F. (2019). Association between circulating leptin concentration and G-2548A gene polymorphism in patients with breast cancer: A meta-analysis. *Archives of Medical Science*, *15*(2), 275–283. https://doi.org/10.5114/aoms.2018.75638

Haslam, D. (2016). Weight management in obesity - Past and present. *International Journal of Clinical Practice*, *70*(3), 206–217. https://doi.org/10.1111/ijcp.12771

Huang, Q. F., Yang, W. Y., Asayama, K., Zhang, Z. Y., Thijs, L., Li, Y., O'Brien, E., & Staessen, J. A. (2021). Ambulatory Blood Pressure Monitoring to Diagnose and Manage Hypertension. *Hypertension*, *77*(2), 254–264. https://doi.org/10.1161/HYPERTENSIONAHA.120.14591

Hussain, A., Ali, I., Kaleem, W. A., & Yasmeen, F. (2019). Correlation between body mass index and lipid profile in patients with type 2 diabetes attending a tertiary care hospital in Peshawar. *Pakistan Journal of Medical Sciences*, *35*(3), 591–597. https://doi.org/10.12669/pjms.35.3.7

Jastreboff, A. M., Kotz, C. M., Kahan, S., Kelly, A. S., & Heymsfield, S. B. (2019). Obesity as a Disease: The Obesity Society 2018 Position Statement. *Obesity*, *27*(1), 7–9. https://doi.org/10.1002/oby.22378

Jensen, M. D., Ryan, D. H., Apovian, C. M., Ard, J. D., Comuzzie, A. G., Donato, K. A., Hu, F. B., Hubbard, V. S., Jakicic, J. M., Kushner, R. F., Loria, C. M., Millen, B. E., Nonas, C. A., Pi-Sunyer, F. X., Stevens, J., Stevens, V. J., Wadden, T. A., Wolfe, B. M., & Yanovski, S. Z. (2014). 2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults. *Journal of the American College of Cardiology*, *63*(25), 2985–3023. https://doi.org/10.1016/j.jacc.2013.11.004

Jiang, Y., Zhang, Y., Jin, M., Gu, Z., Pei, Y., & Meng, P. (2015). Aged-related changes in body composition and association between body composition with bone mass density by body mass index in Chinese Han men over 50-year-old. *PLoS ONE*, *10*(6), 1–15. https://doi.org/10.1371/journal.pone.0130400

Jura, M., & Kozak, L. P. (2016). Obesity and related consequences to ageing. *Age*, *38*(1). https://doi.org/10.1007/s11357-016-9884-3

Kanter, R., & Caballero, B. (2012). Global gender disparities in obesity: A review. *Advances in Nutrition*, *3*(4), 491–498. https://doi.org/10.3945/an.112.002063

Khalid M Salih, & Luma Qasim Ali. (2022). Effect of age and sex on Obesity Average of Population Sample in Baghdad City. *Journal of the College of Basic Education*, *22*(96), 73–84. https://doi.org/10.35950/cbej.v22i96.9016

L, T., M, D., X, W., N, D., J, C., R, D., GJ, R., LA, C., FT, C., J, D., C, M., S, S., A, M., KJ, M., JS, S., GG, M., EA, W., CA, M., & RI., T. (1995). Identification and expression cloning of a leptin receptor, OB-R. *Cell*, *83*(7), 1263–1271. https://www.cell.com/cell/pdf/0092-8674(95)90151-5.pdf?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2F0092867495901515%3Fshowall%3Dtrue%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/8548812

Lee G H, Proenca R, Montez J M, Carroll K M, Darvishzadeh J G, & Lee J I. (1996). Abnormal splicing of the leptin receptor in diabetic mice. *Nature*, *379*(February), 632–635.

Li, Y. Y., Wang, H., Yang, X. X., Wu, J. J., Geng, H. Y., Kim, H. J., Yang, Z. J., & Wang, L. S. (2017). *LEPR* gene Gln223Arg polymorphism and type 2 diabetes mellitus: A meta-analysis of 3,367 subjects. *Oncotarget*, *8*(37), 61927–61934. https://doi.org/10.18632/oncotarget.18720

Loktionov, A. (2003). Common gene polymorphisms and nutrition: Emerging links with pathogenesis of multifactorial chronic diseases (review). *Journal of Nutritional Biochemistry*, *14*(8), 426–451. https://doi.org/10.1016/S0955-2863(03)00032-9

Luft, F. C. (2001). Twins in cardiovascular genetic research. *Hypertension*, *37*(2 II), 350–356. https://doi.org/10.1161/01.hyp.37.2.350

Ma, D., Feitosa, M. F., Wilk, J. B., Laramie, J. M., Yu, K., Leiendecker-Foster, C., Myers,

R. H., Province, M. A., & Borecki, I. B. (2009). Leptin is associated with blood pressure and hypertension in women from the National Heart, Lung, and Blood Institute family heart study. *Hypertension*, *53*(3), 473–479. https://doi.org/10.1161/HYPERTENSIONAHA.108.118133

Maffetone, P. B., Rivera-Dominguez, I., & Laursen, P. B. (2017). Overfat and underfat: New terms and definitions long overdue. *Frontiers in Public Health*, *4*(JAN), 1–10. https://doi.org/10.3389/FPUBH.2016.00279

Manuscript, A. (2017). *Europe PMC Funders Group Europe PMC Funders Author Manuscripts Europe PMC Funders Author Manuscripts Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension*. *48*(10), 1151–1161. https://doi.org/10.1038/ng.3654.Trans-ancestry

McNiven, E. M. S., German, J. B., & Slupsky, C. M. (2011). Analytical metabolomics: Nutritional opportunities for personalized health. *Journal of Nutritional Biochemistry*, *22*(11), 995–1002. https://doi.org/10.1016/j.jnutbio.2011.05.016

MedlinePlus Genetics. (2020). *Genomic Research Center*. https://medlineplus.gov/genetics/understanding/genomicresearch/snp/

MICHA, R. (2017). 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*, *176*(1), 100–106. https://doi.org/10.1177/0022146515594631.Marriage

Mills, K. T., Bundy, J. D., Kelly, T. N., Reed, J., Kearney, P., Reynolds, K., Chen, J., & He, J. (2017). Global Disparities. *Physiology & Behavior*, *176*(3), 139–148. https://doi.org/10.1161/CIRCULATIONAHA.115.018912.Global

Obradovic, M., Sudar-Milovanovic, E., Soskic, S., Essack, M., Arya, S., Stewart, A. J., Gojobori, T., & Isenovic, E. R. (2021). Leptin and Obesity: Role and Clinical Implication. *Frontiers in Endocrinology*, *12*(May), 1–14. https://doi.org/10.3389/fendo.2021.585887

Passos, D. T., Hepp, D., Moraes, J. C. F., & Weimer, T. A. (2007). Effect of polymorphisms linked to *LEP* gene on its expression on adipose tissues in beef cattle. *Journal of Animal Breeding and Genetics*, *124*(3), 157–162. https://doi.org/10.1111/j.1439-0388.2007.00653.x

Petridou, E., Papadiamantis, Y., Markopoulos, C., Spanos, E., Dessypris, N., & Trichopoulos, D. (2000). Leptin and insulin growth factor I in relation to breast cancer (Greece). *Cancer Causes and Control*, *11*(5), 383–388. https://doi.org/10.1023/A:1008903727238

Phillips, C. M. (2013). Nutrigenetics and metabolic disease: Current status and implications for personalised nutrition. *Nutrients*, *5*(1), 32–57. https://doi.org/10.3390/nu5010032

Poirier, P., Giles, T. D., Bray, G. A., Hong, Y., Stern, J. S., Pi-Sunyer, F. X., & Eckel, R. H. (2006). Obesity and cardiovascular disease: Pathophysiology, evaluation, and effect of weight loss: An update of the 1997 American Heart Association Scientific Statement on obesity and heart disease from the Obesity Committee of the Council on Nutrition, Physical. *Circulation*, *113*(6), 898–918.

https://doi.org/10.1161/CIRCULATIONAHA.106.171016

Primo, D., Izaola, O., & Luis, D. De. (2020). *Leptin gene polymorphism (rs 7799039 ; G2548A) is associated with changes in lipid profile during a partial meal-replacement hypocaloric diet*. *2*, 1–8. https://doi.org/10.1111/jhn.12809

Rao, G., Powell-Wiley, T. M., Ancheta, I., Hairston, K., Kirley, K., Lear, S. A., North, K. E., Palaniappan, L., & Rosal, M. C. (2015). Identification of obesity and cardiovascular risk in ethnically and racially diverse populations: A scientific statement from the American Heart Association. *Circulation*, *132*(5), 457–472. https://doi.org/10.1161/CIR.0000000000000223

Rosen, C. J. (2023). Antagonizing the Leptin Receptor in Obesity. *New England Journal of Medicine*, *388*(24), 2291–2293. https://doi.org/10.1056/nejme2301915

Rudkowska, I., & Pérusse, L. (2012). Individualized weight management: What can be learned from nutrigenomics and nutrigenetics? In *Progress in Molecular Biology and Translational Science* (Vol. 108). https://doi.org/10.1016/B978-0-12-398397-8.00014-9

Sánchez-Margalet, V., Martín-Romero, C., Santos-Alvarez, J., Goberna, R., Najib, S., & Gonzalez-Yanes, C. (2003). Role of leptin as an immunomodulator of blood mononuclear cells: Mechanisms of action. *Clinical and Experimental Immunology*, *133*(1), 11–19. https://doi.org/10.1046/j.1365-2249.2003.02190.x

Sanchez-Rodriguez, M., Garcia-Sanchez, A., Retana-Ugalde, R., & Mendoza-Nunez, V. M. (2000). Serum leptin levels and blood pressure in the overweight elderly. *Archives of Medical Research*, *31*(4), 425–428. https://doi.org/10.1016/S0188-4409(99)00079-X

Saydah, S., Bullard, K. M., Chen, Y., Ali, M. K., Edward, W., Geiss, L., & Imperatore, G. (2015). *HHS Public Access*. *22*(8), 1888–1895. https://doi.org/10.1002/oby.20761.Trends

Scarpace, P. J., Matheny, M., Moore, R. L., & Tümer, N. (2000). Impaired leptin responsiveness in aged rats. *Diabetes*, *49*(3), 431–435. https://doi.org/10.2337/diabetes.49.3.431

Shabana, & Hasnain, S. (2016). Leptin promoter variant G2548A is associated with serum leptin and HDL-C levels in a case control observational study in association with obesity in a Pakistani cohort. *Journal of Biosciences*, *41*(2), 251–255. https://doi.org/10.1007/s12038-016-9612-2

Sitinjak, B. D. P., Barliana, M. I., Murdaya, N., Rachman, T. A., & Zakiyah, N. (2023). The Potential of Single Nucleotide Polymorphisms (SNPs) as Biomarkers and Their Association with the Increased Risk of Coronary Heart Disease: A Systematic Review. *Vascular Health and Risk Management*, *19*(May 2023), 289–301. https://doi.org/10.2147/VHRM.S405039

Sugawara, E., & Nikaido, H. (1972). Estimationofthe Concentrationof Low-Density LipoproteinCholesterolin Plasma,Without Useof the PreparativeUltracentrifuge William. *Clinical Chemistry*, *18*(6), 499–502.

https://journals.asm.org/doi/10.1128/AAC.03728-14

Takizawa H, Ura N, and Saitoh S. Gender difference in the relationships among hyperleptinemia, hyperinsulinemia, and hypertension. Clin Exp Hypertens 2001, 23: 357-68.

Tanzil, S., & Jamali, T. (2016). Obesity, An Emerging Epidemic In Pakistan-A Review Of Evidence. *Journal of Ayub Medical College, Abbottabad : JAMC*, *28*(3), 597–600.

White, D. W., & Tartaglia, L. A. (1996). Leptin and OB-R: Body weight regulation by a cytokine receptor. *Cytokine and Growth Factor Reviews*, *7*(4), 303–309. https://doi.org/10.1016/S1359-6101(96)00040-8 Gu P, Jiang W, Chen M, Lu B, Shao J, Du H, and Jiang S. Association of leptin receptor gene polymorphisms and essential hypertension in a Chinese population. J. Endocrinol. Invest. 35: 859-865, 2012

Wu, L., & Sun, D. (2017). Leptin receptor gene polymorphism and the risk of cardiovascular disease: A systemic review and meta-analysis. *International Journal of Environmental Research and Public Health*, *14*(4). https://doi.org/10.3390/ijerph14040375

Yadav, N. K., Thanpari, C., Shrewastwa, M. K., & Mittal, R. K. (2012). Comparison of lipid profile in type-2 obese diabetics and obese non-diabetic individuals. A hospital based study from Western Nepal. *Kathmandu University Medical Journal*, *10*(39), 44–47. https://doi.org/10.3126/kumj.v10i3.8017

Zandoná, M. R., Rodrigues, R. O., Albiero, G., Campagnolo, P. D. B., Vitolo, M. R., Almeida, S., & Mattevi, V. S. (2013). Polymorphisms in *LEPR*, PPARG and APM1 genes: associations with energy intake and metabolic traits in young children. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *57*(8), 603–611. https://doi.org/10.1590/s0004-27302013000800004

Zhang, F., Basinski, M. B., Beals, J. M., Briggs, S. L., Churgay, L. M., Clawson, D. K., DiMarchi, R. D., Furman, T. C., Hale, J. E., Hsiung, H. M., Schoner, B. E., Smith, D. P., Zhang, X. Y., Wery, J. P., & Schevitz, R. W. (1997). Crystal structure of the obese protein leptin-E100. *Nature*, *387*(6629), 206–209. https://doi.org/10.1038/387206a0

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, & Friedman JM. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, *372*(DECEMBER), 425–432. https://www.nature.com/articles/372425a0.pdf

(Szapary, P.O.; Hark, L.A.; Burke, F.M. The metabolic syndrome: A new focus for lifestyle modification. (CHD prevention). Patient Care 2002, 13, 75–88).

Appendices

Table 1.1: Chemicals and Reagents used in the study

Reagents	Provider
QIAamp DNA Mini Kit	Qiagen/ Germany
ELIZA kit	DRG
Ethanol absolute anhydrous	Carlo Erba Reagent
Master Mix	Promega
Nuclease-free water	Promega
Hha1	New England BioLabs
Msp1	New England BioLabs
Hha1Buffer	New England BioLabs
Msp1 Buffer	New England BioLabs
Agarose powder	Invitrogen
Ethidium bromide(20ml)	Hylabs
TBE (10X)	Sigma
Ladder 50bp	Promega

Table 1.2: Materials and Consumables

Materials	Provider
Sterile PLAN vacutainer tubes	Vacuumed
Sterile EDTA vacutainer tubes	Vacuumed
Needles	BD Microlance
Syringes	Consultant medical company
Centrifuge	Hettich
Sterile urine cup	Consultant medical company
Microcentrifuge	HERMLE
Mini microcentrifuge	Qik Spin
Micropipettes	Human
Sterile aerosol pipet tips	Labcon

Vortex	Stuart
Heating block	Labmet
Microwave	Mega
Flask	SCHOTT
Electrophoresis	BIORAD
Gel tray	BIORAD
Thermo cycler	Biometrat advanced
NanoDrop Analyzer	Implen
Analytical balance	Adam Equipment

العلاقة بين تعدد الأشكال الجينية للببتين ومستقبلات اللبتين ومستوى اللبتين في المصل لدى مرضى

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ملخص

الخلفية:

يُعتبر الهرمون الصمّاوي المسمى ليبتين(LEPTIN) ، وهو النظير البشري لجين LEP، أحد الجينات المرتبطة بالسمنة. يلعب دورًا مهمًا في تنظيم تناول الطعام، والتحكم في إفراز الهرمونات العصبية، والتمثيل الغذائي، وتراكم الدهون. كما تُظهر بعض الدراسات التجريبية أن الليبتين قد يؤثر على ضغط الدم من خلال التنشيط الودي سواء على مستوى الدورة الدموية أو الكلى، مما قد يساهم في تطور ارتفاع

ضغط الدم. بالإضافة إلى ذلك، أشارت حقن الليبتين الدقيقة إلى أن المستويات المرتفعة منه تؤدي إلى تأثيرات أكبر على التنشيط الودي.

الأهداف:

تهدف الدراسة إلى التحقيق في العلاقة بين تعدد أشكال الجينين *LEP* و *LEPR*وحدوث ارتفاع ضغط الدم.

المنهجية:

أُجريت الدراسة على 45 مريضًا يعانون من ارتفاع ضغط الدم ويمتلكون مؤشر كتلة جسم < (BMI) 30في مراكز وزارة الصحة بجنين، بالإضافة إلى 45 مريضًا آخرين يعانون من ارتفاع ضغط الدم ولكن لديهم مؤشر كتلة جسم .25 ≥ (BMI) تم جمع عينات الدم في أنابيب EDTA وأنابيب PLANE لجميع المرضى، واستخراج الحمض النووي (DNA) من عينات الدم في أنابيب .EDTA جرى تحديد تعدد أشكال الجينين *LEP* و *LEPR*باستخدام تقنية تعدد الأشكال لطول القطع الناتجة عن التقييد . (RFLP) كما وتم استخدام مصل الدم لقياس تركيز هرمون اللبتين في الجسم ومقارنته بين مجموعات المرضى.

النتائج:

بالنسبة لنتائج تقنية PCR-RFLPلجين LEP، كانت نسبة المرضى الذين يحملون النمط الجيني المتماثل للحالة الطبيعية 0% (wild type) مقارنة بـ 20% في المجموعة الضابطة. أما النمط الجيني غير المتماثل فتم رصده بنسبة 27% لدى المرضى مقارنة بـ 23% في المجموعة الضابطة. وتم الإبلاغ

عن النمط الجيني المتماثل بنسبة 18% لدى المرضى مقارنة بـ 13% في المجموعة الضابطة. كانت هذه النتائج ذات دلالة إحصائية عالية (P=0.006). بينما لم تظهر دلالات إحصائية لتعدد أشكال *LEPR*.

بلغ متوسط تركيزات الليبتين 13.7 ± 12.5 (متوسط ± انحراف معياري) لدى 45 مريضًا بمؤشر كتلة جسم 30 <، مقابل 41.3 ± 17.7 لدى 45 مريضًا بمؤشر كتلة جسم 25 >. وكانت هذه النتائج ذات دلالة إحصائية عالية (P<0.001).

الخلاصة:

تشير نتائج الدراسة الحالية إلى دليل قاطع بأن تعدد أشكال جين *LEP* مرتبط بقابلية الإصابة بارتفاع ضغط الدم. وتُظهر الدراسة أن النمط الجيني *LEP* AA يُعد عامل خطر مستقل لتطور ارتفاع ضغط الدم لدى الأفراد المصابين بالسمنة. ولإثبات العلاقة المؤكدة بين هذه التعددات الجينية وخطر الإصابة بارتفاع ضغط الدم، يلزم إجراء المزيد من الدراسات.

الكلمات المفتاحية: هرمون اللبتين، مرض ارتفاع ضغط الدم، معدل كتلة الجسم.