RESEARCH ARTICLE

The Association of Angiotensin-converting Enzyme (*ACE*) Gene polymorphism and Some Biomarkers in Hemodialysis Patients

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ABSTRACT

The Aim: Chronic kidney disease (CKD) is clarified by constant structural abnormalities, urine abnormalities, or damaged excretory renal function indicative of a failure of functional nephrons. One of the treatments for renal failure is hemodialysis. The aim was assessing the potential association between *ACE* gene I/D polymorphism with renal failure and their correlation with some biochemical parameter as Insulin-like growth factor 1 (IGF-1) and symmetric dimethyl arginine (SDMA) and kidney function test (KFT) "urea" and "creatinine" in Iraqi hemodialysis patients.

Materials and Methods: The study was contained 60 hemodialysis patients (30 Male and 30 Female).The control group was 30 healthy subjects (15 Male and 15 Female). The ages of hemodialysis patients and control groups ranged between (11–75 years). The information was taken from a questionnaire which included age, sex, smoking, duration of disease, duration of dialysis, number of dialyzes, other diseases, and family history. Also, body mass index (BMI), urea, creatinine, IGF-1, and SDMA were measured. After extraction of DNA from white blood cells and carrying out of PCR, characterizing ACE I/D polymorphism by applying an amplification refractory mutation system (ARMS) technique.

Results: The demographic study showed that there were more hemodialysis patients had age ≥ 50 years old (40%)when compared with the control group. The most hemodialysis patients had BMI were 18–24.9 (48.33%)in comparison with the control group, which was 18–24.9 (100 %). The smoking percentage was (16,67%) in hemodialysis patients compared to the control group (10%). The presence of another disease in the hemodialysis patients were hypertension (70 %) followed by diabetes mellitus (25 %), arthritis (23.33%), and cardiovascular disease (20 %). Concerning a family history of CKD, (25%) of hemodialysis patients have a family history while the family history of the control group was (6.67%). In the hemodialysis patients, the genotype I/I, I/D, and D/D distribution percentage were 18.33 %, 65 %, and 16.67% respectively compared with the control group which was 33.33 %, 36.67%, and 30% respectively.

Conclusion: There was a high association between heterozygous genotype (I/D) with the occurrence of CKD. Serum urea level increased in hemodialysis patients had ID genotypes in comparison with II and DD genotypes. Also, IGF-1 and SADMA have importance as a risk factor or prognosis indicator in adult CKD hemodialysis patients.

Keyword: *ACE* gene polymorphism, ARMS, Hemodialysis, IGF-1, Iraq, SDMA.

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INTRODUCTION

The (CKD) is a syndrome clarified as constant modifications in the function, the composition of kidney or both with a consequence for the health of the individual.[1] Kidneys in good health make clean blood and eliminate extra fluid in the shape of urine. As well, they synthesize hormones which necessarily for some important functions of the body. If the failure of the kidney takes place, treatment is essential to replace some of the essential functions of kidneys. There are five stages of CKD, and the stage of kidney disease is determined by dependence on the existence of damage in kidney and measurement of kidney function level by *glomerular filtration rate (GFR).* The first stage is damage of kidney (e.g., protein in the urine) accompanied by a normal GFR "90" or above, the second stage is damage of kidney with mild decline in GFR "60" to "89", the third stage is moderate decline in GFR 30 to 59, the fourth stage is severe reduction in GFR 15–29 and the final stage is kidney failure or end-stage renal disease when the GFR little than 15.[2]

IGF-1 is a polypeptide hormone consisting of 70 amino acid with a partial weight estimated at 7,649 Dalton.[3] Growth hormone (GH) secretes by the anterior pituitary gland that

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acts on the liver to IGF-1 synthesis then function on bone and kidney to intermediate longitudinal growth.[4] In addition, IGF-1 is fundamental for growth, cell differentiation, development, survival, and proliferation besides metabolic influence similar to insulin in the main cell and tissues kinds. Specifically, IGF-1 is influential for the normal pre-and postnatal progression of the kidney. Also, IGF-1 intermediatenumerousGH activities and both GH deficiency and excess are connected with the disturbed function of kidney and influence hemodynamics of kidney both indirectly and directly by interaction with the renin-angiotensin system (RAS).[5]

Symmetric dimethylarginine (SDMA) is a product from catabolism of arginine methylated proteins and was revealed as kidney function biomarker [6] and it is supposed uremic toxins that may cause toxicity by many mechanisms, involving a damaged synthesis of nitric oxide and reactive oxygen species generation.[7] In hemodialysis patients with kidney diseases, SDMA is elevated and related with GFR. Additionally, it is related to raised mortality in the general population.[8]

Angiotensin-converting enzyme (ACE) encoded by a single gene existing on chromosome 17 at q23. The length *of the ACE* gene is 21 kb and includes25 introns and 26 exons[9] and is one of the influential genes included in the RAS. In cardiovascular disease (CVD), the RAS is increased and has been hypothesized to impart to the progression of CKD,[10] and is an influential modulator for kidney disease and blood pressure.[11]

MATERIALS AND METHODS

The study was contained 60 hemodialysis patients (30 Male and 30 Female) from Merjan hospital in Babylon Province/ Iraq. The control was 30 healthy subjects (15 Male and 15 Female). The ages of hemodialysis patients and control were 11–75 years. The information was taken from a questionnaire which included age, sex, weight, length, smoking, duration of disease, duration of dialysis, number of dialyzes, other diseases, and family history. Also, BMI was measured, and the biochemical study included IGF-1 (Elabscience ELISA Kit), SDMA (Bioassay technology Sandwich ELISA Kit, urea, and creatinine levels (BioMérieuxELISA Kits).The genetic research includes DNA extraction according to the Favorgene Biotech Kit for extraction of DNA from white blood cells. Genotyping of *ACE* gene polymorphism was observed by polymerase chain reaction (PCR) applying the amplification refractory mutation system (ARMS) technique.

For polymorphism (I/D) for *ACE* gene, a sense primer 5'GACCTGCTGCCTATAGACT3' and anti-sense primer 5'GGGTAAAACTGGAGGATGGGT3' that developed a 521 bp band for *I and* 233 bp band for *D* [12]. In case of a heterozygote, both 233 bp and 521bp bands must be noticed. To preventing mistyping of the DD genotype, an additional specific pair of primer for *I*: a sense primer 5'GATTACAGGCGTGATACAGT3' and antisense primer 5'GGGTAAAACTGGAGGATGGGT3' were utilized.

Statistical Analysis

The Statistical Package for Social Science (SPSS), version 23 was employed to the analysis of different biomarkers in the study. Chi-square analysis was utilized to a comparison between percentage and analysis of variance (ANOVA), least significant (statistically accepted) difference and Duncan test or t-Test was employed to a comparison between means. The correlation coefficient between variables in this study also estimated.

RESULTS

The results of the demographic study recorded that the percentage of hemodialysis patients age group ≥ 50 years old was higher (40.0%) compared to the control group which was equal (33.33%) for different age groups. The ratio between males and females was equal in patients and control groups, and there were no significant differences between them in age and smoking. Differences between hemodialysis patients and control groups were significant($p \leq 0.01$) in BMI where the most hemodialysis patients were in 18-24.9 (48.33%)in comparison with the control group (100%). Regarding the presence of a history of previous CKD (OR = 4.67; 95% CI: 0.99–21.97), (25.0%) of hemodialysis patients have a family history in comparison with the control group (6.67%). Also, the presence of another disease in hemodialysis patients were hypertension (70%) followed by diabetes mellitus (25%), arthritis (23.33%), and cardiovascular disease (20%) as displayed in Tables 1 and 2.

This study showed significant differences ($p \leq 0.01$) in urea and creatinine between hemodialysis patients and the control groups (Figure 1 A and B) and there were significant differences for IGF-1 and SDMA ($p \leq 0.05$) as shown in (Figure 1 C and D). When comparing hemodialysis patients and controls groups in age and BMI, there is a significant difference in the BMI($p \leq 0.05$) only Table 3.

Statistical analysis of biochemical parameters in hemodialysis patients according to age, there was a significant decrease in IGF-1 and significant increase ($p \leq 0.05$) in SDMA in 31–49 years group (Table 4) and there is a significant elevation in SDMA in 18–24.[9] category according to the BMI group as shown in (Table 5).

The impact of duration of disease on biochemical parameters of hemodialysis patients showed a significant decline ($p \leq 0.05$) in IGF-1when the duration of CKD increased and nonsignificant accepted increment ($p < 0.05$) in SDMA when the duration of CKD increased (Table 6). Also, significant increment ($p \leq 0.05$) in SDMA with increased the duration of dialysis (Table 7), while according to the number of dialyzes, there is significant elevation ($p \leq 0.05$) in creatinine, IGF-1, and SDMA (Tables 8). The study of the effect of sex in biochemical parameters showed a significant difference ($p \leq 0.05$)in creatinine level only between male and female (Table 9).

The correlation coefficient between parameters in hemodialysis patients showed a significant correlation between

Table-1: Demographic characteristics of hemodialysis and control groups.

Variables	*Control group*	*Hemodialysis group*
Total Number	*30 (%)*	*60 (%)*
Age (years)		
(≤ 30) years	10 (33.33%)	14 (23.33%)
(31–49) years	10 (33.33%)	22 (36.67%)
(≥ 50) years	10 (33.33%)	24 (40.0%)
Sex		
Male	15(50.0%)	30(50.0%)
Female	15(50.0%)	30(50.0%)
BMI Kg/m^2		
Underweight < 18	0 (0%)	5 (8.33%)
Normal weight 18-24.9	30 (100%)	29 (48.33%)
Overweight 25–29.9	0 (0%)	16 (26.67%)
Obesity 30–39.9	0 (0%)	10 (16.67%)
Family history		
Present	2 (6.67%)	15 (25.0%)
Absent	28 (93.33%)	45(75.0%)
Smoking		
Yes	3(10.0%)	10(16.67%)
No	27(90.0%)	50(83.33%)
Hypertension		
Present	0	42 (70.0%)
Absent	0	18 (30.0%)
Diabetes mellitus		
Present	0	15 (25.0%)
Absent	0	45 (75.0%)
Arthritis		
Present	0	14 (23.33%)
Absent	0	46 (76.67%)
Cardiovascular disease		
Present	0	12 (20.0%)
Absent	0	48 (80.0%)

Table 2: :The association of study groups by different variables

Variables	*Control groups*	*Hemodialysis groups*	*χ2*	*Sig.*	*Odds ratio*	*95% CI*
Age(Year)						
≤ 30	10 (33.33%)	14 (23.33%)				
(31–49)	10 (33.33%)	22 (36.67%)	1.05	0.59		
≥ 50	10 (33.33%)	24 (40.0%)				
Sex						
Male	15 (50.0%)	30(50.0%)	0.001	1.000	1.000	0.42–2.40
Female	15 (50.0%)	30(50.0%)				
BMI Kg/m^2						
< 18	0 (0%)	5 (8.33%)				
18–24.9	30 (100%)	29 (48.33%)	23.65	0.001**		
25–29.9	0 (0%)	16 (26.67%)				
30–39.9	0 (0%)	10 (16.67%)				
Family history						
Present	2 (6.67%)	15 (25.0%)	4.39	0.03*	4.67	0.99–21.97
Absent	28 (93.33%)	45 (75.0%)				
Smoking						
Present	3 (10.0%)	10(16.67%)	0.71	0.39	1.8	0.46–7.10
Absent	27 (90.0%)	50(83.33%)				

**p ≤0.01, S.E: Standard error

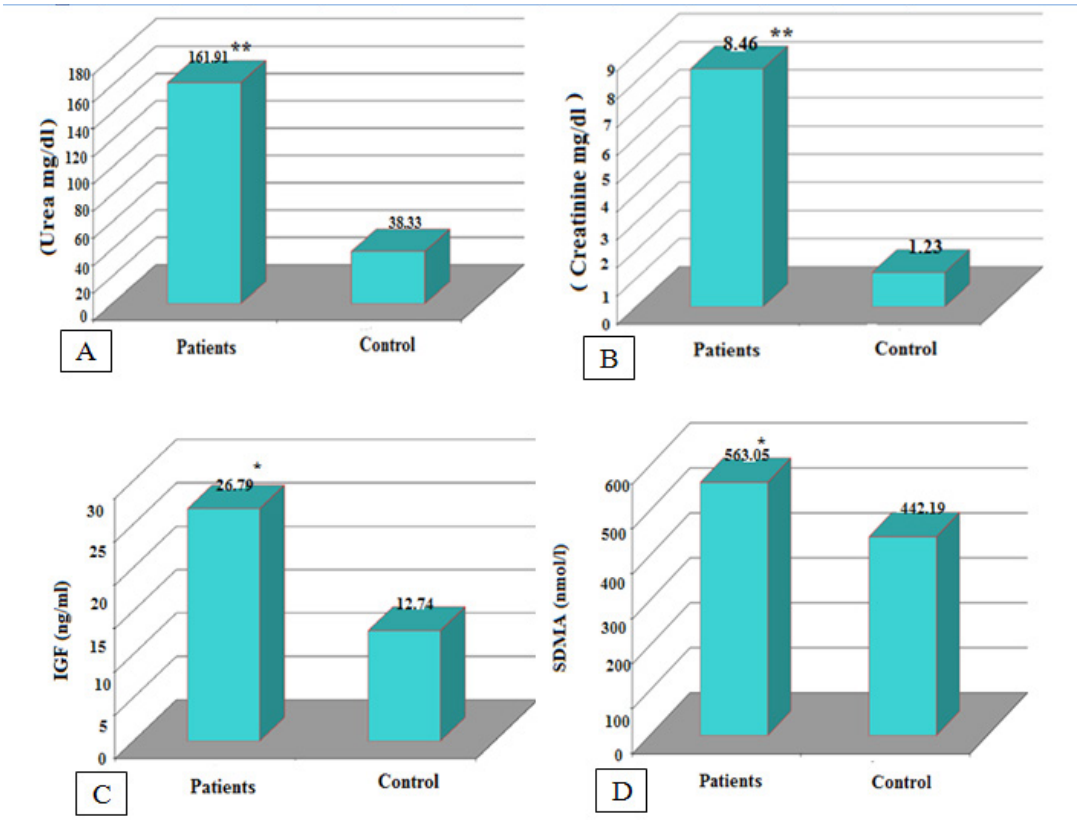

Figure 1: Comparison of urea (A), creatinine (B), IGF (C), and SDMA (D) concentration between hemodialysis and control groups.

Table 3: The comparison ofage and BMI between hemodialysis and control groups.

Group / *Parameters*	*Control (Mean ± S.E) n = 30*	*Hemodialysis hemodialysis patients (Mean ± S.E) n = 60*	*Sig ***
Age (year)	37.77 ± 2.52	43.68 ± 1.93	0. 77
BMI (kg/m^2)	23.20 ± 0.30	25.46 ± 0.73	0.0001

t-test, **P ≤ 0 .01, S.E: Standard error

Table 4: The impact of age groups on biochemical parameters of hemodialysis group.

Age Group / *Parameters*	*Mean ± S.E* ≤30 n = 14	*31-49 n = 22*	≥ *50 n = 24*
Urea (mg/dL)	170.14 ± 14.93**a**	160.54 ± 10.99 **a**	158.37 ± 10.25**a**
Creatinine (mg/dL)	8.92 ± 0.74**a**	9.08 ± 0.78 **a**	7.63 ± 0.67**a**
IGF-1 (ng/mL)	30.74 ± 2.14 **a**	22.96 ± 2.06**b**	27.98 ± 2.22**ab**
SDMA (nmol/L)	476.91 ± 43.30**b**	608.35 ± 83.10**a**	571.78 ± 77.89**ab**

Different letters in the same raw refer to significant differences (p≤ 0.05), S.E: Standard error.

Table 5. The impact of BMI groups on biochemical parameters of hemodialysis group.

BMI(Kg/m²) / *Parameters*	*Mean ± S.E* <18 n = 5	*18-24.9 n= 29*	*25.29.9 n = 16*	*30-39.9 n = 10*
Urea (mg/dL)	159 ± 12.96a	172.55 ± 9.66 a	157.81 ± 14.93 a	138.90 ± 12.59a
Creatinine (mg/dL)	8.20 ± 0.78 a	9.24 ± 0.60 a	7.74 ± 0.95 a	7.49 ± 1.09a
IGF-1 (ng/mL)	29.12 ± 3.23 a	26.23 ± 2.06 a	28.22 ± 2.11 a	24.95 ± 3.78a
SDMA (nmol/L)	335.55 ± 20.67b	649.85 ± 75.86a	478.85 ± 62.71c	510.84 ± 103.71ac

Different letters in the same raw refer to significant differences (p ≤0.05), S.E: Standard error.

Table 6: The impact of duration of disease on biochemical parameters of hemodialysis group.

Parameters \ Group(years)	Mean ± S.E		
	Less than 1 n = 34	1-5 n = 22	More than 5 n = 4
Urea (mg/dL)	169.32 ± 8.45a	149.54 ± 9.65a	167 ± 47.72a
Creatinine (mg/L)	9.05 ± 0.58a	7.60 ± 0.70a	8.15 ± 1.52a
IGF-1 (ng/mL)	29.56 ± 1.77a	24.05 ± 1.96ab	18.26 ± 2.78 b
SDMA (nmol/L)	506.59 ± 38.90a	648.79 ± 101.67a	571.44 ± 154.71a

Table 7: The impact of duration of dialysis on biochemical parameters of hemodialysis group.

Parameters \ *Group(years)*	*Mean ± S.E*		
	Less than 1 *n = 39*	*More than 1* *n = 21*	*Sig**
Urea (mg/dL)	166.64 ± 8.07	153.14 ± 11.62	0.89
Creatinine (mg/dL)	8.41 ± 0.57	8.54 ± 0.63	0.32
IGF-1 (ng/mL)	28.48 ± 1.62	23.63 ± 2.14	0.43
SDMA (nmol/L)	529.59 ± 42.54	625.21 ± 100.29	0.02*

t-test, *p≤ 0.05, S.E: Standard error.

Table 8: The impact of many dialyzes weekly on biochemical parameters of hemodialysis group.

Parameters \ *Group(weekly)*	*Mean ± S.E*		
	1 *n = 3*	*2* *n*	*= 46* *3* *n = 11*
Urea (mg/dL)	144.00 ± 29.48a	161.08 ± 7.00 a	170.27 ± 20.76 a
Creatinine (mg/L)	6.80 ± 0.60b	8.42 ± 0.52ab	9.08 ± 0.85a
IGF-1 (ng/mL)	19.68 ± 3.03 b	27.47 ± 1.51a	25.88 ± 3.20ab
SDMA (nmol/L)	492.17 ± 16.79b	517.44 ± 42.33ab	773.13 ± 157.35a

Different letters in the same raw refer to significant differences (p≤ 0.05).S.E: Standard error.

Table 9: The impact of sex on biochemical parameters of hemodialysis group.

Parameters \ *Group(years)*	*Mean ± S.E*		
	Male *n= 30*	*Female* *n= 30*	*Sig**
Urea (mg/dL)	167.53 ± 9.61	156.30 ± 9.19	0.76
Creatinine (mg/dL)	9.20 ± 0.55	7.72 ± 0.65	0.02*
IGF-1 (ng/mL)	28.25 ± 2.08	25.32 ± 1.58	0.07
SDMA (nmol/L)	545.13 ± 64.12	580.98 ± 62.83	0.97

t-test, *p ≤ 0 .05, S.E: Standard error.

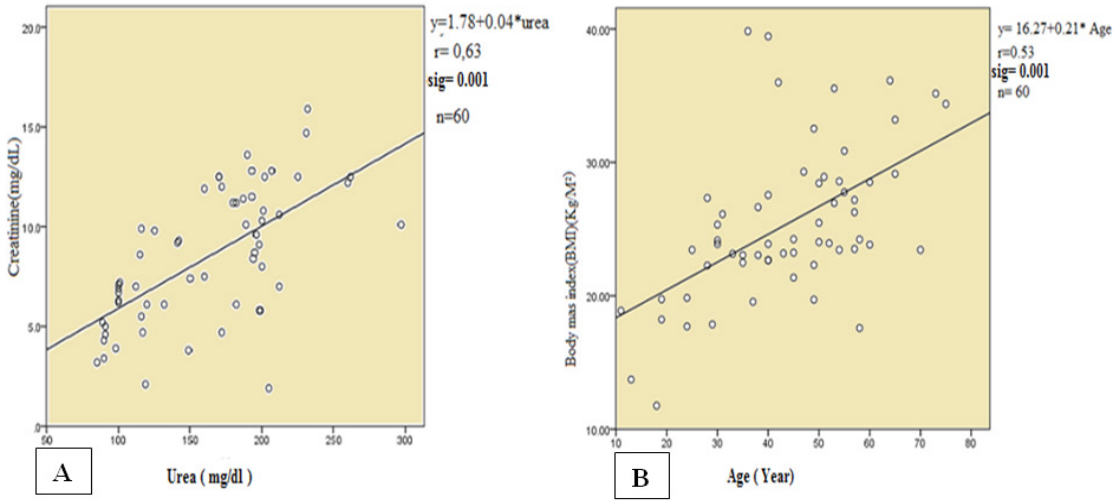

Figure 2: The correlation coefficient between urea(mg/dl) and creatinine (mg/dL) (A) and between BMI (Kg/m²) and age (year)(B) in Hemodialysis group.

urea and creatinine in addition to age and BMI ($p \leq 0.05$) as shown in Figures (2A and B).

MOLECULAR ANALYSIS

Deoxyribo nucleic acid (DNA) extraction

The DNA was extracted from the white blood cells of hemodialysis patients and control groups as in (Figure 3) which showed the electrophoresis pattern of DNA extracted.

The Genotype of *ACE* gene polymorphism using PCR-ARMS

The PCR product of *ACE* gene amplification was 521,233, 234 bp in control, and hemodialysis groups (Figures 4 and 5).

Respecting the *ACE* genotype distribution, I/I, I/D, and D/D distribution in hemodialysis patients were 18.33%, 65%, and 16.67% respectively while in the control group the genotypes were 33.33 %, 36.67%, and 30% respectively. Frequencies of alleles for the *ACE* gene were 50.83% and 49.17% for *I* and *D* in hemodialysis patients and 51.67% and 48.33% for control. The results exhibit significant differences ($p \leq 0.05$) between I/I and I/D genotype in hemodialysis patients (OR = 0.31, CI:0.10-0.92) and I/D-D/D genotype (OR = 0.32,CI:0.11-0.96) as compared with control group, while there were no significant differences in allele frequency (I, D) (Table 10).

The table 11 refers to the significant increasing($p \leq 0.05$) in the serum of urea level in heterozygous I/D genotype in hemodialysis groups, while there is no appearance of

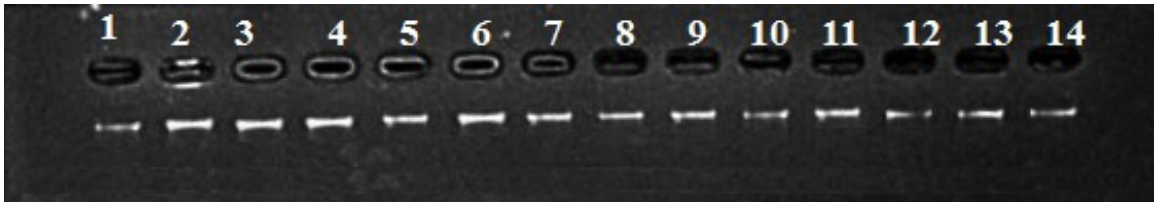

Figure 3: The electrophoresis pattern of DNA extracted from white blood cell of hemodialysis patients(lane 1–7) and control group (lane 8–14), 1% agarose, 75 V, 20 Am for 30 minutes.

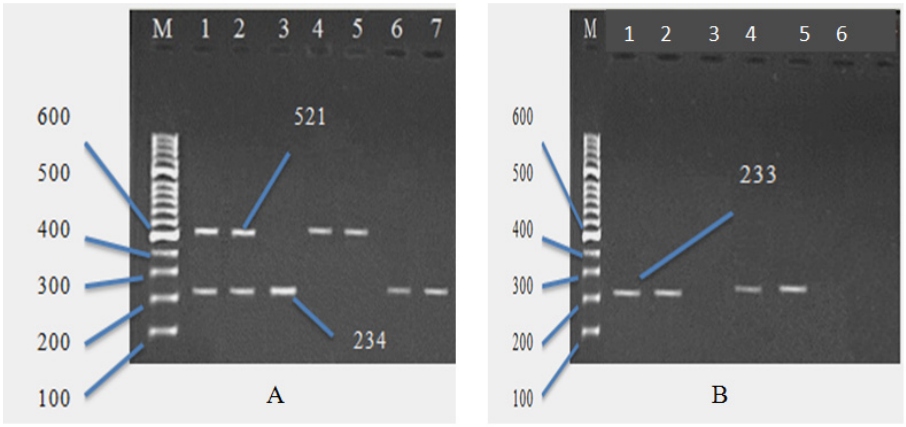

Figure 4: A- Electrophoresis pattern of PCR product of the *ACE* gene in control group, lane M: DNA ladder. Lanes (1-2) show heterozygotes (I/D) genotype yielded a 521 bp and 234bpbands. Lanes (3,6 and 7) show (D/D) genotype yielded a 234bp band. Lanes (4,5) show (I/I) genotype yielded a 521bp band. B- Display a 233 bp band which appears as a result of confirming primers for insertion for the same specimens.3% agarose, 70 V, 20 mA for 90 minutes.

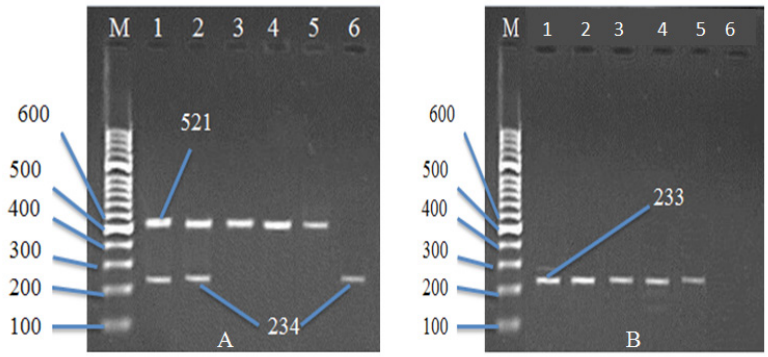

Figure 5: Electrophoresis pattern of PCR product of the *ACE* gene in hemodialysis patients. Lane M: DNA ladder. Lanes (1–2) show heterozygotes (I/D) genotype yielded a 521 bp and 234bpbands. Lanes (3,4, and 5) show (I/I) genotype yielded a 521 bp band. Lanes (6) show (D/D) genotype yielded a 234bp band. B Displays 233 bp band which appears as a result of confirming primers for insertion for the same specimens. 3% agarose, 70 V, 20 mA for 90 minutes.

Table11: Association of *ACE* gene polymorphisms in Hemodialysis group with biochemical parameters.

Parameters \ *Genotype*	*Mean ± S.E* *II* *n = 11*	*ID* *n = 39*	*DD* *n = 10*
Urea (mg/dL)	148.09 ± 12.36 ab	173.21 ± 8.58 a	133.10 ± 12.75b
Creatinine (mg/L)	7.19±1.02 a	9.11±0.53 a	7.34±0.97 a
IGF-1 (ng/mL)	27.04±3.18 a	26.29±1.64 a	28.46±3.32 a
SDMA (nmol/L)	558.59 ± 84.17 a	559.70 ± 52.53 a	581.05 ± 154.87 a

Different letters in the same raw refer to the significant difference ($p \leq 0.05$).

Table 10: Genotype distribution of ACE gene polymorphism of patient and control groups.

Genotype	*Control* *%*	*Study Group* *Hemodialysis group* *%*	*χ2*	*Sig.*	*Odds ratio* *(95% CI)*
IIa	10 (33.33%)	11 (18.33%)	4.66	0.03*	0.31 (0.10–0.92)
I/D	11(36.67%)	39 (65.0%)			
DDa	9 (30.0%)	10 (16.67%)	0.001	0.97	0.98 (0.29–3.43)
I/D	11(36.67%)	39 (65.0%)	4.30	0.04*	0.32 (0.11–0.96)
Total	30	60			
Allele frequency					
I	31(51.67%)	61 (50.83%)			
D	29 (48.33%)	59 (49.17%)	0.11	0.9	0.95 (0.52-1.79)

[a]reference group, *P ≤ 0 .05.

significant differences ($p > 0.05$) in the levels of creatinine, IGF-1, and SDMA in comparison with homozygous genotypes II and DD.

DISCUSSION

The outcome of the current data revealed that the hemodialysis patients had higher urea and creatinine levels with a significant increase in comparison with the control group. In CKD, the kidneys are damaged or cannot filter blood as healthy kidneys. Because of this damages, excess fluid and waste from the blood remain in the body and may cause other health problem,[13] these alterations or damages are most frequently identified by elevated serum levels of blood urea nitrogen or creatinine in CKD hemodialysis patients.[2]

According to the level of IGF-1, a significant increment ($p \leq 0.05$) was shown in hemodialysis patients in comparison with control. Previous studies demonstrated that the CKD cause numerous alterations of the GH/IGF-1 system which collectively cause GH and IGF-1 resistance, these alterations bring about significant clinical outcomes, involving growth retardation in children while in adults result in malnutrition and catabolism. This result is not consistent with previous studies which recorded less than normal level of IGF-1 in adult CKD hemodialysis patients with stage 5 directing to the risk of mortality connected with a less than normal IGF-1. Earlier investigations demonstrated that widespread hemodialysis patients who died have lessened levels of IGF-1 from survivors.[14,15] The mortality prognosis and the function of IGF-1 analyzed in non-dialyzed CKD hemodialysis patients at stage 5 and the risk of mortality connected with the alteration of IGF-1 levels after the beginning of treatment with dialysis in these patients. In addition, there was a meaningful elevation of IGF-1throughout the dialysis therapy (first 1 year), and those hemodialysis patients who appeared to had a persistently raised level of IGF-1 had a lessen risk of mortality from all cases.[16]

In regarding the effect of the age group in biochemical parameters, there was a significant decline in IGF-1 between groups 31–49 and ≤30 age groups.[17]explained that IGF-1 level linked in a negative manner with age, taking into account that depletion of IGF-1 level in CKD hemodialysis patients is connected to the structure of the body,specificallymusclewea kening[17,18] and lower bone mineral density(BMD).[19]

When studying the effect of duration disease, there is significant decrease ($p \leq 0.05$)in IGF-1 level in hemodialysis patients with increased duration of disease and showed the high level of the IGF-1 in dialysis patients had dialysis two to three times a week compared to patients who use dialysis once a week (Table 8). This explained by the baseline level of serum IGF-1and the alteration of IGF-1throughout 1 year in CKD hemodialysis patients in stage 5 beginning dialysis in connection with bone mineral density (BMD), metabolic parameters, nutritional status, and mortality.16 Also, the IGF-1 may be considered as a novel biomarker in ESRD patients getting hemodialysis two or three times a week.[20] On the other hand, levels of IGF-1 in CKF are normal, whereas, in ESRD, the IGF-1 levels were slightly reduced.[21]

With regard to the level of SDMA, there was a significant increase ($p \leq 0.05$) in hemodialysis patients in comparison with control. SDMA is believed an inert metabolite, but since it can transfer into cells, SDMA effect on glomerular endothelial cells, in addition, circulating SDMA is raised in hemodialysis patients with CKD[22] and SDMA is a catabolic

outcome of arginine methylated proteins.[6] Also, it revealed as kidney function biomarker while the level of SDMA raised and linked with GFR in hemodialysis patients.[23]

According to the age, these results recorded a significant increment in the age group 31–49 in comparison with age groups ≤ 30 and nonsignificant increment in comparison with the age group ≥ 50. These results not consistent with previous studies which recorded that SDMAis not being affected by non-renal agents which are verified to affect creatinine like diet, muscle mass, diabetes, and inflammation. Moreover, SDMA is slightly affected by obesity, age, and another reason[24].

According to BMI, the significant decrease in SDMA in the < 18 when compared with other BMI groups. These results not consistent with previous studies illustrated SDMA is minimally influenced by obesity.[24] Another study recorded the absence of the correlation between BMI and level of SDMA when studying the relation between the level of SDMA and level of arginine in hemodialysis patients group.[25] Malnutrition capable to share in the reduction of nitric oxide (NO) production as a result of a deficiency of arginine, particularly hemodialysis patients endures from malnutrition.[26] The deficiency in proteins can be brought about by a reduction of protein intake, and hemodialysis treatment worsens this situation.[28]

Furthermore, this results disagreed with another study which showed no correlation between BMI and level of SDMA.[28] The ESRD effects on the patient body, including peripheral nerves and muscles, differently from one patient to another, which is reflected on physical activity and affect the independence of the patient and the physical activity of healthy people is much better than people under hemodialysis. This reflects the effect of the disease on the patient.[29]

According to the duration of dialysis and the number of dialyzes, there is a significantincrease ($p \leq 0.05$) in the level of SDMAwithincreasing of number and duration of dialysis. The level of SDMAwas about four-fold larger in CRF and about 5.5-fold are larger in hemodialysis patients.[25] The increase was more noticeableforSDMA, where its level increased about fourfold in CKD and about sixfold in dialysis hemodialysis patients.[30] This result explains the increased level of SDMA as the duration of disease and the number of dialysis in hemodialysis patients.

According to the effect of sex in biochemical parameter studied, there was a statistically accepted increase in creatinine levels of males than females. Serum creatinine level is the largest usually employed biomarker for prediction the degree of kidney function, except it can be influenced by different factors as a dietary habit, muscle mass, gender, age, ethnicity, and specific drug treatment. Furthermore, the muscle mass is a great determinant for the level of serum creatinine, and females have little mass of muscle in comparison with males.[31] Moreover, the variation between males and females in glomerular hemodynamics, glomerular structure, and the metabolism of the hormone could perform an important function in the gender dissimilarity.[32]

There were little studies in adult CKD hemodialysis patients with stage 5 directing to the mortality risk connected with IGF-1 or SADMA in the Iraqi population. To our acknowledgment, this the first study analyzed the importance of IGF-1 and SADMA as a risk factor or prognosis indicator? And their association with I/D polymorphism of *ACE* gene polymorphism in adult Iraqi CKD hemodialysis patients.

In hemodialysis patients, the positive correlation between urea and creatinine in addition to age and BMI ($p \leq 0.05$) may be illustrated as hemodialysis plays a vital role in the elimination of waste products such as free water, urea, and creatinine from the blood as the kidneys are impaired. Hemodialysis is usually performed with uremic patients for two to three times a week, and the required times for dialysis vary from two to four hours.[33] The results of this study agreed with the previous study reported the rang of serum creatinine, and urea was significantly high before hemodialysis and reduced significantly after hemodialysis and noted among many biochemical parameters in blood, serum creatinine and urea are emerging as a source of more sensitive markers for the detection of the renal failure[34].

In terms of age and BMI, obesity is linked with raising the risk of occurrence CKD,[35,36] ESRD,[37] and mortality.[38] The gradual deprivation of kidney role like with the diversely combined age-BMI groups, older patients had a larger risk of gradually deprivation of kidney role, independent of BMI levels. The correlation between BMI and a faster decrease in kidney role was larger in patients older than 40 years old, with a noticeable accentuation of the risk linked with higher BMI as age increased[39]. In older patients with larger BMI had higher medication usage, the higher predominance of cardiovascular disease, hypertension, and diabetes mellitus. Also, aging itself result in raised glomerular permeability, the decline in individual glomerular volume decreased nephron numbers, and glomerular sclerosis.[40] All these influences could superimpose on the alterations induced by obesity and cause a more observed effect on the function of kidney in older individuals.[39]

In *ACE*genotyping study, showed that there were significant differences of study groups in the *ACE* gene ($p \leq 0.05$), I/D genotype was higher in hemodialysis patients 39 (65%) than control 11(36.67) with OR = 0.31 and 95% CI: 0.10–0.92.

The role of RAS is familiar for its maintenance and regulation of glomerular and systemic blood pressure as well as salt balance. The hyperactivity of this system cause raising in glomerular and systemic blood pressure results in fibrosis and progressive failure of the renal function. The result of this study agreed with results obtained by who recorded the ACE I/D polymorphisms association with essential hypertension that leads to CKD [41-43] while this study disagreed with earlier studies which concluded that ACE-DD genotype might be a potential risk factor for causing and progression of CKF among hypertensive hemodialysis patients.[44-46].

This study showed that subjects having I/D genotype had a higher risk for the development of CKD compared with II and DDgenotypebut statistically no significant differences

between I and D allele frequency. Another previous result recorded the ACE I/D polymorphism is liked with female CKD hemodialysis patients rather than male CKD hemodialysis patients [47]while [11]found that DD genotype is associated with female CKD hemodialysis patients. The kidney is affected by many factors as recorded by the molecular study of DNA, which showed fragmentation in DNA extracted from the renal cortex in the diabetic group compared to the control group.[48] There is another polymorphism in other genes associated with kidney diseases as a link between polymorphism of promoter region of -634 G/C with levels of systolic blood pressure (SBP), GFR, type IV collagen and increased expression of

Vascular endothelial growth factor (VEGF) in blood and urine. The increase in VEGF in the urine reflects the activation of angiogenesis and endothelial dysfunction, aggravated during the development and progression of chronic kidney disease.[49]

In conclusion, subjects having I/D genotype may be a potential risk factor for the development of CKD in hemodialysis patients as compared with II and DD genotype. Also, level of serum urea statistically increases in hemodialysis patients had ID genotypes in comparison with II and DD genotypes as well as, the importance of IGF-1 and SADMA as a risk factor or prognosis indicator in adult CKD hemodialysis patients.

Authors contributions

Maysaa Adil Hadi conceived of the presented idea, contributed in design, planning methodology, and supervision. Wisam Abed Al-Amer Rady carried out the experiments, processed the experimental data using the analysis by the statistical methods, designed the figures and performed the drafted manuscript. Both Maysaa Adil Hadi and Wisam Abed Al-Amer Rady authors contributed to the interpretation of the results and completed writing of the final version of the manuscript with support from Ali Hmood Al. Saadi. Both Ali Hmood Al. Saadi and Maysaa Adil Hadi supervised the project.

REFERENCES:

1 Zoccali, C.; Vanholder, R.; Massy, ZA.; Ortiz, A.; Sarafidis, P.; Dekker, FW.; Fliser, D. and Fouque, D. (2017). The systemic nature of CKD. Nat. Rev. Nephrol. 13: 344-358.

2 Kidney Disease Improving Global Outcomes (KDIGO). (2013). Clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. Suppl. 3: 1– 150.

3 Laron, Z. (2001). Insulin-like growth factors–I: a growth hormone. J. Clin.Pathol: MolPathol. 54:311-316.

4 Rogers, S.; Ryan, G. and Hammerman, M. (1991). Insulin-like growth factors I and II are produced in the metanephros and are required for growth and development in vitro. J.Cell Biol.113:1447-1453.

5 Leon, A. B.; Lorna J. H. (2014). Insulin-like Growth Factors and Kidney Disease. Am J Kidney Dis. by the National Kidney Foundation, Inc.

6 El-Khoury, J.M.; Dustin, R.B.; Bo, H.; Edmunds, Z.R., and Sihe, W. (2016). Comparison of symmetric dimethylarginine with creatinine, cystatin C and their eGFR equations as markers of kidney function. Clin.Biochem.49 (15):1140-1143.

7 Shafi, T.; Hostetter, T.H.; Meyer, TW.; Hwang, S.; Hai, X.; Melamed, M. L.; Banerjee, T.; Coresh, J. and Powe, N. R. .(2017). Serum Asymmetric and Symmetric Dimethylarginine and Morbidity and Mortality in Hemodialysis Patients. Am. J. Kidney Dis.70:48-58.

8 Kielstein, J.T.; Salpeter, S.R.; Bode-Boeger, S.M.; Cooke, J.P., and Fliser, D. (2006). Symmetric dimethylarginine (SDMA) as endogenous marker of renal function--a meta-analysis. Nephrol. Dial. Transplant. 21: 2446 -2451.

9 Devansena, T.(2015). Enzymology, published in Indian, Oxford university press. pp 442.

10 Weiner, D.E.; Tabatabai, S.; Tighiouart, H.; Elsayed, E.; Bansal, N.; Griffith, J.; Salem, DN.; Levey, A. S. and Sarnak, M. J. (2006). Cardiovascular outcomes and all-cause mortality: Exploring the interaction between CKD and cardiovascular disease. Am. J. Kidney Dis. 48:392- 401.

11 Nagamani S.; Shanmuga, P.M.; Shanmuga, P.R. L. Chandrasekhar, K., and Kartikeyan, M. (2015). ACE DD genotype associated with the female Chronic Kidney Disease hemodialysis patients of Tamilnadu population. The Egyptian Journal of Medical Human Genetics.16: 29–33.

12 Sakhteh, M.; Behzad, P.; Naser, A.; Ahmadreza, S.; Abdolhamid, B. and Mohammad, F. (2015). Polymorphism and synergism of angiotensin-converting enzyme (ACE) and plasminogen activator inhibitor-1 (PAI-1) genes in coronary artery disease. 16(4) :1168– 1174.

13 National Chronic Kidney Disease Fact Sheet. (2017). National Centers for Chronic Disease Prevention and health promotion.

14 Fernandez-Reyes, M.J.; Alvarez-Ude, F.; Sanchez, R.; Mon, C.; Iglesias, P.; Diez, J.J., and Vazquez, A. (2002). Inflammation and malnutrition as predictors of mortality in hemodialysis patients on hemodialysis. J Nephrol 15: 136–143.

15 Qureshi, A.R.; Alvestrand, A.; Divino-Filho, J.C.; Gutierrez, A.; Heimburger, O.; Lindholm, B. and Bergstrom, J.(2002). Inflammation, malnutrition, and cardiac disease as predictors of mortality in hemodialysis patients. J. Am. Soc. Nephrol. 13(1): 28–36.

16 Jia, T.; Gama, A. T.; Heimburger, O.; Bárány, P.; Lindholm, B.; Stenvinkel, P. and Qureshi , A. (2014). IGF-1 and survival in ESRD. Clin. J. Am. Soc. Nephrol .9(1):120-127.

17 Zheng, Z.; Messi, M.L., and Delbono, O. (2001). Age-dependent IGF-1 regulation of gene transcription of Ca2+ channels in skeletal muscle. Mech. Ageing Dev.122: 373–384.

18 Scicchitano, B.M.; Rizzuto, E. and Musaro, A. (2009). Counteracting muscle wasting in aging and neuromuscular diseases. The critical role of IGF-1. Aging. Albany, NY. Online. 1: 451–457.

19 Park, S.; Jia, T.; Qureshi, A.; Barany, P.; Heimburger, O.; Larsson, T.; Axelsson, S. P. and Lindholm, B. (2013). Determinants and survival implications of low bone mineral density in end-stage renal disease hemodialysis patients. J .Nephrol 26: 485–494.

20 Prelevic, V.; Radunovic, D.; Ratkovic, M.; Gledovic, B.; and Antunovic, T. (2018). IGF1 can be considered as novel biomarker for assessment of cognitive function in CKD patients? Nephrology Dialysis Transplantation, 33(1): 564.

21 Tonshoff, B.; Powell, D.; Zhao, D.; Durham, S.; Coleman, M.; Domené, H.; Blum, W.; Baxter, R.; Moore, L. and Kaskel, F. (1997). Decreased hepatic insulin-like growth factor (IGF-I) and increased IGF-1 binding protein-1 and -2 gene expression

in experimental uremia. Endocrinology.138 (3):938-946.
22 Feliers, D.; Duck-Yoon, L.; Yves, G. and Balakuntalam, S. K. (2015). Symmetric dimethylarginine alters endothelial nitric oxide activity in glomerular endothelial cells. Cellular Signalling. 27: 1–5.
23 Schwedhelm, E. R., and Boger, H. (2011). The role of asymmetric and symmetric dimethylarginines in renal disease. Nat. Rev. Nephrol. 7: 275–285.
24 Hokamp, J.A. and. Nabity, M.B.. (2016). Renal biomarkers in domestic species. Vet. Clin. Pathol. 45: 28-56.
25 Christian, F.; Frank, S.; Elke, K.; Martin, B. and Gunter, S. (2003). Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in hemodialysis patients with chronic kidney diseases. Clinica Chimica Acta. 336: 1-12.
26 Suliman, ME.; Stenvinkel, P.; Heimburger, O.; Barany, P.; Lindholm, B. and Bergstrom, J.(2002). Plasma sulfur amino acids in relation to cardiovascular disease, nutritional status, and diabetes mellitus in hemodialysis patients with chronic renal failure at start of dialysis therapy. Am. J. Kidney Dis.40: 8-480.
27 Ritz, E. and Nowicki, M. H. (1994). Kardiovaskulare und infektions –bedingteMortalitatbeiDialyse. Ist ADMA (asymmetrisches Dimethyl-L Arginine) involviert? Nieren – Hochdruckkrankh .23:20 -116.
28 Flecka, C.; Frank, S.; Elke, K.; Martin, B. and Gunter, S. (2003). Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in patients with chronic kidney diseases. Clinica Chimica Acta. 336: 1–12.
29 Baqer, H. M.; Jabur, F.and Kadhum, S. (2018). Impact of End Stage Renal Disease upon physical activity for Adult Patients Undergoing Hemodialysis at AL-Najaf Governorate Hospitals. J. Pharm. Sci.& Res.10(5):1170-1174.
30 Morescau, B.; Nagels, G.; Posseniers, I.; Debroe, M.E.; Because, I.; Bllioux, J.M.; Lornoy, W. and DE, D. P.P. (1997). Guanidino compounds in serum and urine of non dialyzed hemodialysis patients with chronic renal insufficiency. Metabolism 46: 1024-1031.
31 Heymsfield, S.B.; Arteaga, C.; Mc, M. C.; Smith, J. and Moffitt, S. (1983). Measurement of muscle mass in humans. the validity of the 24-hour urinary creatinine method. Am. J.Clin.Nutr. 37:478-494.
32 Silbiger, SR. and Neugarten, J. (2003). The role of gender in the progression of renal disease. Adv.Ren. Replace Ther.10:3-14.
33 Wendy, E.; Bloembergen, D.C. and Friedrich, K. (1996). Relationship of dose of hemodialysis and cause-specific mortality. Kidney Int. 50:557-565.
34 Nisha, R.; Kannan, S.S.R.; Mariappan, T.K., and Jagatha, P. (2017). Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. Journal of Clinical Pathology and Laboratory Medicine .1(2): 1-5.
35 Fox, C.S.; Larson, M.G.; Leip, E.P.; Culleton, B.; Wilson, P.W., and Levy, D. (2004). Predictors of new-onset kidney disease in a community-based population. JAM,.291(7):844–850.
36 Foster, M.C.; Hwang, S.J. and Larson, M.G. (2008). Overweight, obesity, and the development of stage 3 CKD: the Framingham Heart Study. Am J Kidney Dis. 52(1):39–48.
37 Vivante, A.; Golan, E.; Tzur, D.; Leiba, A.; Tirosh, A.; Skorecki, K.; Calderon-Margalit, R. (2012). BMI in 1.2 Million Adolescents and Risk for End-Stage Renal Disease. Arch Intern Med.172(21):1644-1650.
38 De-Gonzalez, A.B.; Hartge, P. and Cerhan, J.R. (2010). Body-Mass Index and Mortality among 1.46 Million White Adults. N Engl. J. Med. 363(23): 2211–2219.
39 Ling, J.L.U.; Molnar, M. Z.; Naseer, A.; Mikkelsen, M.K.; Kalantar-Zadeh, K. and Kovesdy, C.P. (2015). Age and Association of BMI with Loss of Kidney Function and Mortality. Lancet Diabetes Endocrinol. 3(9): 704–714.
40 Mcnamara, B.J.; Diouf, B.; Hughson, M.D.; Hoy, W.E., and Bertram, J.F. (2009). Associations between age, body size and nephron number with individual glomerular volumes in urban West African males. Nephrol Dial Transplant. 24(5):1500–1506.
41 Glavnik, P. (2007). M235T polymorphism of the angiotensinogen gene and insertion/deletion polymorphism of the angiotensin-1 converting enzyme gene in essential arterial hypertension in Caucasians. Folia Biol. 53:69–70.
421 Gupta, S.; Agrawal, B.K.; Goel, R.J., and Sehajpal, P.K. (2009). Angiotensin-converting enzyme gene polymorphism in hypertensive rural population of Haryana. India. J .Emerg Trauma Shock, 2:150–154.
431 Patnaik, M.; Pati, P.; Swain, S.N.; Mohapatra, M.K.; Dwibedi, B.; Kar, S.K. and Ranjit, M. (2014). Association of angiotensin-converting enzyme and angiotensin-converting enzyme-2 gene polymorphisms with essential hypertension in the population of Odisha, India. Ann Hum Biol. 41:143–150.
44 Tripathi, G.; Dharmani, P.; Khan, F.; Sharma, RK.; Pandirikka, V. and Agrawal, S. (2006). High prevalence of ACE DD genotype among north Indian end-stage renal disease hemodialysis patients. BMC Nephrol.7:15.
45 Tripathi, G.; Sharma, R.; Baburaj, V.; Sankhwar, S.; Jafar, T. and Agrawal, S. (2008). Genetic risk factors for renal failure among North Indian ESRD hemodialysis patients. Clin. Biochem. 41:525–531.
46 Sarkar, T.; Narinder, P. S.; Premashish, K.; Syed, A. H.; Seema, K.; Sunil, K. P.; Anish, K.andNeena, G. (2016). Does angiotensin-converting enzyme-1 (ACE-1) gene polymorphism lead to chronic kidney disease among hypertensive hemodialysis patients? Renal Failure. 38(5): 765–769.
47 Mansoor, Q.; Bilal, N.; Qureshi, S.; Qureshi, Q.; Javaid, A. and Ismail, M. (2010). Gender-based disparities in ACE I/D polymorphism associated with progression of diabetic nephropathy in Pakistani hemodialysis patients with type 2 diabetes mellitus. Int. J. Diab.Metab.18:67–71.
48 Al-Joubori, M. A. Ali Hmood Al-Saadi, A.H. and Haider Kamil Zaidan, H. Z. (2013). Effect of a Mixture of Plants Extracts on Genomic DNA, Insulin Receptor, and Insulin Receptor Substrate-1 Genes in Alloxan-Induced Diabetic Male Rats. Journal of Biology, Agriculture, and Healthcare,3(2): 140-149.
49 Shulkina, S. G.; Smirnova, E.N.; Osadchuk, M.A., and Trushin, M.V.(2018). Obesity and functional state of kidney depends on VEGF polymorphism. J. Pharm. Sci. & Res.10(5): 1259-1262.