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Original Research Article

Genetic Variation of Leptin Hormones in Hemodialysis Patients in Thi-Qar Province/ Iraq

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Name of Author:
Ali Manhal Ibrahim[1], Mohammed Ajah Aouda[2], Kadhim M Manhil[3]

Affiliation: *[1]Department of chemistry, College of sciences, university of Thi-Qar ,Thi-Qar ,64001, Iraq*
[2]university of Thi-Qar, college of sciences,Department of chemistry.
[3]university of Thi-Qar, college of Medicine.

Corresponding Author:
Mohammed Ajah Aouda, Kadhim M Manhil

Abstract: Chronic kidney disease (CKD) is a complex, progressive chronic condition that is defined by either abnormalities of kidney structure or function present for ≥3 months, it is end-stage renal disease (ESRD). Either markers of kidney damage or decreased glomerular filtration rate may be present. dialysis procedures stimulate protein degradation and reduce protein synthesis; these responses persist following dialysis, which might lead to loss of muscle mass. Having a specific genetic polymorphism can be a predisposing factor to developing some diseases. This study aimed to investigate the linked between Leptin gene SNP with the development of CKD. Genomic DNA from blood samples were extracted by using DNA extraction kit (Geneaid, Uk), then Leptin gene was amplified through conventional PCR and SNP was discover using restriction enzyme SmaI. The present study recorded the CC and TC genotypes at 370 positions increased non-significantly in control group, while the TC genotype increased non-significantly in dialysis patients than control group. The frequency of C allele increased non-significantly in control group than dialysis patients, while the T allele increased non-significantly in dialysis patients than control group, also recorded by odds ratio a non-significant between patients and control at p. value < 0.05.

Keywords: Leptin gene, SNP, CKD, Dialysis, RFLP..

INTRODUCTION

The prevalence of chronic kidney disease (CKD) is increasingly becoming recognized as a global health concern as well as a critical determinant of poor health outcomes. Decreased access to health care and low socioeconomic status (SES) worsen the adverse effects of biologic or genetic predisposition to CKD[1]. Glomerulonephritis (GN) are relatively rare kidney diseases with substantial morbidity and mortality, can lead to chronic kidney disease (CKD) and end stage kidney disease (ESKD)[2]. It is typically defined as a reduction in kidney function, estimated glomerular filtration rate [eGFR] <60 ml/min/1.73m2, or markers of kidney damage, such as albuminuria, hematuria or abnormalities detected on imaging, present for at least 3 months[3]. Although creatinine clearances can be calculated from urine creatinine concentration measured in a 24-hour urine collection and a concomitant serum creatinine concentration, a more practical approach in the office is to estimate GFR (estimated GFR or eGFR) from the serum creatinine concentration, using either the Cockcroft-Gault or the Modification of Diet in Renal Disease (MDRD) Study estimating equations[4] .There are many causes of CKD, including those that more common and well-researched such as older age, diabetes, Hypertension and cardiovascular disease , In addition to that accumulated uremic toxins, increased

oxidative stress, endothelial dysfunction, and mineral and bone disorders[5]. Creatinine and urea levels are increased in both males and females in chronic kidney disease[6]. Serum creatinine as well as urea are well-known indicators of GFR, although serum creatinine is the most sensitive indicator of renal dysfunction. Every time there was poor kidney function or kidney damage, the urea value increased. Kidney damage resulting from increased blood sugar levels when the urea concentration rises with the sugar level[7].

Leptin is a peptide hormone secreted by adipose tissue that was identified by positional cloning of the ob gene responsible for the development of obesity[8]. It is a product of the ob gene and is associated with obesity because increasing adipose tissue mass leads to higher leptin levels[9]. Leptin levels increase in proportion to the body's energy reserves in the form of stored fat, and leptin thus serves as an indicator of energy availability[10]. leptin then increases metabolic rate[11]. Leptin gene is located on chromosome 7q32.1, spanning approximately 20 kb with three exons separated by two introns[12]. The level of the hormone leptin increases in patients with type 2 diabetes[13].

LEP, and LEPR genes are involved in the hypothalamic leptin-melanocortin regulation pathway, which is important for energy homeostasis[14]. Leptin is helps to regulate the hypothalamic-pituitary-gonadal axis[15]. A study showed no significant alterations in the serum leptin concentrations, which reflects that peripheral signals released from adipocytes are not important in this group[16]. Thus, a decrease in renal function is suspected to be a major cause of hyperleptinemia in CKD. Previous studies have shown that increased serum leptin levels are seen at an early stage of CKD. Moreover, serum insulin seems to be more strongly related to serum leptin rather than to GFR per se[17,18]. Leptin plays an important role in the development of hypertension[19]. It regulates energy homeostasis through signal transduction in the arcuate nucleus of the hypothalamus where it interacts with NPY and AgRP, acting as an antagonist to ghrelin[20]. Leptin suppresses food intake by counteracting the activity of neurons containing NPY and AgRP and by increasing the activity of neurons expressing α-melanocyte stimulating hormone (α-MSH)[21]. In a study of energy homeostasis in hemodialysis (HD) patients, acyl ghrelin levels were low and leptin levels were elevated[22]. Leptin is cleared from the circulation by the kidney by glomerular filtration followed by metabolic degradation in the renal tubules, which accounts for the elevated level of leptin in CKD[23].

Materials and Method

The Primers Used in the Interaction

The primers were lyophilized, dissolved in free ddH_2O to give a final concentration of 100 pmol/L as stock solution, and stored at -20 °C to prepare a 10 pmol/L concentration as work primer suspended, 10 µL of the stock solution in 90 µL of free ddH_2O water to reach a final volume of 100 µL. The molecular weight markers used in this study for DNA and PCR products were from Korea. The range of the markers were (100-2000 bp).

Table (1) The product names, primers used, annealing temperature and the products size for Lep genes.

Product Name	Product size (bp)	Annealing Temperature	Primer	Oligonucleotide sequence (5′-3′)
Lep	242 bp	56 C	Forward	AAAGCAAAGACAGGCATAAAAA
			Reverse	TTTCCTGTAATTTTCCCGTGAG

Table (2) protocol PCR amplification

Step		Temp.	Time	Cycle
Initial Denaturation		95	5.00 min	1 time
	Denaturation	95	30 sec.	**38 cycle**
	Annealing	56-60	30 sec.	
	Extension	72	40 sec.	
Final Extension		72	10.00 min	1 time

Sequencing and Alignment of Genes

The amplified PCR products were sequenced at Macrogen Company (South Korea) by sanger sequencing used " dideoxynucleotides method". Bioinformatics analysis software, such as Bioedit, was used to analyze the sequencing results after receiving them from the company, revealing the polymorphisms of the studied SNPs[24].

When using the blast tool available in GenBank to search for the sequence of the nitrogenous bases of the lEP gene, and using the Clustal omega tool available on the Internet, comparing two different gene sequences for the CC and TT genotypes, Figure (1).

We note from the result of the alignment between the amino acids resulting from the translation of the codes for the studied leptin gene sequence that the type of mutation occurring is due to changing the base T to C at the lep

site. T173C is a missense mutation, as this mutation, which is located in the code for the amino acid (valin), changes it to the amino acid (alanine) at position 58 of the peptide chain of the leptin protein (Figure 2)

```
 amino acid      I  W  E  L  I  S  V  H  I  K  M  K  S  S  K  F  M  F
                                   L
 TT          1
TTATTTGGGAACTGATAAGTGTCCATATTAAAATGAAATCTTCAAAATTTATGTTCCTCT  60

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 CC          1
TTATTTGGGAACTGATAAGTGTCCATATTAAAATGAAATCTTCAAAATTTATGTTCCTCT  85
amino acid      I  W  E  L  I  S  V  H  I  K  M  K  S  S  K  F  M  F
L
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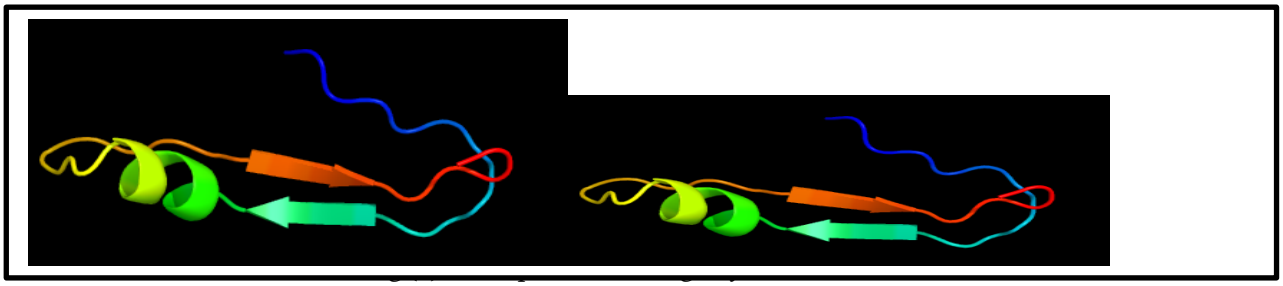
Fig (1) Multiple Alignment (CDs Coding Sequence) between TT & CC polymorphisms for Lep. Gene

With regard to drawing the three-dimensional shape of the peptide chain of the leptin gene and the region studied in the current study, I used the Phyre2 Server tool on the website for the purpose of converting from the amino acid chain as shown in Figure (2) to the PDB file format (Protein Data Bank) for the purpose of drawing the three-dimensional shape of the peptide chain. For the leptin gene and for the region studied in the current study, we obtained a PDB file and used the RasMol program to draw the protein. When comparing the three-dimensional shapes of the three structures (TT & TC) and (CC), the shape did not show any clear difference between the two models, Figure (3), and this may be due to The amino acids (valine) and (alanine) have the same chemical properties, or we guess that both acids are given the same importance through their similar wrapping in both forms. This gives the impression that there are functional differences between these two types of protein, based on the well-known general rule: "The function of the protein depends on "its shape".

The amino acid sequences of the resulting forms of the studied region of the leptin gene were in the International Gene Bank (NCBI), and we obtained independent accession numbers for our current study, which are BET20313 and BET20314, in addition to the sequences of the genotypes of the LEP gene in the International Gene Bank (NCBI), and we obtained independent accession numbers, and the following table shows That table (4):-

```
BET20313.1 (TT,TC) TGIKNYKSIIWELISVHIKMKSSKFMFLCHGSSRSLCSGSLTSCNISALRETEAGGSVQP
                                                          60
BET20314.1 (CC)    TGIKNYKSIIWELISVHIKMKSSKFMFLCHGSSRSLCSGSLTSCNISALRETEAGGSAQP
```



BET20314.1 (CC) **BET20313.1 (TT,TC)**

Genotype and Allele Frequency of Leptin 173.T>C Gene in Dialysis Patients and Control Group

The present study recorded the CC and TC genotypes at 370 position increased non-significantly in control group, while the TC genotype increased non-significantly in dialysis patients than control group. The frequency of C allele increased non-significantly in control group than dialysis patients, while the T allele increased non-significantly in dialysis patients than control group, also recorded by odds ratio a non-significant between patients and control at p. value < 0.05, as in Table 5.

Table 5: Genotype and allele frequency of Leptin 173.T>C gene in dialysis patients and control group

Gene	Genotype	Dialysis P No. 100		Control No. 50		CalX² and p. value	OR 95%CI
		No.	%	No.	%		
Leptin 173.T>C	CC	4	8.89	6	13.33	CalX² 3.21 0.200	Non-OR
	TC	26	57.78	29	64.44		
	TT	15	33.33	10	22.22		
	Allele	**Dialysis P**		**Control**		**CalX²**	**OR 95%CI**
Leptin 173.T>C	C	30	42.25	35	47.30	CalX² 0.50 0.477	0.87 (0.46-1.4)
	T	41	57.75	39	52.70		

DISCUSSION

The clinical outcomes of CKD depend on various factors, including metabolic disorders. Leptin, a peptide hormone, produced in adipose tissues, plays an important role in regulating food consumption and energy expenditure. Leptin also influences the immune system and hematopoiesis. Increased leptin status is observed in CKD, leptin deficiency attenuates the immune response in nephritis. Conversely, leptin inhibits the development of obesity, which is closely associated glomerular disorder. Now, the precise role of leptin in CKD remains elusive. This review will give an integrated understanding of the potential role of leptin and its interactions with other signal molecules in CKD25. Recent studies have drawn attention to the role of leptin (LEP) and leptin receptor (LEPR) gene polymorphisms in the pathogenesis of T2DM26. A few studies have reported on how to identify leptin gene mutation(s)27. developed a gel-free SNP detection for leptin mutation28. In this method, the annealing of the oligonucleotide complementary to the SNP allele led to the cleavage of the oligonucleotide by a cleavage enzyme. The released 5′ arm fragment then served as an invader, leading to a secondary cleavage reaction to release a fluorescent signal from fluorescence resonance energy transfer (FRET) cassettes29. In this study the dominant genotype was TC in both dialysis patients and control group without significant difference p. value 0.20, also, the dominant allele was T in both dialysis patients and control group without significant difference p. value 0.477. the Egyptian study of Abd El-Aziz et al30, not recorded association between LEP gene polymorphism with occurrence of both kidney failure and CVD, while the of Ragin et al31, showed an association between LEP gene polymorphism and disorder of glucose metabolism and CVD. In addition, Wu and Sun32, showed a positive association between leptin gene polymorphism and occurrence of CVD. This study suggested that the occurrence of diabetes, as well as heart disease, is a gateway to kidney failure in the long term. Leptin polymorphisms activate ATP-sensitive potassium channels and cause beta-cell hyperpolarization, which reduces intracellular calcium concentration and inhibits insulin secretion in post-renal transplant patients33. Leptin SNPs rs1137100 and rs1137101 were associated with the risk of obesity, diabetes type 2, gestational diabetes and cardiovascular diseases in various populations34. Also, the study of Dziedziejko et al.35 showed that the polymorphisms in genes involved in leptin and adiponectin function modify long-term outcomes in kidney transplantation. the linking between leptin-related gene polymorphisms to PTDM is not unprecedented. the study of Romanowski et al36, have shown in 323 renal transplant recipients that the rs2167270 SNP of the LEP gene, coding for the leptin protein, was also associated with increased risk of PTDM.

Conclusion

In this study the dominant genotype was TC in both dialysis patients and control group without significant difference p. value 0.20, also, the dominant allele was T in both dialysis patients and control group without significant difference p. value 0.477, not recorded association between LEP gene polymorphism with occurrence of both kidney failure .Further studies are needed to explore the potential mechanisms by which SNP rs2167270 of the LEP gene modulates predisposition to CKD.

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