Association of Angiotensin Converting Enzyme gene polymorphism in anemic patients with regular haemodialysis in Sohag University Hospital

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Abstract

Background: Anemia could be a commonly analyzed complication in patients enduring from chronic kidney infection. Erythropoietin (EPO) stimulating agents (ESAs) and iron supplementation are the foundations within the administration of frailty in Chronic Kidney Disease (CKD) and dialysis. There's a hereditary variety inside angiotensin converting enzyme (ACE), a 278 base combine insertion/deletion (I/D) polymorphism, coming about in three diverse ACE genotypes: I/I, I/D and D/D

Objectives: This study conducted in patients with CKD on regular haemodialysis to assess their ACE gene polymorphism frequencies; look at the levels of serum EPO and haemoglobin (HB) and their relation with ACE polymorphism and give new insights about the conceivable role of ACE gene polymorphism in causes of CKD.

Patients and Methods: Fifty Egyptian adult patients (30 with glomerulonephritis, 20 with hypertensive and diabetic mellitus type2) analyzed at Sohag University Hospital from June 2021 to January 2022 in a prospective study compared with 40 control cases). Serum erythropoietin was assessed by ELISA. Genomic TaqMan genotyping test was utilized to assess DNA genomic for ACE rs 1799752 polymorphism.

Results: Our results revealed that the foremost common ACE genotype in patients and controls was the DD, then the I/D; the last one was I/I genotype (p value =0.006).Significant relationship between causes of renal failure (Chronic Glomerulonephritis (CGN), combined DM 2) with HTN compared to control group) and ACE gene polymorphism (P value =0.006). The impact of serum EPO and HB levels on ACE polymorphism was significant (p=0.000). A more significant rise in serum EPO levels was linked to ID genotype, then DD genotype, at least II genotype (p-value between 3 genes =0.000) .There were no significant differences in HB on using ACE inhibitor therapy or not in DD genotype group .

Conclusion: Serum erythropoietin and haemoglobin levels significantly influenced by the polymorphism of ACE . ACE genotypes significantly alter causes of CKD. D allele significantly increased risk of CGN and non-significantly risk factor of combined DM2 with HTN. HB had non significant relationship with different ACE genotypes.

Keywords: Angiotensin Converting Enzyme (ACE), Erythropoietin (EPO), Gene polymorphism, chronic renal failure (CRF), Erythropoietin stimulating agents (ESAs) .

Introduction

Chronic kidney disease (CKD) is considered as dynamic and irreversible misfortune of the work of kidney. It incorporates prior stages of kidney harm (characterized by proteinuria , electrolyte variations from the normal , and hoisted serum creatinine) that lead to a diminish within the glomerular filtration rate (GFR) and amplifies to total misfortune of kidney function—that is , kidney disappointment or end-stage renal infection (ESRD) (**Drawz and Rahman, 2015**).

Diabetes mellitus (DM) and hypertension(HTN) are the foremost risk factors for CKD. Glomerular Infections too constitute an critical cause of developing plague of CKD. In any case, the predominance and rate of glomerulopathies changes broadly in numerous parts of the world(**Naqvi , 2017**).

Anemia in chronic renal disease is a multifactorial condition, the broadly acknowledged etiology being decreased renal generation of erythropoietin, the hormone that's dependable for the incitement of ruddy blood cells generation. Diminished erythropoietin has late connection with down control of hypoxia-inducible factor (HIF), a translation factor that controls quality expression of erythropoietin. Other instruments incorporate uremia; folate and vitamin B12 insufficiency, iron lack, bleeding due to broken platelets, and seldom blood misfortune from hemodialysis. RBC fracture by harmed renovascular endothelium in chosen conditions such as glomerulopathy and dangerous hypertension exacerbates the iron deficiency, which clarifies why iron deficiency can be especially extreme in renal glomerulopathies, counting glomerulonephritis, diabetic nephropathy, for the degree of excretory disappointment (**Shaikh and Aeddula , 2022**).

Erythropoietin (EPO) considered as glycoprotein hormone, normally delivered by the peritubular cells of the kidney that fortifies ruddy blood cell generation. Renal cortex peritubular cells create most EPO within the human body, in spite of the fact that in a embryo, the liver is the essential location of generation (**Jelkmann ,2016**).

A hypoxia-inducible factor (HIF) stimulates the gene of EPO (**Wang and Semenza, 1993; Huang et al .,1997**) which joins to the EPO gene at hypoxia-sensitive locale to enact it .It is arranged at chromosome 7q11-22 , comprising of five exons and four introns, which

lead to post-transcriptional single polypeptide containing 193 amino acids (**Ng et al., 2003**)

The rate of erythropoiesis may be a work of Erythropoietin Responsive Cells (ERC) and the sum of circulating EPO. Subsequently, it has been reported that as the ERC populace diminishes in its count and/or work, the requirement of EPO will increment to protect its rate. The sum of requirements of EPO to protect the erythropoiesis rate thought to be less in the event that the ERC are expanded. The oxygen supply controls the EPO era by a sensor inside the kidney according to its oxygen need (**Fried et al., 1957; Fried ,2009**) .

Human recombinant erythropoietin: Hematopoietic growth factor that fortifies erythropoiesis (**Papich ,2016).**Four types of Epoetin present : epoetin (α ,β, ω) and darbepoetin α . The longest half life is darbepoetin α. with supersialylated shape of EPO .(**Pharmacokinet et al., 2021**).

Human recombinant erythropoietin (epoetin and darbepoetin) gives successful treatment with an awfully favorable benefits compared to risks in patients with last stage inveterate renal lacking on haemodialysis , Also patients not dialysed with inadequate dynamic renal work (**Adamson and Eschbach,1990**).It makes better life for chronic uremic patients(**Sakaguchi et al., 1999 ; Finelli and Carley,2000**) and is very effective in children with chronic renal graft rejection and anemia (**Aufricht et al., 1998**). It moreover offers improvement in patients with no uremia , with chemotherapy- initiated

iron deficiency, epoetin increments haemoglobin level, decreases transfusion prerequisites, and for better life (**Barosi et al .,1998 ;Csaki et al .,1998 ;Pharmacokinet et al., 2021).**

Angiotensin converting enzyme (ACE) is a glycoprotein present in almost all mammalian tissue and body fluids (**Kleij, 2002**) . ACE considered as a dipeptidyl carboxypeptidase with 2 isoforms. Somatic ACE is communicated in the heart, kidneys, digestive tract, adrenal organ, liver, uterus etc, (**Lieberman and Sastre,1983**), whereas testicular ACE presents in sperm cells (**Ramaraj et al.,1998**) . The two forms contain hydrophobic trans-membrane space and a brief cytoplasmic part(**Wei et al.,1991 ; Ljungberg, 2009**) .

Renin-angiotensin–system (RAS) has many functions with hereditary varieties connecting its components with several impacts physiologically and clinically . It includes (ACE), angiotensinogen (AGT) and angiotensin II sort 1 receptor (AGTR1) which show up its importance to renal malady naturally and clinically. Their polymorphisms give a premise for understanding their hereditary variaties relation to advancement of Cardio Vascular Disease(CVD) and/or kidney harm(**Al-Radeef et al .,2019).**

This study looked at the levels of serum EPO and haemoglobin (HB) and their relationships with ACE polymorphism in CKD patients receiving regular haemodialysis in order to assess their ACE gene polymorphism frequencies. It also provided new information regarding the potential role of ACE gene polymorphism in the causes of CKD.

Patients and methods
Patients

This study was performed at Sohag University Hospital from June 2021 to January 2022 , Fifty Egyptian adult patients (30 with glomerulonephritis , 20 with hypertensive and diabetic mellitus type 2) analyzed in a prospective way compared with 40 control cases. We carried out the sample size of 90 respondents (50 cases and 40 controls) to attain 80% power and a 5% confidence level of significance on 2 tailed test (type 1 error).

All routine assay were recorded:
- Complete blood count.
- Kidney function test (urea ,creatinine, uric acid, BUN)
- -Liver function test (AST ,ALT ,albumin ,globulin ,albumin globulin ratio ,total protein)
- Lipid profile test (HDL, VLDL ,total glyceride).

Ethical Consideration

After endorsement of Sohag Faculty of Medicine written assents were gotten from all people included. All individuals were informed regarding the tests and their clinical meanings before the study.

Inclusion characteristics

1- Treatment with haemodialysis for 3 months or more . 2-Age 18 or above. 3-Injection of either alpha or beta epoetin for anemia due to kidney disease .

Prohibition characteristics

1- Current blood loss that require a blood transfusion. 2- Intense kidney disappointment. 3- Blood diseases. 4-Cancer. 5- Serious infection .

Collection of blood and extraction of DNA

Three mls of venous blood were withdrawn from our participants within early morning after an overnight fast before the session of dialysis. 2 ml were collected in plain tubes for serum analysis and 1 ml was collected in EDTA tubes for genomic DNAextraction. Tests at that point were put away at (–80 C) until the time of the measure .DNA extraction was done utilizing turn column based by The QIAamp blood pack (cat.nos.51104 and 51106) agreeing to encased instructions.

Assay of EPO level

Enzyme–linked Immunesorbent Assay (ELISA) was used to assess serum level of EPO by human EPO ELISA Pack supplied by.(Glory Science Co., Ltd cat 17185) agreeing to encased instructions..

Genotyping

TaqMan SNP genotyping test was utilized for ACE rs1799752 SNP genotyping by Step One real time PCR (Connected Biosystem,, USA). The total reaction volume 25 μl (12.5 μl TaqMan® Genotyping master mix, 1.25 μl specific TaqMan® SNP genotyping test)with its forward and reverse primers and two TaqMan® MGB tests ; One VIC® color for the first allele ,one FAM™ color for the second allele) and 5 μl (20 ng) of DNA. We rise the temperature firstly at 95 °C for 10 minutes, then by 40 amplification cycles. Each cycle comprised of denaturation at 95 °C for 15 s, annealing of primer then extension at 60 °C for 60 s. The consider information were analyzed by The TaqMan® Genotyper™ Program

Statistical analysis

SPSS computer program version 25 was used to assess our data. Our quantitative results were shown as means ± standard deviation, median. Qualitative results were shown as numbers and percentages. Tests of significance such as the CHI square test, independent t-test, one-way ANOVA test was utilized for comparing different parameters in our participants. The level of significance was less than 0.05 in all statistical tests. Also, bivariate correlation analysis was done among clinical and laboratory parameters which results showed that r means Pearson correlation coefficient had degrees (r- means negligible correlation, 0.2 to 0.4 , means mild correlation ,r=0.4 to 0.6 means moderate correlation ,r=0.6 to 0.8 means high correlation and > 0.8 means excellent correlation .The genotype distribution of ACE rs 1799752 showed no deviation from Hardy Weinberg equilibrium (p=0.242 >0.05).

Results

Our results revealed clinical characteristics of our study population and sub types of cases with no significant differences in age with high significant difference of sex between cases and controls (**Table 1**).

Table 1. Sex distribution in the study

Parameter of Sex	Cases n=50	Controls N=40	P value by Pearson's Chi-square
Male	**35(38%)**	**15 (16.7%)**	**0.002****
Female	**15 (16.7%)**	**25 (27.8%)**	
Total	**50 (55.6%)**	**40 (44..4%)**	**90 (100%)**

*P < 0.05 (significant) **P < 0.01 (highly significant) ***P < 0.001 (very highly significant) NS: Non significant p >0.05

The percentage of CGN cases was 60% and the percentage of combined HTN and DM2 was 40% . Haemoglobin and hematocrit levels were significantly lower in CKD patients than control group ,Also significant differences in laboratory parameters plasma urea, platelets count, WBC comparing cases to controls**,** no significant difference in triglyceride level and body mass index(BMI) (**Table 2**). Levels of HB were significantly different for cases and controls among differing ACE genotypes ,Also erythropoietin levels were significantly different for controls and patients among diverse ACE genotypes (**Table 3**).No significant differences in demographic data (age , sex)between the three genotype groups and significant difference according to serum ferritin between the three genotype groups(**Table 4**) .The foremost common genotype in cases and controls was DD , then the I/D; the last one was I/I with significant differences in these genotypes between CKD and controls (p value =0.006) and D allele was the most

common in cases and controls(**Tables 5,6**). Significant relationship between causes of renal failure (CGN), combined DM 2 with HTN compared to control group) and ACE gene polymorphism (P value =0.006)(**Table 7**). D allele is significant risk factor for occurrence of CGN (p value =0.018)(**Table 8**) . D allele is non significant risk for occurrence of combined diabetic and hypertensive nephropathy (p value =0.343)(**Table 9**). Serum EPO and HB levels significantly affected by ACE polymorphism (**Table 10**).

Table 2. Mean difference of clinical characteristics of the cases and controls

Parameter	Cases n=50	Controls N=40	P value by independent t test
Age (mean±SD)	48.1 ± 9.49	43.1 ± 19.76	0.114 (NS)
PTH (pg/mL)	101.5 ± 293.4	38.05± 7.6	0.018*
Duration of dialysis years	6.18 ± 2.97	0	-----
Creatinine (mg/dl)	9.57± 2.04	0.9± 0.15	0.000***
BUN (mg/dl)	57.24± 13.52	13.2 ± 3.7	0.000***
Albumin (g/ dl)	3.9± 0.3	4.2 ± 0.5	0.014*
Globulin (g/ dl)	3.47± 0.77	2.7 ± 0.38	0.000***
A/G ratio	1.19± 0.26	1.52± 0.106	0.000***
Uric acid (mg/dl)	6.52± 2.3	5.1± 1.14	0.000***
Calcium (mg/dl)	9.28 ± 1.28	9.5 ± 0.5	0.348NS
Urea (mg/dl)	140.1 ± 50.87	25.8 ± 6.2	0.000***
AST (U/L)	29.9 ± 17.4	24.9 ± 3.4	0.729 NS
ALT (U/L)	35.46 ± 41.4	24.6± 3.3	0.513 NS
Ferritin (µg/l)	95.34 ± 31.33	86.2± 34.2	0.07 NS
EPO (mU/mL)	5.1 ± 1.54	4.86 ± 0.7	0.26 NS
HB (g/dl)	10.1 ± 0.9	14.2 ± 1.36	0.000***
BMI (Kg/m^2)	28.9 ± 2.5	29.07± 2..3	0.76 NS
WBCs (10^9/L)	7.15 ± 2.57	7.99± 1.24	0.006**
RBCs(10^{12}/L)	3.85 ± 0.839	5.36 ± 0.5	0.000***
PLTs (10^9/L)	229± 29.2	304.8 ± 57.1	0.000***
MCV (fl)	83.13±8.25	87.8 ± 4.1	0.002**
MCH (pg)	25.8 ± 3.58	29.1 ± 1.2	0.000***
MCHC (g/dl)	30.9 ± 1.57	33.38±0.8	0.000***
HCT (%)	31.17 ± 6.68	42.6 ± 0.9	0.000***
TG (mg/dl)	176.9 ± 90.5	129.7 ± 8.08	0.2 NS
HDL (mg/dl)	35.8 ± 7.5	46.03 ± 3.06	0.000***
VLDL (mg/dl)	36.4 ± 176	22.5± 4.6	0.000***
Random glucose (mg/dl)	121.8± 22.7	121.4 ± 32.8	0.000***
Systolic blood pressure (mmHg)	144.5± 24.9	126.25± 16.2	0.000***
Diastolic blood pressure (mmHg)	101.8± 23.09	84.5± 13.57	0.000***

*P < 0.05 (significant) **P < 0.01 (highly significant) ***P < 0.001 (very highly significant)
NS: Non significant p >0.05. **Abbreviations:** PTH para thyroid hormone ,BUN blood urea nitrogen , A/G albumin /globulin ratio ,AST aspartate transaminase, ALT alanine transaminase , EPO erythropoietin , HB haemoglobin,BMI body mass index ,WBCs white blood cells ,RBCs red blood cells , PLTs platelets ,MCV mean corpuscular volume , MCH mean corpuscular haemoglobin , MCHC mean corpuscular haemoglobin concentration , HCT haematocrit value , TG triglyceride ,HDL high density lipoprotein , VLDL very low density lipoprotein .

Table 3. ANNOVA test mean difference of important clinical parameter between cases and controls according to ACE genotypes

Parameters	Control (n=40)			Cases (n=50)			
	DD (n=28) mean± SD	ID(n=12) mean± SD	P value	DD (n=45) mean± SD	ID(n=3) mean± SD	II (n=2) mean± SD	P value
PTH (pg/mL)	33.1±7.1	29.6±8.3	0.26	108.02±308.9	36.33±5.7	52±0.0	0.897
Creatinine (mg/dl)	0.8±0.2	0.9±0.14	0.09	9.4±2.1	10.8±0.68	9.6±0.0	0.537
Albumin (g/ dl)	4.1±0.5	4.4±0.5	0.109	3.9±0.38	4.06±0.11	3.4±0.0	0.126
Ferritin (µg/l)	85.0±31.2	88.9±41.7	0.848	98.36±28.76	91.6±41.6	33±0.0	0.012*
EPO (mU/mL)	4.6±0.6	5.3±0.6	0.013*	5.2±1.3	5.6±1.37	1.5±0.0	0.002**
HB (g/dl)	13.7±.0.9	15.1±1.6	0.002**	10.22±0.8	10.23±0.9	8.05±0.07	0.003**
BMI (Kg/m^2)	28.6±2.3	29.9±2.2	0.112	28.8±2.6	29.9±2.4	29.8±0.2	0.712
TG (mg/dl)	130.7±9.0	127.8±37.3	0.342	184.6±91.08	138±39.8	63±0.0	0.132
HDL (mg/dl)	46.5±3.1	42.5±1.1	0.108	36.0±7.5	30.3±9.2	41±0.0	0.287
VLDL (mg/dl)	21.2±3.9	25.6±5.03	0.01*	38.1±17.56	26.9±0.0	12.6±0.0	0.083

Table 4. Characteristic and results (mean±SD) of biochemical parameters among study group (90 person) according to ACE genotypes

Parameters	ACE gene polymorphism			P value by ANNOVA
	DD	ID	II	
Age (years)	47.51±14.5	39.33±16.85	38	0.120 NS
Sex-male	40	8	2	0.439NS by chi-square
-female	33	7	0	
blood pressure(mmHg) -Systolic	138.08±25.25	129.3±9.61	130	0.389 NS
- diastolic	95.75±23.02	86.6±7.23	90	0.310 NS
-Serum triglyceride (mg/dl)	163.9±76.17	129.47±16.19	63	0.039*
-HDL-C (mg/dl)	40.04±8.01	41.93±7.39	41	0.696 NS
-LDL-C (mg/dl)	31.63±16.2	25.9±5.53	12.6	0.102 NS
Duration of dialysis (years)	3.89±3.921	1.0±2.17	5	0.021*
Ferritin (µg/l)	93.25±30.25	89.49±40.26	33	0.035*

Table 5. ACE gene frequency in cases and controls

Parameters	ACE gene polymorphism			Total	P value by Pearson's Chi-square
	DD	ID	II		
Cases	45	3	2	50	0.006**
Controls	28	12	0	40	
Total	73	15	2	90	

*P < 0.05 (significant) **P < 0.01 (highly significant) ***P < 0.001 (very highly significant). NS: Non significant p >0.05.

Table 6. Distribution of allele gene among cases and controls:

Variables			ACE gene polymorphism			P value
			DD	ID+II	Total	
Group	Cases	Count	**45**	**5**	**50**	
		% within Group	90.0%	10.0%	100.0%	0.016*
	Controls	Count	28	12	40	
		% within Group	70.0%	30.0%	100.0%	
Total		Count	73	17	90	
		% within Group	81.1%	18.9%	100.0%	

Table 7. Chi-square relationship between cause of renal failure and ACE gene polymorphism

Parameter	ACE gene polymorphism			Total	P value by Pearson's Chi-square
	DD	ID	II		
Glomerulonephritis	28 (38.3%)	0 (0%)	2 (11.8%)	30	0.006**
Hypertensive and diabetic nephropathy	17 (23.3%)	3 (17.6%)	0 (0%)	20	
Controls	28(38.3%)	12 (70.6%)	0 (0%)	40	
Total	73 (100%)	15 (88.2%) 17(100%)	2 (11.8%)	90	
P value by Pearson's Chi-square	0.000***	0.000***			

Table 8. Odds ratio for occurrence of glomerulonephritis and controls

Parameters	ACE gene polymorphism		Total	ODDs ratio with Confidence interval 95% and p value
	D allele	I allele		
Glomerulonephritis	28	2	30	6.0 (CI 1.23-29.30)
No disease	28	12	40	P value: 0.018*

Table 9. Odds ratio for occurrence of diabetic nephropathy with HTN and controls

Parameters	ACE gene polymorphism		Total	ODDs ratio with Confidence interval 95% and p value
	D allele	I allele		
HTN and diabetic nephropathy type 2	17	3	20	2.4 (CI 0.59-9.8) P value: 0.343NS
No disease	28	12	40	

Table 10. Distribution of HB and erythropoietin levels (mean±SD) among study group according to ACE genotype

Parameters	ACE gene polymorphism			P value by ANNOVA
	DD	ID	II	
HB levels (g/ dl)	11.59±1.96	14.18±2.53	8.05±0.07	<0.001***
Erythropoietin (mU /ml)	5.04±1.2	5.4±0.79	1.52±<0.0	<0.001***

Genotype (ID) had the highest level of serum EPO and HB levels then DD genotype, at least II genotype (**Fig. 1 ,2**).

In the controls, Non significant spearman correlation between EPO and HB as overall (p-value 0.460) , Also according to   different ACE genotype groups and in patients group the correlation between EPO and HB was not significant as overall, however, only ID genotype had high negative significant correlation regarding (Tables 12,13). No significant difference of HB of DD group in using ACEI therapy or not (p value =0.57(**Fig.3**).
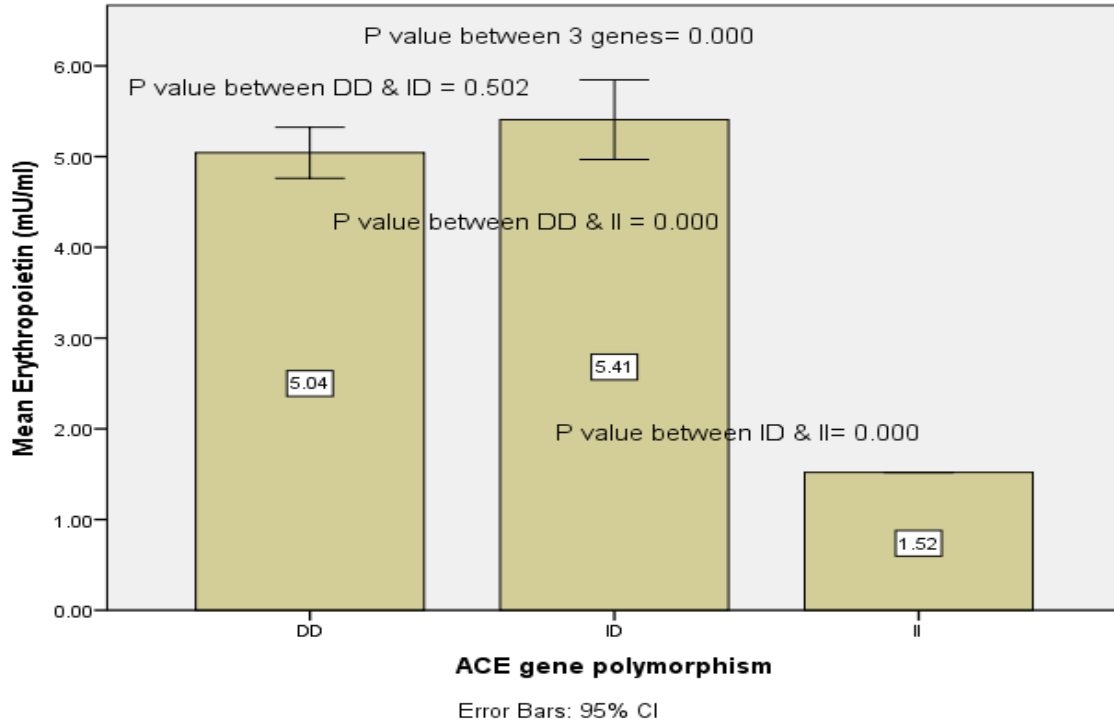
Fig.1. Bar chart shows Relationship between ACE gene polymorphism and EPO level. Very high significant relation between three genes of ACE and EPO level
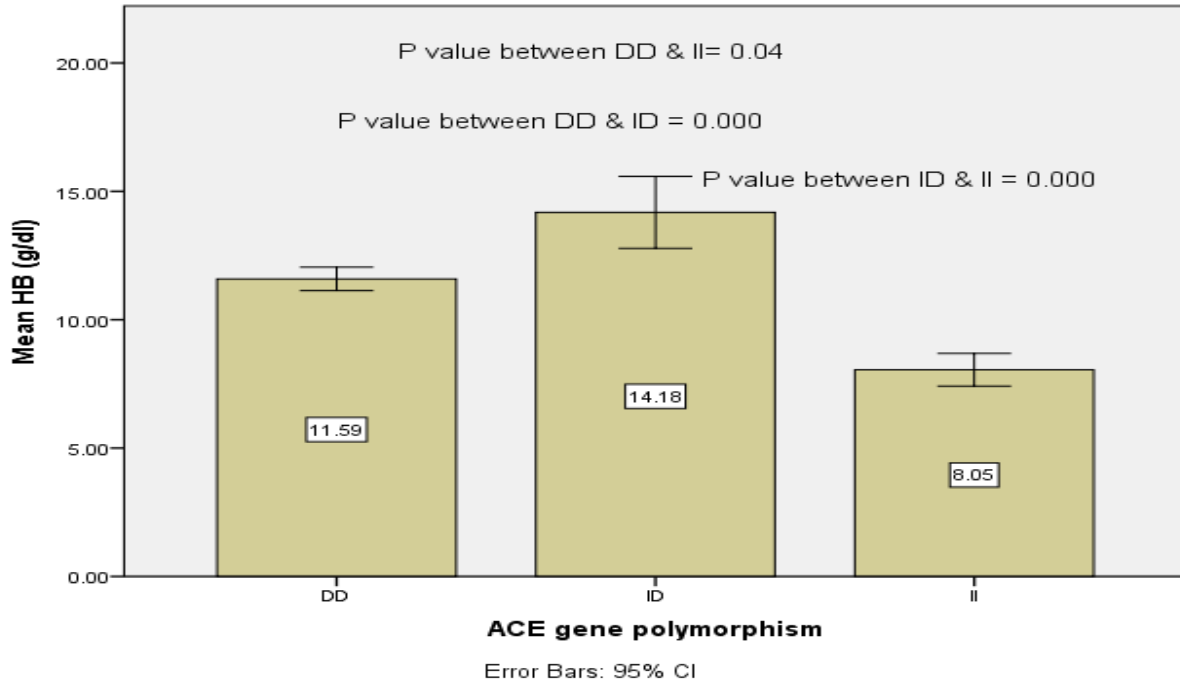

Fig.2. Bar chart shows Relationship between ACE gene polymorphism and HB level. Very high significant relation between three genes of ACE and HB level.
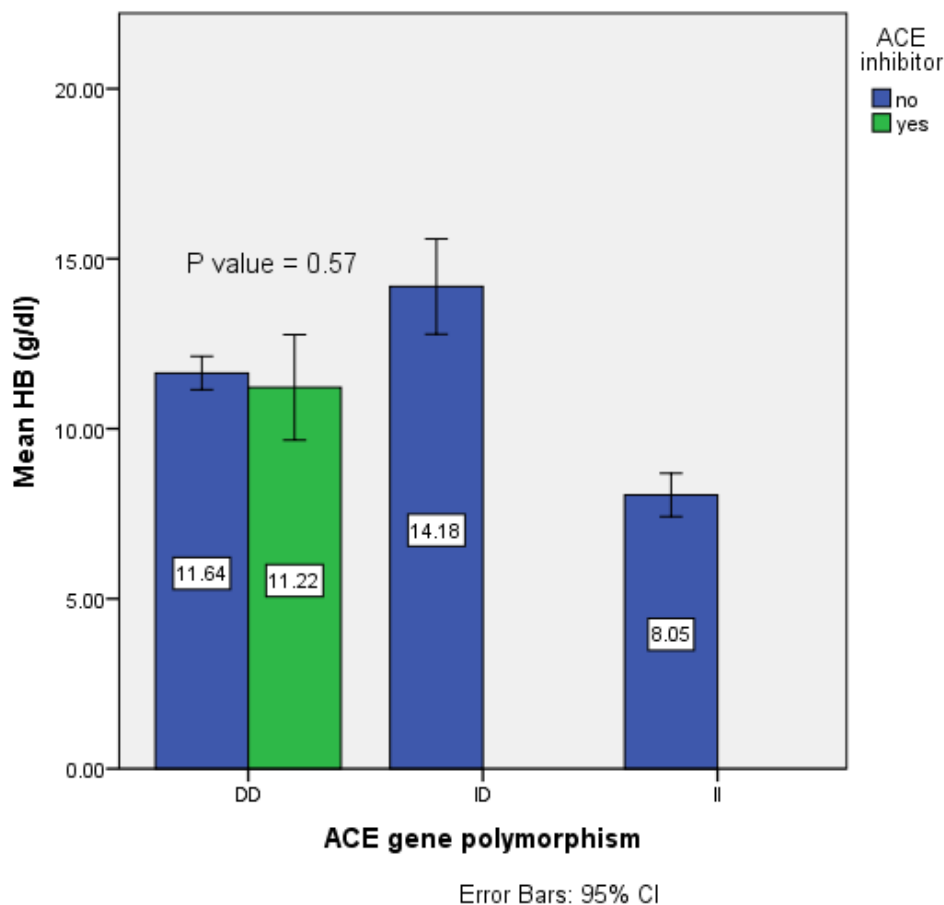
Fig.3. Bar chart showing relation of ACE gene polymorphism and HB in using ACEI therapy or not using ANNOVA test. Figure showed non significant difference of HB of D group in using ACE inhibitor therapy or not using ANNOVA test to get the p value

There was high negative significant correlation in ID gene while no correlation between them in DD and II gene in cases group (**Table.11**). No significant correlation was found between EPO and HB in controls group in ID &DD gene (**Table.12**).

Table 11. Correlation (spearman) between EPO and HB in cases group

Correlation between EPO and HB	Correlation Coefficient	P-value
Overall	**0.131**	**0.365**
DD	**0.105**	**0.491**
ID	**-1.0**	**0.000**
II (can't conduct as only 2 cases in this group)		

Table 12. Correlation (spearman) between EPO and HB in control group

Correlation between EPO and HB	Correlation Coefficient	P-value
Overall	0.120	0.460
DD	0.004	0.98
ID	-0.35	0.26

Discussion

The location of ACE gene is chromosome 17q23.3 and contains many variable polymorphic locales. The changes in ACE levels between people could be a result of polymorphism (rs1799752) that comprises of the appearance (Insertion I) or nonappearance (Deletion D) of a 287-bp Alu repeat sequence in intron 16 of this gene .The activity of ACE is lower in I allele than D allele. The changes in a non-coding locale of this gene , leads to impossibility of being a useful variation. Solid linkage disequilibrium (LD) on this little chromosomal locale of ACE gene leads to restricted haplotypes. Many diseases with inflammatory conditions may be due to ACE gene polymorphism (**Bartakova et al ., 2022**) .

In our study, the whole cohort, males compared to females (54.7% vs 44.5%), mean age was 48.1±9.49 for cases and 43.1±19.76 for controls and median of patients on dialysis therapy by years (6.18±2.97).

Clinical Characteristics of our study population show no significant differences in demographic data (age , sex) between cases and control which agree with (**Al-Radeef et al ., 2019)** and not agree with (**Almaeen and Gomaa , 2021**) who found it significant(Table 1)

In controls, Significant higher levels of haemoglobin (p<0.000) and hematocrit (p<0.000) than in cases. By comparison of cases and controls, cases had low HB levels and high serum creatinine which agree with (**Panjeta et al., 2017 ;Almaeen and Gomaa,2021** , and not agree with (**Al-Radeef et al., 2019)** who found non significant difference in HB between CKD patients and controls . Significant differences in laboratory parameters plasma urea, platelets count, WBC which agree with (**Almaeen and Gomaa, 2021**). No significant difference in triglyceride which agree with (**Park et al., 2005**). No significant difference in BMI which agree with (**Al-Radeef et al., 2019).**

Patients and controls showed significant differences (p- value<0.05) in HB levels among different ACE genotypes which not agree with (**Al-Radeef et al ., 2019**) who found it

insignificant. Moreover in patients and controls significant differences (p-value<0.05) in erythropoietin levels among distinctive ACE genotypes.

No significant differences in demographic data (age , sex)between the three genotype groups which agree with (**Kiss et al., 2015**) and significant difference according to serum ferritin between the three genotype groups which agree with(**Almaeen and Gomaa, 2021**).

The most common genotype in patients and controls was DD genotype then the I/D ; the lowest common was I/I in patients and completely missed within the controls ,with significant difference in the three genotypes among cases and controls (p value =0.006) which agree with (**Elshamaa et al .,2011**) in a haemodialysed Egyptian children ;(**Elhawary et al., 2011)** in astudy on patients with type 1 diabetic nephropathy in Egyptian population, Also (**González et al., 2006**) in a study on the athletes and not agree with (**Al-Radeef et al ., 2019 ; Almaeen and Gomaa, 2021 ;Kiss et al., 2015**) who found the most common ID then DD then II . This explained occurrence of more diseases in specific areas than other areas due to human hereditary varieties.

A significant difference among cases and controls in the gene allele distribution: In cases: DD compared to ID+II 45/5 , in controls: DD compared to ID +II

(28/12) p = 0.016 . D allele is the foremost predominant in cases and controls which agree with (**Almaeen and Gomaa, 2021; van et al ., 2005);(Hočevar et al., 2018)** in a study on spontaneous preterm infants and(**Salem and Batzer , 2009**) in a study in Arab population,and not agree with (**Malueka et al., 2018**) who found the D allele was 37.7%.

Odds ratio of D allele carriers 6.0 (%95 CI 1.23-29.30 P value =0.018) with significant risk of chronic glomerulonephritis compared with I allele carriers, which agree with (**Kalievet al., 2005**)who found that D allele is arisk factor for CGN, as he noted DD subjects had total cholesterol in a high level   and glomerular filtration rate in a low level in comparison to II and ID genotypes (P<0.050), and not agree with (**Kamysheva et al ., 2004**) who found no association was detected between polymorphic markers of genes ACE and onset of CGN .

Also , Odds ratio of D allele carriers 2.4 (%95 CI 0.59-9.8 P value =0.343) with in significant risk of combined diabetic nephropathy type 2 and hypertension compared with I allele carriers which partially agree with (**lin et al .,2014**) who found D allele had significant direct impacts for (Hypertensive nephropathy) but in Diabetic nephropathy not significant and clarified this as people carrying the D allele appeared higher CKD hazard as carriers of D allele had higher levels of ACE than carriers of I allele, which lead to conversion of angiotensin I to

angiotensin II, coming about in (Hypertensive nephropathy) .This due to an added impact of hypertension and the D allele. An added impact was critical within the non diabetic group but not within the diabetic nephropathy subgroups. The advanced glycation end items (AGE) in diabetic patients may be high, increments blood weight So in our study DM2 may have the upper hand , and not agree with **(Panjaliya et al., 2013)** who declared that the I allele and ID genotype significantly increment the hazard for T2DM and Hypertension.

Separately , According to HTN**(El-Mahdy and Morsy, 2002**) showed that the occurrence of essential hypertension was more common with DD genotype (42%) as compared to the controls (30% ; p<0.05) who clarified his contrast with others as the causes of these errors are numerous. The foremost critical cause is that hypertension is polygenic clutter. Racial and natural components may be mindful causes. The utilize of diverse research facility strategies may be other factor (**Tsai et al., 2002)** , In contrast (**Shanmuganathan et al.,2015)** found that ID genotype is markedly risk factor for CKD patients with hypertension . According to DM 2 (**Park et al., 2005 ; Deepashree et al., 2021**)found the DD genotype expanded the hazard of Diabetic nephropathy type 2.These ORs were divergent in numerous ethnicities .This bolsters noteworthy relationship between causes of renal disappointment (CG N , combined DM 2 with HTN compared to control gather) and ACE polymorphism(P value =0.006) .

By assaying serum levels of EPO, HB, and ACE genotype in patients on haemodialysis receiving epoetin injection , DD and ID genotypes all through the consider had significant increase in serum EPO levels (ie, a critical impact of ACE polymorphism on serum EPO levels p=0.000) which agree with (**Al-Radeef et al ., 2019**) , not agree with (**Almaeen and Gomaa, 2021**) who found non significant differences between different ACE G2350A (rs4343) I/I, I/D, or D/D genotypes to serum EPO. We found that ID had more EPO level followed by DD then II which not agree with (**Jeong et al .,2008)** who found DD had more EPO level and clarified that ACE DD genotype brings down the EPO necessity due to the high level of angiotensin II , an critical boost of erythropoiesis. This contrast may be as most DD genotypes gotten ACEI treatment.

A significant difference among the three categories of ACE gene polymorphism and haemoglobin level(ID the highest HB followed by DD then II).Which agree with (**Kiss et al., 2015**)who found(ID the highest HB followed by DD then II) in non using ACE I therapy.

Almaeen and Gomaa, 2021 showed that angiotensin II (Ang II) levels in D/D and I/D genotypes were higher than the II genotype leads to their high HB levels. Ang I converted to Ang 11 by ACE- proteolytic cleavage .The latter is the most important in RAS which acts through AT1 receptor. RAS plays a imperative part in formation of blood cellular components and infections. Among the

other conceivable clarifications is Ang II– actuated Epo discharge inhibition and pluripotent hematopoietic stem cells prevention. ACE coordinates stem cell separation to erythroid progenitors' amalgamation. The Ang II level affected by ACE , specifically expanding erythroid progenitors' in vitro multiplication.The difference from our study most our DD genotypes use ACEI therapy) ,and not agree with (**Al-Radeef et al ., 2019 ; González et al., 2006 ;Saeed and Adam,2018**) who found it insignificant.

Our results showed, In controls, non significant spearman correlation between EPO and HB as overall (p-value 0.460) , Also according to different ACE genotype groups, which not agree with (**Al-Radeef et al ., 2019**)who found it significant as overall , may be as in our study we didnot have II genotype in controls . In patients, non significant spearman correlation between EPO and HB as overall (p-value =0.365) which agree with (**Al-Radeef et al ., 2019)** , however, only ID genotype had high negative significant correlation .

Conclusion

Serum erythropoietin and haemoglobin levels significantly influenced by the polymorphism of ACE . ACE genotypes significantly alter causes of CKD. D allele significantly increased risk of CGN and non significantly risk factor of combined

Kiss et al. (2015) analyzed the HB levels in patients not getting ACEI were higher than who got the treatment . We watched a drift towards diminishing HB levels in patients getting ACEI treatment in DD genotype(p value in groups in DD gene 0.57.(un fortunately we did not have ACEI therapy in ID or II groups due to small sample size (figure 3).**Ajmal et al ., 2013**reported that generation of (EPO) affected by ACEI . Patients who have had kidney transplantation reported that ACEI related diminishment in heamoglobin due to different components connection with erythroid marrow begetters. An affiliation between ACEI treatment, diminish in HB and concealment of EPO generation has moreover been reported . Ang 2 increments multiplication of early erythroid precursors but not the precursors of other cell lines. These perceptions point to a conceivable inhibitory impact of this medicine on bone marrow.

DM2 with HTN. HB had non significant relationship with different ACE genotypes on using Angiotensin converting enzyme inhibitors (ACEI) therapy or not .

References

- **Adamson JW, Eschbach JW(1990).** The use of recombinant human erythropoietin (rHuEpo) in humans. Cancer Surv ,9(1):157–67.
- **Ajmal A, Gessert CE, Johnson BP, Renier CM ,Palcher JA(2013).**Effect of angiotensin converting enzyme inhibitors and angiotensin receptor blockers on hemoglobin levels. BMC Res Notes ,6(1), 443.
- **AlmaeenAbdulrahman Hamdan, Gomaa Mostafa-Hedeab(2021**). Haematological Indicators of Response to Erythropoietin Therapy in Chronic Renal Failure Patients on Haemodialysis: Impact of Angiotensin-Converting Enzyme Rs4343 Gene Polymorphism. Pharmacogenomics and Personalized Medicine ,(2021):1055–68.
- **Al-Radeef Mohanad Yasir, Hayder Adnan Fawzi, Ali Abdulmajid Allawi(2019).** "ACE Gene Polymorphism and Its Association with Serum Erythropoietin and Hemoglobin in Iraqi Hemodialysis Patients." Application of Clinical Genetics ,12: 107–12.
- **Aufricht C, Marik JL, Ettenger RB(1998).**Subcutaneous recombinant human erythropoietin in chronic renal allograft dysfunction. Pediatr Nephrol,12(1):10–13.
- **Barosi G, Marchetti M, Liberato NL(1998).** Cost-effectiveness of

recombinant human erythropoietin in the prevention of chemotherapy-induced anaemia. Br J Cancer,78(6):781–7.
- **Bartakova J, Deissova T, Slezakova S, Bartova J, Petanova J, Kuklinek P., et al ;(2022**) Association of the angiotensin I converting enzyme (ACE) gene polymorphisms with recurrent aphthous stomatitis in the Czech population: case-control study. BMC Oral Health,19:22(1):80.
- **Csaki C, Ferencz T, Schuler D, Borsi JD(1998).** Recombinant human erythropoietin in the prevention of chemotherapy- induced anaemia in children with malignant solid tumours. Eur J Cancer,34(3):364–7.
- **Deepashree G A , Ramprasad E , Jayakumar M , Solomon F D Paul , Gnanasambandan R(2021).**ACE ID gene polymorphism contributes to chronic kidney disease progression but not NOS3 gene among Type 2 diabetes with nephropathy patients. Endocrine and Metabolic Science, (4):100100.
- **Drawz P, Rahman M(2015).** Chronic Kidney Disease. Annals of Internal Medicine, Ninth edit 162(11):ITC1-16.
- **Elhawary NA, Bogari N, Rashad M, Tayeb MT(2011).**Null genetic risk of ACE gene polymorphisms with nephropathy in type 1 diabetes among Egyptian population. Egyptian Journal of Medical Human Genetics, 12(2): 187–

192.

- **El-Mahdy Magda IM, Morsy Mona M (2002)**. "Angiotensin-Converting Enzyme Gene Polymorphism in Patients with Essential Hypertension." The Egyptian Journal of Hospital Medicine, 9(1): 65–73.

- **Elshamaa MF, Sabry SM, Bazaraa HM, Koura HM, Elghoroury EA, Kantoush NA., et al(2011).**Genetic polymorphism of ACE and the angiotensin II type1 receptor genes in children with chronic kidney disease. J Inflamm (Lond),8(1):20.

- **Finelli PF, Carley MD(2000).**Cerebral venous thrombosis associated with epoetin alfa therapy. Arch Neurol,57(2):260–2.

- **Fried W, Plzak LF, Jacobson LO, Goldwasser E(1957).** Studies on erythropoiesis III. Factors controlling erythropoietin production. Proc Soc Exp Biol Med,(94):241–243

- **Fried W(2009) .**Erythropoietin and Erythropoiesis." Experimental Hematology ,37(9): 1007–15.

- **González AJ, Hernández D, De Vera A, Barrios Y, Salido E, Torres A.,et al (2006).** ACE gene polymorphism and erythropoietin in endurance athletes at moderate altitude. Med Sci Sports Exerc,38(4):688-93.

- **Hočevar K, Peterlin A, Jovanović AM, Božović A, Ristanović M, Tul N., et al (2018).**Association between angiotensin-converting enzyme gene insertion/deletion

polymorphism and susceptibility to preterm birth: A case-control study and meta-analysis. Eur J Obstet Gynecol Reprod Biol,(231):122-128.

- **Huang LE, Ho V, Kraine D(1997).** Erythropoietin gene regulation depends on heme dependant oxygen sensing and assembly of interacting transcription factors. Kidney Int,(51):548–522.

- **Hubert C, Houot AM, Corvol P, Soubrier F(1991).**Structure of the angiotensin I- converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. J Biol Chem,(266):15377-83.

- **Jelkmann W(2016).** Erythropoietin. Front Horm Res,(47):115-27.

- **Jeong K, Lee T, Ihm C, Lee S, Moon J(2008).**Polymorphisms in two genes, IL-1 B and ACE, are associated with erythropoietin resistance in Korean patients on maintenance hemodialysis. Exp Mol Med,40(2):161–6.

- **Kaliev RR, Budaĭchiev AB, Aldashev AA, Mirrakhimov MM(2005).**I/D relationship between polymorphism of ACE gene and progression of chronic glomerulonephritis. TerArkh,77(6):12-5.

- **Kamysheva ES, Kutyrina IM, Nosikov VV, Shvetsov MIu, Gorashko NM, Ignat'ev IV., et al(2004).**Association of the complex of polymorphic markers of ACE genes, aldosteron synthetase and endothelial synthetase of nitric oxide with progression of chronic

glomerulonephritis. Ter Arkh,76(9):16-20.

- **Kiss Z, Ambrus C, Kulcsár I, Szegedi J, Kiss I(2015**). Effect of angiotensin-converting enzyme gene insertion/deletion polymorphism and angiotensin-converting enzyme inhibition on erythropoiesis in patients on haemodialysis. J Renin Angiotensin Aldosterone Syst,16(4):1021-7

- **Kleij Franciscus Gerardus Hendricus van Der (2002).** Angiotensin-Converting Enzyme Gene Insertion / Deletion Polymorphism and Renal Disease**.**

- **Lieberman J, Sastre A(1983).** Angiotensin-converting enzyme activity in postmortem human tissues. Lab Invest,(48):711-7.

- **Lin C, Yang HY, Wu CC, Lee HS, Lin YF, Lu KC., et al (2014).**Angiotensin-converting enzyme insertion/deletion polymorphism contributes high risk for chronic kidney disease in Asian male with hypertension--a meta-regression analysis of 98 observational studies. PLoS One 31,9(1):e87604.

- **Ljungberg l(2009).** Angiotensin-Converting Enzyme effects of smoking and other risk factors for cardiovascular diseases.Linköpings Tryckeri AB,(92):1100-6013.

- **Malueka RG, Dwianingsih EK, Sutarni S, Bawono RG, Bayuangga HF, Gofir A ., et al (2018).** "The D Allele of the Angiotensin-Converting Enzyme (ACE) Insertion/Deletion (I/D)

Polymorphism Is Associated with Worse Functional Outcome of Ischaemic Stroke." International Journal of Neuroscience ,128(8): 697–704

- **Naqvi R(2017).** Glomerulonephritis Contributing to Chronic Kidney Disease. Urology & Nephrology Open Access Journal ,5(4): 1–2.

- **Ng T, Marx G, Littlewood T, Macdougall I(2003).** Recombinant erythropoietin in clinical practice. Postgrad Med J, 79(933):367–76.

- **Panjaliya Rakesh K, Surbhi Sethi, Minakashee Sharma, Ravi Sharma, Parvinder Kumar (2013).** Association of Insertion / Deletion Polymorphism of Alu Angiotensin Converting Enzyme Insertion / Deletion Genotype with Type 2 Diabetes Mellitus and Hypertension in J & K Population : A Case Control Study." Journal of Medical Genetics and Genomics ,5(1): 1–5.

- **Panjeta M, Tahirović I, Sofić E, Ćorić J, Dervišević A (2017).** Interpretation of Erythropoietin and Hemoglobin Levels in Patients with Various Stages of Chronic Kidney Disease. J Med Biochem, 36(2):145-152.

- **Papich Mark G (2016).** Epoetin Alfa (Erythropoietin). Saunders Handbook of Veterinary Drugs,293–94.

- **Park HC, Choi SR, Kim BS, Lee TH, Kang BS, Choi KH., et al (2005).**Polymorphism of the ACE Gene in dialysis patients: overexpression of DD genotype in type 2 diabetic end-stage renal

failure patients. Yonsei Med J, 46(6):779-87.

- **Pharmacokinet D, Do L, Cd L, Jc P, Chil R, Med A .,et al (2021)**Erythropoietin , Epoetin Alfa , Epoetin Beta , Epoetin Gamma , and Darbepoetin.. 24(3).

- **Ramaraj P, Kessler SP, Colmenares C, Sen GC (1998).** Selective restoration of male fertility in mice lacking angiotensin-converting enzymes by sperm-specific expression of the testicular isozyme. J Clin Invest ,(102):371-8.

- **Saeed T,Adam M(2018).**Erythropoietin Responsiveness in Hemodialysis Patients; Role of Angiotensin Converting Enzyme Insertion /Deletion (ACEI/D) Polymorphism .Ommdurman Islamic university,(2013)

- **Sakaguchi M, Kaneda H, Inouye S (1999).** A case of anaphylaxis to gelatin included in erythropoietin products. J Allergy Clin Immunol ,(103)(2 Pt 1):349–50

- **Salem AH, Batzer MA (2009).** High frequency of the D allele of the angiotensin-converting enzyme gene in Arabic populations. BMC Res Notes,2(99):1756-0500.

- **Shaikh H, Aeddula NR(2022).**Anemia Of Chronic Renal Disease. StatPearls [Internet]. Treasure Island (FL): StatPearls,

- **Shanmuganathan R, Kumaresan R, Giri P (2015).** Prevalence of angiotensin converting enzyme (ACE) gene insertion/deletion polymorphism in South Indian population with hypertension and chronic kidney disease. J Postgrad ,61(4):230-4.

- **Tsai C, Fallin D, Chiang F, Hwang J, Lai L, Hsu K., et al (2002).** Angiotensinogen gene haplotype and hypertension interaction with ACE gene I allele. Hypertension,(41): 9-15.

- **van der Sman-de Beer F, Verhagen C, Rombach SM, Boorsma P, van Manen JG, Korevaar JC., et al (2005).**ACE I/D polymorphism is associated with mortality in a cohort study of patients starting with dialysis. Kidney Int.,68(5):2237-43.

- **Wang GL, Semenza GL (1993).** General involvement of hypoxia inducible factor in transcriptional response to hypoxia. Proc Soc Natl Acad Sci U S A,(90):4304–4308.

- **Wei L, Alhenc-Gelas F, Corvol P, Clauser E(1991).** The two homologous domains of human angiotensin I-converting enzyme are both catalytically active. J Biol Chem, (266):9002-8.