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RESEARCH ARTICLE

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## MOLECULAR INVESTIGATION OF LEPTIN PROTEIN IN THE REVERSAL OF TYPE-II DIABETES MELLITUS

Maha Amin<sup>1</sup>, M Ali<sup>2</sup>, Ahmad Naveed Bhatti<sup>2</sup> and Samreen Riaz<sup>1\*</sup>

<sup>1</sup>Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore. Pakistan; <sup>2</sup>Pakistan Academy of Family Physician, Pakistan

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\*Corresponding author: Samreen Riaz

### ABSTRACT

Insulin resistance and poor glucose control are two features of type 2 diabetes mellitus (T2DM), a chronic metabolic condition. Leptin, a hormone generated from adipose tissue, has become recognized as a possible therapeutic target in the treatment of type 2 diabetes. The current understanding of leptin's role in T2DM reversal is summed up in this abstract. Leptin has been discovered to have a variety of metabolic functions in addition to its traditional recognition for controlling hunger and energy balance. Leptin resistance frequently coexists with high levels of circulating leptin in people with obesity and type 2 diabetes, resulting in decreased responsiveness to its metabolic effects. Recent research, however, has shown that exogenous leptin injection can significantly enhance insulin sensitivity and glycemic control in some subgroups of T2DM patients.

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## INTRODUCTION

Diabetes is a medical illness, where body fails to regulate normal glucose concentration in blood (Zimmet *et al.*, 2014). Depending upon the disrupted body functions, diabetes has two main types diabetes insipidus, diabetes mellitus. Diabetes mellitus have further two types depending upon the age of onset of disease. Diabetes Mellitus Type-I is a rare disease usually start at younger age, resulting because of mutation that damage the beta cells of pancreas and affecting the normal insulin production (Katsarou *et al.*, 2017; Afifa, 2018). Whereas Diabetes Mellitus Type-II diabetes usually develops later in life and is primarily caused by poor lifestyle choices. It represents 90% of diabetes cases and has a rapidly increasing global prevalence (Harrison *et al.*, 2023). Type-II diabetes is often associated with abnormalities in lipoproteins, leading to macrovascular and microvascular complications. Diabetic patients have a higher incidence of vascular disease compared to non-diabetic individuals. According to the International Diabetes Federation, the incidence of diabetes is projected to affect 7.6% of the world's population by 2015, with an estimated 438 million people being diabetic in the 2030s. The prevalence of both Type-I and Type-II diabetes is increasing, with Type-II diabetes rising dramatically due to factors such as physical inactivity and obesity, especially in urban areas.

In 2020, India had the highest number of diabetes cases in the world, with an estimated 35 million patients, expected to reach 75 million by 2030 (Shojima and Yamauchi, 2023). Insulin resistance is the main cause of type-II Diabetes mellitus but some common features are hypertension, obesity, hyperinsulinemia, atherosclerosis and abnormal lipid profile as increase in cholesterol, triglyceride and decrease in HDL level, anti-diabetic treatment along with aggressive management of lipid profile not only cure the type-II DM complications but also control the mortality rate (Kasper *et al.*, 2015). Symptoms of Diabetes mellitus includes fatigue, slow healing of wounds, pain, tingling, numbness in hands and feet, blurry vision, excessive hunger and thirst and urination (Alidu *et al.*, 2023). The diagnosis of Type-II DM can be made when symptoms are missing. It is done when OGTT is  $\geq 200$ mg/dL or when FPG is  $\geq 126$ mg/dL or when HbA1c is  $\geq 48$  mmol/mol (Inzucchi, 2012). On the other hand, when symptoms are present, diagnosis is done when blood glucose level is  $\geq 200$  mg/d. A chronic hyperglycemia increased the risk of micro and macrovascular complications including neuropathy, retinopathy and nephropathy. While longstanding Diabetes include cardiovascular disease, congestive heart failure, myocardial infarction, stroke (ElSayed *et al.*, 2023). Diabetes mellitus type 2 is not curable but it can be prevented by diet and weight loss (Taylor *et al.*, 2019). Adapting healthy lifestyle including (exercise and low-fat diet) can reduce the risk of this disease by 95%. Anti-diabetic drugs are available in market that can keep the glucose level within limits.

Proteins obtained from animals are mostly associated with fats, while proteins from plant sources are less associated with fats (as in legumes), therefore they are used to reduce the progression of Type 2 Diabetes mellitus. These proteins act as enzyme that cause lipolysis of fats in liver, fat degradation play a role in controlling obesity that is a strong risk factor of diabetes, it may act to repair the defects in insulin secretion pathways hence improving insulin concentration in blood. Some proteins may act as antioxidants to improve the cell's sensitivity to insulin (Harvey and Stephens, 2023). Leptin is a protein produced in adipose tissue and involved in maintaining normal blood glucose level in body (Meek and Morton, 2016). Genes encoding leptin are located on chromosome number 7, it encoded a protein of molecular weight 16 kDa. It is released into blood stream and acts on hypothalamus and play role in reducing glucose concentration, hunger, blood, insulinemia, insulin resistance (Srivastava *et al.*, 2023). Leptin is a body secretion that is secreted by white adipose tissues (fat cells). It plays a vital role in controlling fats and body metabolism. Its major site of action is brain where it reduces the sensation of hunger when there is enough energy available to body. In patients with Diabetes Leptin plays an imperative role in adjusting sugar level of blood and sensitivity to insulin (Fathy *et al.*, 2023).

### METHODOLOGY

Performed Blood screening of patients with Diabetes mellitus Type-II, isolated genomic DNA and performed Polymerase chain reaction using gene specific primers. Performed Cloning of gene encoding Leptin into cloning vector pTZ57R/T.

After confirmation of cloning of gene encoding Leptin in cloning vector, the template was transferred to expression vector pET21a in order to check the gene expression of Leptin in Diabetes patients. Performed Bradford assay to quantify the protein produced in patients. The level of gene expression of Leptin was checked through high performance liquid chromatography. Performed statistical analysis to check various physical and biochemical parameters in patients with Diabetes mellitus type-II. In order to check the effect of diabetes on behavior and physical activities of patients performed Chi-square test and T-test.

### Gene Sequence of Leptin Protein

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GTAGGAATCGCAGCGCCAGCGGTTGCAAGGTAAGGCCCCG
GCGCGCTCCTTCCTCTCTCTGCTGGTCTTTCTTGGCAGGC
CACAGGGCCCCACACAACCTCTGGATCCCAGGGAAACTGAG
TCAGGAGGGATGCAGGGCGGATGGCTTAGTCTGGACTAT
GATAGCTTTGTACCGAGTTCTAGCCAGATAGAAGGTTACCG
GGAGCTGGGGAGCGTTGGATTTGCTGCTGGGCTGTGCCGGT
GCCAGAAGGCAGGACCTTGCAGAACCAGCCAGGTCCCTG
GGAGACTGTCAGACCCACCAACCTGGTGGCATTTCGCAGAG
CTGAGATGCATTGGAAATTGCCTTGGGCACATCCCCAAGA
TCAGGATGTCCCACCCAGTCTGAAGGAGATAAAGTTGGG
GGTAGGAGAGACGCAGATGCAAGTGATCAGTCTCAGTCCC
AGACATTGCCTTGCTCTGCGGGTAGGAATTCAGGATTCATT
TTCCAGGGAAGTTCCTGACCTCTGAATGAGAGGGGGCTGTG
AAGGCCAATGCCTGGGAGGAAGGCAAGGATGAGTAGAGGT
GGGGGAAACAAGTGTGAGGAAGACTCAAAATCTTCCAGA
GAAATTGTGCAGGGTCTTACCAGATCTGTCTCAAAGCCAT
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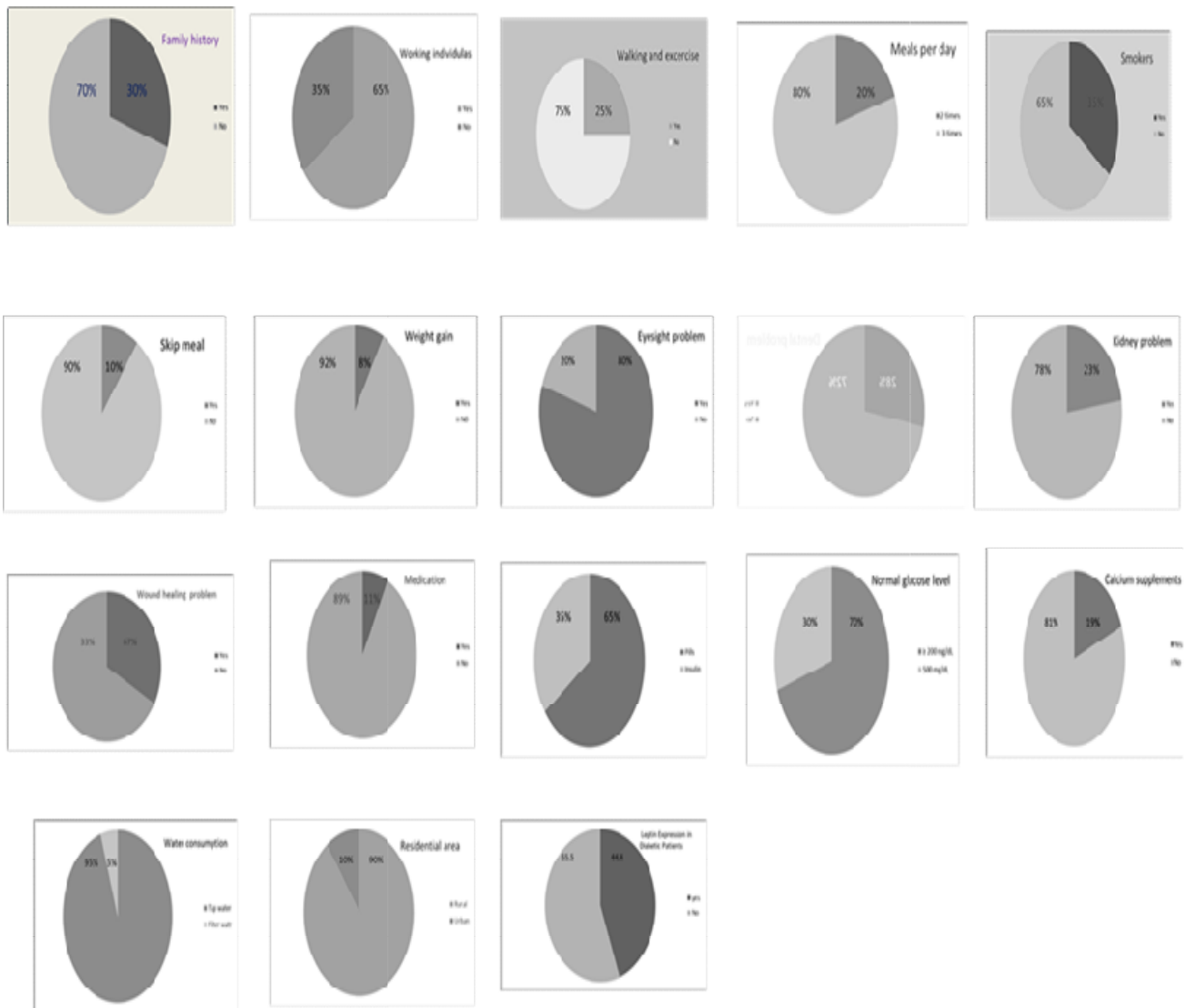
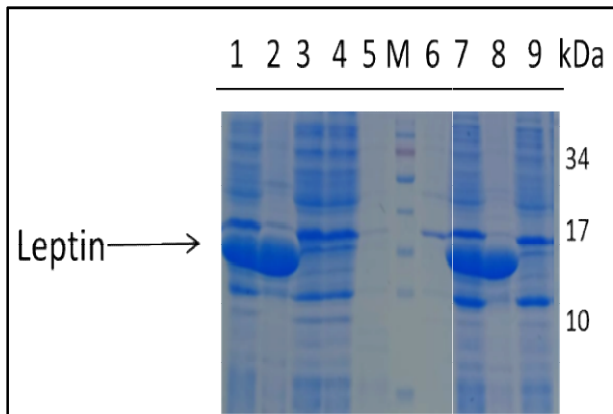
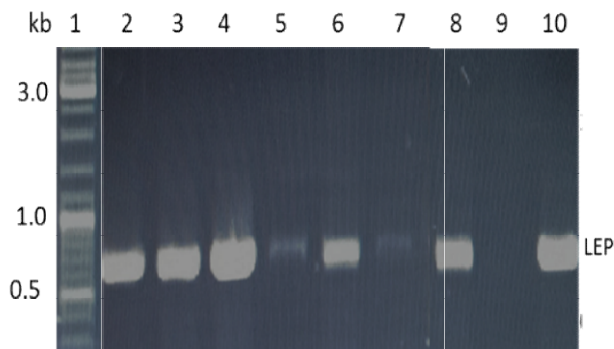


Figure 1: Showing the analysis of physical and biochemical parameters of patients affected by Diabetes. Family History, low physical activity, smoking, obesity were found to be the major risk factors for diabetes while major areas affected by diabetes were eyesight, kidneys, dental health. Most of the diabetes patients preferred diabetes pills over insulin injection

GCAAATTGCCTTCTTTGCAATGCATACAATGAGGTGTCTCT  
GGGGGTCAGAACTGGTTATTAGGGAACCTTAGCCAGGACT  
GCTAAATACGCTAG.



**Figure 2. SDS-PAGE showing Gene expression of Leptin in Patients with Diabetes mellitus Type-II, Lane 6 showing prestained protein marker while remaining Lane 1-5 and Lane 7-10 showing Gene expression of Leptin. A bright band of gene expression is present at position of 15 kDa, out of 9 patients gene expression is found in only 4 patients**



**Figure 3. Showing colony PCR of transformants.LEP-3952-pTZ Lane 1-9 transformed colonies of ligated LEP-3952-pTZ. Lane 10, Positive control: Lane 11, Negative control. Lane M, DNA Ladder mix (Thermo fisher Scientific Cat # SM0331).**

## CONCLUSION

Diabetes mellitus Type-II is a severe metabolic disease that cannot be cured but it can be prevented through lifestyle modification and diet changes. Leptin play an important role in reversal of Diabetes, cloning of gene encoding Leptin is performed in this research work. Gene encoding Leptin was cloned in Cloning vector and then in expression vector. Gene was cloned successfully in both cloning and expression vectors but gene expression was not found equally in all patients under study. Gene expression was induced using IPTG as inducer, which work under T7 promoter. Poor gene expression was attributed to obesity, gene mutation, insulin resistance and other congenital diseases. While overproduction of protein was due to chronic kidney disease in Diabetic Patients. A number of Demographic features were found abundant in Diabetic patient, the most common of them is unhygienic living conditions including Poor lifestyle, taking fatty diets, lack of exercise, consumption of contaminated water, living in unsanitary conditions. Malformations associated with diabetes include Eyes and dental problem while kidney, heart and wound healing problem was not found abundantly in my research participants.

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