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Relationship between LSP1 polymorphisms and the susceptibility to chronic kidney disease with hypertensive

Ahmed Mudher AL khaykanee[1], Thulfeqar Ahmed Hamza[2], Soura Alaa Hussein[3]

[1] General Directorate of Education of Babylon Governorate, Iraq

[2] Chemistry Department- College of Science for Women – University of Babylon, Iraq

[3]Al-Rafidain University College, Iraq

Correspondence: Ahmed Mudher Al Khaykanee, A.M. **Address:** Babylon - Hilla - Street 40, Iraq. **Email:** ahmed muder14@gmail.com

Email ahmed.mudhhir23@bab.epedu.gov.iq **Phone:**009647811602922

Email: dolfikarbiochemist@gmail.com , tholfikar_ahmed@uomus.edu.iq

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ABSTRACT

Background: LSP1 gene *polymorphisms have* been tied with some diseases as well as some types of cancer. **Aims:** aimed to Evaluate the link of *LSP1gen* rs569550 and rs592373 linked with CKD with hypertension. **Methods:** 100 patients with CKD without hypertension, 100 patients with CKD with hypertension, and 100 controls were genotyped for *LSP1gen* rs569550 and rs592373 using allele-specific Real-Time PCR analysis.

Results: genotype TT and GT of the *LSP1* rs569550 were associated with a significantly lower risk of CKD with hypertension in patients with CKD without hypertensive [OR (95 % CI) = 0.2 (0.08 – 0.4), P < 0.001and[OR (95 % CI) = 0.38 (0.28 – 1.23), P < 0.001*]. Patients less likely to be affected were carriers of the allele T . CKD with hypertensive than those they have the G allele [OR (95 % CI) = 0.43 (0.29 – 0.67), P < 0.001*].

Related *LSP1*rs592373 the variant genotypes, TC and CC were significantly associated with increased risk of CKD with hypertensive [OR (95 % CI) =4.01 (1.98 – 6.5), P < 0.001 and OR (95 % CI) =8.7 (3.01 – 25.65), p <0.001 respectively]. Similar trends were observed at the allele levels, carriers of the C allele were at a higher risk for developing CKD with hypertensive [OR (95 % CI) = 3.02 (2.05 – 3.98), p< 0.001].

Conclusions: LSP1 rs569550 and rs592373 genes were associated with CKD with hypertensive sensitivity suggesting its inclusion in CKD with hypertensive in CKD without hypertensive patients.

Keywords: Chronic kidney disease, hypertensive, polymorphism, *LSP1*.

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1. INTRODUCTION

The main cause of chronic kidney disease is Hypertension [1]. Hypertension pressure is one of the most important problems that the world suffers from, so it is considered one of the most important causes of death around the world[2, 3], Hypertension is considered a major factor in chronic kidney diseases, blood vessels and the heart, and hypertension pressure is one of the most important causes of chronic kidney disease and a very risk factor for heart disease and the cause of death [4–5] Chronic kidney disease is one of the diseases spread around the world and one of the traditional diseases associated with heart disease and hypertension[6]Chronic kidney disease is defined as a decrease in the glomerular filtration rate, as well as a decrease in

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kidney function, as well as signs of kidney damage such as hematuria, albuminuria, or abnormalities that are detected by laboratory analyzes. Chronic kidney disease is considered an advanced case of kidney disease and is followed by functional changes in the kidneys. [7] High risks of blood vessel and heart diseases such as coronary artery disease, sudden cardiac death, and arrhythmia appear in people with chronic kidney disease, and cardiac risks are greater for people with long-term chronic kidney disease [8].

Leukocyte-specific protein 1 (*lsp1*, located on 11p15.5) gene encoding an F-actin binding protein that is expressed in all hematopoietic cells and endothelial cells which regulates neutrophil morphology and motility, adhesion to fibrinogen matrix proteins, and transendothelial migration [9-10]. (LSP1), an F-actin binding protein and a major downstream substrate of p38 mitogen-activated protein kinase as well as protein kinase C, has been reported to be important in leukocyte chemotaxis. Although its distribution has been thought to be restricted to leukocytes[11]. Studies have found that LSP1 is overexpressed with leukemia, lymphoma, and breast cancer.[12] The study aimed to determine the relationship between nucleotide polymorphisms rs569550 and rs592373 of exon 2 of the LSP1 gene and hypertension in CKD and the correlation between biochemical parameters.

2 - Methods

2.1. Population study

This study was conducted on 300 Iraqis and divided into three groups:

The first group, the control group: included 100 healthy subjects

The second group: included 100 patients CKD with and hypertensive

The third group: included 100 patients with CKD without hypertension.

The patients were attendants at Marjan Specialist Hospital in Babil Governorate during the period from March 2021 to March 2022. Besides.

The diagnosis of patients who suffer from hypertension without CKD as well as people who suffer hypertension with CKD was made by conducting laboratory analyses as well as laboratory tests, Chronic kidney disease is called if the glomerular filtration rate is about 60 ml/min, and the albumin to creatinine ratio is approximately 30 microns per gram. Blood pressure was measured after resting for about 15 minutes.

The control group included 46 men and 54 women, CKD with hypertensive 58 men and 42 women. Patients with CKD without hypertension included 54 men and 46 women.

The Ethics Committee at the University of Babylon approved the study, as well as all patients agreed to the study before it began

Many clinical tests were performed on the control group and the patients, such as cardiovascular tests, Renal function tests, and liver function tests(LFT). and a full history of hypercholesterolemia was taken for the patients.

2.2-Sample and collection

The blood was collected from the subjects in this study after fasting for about 12 hours and the blood was divided into two groups, the first part was taken from the blood and placed in tubes containing EDTA to extract the DNA from it, and the second part, the serum was separated from it by a centrifuge at a speed of 3000 revolutions per minute and for 10 minutes to conduct many clinical tests such as tests of kidney functions, liver functions and Lipid profile. The technique of Pcr by which DNA extraction and genotyping were carried out at the Babil University Faculty of Science, Department of Chemistry, Biochemistry Laboratory

2.3.SNP Genotyping of LSP1 rs569550 and rs592373 by Real-time PCR

Using Quick-genomic DNA™ Mini DNA extracted from blood as well as LSP1 SNPs (rs 569550 and rs592373) were genotyped using Real-Time PCR

The genotype reaction mix was prepared using Taq Man universal master mix II (2x), supplied by Applied Biosystems, Foster City, USA, 2010. The manufacturer described probes: [VIC/FAM] for **SNP2** (rs569550) was: 5’-

CTGTCACCTGCTCACACCTC-3' [G/T] 5'-GGAGAGGTGTGAGCAGGTGA-3'
For **SNP1** (rs592373) was: 5'-GTGCTCCAAAGACGGGCGGT-3' [T/C] 5'-CTCCTACCGCCCGTCTTTGG-3'. Both primers and probes were purchased from an Applied Biosystem, Foster City, USA 2010.

2.4.Statistical Analysis

The SPSS program was used to analyze the obtained data The average ± SD was used to express the results We used a test Post Hoc To compare the pairs of each two groups And to determine the difference between the control and test groups, using the ANOVAF test. The allocation of genotypes for LSP1569550 and rs592373 genes were assessed for the Odds ratio and 95% confidence intervals Hardy-Weinberg heredity equilibrium using the $\chi 2$ test or Fisher's exact test.

3-Results

3.1. clinical and biochemical characteristics of the studied groups:

The study included 300 samples [100 patients with CKD without hypertension, 100 patients with CKD with hypertensive and the study contained 100 samples of healthy people. Clinical and biochemical characteristics of Table 1 shows the studied aggregates: This table shows a significant between group II and group III compared to controls as, regards Total Cholesterol, TG, HDL, ALT, AST, GGT, Albumin, Uric acid, Urea, Creatinine and GFR. While the other parameters did not have clear differences

3.2. Genotypes and alleles allocation

The genotypic and allelic allocation for the two SNPs (*LSP1* rs569550 and *LSP1*rs592373) are shown in **Table 2**. Genotype allocation of *LSP1* rs569550 gene G/T polymorphism showed a significant statistical difference among the three studied groups. The GT genotype was more allocated among CKD without hypertensive and CKD with hypertensive patients, while the GG genotype was the most prevalent in the control group (< 0.001). Allelic allocation showed that the T allele was significantly higher in CKD without hypertensive group compared to CKD with hypertensive group and controls (< 0.001).

There were marked differences in genotype allocation of *LSP1*rs592373 T/C polymorphism between the three studied groups. The TC genotype was the prevalent genotype among CKD with hypertensive patients, while the TT genotype was more allocated in CKD without hypertensive group and controls (< 0.001), and the C allele was the most frequent in CKD with hypertensive group compared to CKD without hypertensive group and controls (< 0.001).

The observed genotype frequencies for the two polymorphisms in CKD without hypertensive group, CKD with hypertensive group, and control group were all in Hardy–Weinberg equilibrium except for *LSP1* rs592373 in CKD without hypertensive group as shown in **Table 3**.

3.3-Association. of genotypes. and allele polymorphisms *in LSP1* rs569550 and *LSP1*rs592373 *with* CKD with hypertensive *risk*.

Table 4 As shown**,** the variant genotype TT and GT of the *LSP1* rs592373 was associated with a significantly lower risk of CKD with hypertensive in patients with CKD without hypertensive [OR (95 % CI) = 0.2 (0.08 – 0.4), P < 0.001 and[OR (95 % CI) = 0.38 (0.28 – 1.23), P < 0.001*]. and patients carrying the T allele were at lower risk of developing CKD with hypertension than those carrying the G allele [OR (95 % CI) = 0.43 (0.29 – 0.67), P < 0.001*].

Regarding *LSP1*rs592373, the variant genotypes TC and CC were significantly associated with increased risk of CKD with hypertensive [OR (95 % CI) =4.01 (1.98 – 6.5), P < 0.001 and OR (95 % CI) =8.7 (3.01 –25.65), p <0.001 respectively]. Similar trends were observed at the allele levels, carriers of the C allele were at a higher risk for developing CKD with hypertensive [OR (95 % CI) = 3.02 (2.05 – 3.98), p< 0.001].

Table 1: clinical and biochemical characteristics of the studied groups.

Variable Mean ± SD.	**Group I (n = 100)**	**Group II (n = 100)**	**Group III (n = 100)**	**p-value**
Gender:				
Male n. (%)	60	57	55	0.235
Female n. (%)	40	43	45	
Age (years)	49.54 ± 9.07	54.90 ± 7.14	58.62 ± 6.32	0.075
Fasting blood sugar (mg/dl)	100.03 ± 10. 7	99.6 ± 8.9	97.8 ± 10.81[a]	<0.072
MDA (µmol/L)	4.9 ± 1.6	5.3 ± 1.8 [a]	4.8 ± 1.8[a]	<0.093*
Total Cholesterol (mg/dl)	178.01 ± 25.67	235.1 ± 35.11 [a]	216.6 ± 32.03[a]	<0.001*
TG (mg/dl)	137.5 ± 24.41	261.1 ± 48.2 [a]	205.2 ± 51.02[a]	<0.001*
HDL (mg/dl)	58.5 ± 5 .6	35.71 ± 4.35 [a]	39.21 ± 5.45[a]	<0.001*
ALT(IU/L)	19.2 ± 4.8	19.2 ± 6.01 [a,b]	20.5 ± 6.4 [a]	<0.001*
AST (mg/dl)	19.1 ± 4.81	22.5 ± 7.2 [a]	24.01 ± 7.3[a]	<0.001*
GGT (IU/L)	34.07 ± 14.15	158.9 ± 11.07 [a,b]	86.55 ± 17.09[b]	<0.001*
Albumin (mg/dl)	4.65 ± 0.33	3.11 ± 0.73 [a]	3.41 ± 0.63[b]	<0.001*
Uric acid(mg/dl)	6.53 ± 1.8	9.33 ± 2.15 [a]	8.99 ± 1.35	<0.001*
Urea (mg/dl)	31.6 ± 6.1	71.13 ± 10.5 [a]	67.5 ± 11.3[a]	<0.001*
Creatinine (mg/dl)	1.02 ± 0.07	7.5 ± 1.09 [b]	7.12 ± 1 .1[a]	<0.001*
GFR (mg/dl)	82.5 ± 6.6	28.7 ± 10.5[a]	27.5 ± 10.93[b]	<0.001*
Diastolic BP	80.02 ± 3.11	98.5 ± 11.03[a]	80.02 ± 3.11[a]	<0.001

* Statistically significant at $p \leq 0.05$.
a significantly differs from the control group.
[b] significantly differs from group II.

Table 2: Genotypes and alleles allocation.

	Group I (n = 100)		**Group II (n = 100)**		**Group I II (n = 100)**		χ^2	**p**
	No.	%	**No.**	**%**	**No.**	**%**		
LSP1 **rs 569550**								
GG	55	(55%)	24	(24%)	12	(12%)		
GT	34	(34%)	57	(57%)	50	(50%)	51.48*	<0.001*
TT	11	(11%)	19	(19%)	38	(38%)		
Allele								
G	144	(72%)	105	(52.5%)	74	(37 %)	49.73*	<0.001*
T	56	(28%)	95	(47.5%)	126	(63%)		
LSP1**rs 592373**								
TT	78	(78%)	20	(20%)	52	(52%)		
TC	18	(18%)	62	(62%)	42	(42%)	71.42*	<0.001*
CC	4	(4%)	18	(18%)	6	(6%)		
Allele								
T	174	(87%)	102	(51%)	146	(73%)	67.25 *	<0.001*
C	26	(13%)	98	(49%)	54	(27%)		

*: Statistically significant at $p \leq 0.05$

Table 3: Distribution of observed and **Expected** genotype frequencies and their consistency with Hardy-Weinberg.

	Observed	Expected	χ^2	p
LSP1 **rs 569550**				
GroupI (n= 100)				
GG	55	52.3		
GT	34	39.4	1.152	0.279
TT	11	8.3		
Group II (n= 100)				
GG	24	32.1		
GT	57	45.1	1.93	0.181
TT	19	22.8		
Group III (n=100)				
GG	12	12.1		
GT	50	49.7	0.036	0.872
TT	38	38.2		
LSP1 **rs592373**				
GroupI (n= 100)				
TT	78	75.2		
TC	18	22.1	5.787	0.131
CC	4	2.7		
GroupII (n= 100)				
TT	20	21.0		
TC	62	54.5	2.55	0.017*
CC	18	24.5		
Group III (n=100)				
TT	52	52.9		
TC	42	39.3	0.822	0.358
CC	6	7.8		

*: Statistically significant at $p \leq 0.05$

Table 4: **Association. of genotypes. and allele polymorphisms *in LSP1* rs569550 and *LSP1*rs592373*with* CKD with hypertensive *risk***

	p_1	OR_1 (CI. 95%)	p_2	OR_2 (CI. 95%)	p_3	OR_3 (CI. 95%)
LSP1**rs 569550**						
Genotype						
GG		1		1		1
GT	0.001	0.38 (0.28 – 1.23)	<0.001*	3.2 (1.61 – 5.57)	<0.001*	4.14 (2.46 – 10.55)
TT	<0.001*	0.2 (0.08 – 0.4)	0.012*	3.11 (1.26 – 7.55)	<0.001*	7.9 (5.87 – 35.41)
GT+TT	0.014*	0.32 (0.21-0.85)	<0.001*	3.12 (1.68-5.43)	<0.001*	6.99 (3.6-13.44)
Allele						
G®		1		1		1
T	<0.001*	0.43 (0.29 – 0.67)	<0.001*	3.02 (1.6 – 1.01)	<0.001*	3.9 (1.9 – 5.87)
LSP1 **rs592373**						
Genotype						
TT®		1		1		1
TC	<0.001*	4.01 (1.98 – 6.5)	<0.001*	12.55 (5.42 – 25.96)	<0.001*	2.95 (1.64 – 6.54)
CC	<0.001*	8.7 (3.01 –25.65)	<0.001*	20.32 (6.34 – 67.34)	0.21	2.41 (0.82 –7.52)
TC+CC	<0.001*	3.95 (2.55-7.95)	<0.001*	15.22 (7.52 – 27.56)	<0.001*	3.07 (1.92-5.62)
Allele						
T®		1		1		1
C	<0.001*	3.02 (2.05 – 3.98)	<0.001*	5.02 (2.83 – 9.43)	<0.001*	2.01 (1.04 –3.44)

p_1: p-value for Group IIvs Group I
p_2: p-value for Group II vs. Group III
p_3: p-value for Group I vs. Group III

OR1: Odds ratio for Group II vs. Group I
OR2: Odds ratio for Group II vs. Group III

OR3: Odds ratio for Group I vs. Group I II

CI: Confidence interval
*: Statistically significant at $p \leq 0.05$

4. Discussion

Chronic kidney disease always occurs in people with high blood pressure and diabetes, and it is considered one of the advanced diseases due to the difficulty of its treatment, its rates are increasing, and the resulting deaths are increasing [13] Patients with chronic kidney disease do not show symptoms early, and when the condition progresses, symptoms appear late in the disease [14].

In this study, we examined gene LSP1 which is associated with different types of disease and is associated with the process of hypertension as well as chronic kidney disease, namely, LSP1 rs 569550 and LSP1rs 592373, and to the best of our knowledge, this study is the first attempt to investigate the association. Polymorphisms of this gene are associated with the risk of chronic kidney disease in hypertensive patients.

LSP1 is a marker for fibrocytes and has been reported to be important in leukocyte chemotaxis taxis LSP1 is a substrate of p38 mitogen-activated protein kinase and protein kinase C [15]

It contains LSP1 in humans a large number of amino acids. Human LSP1 has two putative Ca2-binding isoforms and is distributed at three different sites 60% in the cytosol 15% in the cytoskeleton and 25% on the cytoplasmic face of the plasma membrane. [16]

a study conducted by Jian Chen et al showed LSP1 genes associated with BC risk and increased risk for Caucasians and Asians [17]

Chen, Hai, et al., showed The genotype distribution of the TT genotype and the T allele in the rs569550 genotype, CC and C allele are correlated in 592373 rs in the gene LSP1 associated with BC risk [18]

in this study, we observed a significant difference in the distribution of LSP1 rs592373 genotypes and alleles among the studied groups The genotype TC was the highest frequency, CKD with hypertensive patients. While was TT genotype in CKD without hypertension is the most frequent group and control group. The dominant allele in the genetic distribution was the C allele in CKD with hypertensive patients group compared to CKD without hypertensive group and control group. There was a significant correlation between LSP1 rs592373 and CKD in hypertensive patients, occurrence, specifically, the TC and CC genotypes and C allele.

In our study, the importance of LSP1 gene rs569550 G/T Genetic polymorphisms in CKD with hypertension and CKD without hypertension was evaluated. In our study, the statistical analysis showed that there were statistically significant differences in the allele frequencies and the genotype of the studied groups of patients CKD without hypertensive Caused by CKD with hypertensive patients are suffering CKD without hypertensive, a major risk of CKD with hypertensive in Iraq and control group with increasing frequency of GT genotype among CKD without hypertensive and CKD with hypertensive patients whereas GG The genotype was the most common in the control group, and the genotype of TT of LSP1 rs569550 was associated with a significant reduction in risk of CKD with chronic hypertension CKD without hypertensive patients The T allele of LSP1 rs569550 It is a protective agent against CKD with hypertensive development in CKD without hypertensive Through the tests, the level of fats in the blood was high in people with hypertensive [19] higher LSP1 levels have been associated with a decrease in the glomerular filtration rate (GFR) and when it happens CKD in the elderly, it leads to activation of infections, as well as weakness in kidney functions in the early stages of the disease. Hypertension, the presence of more conventional cardiovascular risk factors, and no demonstrated high risk of hypertension and systolic hypertension. In people who suffer from chronic kidney disease[20]

5. conclusion

Our study shows that the risk of chronic kidney disease and hypertension is associated with

genotype TT and GT and T allele of LSP1 rs569550, While the study also showed that genotypes TC and CC genotypes and C allele of*LSP1* rs592373 are associated with the risk of developing chronic kidney disease in patients with hypertensive is preferable to increase the number of studies dealing with more discoveries of the role of this SNP in CKD with hypertensive as well as in CKD without hypertensive.

Conflict of interest:

None

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