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Genetic and Biochemical Association to Renal Failure

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Abstract

This study aims to valuate SNPs in *APOL1* and *HAVCR1* genes and levels of serum biomarkers in renal failure (RF) patients with acutely kidney injury (AKI) and chronically kidney disease (CKD). A total of 60 RF-diseased (30 AKI and 30 CKD) and 30 healthy control (HC) individuals, were subjected to sampling of venous blood that tested molecularly by PCR serologically by ELISA to measurement KIM-1, suPAR, TNFR1 and TNFR2. Molecularly, the levels and risk of genotyping frequency for CG in *APOL1* rs136161 were higher in AKI and CKD patients than healthy ones. Additionally, RF patients showed a lower frequency of C allele and higher risk of G. For *HAVCR1* rs6555820 C>A, polymorphism and harm effects of CA and AA genotypes in RF patients were higher; while, frequency and risk of C was lowered and A allele was elevated in RF patents. Significantly, gradual increases in serum KIM-1, suPAR, TNFR1, and TNFR2 were reported markedly in CKD followed by AKI when compared to HC.

Keywords: Allele frequency, Genotyping frequency, KIM-1, SNPs, suPAR, Iraq.

الارتباط الجيني والكيميائي الحيوي بالفشل الكلوي

زينب ضياء الخطيب
قسم الاحياء, كلية العلوم, جامعة واسط, واسط, العراق

الخلاصة

هدفت الدراسة إلى تقييم تعدد أشكال النوكليوتيدات المفردة في جينات *APOL1* و*HAVCR1* ومستويات المؤشرات الحيوية لدى مرضى الفشل الكلوي بالإصابات الكلوية الحادة ومرض الكلى المزمن . خضع 60 مريضًا بالفشل الكلوي (30 مصابًا بإصابة كلوية حادة و30 مصابًا بمرض الكلى المزمن) و30 فردًا سليمًا كمجموعة ضابطة، لجمع الدم الوريدي وفحصه جزيئيًا باستخدام تفاعل البوليميراز المتسلسل ، ومصليًا باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم لقياس مستويات KIM-1 وsuPAR وTNFR1 وTNFR2. اظهرت النتائج الجزيئية في جين *APOL1* rs136161 ارتفاعا في مستوى النمط الجيني CG لدى مرضى الإصابة الكلوية الحادة المزمنة مقارنةً بالأصحاء. بالإضافة إلى ذلك، أظهر مرضى الفشل الكلوي انخفاضًا في تردد الأليل C وزيادة في خطر الإصابة بالأليل G. بالنسبة للمتغير الجيني *HAVCR1* rs6555820 C>A، كان تعدد الأشكال وتأثيرات الضرر للنمطين الجينيين CA وAA أعلى لدى مرضى الفشل الكلوي؛ بينما انخفض تردد الأليل C وخطر الإصابة به، وارتفع تردد الأليل A لدى هؤلاء المرضى. ومن الجدير بالذكر أنه تم الإبلاغ عن زيادات تدريجية ملحوظة في مستويات KIM-1 وsuPAR وTNFR1 وTNFR2 في مصل الدم لدى مرضى القصور الكلوي المزمن الذين تلاه قصور كلوي حاد، مقارنةً بالأصحاء.

1. Introduction

Renal failure (RF) is a disease condition characterized by inability of the kidneys to adequately perform their essential physiological functions including filtration of metabolic waste products, regulation of electrolyte balance, maintenance of acid-base homeostasis, and control of fluid volume [1, 2]. Clinically, RF is classified into two major categories including AKI that characterized by rapid decline in renal function occurring over hours to days; and CKD that manifested typically by irreversible deterioration in kidney function lasting more

than three months [3, 4]. Although, the etiology of RF is multifactorial and varies depending on whether the condition is acute or chronic, certain causes are more predominant across population such as diabetes mellitus, hypertension, obstructive uropathy, glomerulonephritis, and polycystic kidney disease [5, 6]. Additionally, the pathophysiology of RF involves complex mechanisms that induce the renal vasculature and glomeruli, leading to progressive nephron loss and impaired renal function [7-9]. In Iraq, RF represents a growing public health concerns due to increasing the lifestyle-related risk factors. Subsequently, several studies demonstrated that diabetes mellitus and hypertension have consistently identified as the leading causes of RF particularly CKD among approximately 33-49.9% and 22.6-36.5%, respectively [10-12].

Worldwide, despite advances in clinical diagnostic and therapeutic strategies, the heterogeneity in disease onset, progression, and response to treatment remains a major obstacle in effective disease management [13, 14]. In last three decades, the integration of genotyping technologies into nephrology has provided new insights into the molecular mechanisms underlying renal diseases and has opened promising avenues for early diagnosis, risk stratification, and prognostic prediction [15-17]. In general, genotyping refers to the process of determining differences in the genetic makeup of an individual by examining specific DNA sequences including SNPs, insertion, deletion, and structural variation by application the highly throughput genomic technologies such as polymerase chain reaction (PCR) which become possible to identify genetic variants associated with susceptibility to renal failure and its progression [18, 19]. These advances have transformed the understanding of renal pathophysiology from a purely clinical perspective to a more comprehensive molecular and genetic framework [20]. Hence, this study aims to evaluate the polymorphisms of *APOL1* and *HAVCR1* genes in acutely and chronically RF-diseased individuals in comparison with healthy individuals with measurement of related serum markers (KIM-1, suPAR, TNFR1, and TNFR2) to investigate their associations in progression of disease.

2. Materials and Methods

2.1 Samples

The study population was involved a total of 90 individuals who categorized into three groups as following:

1. AKI Group that includes 30 acutely renal failure patients who attended to some private clinician specialists, diagnosed based on clinical and laboratory tools, and subjected to collection of blood samples within 24-48 hours after the diagnosis.
2. CKD Group that includes 30 chronically renal failure patients who attended to some private clinician specialists, diagnosed based on clinical and laboratory tools, and subjected to collection of blood samples within 3-4 weeks after the diagnosis.
3. Healthy control group (HC) that includes 30 individuals who attended to some private clinician specialists, tested clinically and based on laboratory tools, diagnosed as healthy or with simple issues, and subjected to direct collection of blood samples.

In this study that done in Wasit province (Iraq) during September and December (2025), 5ml of venous blood was sampled from each study individual under aseptic conditions to be divided equally into EDTA-anticoagulant (whole blood) and free-anticoagulant tubes, and transferred to the laboratory until cooled conditions. For serology, the free-anticoagulant blood tubes were centrifuged, and the obtained sera were pipetted into labeled 1.5ml Eppendorf tubes. Then, all whole blood and serum tubes were kept frozen (-20°C) until be tested molecularly and serologically, respectively.

2.2 Molecular examination

The whole blood samples of all study individuals were thawed initially at room temperature and subjected to extraction of DNAs following the manufacturer instructions of the gSYNC^{TM} DNA Extraction Kit (Genaid, Taiwan). After checking of purity and concentration of DNA samples, in addition to AccuPower® PCR PreMix kit (Bioneer, Korea) and two sets of primers were utilized to preparation of MasterMix tubes at 25µl final volume. The primers used for the PCR were *APOL1* rs136161 C>G [F (5′-CTC TCT TGC TGG CTT ATG GAA-3′) and R (5′-GCTGTGATGTGGGACTTGTTT-3′)] and *HAVCR1* rs6555820 C>A [F (5′-TTC AAT TTA CAA ATT AGG CAC AGA A-3′) and R (5′-AGA TAT TAA CAG CAA TAA ATA ATA TA-3′)]. In Thermal Cycler system, the modified amplification conditions consisted of 1 cycle initial denaturation (94°C/7min), 35 cycles for denaturation (94°C/30sec), annealing (58°C/30sec) and extension (72°C/30sec), and 1 cycle final extension (72°C/7min). Electrophoresis of agarose-gel (1.5%) stained with ethidium bromide was done at 100volt and 80mA for 90min, and the products size for genotyping of *APOL1* rs136161 C>G and *HAVCR1* rs6555820 C>A SNPs were identified under the UV transilluminator at 128bp and 164bp, respectively [21-23].

2.3 Serological examination

Specific quantitative ELISAs' kits (SunLong Biotech, China) were utilized in the present study to measurement of serum markers including KIM-1, suPAR, TNFR1, and TNFR2. Briefly, the serum samples and kits contents were prepared at room temperature, processed following the manufacturer instructions, and the absorbance was measured at optical density (OD) of 450nm. Then, the ODs for samples in addition to ODs and their respective concentrations of Standard Solution diluents were plotted on the Standard Curve to obtain the concentration of each marker [24].

2.4 Statistical analysis

The obtained data were analyzed statistically using the Chi-square (x^2) in the GraphPad Prism software while the risk was calculated by the Odds ratio (OR) and relative risk (RR) in the MedCalc software. Values were represented as mean±standard error (M±SE) and differences between horizontal large and vertical small letters referred to significant variation at $p<0.05$ [25, 26].

3. Results

3.1 Molecular genotyping

The findings of *APOL1* rs136161 C>G gene polymorphism revealed that the genotyping frequency of CC genotype was increased markedly ($p<0.023$) in values of HC (53.33%) but decreased in CKD (23.33%) when compared to AKI (33.33%). Although, the CG genotype was shown the absence of significant variation ($p>0.05$) between values of HC and AKI, polymorphism was elevated significantly ($p<0.0467$) in CKD (43.33%). The findings of GG genotype were significantly higher ($p<0.0325$) in AKI (30%) and CKD (33.33%) than HC (10%); however, no marked variation ($p>0.05$) between values of AKI and CKD. In comparison between the frequency of genotype(s) in each study group, the findings observed

that CC genotype was increased significantly (p<0.0118) in HC; while, CG was elevated apparently (p<0.0473, 0.0287) in RF AKI and CKD patients (Table 1).

Table 1- Genotyping frequency of *APOL1* rs136161 C>G among individuals of study population

Gene	HC	AKI	CKD	p-value	95% CI
CC	16 (53.33%) Aa	10 (33.33%) Bab	7 (23.33%) Cc	0.023	1.282 - 74.61
CG	11 (36.67%) Bb	11 (36.67%) Ba	13 (43.33%) Aa	0.0467	29.34-48.44
GG	3 (10%) Bc	9 (30%) Ab	10 (33.33%) Ab	0.0325	6.903-55.79
p-value	0.0118	0.0473	0.0287	-	-

Regarding risk (OR, RR) of *APOL1* rs136161 C>G gene polymorphism, risk effect of CG (1.1515, 1.0282) and GG (4.1707, 1.232) genotypes were increased significantly (p<0.0001) in both RF patient groups (p<0.0001) when compared to HC (0.3459, 0.7907), (Table 2).

Table 2- Risk of *APOL1* rs136161 C>G gene polymorphism among individuals of study population

Gene	Total	HC	AKI + CKD	OR	RR	NNT	95% CI
CC	*33*	16 (48.49%)	17 (51.52%)	0.3459	0.7907	11.111 (Benefit)	13.21-3.91
CG	*35*	11 (31.43%)	24 (68.57%)	1.1515	1.0282	89.483 (Harm)	2.35-14.27
GG	*22*	3 (13.64%)	19 (86.36%)	4.1707	1.232	11.459 (Harm)	3.81-11.35
p-value		0.0118	0.0207	0.0001	0.0001	-	-

Allele frequency for *APOL1* rs136161 C>G demonstrated that the C allele was significantly lowered (p<0.0381) in RF study patients, AKI (30.69%) and CKD (26.73%); whereas in contrast, occurrence of G allele was apparently higher (p<0.0317) in RF study patients, AKI (36.71%) and CKD (41.77%) compared to values of HC (21.52%). In comparison to G allele, though the occurrence of C allele was significantly higher (p<0.0202) in HC, it lowered significantly (p<0.0443, p<0.0273) in study patients, AKI and CKD (Table 3). In comparison to HC, the risk (OR, RR) of G allele (2.7039, 1.2054) in RF patients was apparently (p<0.0001) higher than seen in C allele (0.3698, 0.8296), (Table 4).

Table 3- Allele frequency of *APOL1* rs136161 C>G among individuals of study population

Gene	Total	HC	AKI	CKD	p-value	95% CI
C	*101*	43 (42.57%) Aa	31 (30.69%) Bb	27 (26.73%) Cb	0.0381	12.85-53.81
G	*79*	17 (21.52%) Cb	29 (36.71%) Ba	33 (41.77%) Aa	0.0317	7.15-59.51
p-value		0.0202	0.0443	0.0273	-	-

Concerning the *HAVCR1* rs6555820 C>A gene polymorphism, the genotyping frequency of CC genotype was significantly raised (p<0.0305) in HC (56.67%) but reduced in AKI (36.67%) followed more obviously by CKD (20%). On other hand, the polymorphism of CA and AA genotypes was elevated significantly (0.0385, 0.0394) in CKD (43.33% and 36.67%, respectively) and to less extent in AKI (33.33% and 30%, respectively) individuals when compared to HC (30% and 13.33%, respectively), (Table 5). In comparison to CC genotype,

the polymorphisms of CA and AA was decreased markedly in HC (p<0.0118), and elevated obviously in AKI (p<0.0473) and CKD (p<0.0103).

Table 4- Risk of allele frequency in *APOL1* rs136161 C>G among individuals of study population

Gene	Total	HC	AKI + CKD	OR	RR	NNT	95% CI
C	*101*	43 (42.57%)	58 (57.43%)	0.3698	0.8296	13.345 (Benefit)	27.92-5.388
G	*79*	17 (21.52%)	62 (78.48%)	2.7039	1.2054	13.345 (Harm)	5.385-27.92
p-value		0.0202	0.0232	0.0001	0.0001	-	-

Table 5- Genotyping frequency of *HAVCR1* rs6555820 C>A among the individuals of study population

Gene	HC	AKI	CKD	p-value	95% CI
CC	17 (56.67%) Aa	11 (36.67%) Ba	6 (20%) Cc	0.0305	7.829-83.39
CA	9 (30%) Bb	10 (33.33%) Bab	13 (43.33%) Aa	0.0385	18.32-52.79
AA	4 (13.33%) Cc	9 (30%) Bb	11 (36.67%) Ab	0.0394	3.197-56.53
p-value	0.0118	0.0473	0.0103	-	-

Relation to the risk (OR, RR) of *HAVCR1* rs6555820 C>A gene polymorphism, the harm effects of CA (1.4505, 1.0737) and AA (3.25, 1.2045) genotypes in AKI and CKD patients were appeared largely when compared to HC (0.2558, 0.7674). In comparison to results of CC genotype, significant lowered values of CA and AA was seen in HC (p<0.0118), while marked increases were identified in RF study population (p<0.0198), (Table 6).

Table 6- Risk of *HAVCR1* rs6555820 C>A gene polymorphism among individuals of study population

Gene	Total	HC	AKI + CKD	OR	RR	NNT	95% CI
CC	*34*	17 (50%)	17 (50%)	0.2558	0.7674	9.900 (Benefit)	15.70-3.763
CA	*32*	9 (28.125%)	23 (71.875%)	1.4505	1.0737	34.833 (Harm)	5.226-7.467
AA	*24*	4 (16.67%)	20 (83.33%)	3.25	1.2045	12.956 (Harm)	4.017-10.57
p-value		0.0118	0.0198	0.0001	0.0001	-	-

Significantly, the frequency of C allele was higher (p<0.0238) in HC (43%) than the values of AKI (32%) and CKD (25%); whereas, A allele was higher (p<0.0365) in CKD (43.75%) and to less extent in AKI (35%) when compared to HC (21.25%). In comparison to C allele, the frequency of A allele was lowered (p<0.0207) in HC but elevated in CKD (p<0.0169) with no variation (p<0.0825) in AKI (Table 7). Subsequently, the risk (OR, RR) of allele frequency in *HAVCR1* gene reported a significant (p<0.0001) harm effect of A allele in RF study patents (2.7957, 1.2135) when compared to C allele (0.3577, 0.8241), (Table 8).

Table 7- Allele frequency of *HAVCR1* rs6555820 C>A among individuals of study population

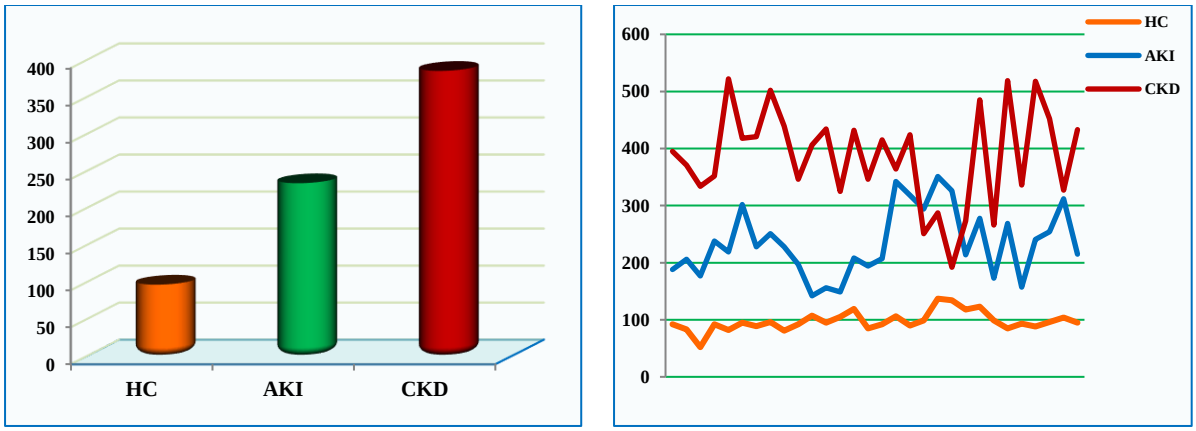
Gene	Total	HC	AKI	CKD	p-value	95% CI
C	*100*	43 (43%) Aa	32 (32%) Bb	25 (25%) Cb	0.0238	10.79-55.87
A	*80*	17 (21.25%) Cb	28 (35%) Bb	35 (43.75%) Aa	0.0365	5.158-61.51
p-value		0.0207	0.0825	0.0169	-	-

Table 8- Risk of allele frequency in *HAVCR1* rs6555820 C>A among individuals of study population

Gene	Total	HC	AKI + CKD	OR	RR	NNT	95% CI
C	*100*	43 (43%)	57 (57%)	0.3577	0.8241	12.903 (Benefit)	30.169-5.315
A	*80*	17 (21.25%)	63 (78.75%)	2.7957	1.2135	12.903 (Harm)	5.315-30.169
p-value		0.0207	0.0194	0.0001	0.0001	-	-

3.2 Serum markers

Significant gradual elevation (p<0.0001, 95%CI: 119.4 to 598.1) in values of serum KIM-1 was reported in AKI (234.47±10.83pg/ml) and CKD (386.17±15.39pg/ml) when compared to results of HC (97.47±3.07pg/ml), (Figure 1).



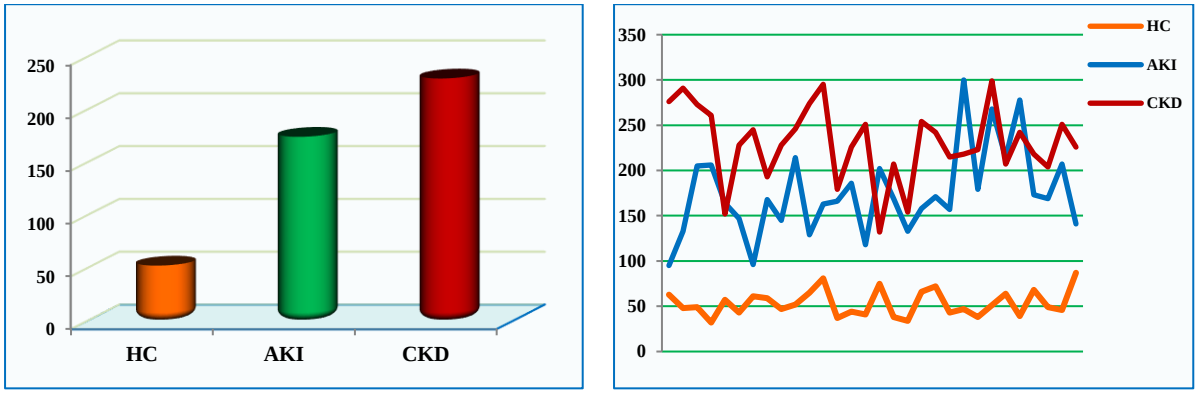
(a) **(b)**

Figure -1 Levels of serum KIM-1 marker among the groups of study population (a) Mean value (b) ODs

For suPAR marker, the findings of AKI (175.13±8.79pg/ml) and CKD (230.33±7.55pg/ml) were obviously (p<0.0001, 95%CI: 72.26 to 137.8) higher than obtained in HC (53.2±2.62pg/ml) population (Figure 2).

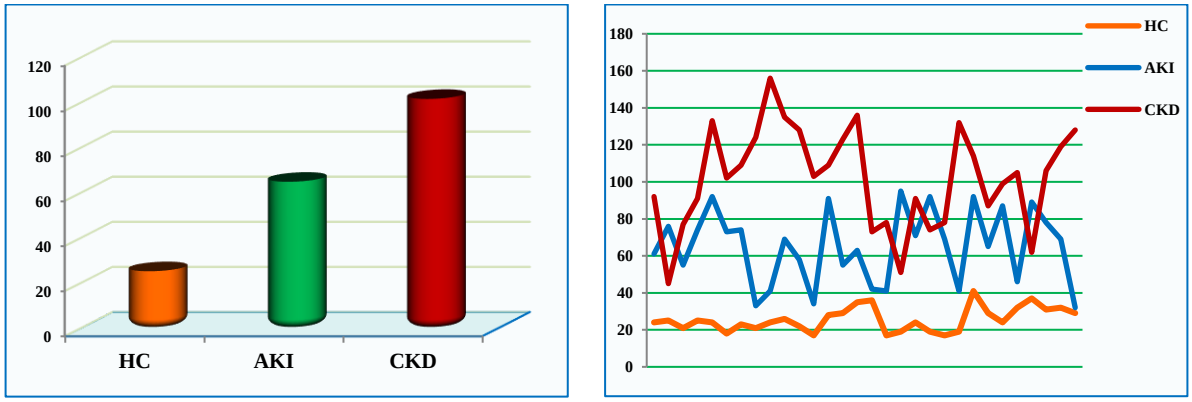
The results of TNFR1 have recorded a significant elevation (p<0.0001; 95%CI: 30.63 to 159.2) in values of CKD (102±4.95ng/ml) followed by the AKI (65.27±3.62ng/ml) individuals when compared to values of HC (25.6±1.18ng/ml) population (Figure 3).

For TNFR2, there were significant increases (p<0.0001; 95%CI: 41 to 177.3) in values of CKD (110.67±5.32pg/ml) and to less extent in AKI (70.77±3.96pg/ml) when compared to those of HC (22.93±1pg/ml), population (Figure 4).



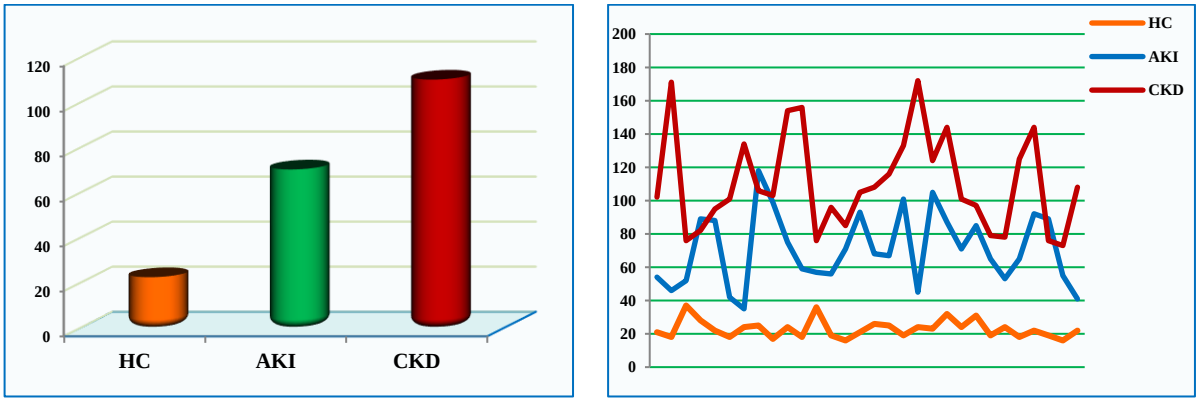
(a) **(b)**

Figure -2 Levels of serum suPAR marker among the groups of study population (a) Mean value (b) ODs



(a) **(b)**

Figure -3 Levels of serum TNFR1 marker among the groups of study population (a) Mean value (b) ODs



(a) **(b)**

Figure -4 Levels of serum TNFR2 marker among the groups of study population (a) Mean value (b) ODs

4. Discussion

Many countries as Iraq characterize by increasing the occurrence of RF. Thus, genotyping into healthcare systems could markedly support the disease prognosis. In the present study, molecular data of *APOL1* rs136161 C>G gene polymorphism revealed the lowered genotyping frequency of CC but a higher frequency of GG genotype in AKI and CKD patients. Concerning the CG genotype, no significant differences were shown between HC and AKI; however, polymorphism was elevated in CKD. Subsequently, the harm effect of

CG and GG genotypes were increased significantly in both RF patient groups when compared to HC. The allele frequency of demonstrated that the marked lower occurrence of C allele in RF study patients but higher prevalence and risk of G allele in RF study patients. Comparatively, several molecular and epidemiological studies demonstrated that *APOL1* variants are strongly associated with increased RF especially in populations of African ancestry [27-29]. Worldwide, large scale genetic studies investigating nephropathy susceptibility have analysed the SNPs across the APOL1 locus. For example, Palmer *et al.* [30] mentioned the nephropathy genes in patients with type 2 diabetes mellitus associated with the end-stage renal disease (ESRD) and included in thousands of SNPs within candidate genes as APOL1. Similarly, Zhao *et al.* [21] examined polymorphisms within the *MYH9-APOL1* genomic region and reported significant relationship with diabetic kidney disease, highlighting the importance of this chromosomal locus in renal pathology. Mechanistically, Bruggeman *et al.* [31] reported that *APOL1* variants induce podocyte injury, mitochondrial dysfunction, and inflammatory responses within the kidney, which leading to chronic structural damage and functional impairment.

For *HAVCR1* rs6555820 C>A gene polymorphism, our findings determined that the genotyping frequency of CC genotype was significantly reduced in AKI and CKD; whereas, the polymorphism and harm effects of CA and AA genotypes were elevated significantly in CKD and to less extent in AKI. Additionally, the frequency of C allele was lowered in AKI and CKD; while, A allele was elevated significantly in both patients groups. The harm effect of A allele was higher in RF study patents than C allele in study patients. One of the earliest important studies involving rs6555820 was done by McMahon *et al.* [32] who investigated genetic determinants of AKI biomarkers, described an intronic C→A substitution in *HAVCR1*, and showed a very strong association with KIM-1 level. A later population-based Sweden study has further strengthened interest in the *HAVCR1*/KIM-1 axis in CKD, and found that plasma KIM-1 predicted future decline in estimated glomerular filtration rate (eGFR) and risk of CKD over long-term follow-up in middle-aged individuals [33]. In contrast to our findings, a recent Thai study involved 250 CKD patients assessed relationship between eGFR and CKD severity, and found the absence of an obvious direct association with KIM-1 levels or eGFR [23].

Significant gradual increases in values of serum markers including KIM-1, suPAR, TNFR1, and TNFR2 were reported markedly in CKD followed by AKI when compared to HC. Globally, many studies have supported the use of blood/serum KIM-1 that emerged clinically as a relevant biomarker for detection of renal injury because it reflects proximal tubular epithelial damage in both AKI and CKD [34-36]. In agreement with our data, Sabbisetti *et al.* [37] demonstrated that the blood KIM-1 is elevated in both AKI and CKD and moreover observed the prognostic importance in diabetic disease, where higher baseline serum KIM-1 was strongly associated with faster eGFR decline and progression to ESRD in type 1 diabetes. In a recent systemic review and meta-analysis study, Su *et al.* [38] indicated that blood KIM has real diagnostic value through evaluation 41 studies with 1790 patients; in which, both urinary and blood KIM-1 in adults with AKI. Subsequently, the study recorded that several individual blood KIM-1 studies in adult AKI cohorts including sepsis-related AKI and perioperative AKI, which supports the applicability of serum KIM-1 across different AKI etiologies.

In agreement with our results, multiple observational pediatric and / or adult disease-specific studies have supported the concept that elevated suPAR is associated with incident kidney dysfunction, faster decline in estimated eGFR, CKD progression, and AKI across several clinical settings [39-42]. Another study demonstrated that, unlike serum creatinine that usually rises only after a substantial decline in glomerular filtration, suPAR may increase earlier and may therefore help identify renal injury before conventional functional markers become abnormal [43]. (Sudhini *et al.*, 2022). In an early landmark study, the strongest early evidence for suPAR as a renal biomarker have been provided by Hayek *et al.* [44] who detected the risk of progression to CKD in the highest quartile of suPAR levels was 3.13 times as high as that in the lowest quartile, with concluding that the elevated level of suPAR was independently associated with incident CKD and an accelerated decline in the eGFR in the groups studied. Later, Hayek *et al.* [39] discovered the high suPAR levels are associated with AKI in several clinical contexts, with concluding that patients having elevated preprocedural suPAR are more likely to develop AKI, suggesting a role for suPAR in early risk identification before overt injury occurs.

As identified in current study, several studies demonstrated that the levels of serum TNFR1 and TNFR2 have reflected a higher activation in both AKI and CKD individuals of RF population [45-47]. Although, various reports observed the strongest evidence for TNFR1 is in the progressive of RF and CKD rather than in classic early diagnosis of AKI [46, 49], other studies showed that circulating TNFR1 and TNFR2 are most strongly associated with progression of ESKD, and patients in the highest TNFR1 and TNFR2 quartiles have a marked greater long-term cumulative incidence than those in the lower quartile [49, 50]. In another recent study, Chen *et al.* [51] reported that longitudinal increases in TNFR1 and TNFR2 were associated with progressive CKD independent of baseline biomarker levels and kidney function, suggesting that serial TNFR1 measurement may be more informative than a single baseline measurement for monitoring CKD trajectory.

5. Conclusion

This study demonstrated the high possible role of *APOL1* and *HAVCR1* genes in RF, with suggesting that genotyping of study genes can provide a powerful tool in identification of RF, enhancing ability to diagnose the disease at early stage, and predicting clinical outcomes of AKI and CKD. This study suggests that the using of molecular genotyping tools in addition to serum biomarkers can definitely supporting the diagnosis and/or prognosis of acute and chronic RF stages, while, the application of solely technique might be supportive rather than definitive. However, the lack of consistency is common in complex renal traits, and furthermore studies can provide more insights for prevention and medication

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Ethical responsibilities of authors

This study was performed under the license of the Scientific Committee in the Department of Biology (Collage of Science, University of Wasit).

Disclosure and conflict of interest

Author declared that they have no conflicts of interest.

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