

Angiotensin Converting Enzyme Genotype Prevalence among Egyptian Primary Nephrotic and End Stage Renal Diseases Patients

Zahran Faten¹, Hamed Sahar², Keshta Akaber, ¹Hussien Mohamed¹

¹ Biochemistry Division, Dept. of Chemistry, Faculty of Science, Zagazig University; ²Urology & Nephrology center, Mansoura University, Egypt.

*Running title: Angiotensin Converting Enzyme Genotype in renal diseases

To whom correspondence should be addressed: Faten Zahran, Biochemistry Department, Faculty of Science, Zagazig University, Egypt.

Background: Renin-Angiotensin system is a key regulator of both blood pressure and kidney functions and their interaction.

Results: Gene polymorphism appears to be an important genetic determinant in causation and progression of renal diseases.

Conclusion: Genotype implicates a strong possible role in the in renal damage among Egyptians.

Significance: The Angiotensin converting enzyme-1 helps in predetermining the timing, type and doses of therapy for end stage renal disease.

ABSTRACT

The Renin-Angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and their interaction. In such a situation, genetic variability in the genes of different components of RAS is likely to contribute for its heterogeneous association in the renal disease patients. Angiotensin converting enzyme-1 (ACE-1) is an important component of RAS which determines the vasoactive peptide Angiotensin-II. In the present study, we have investigated 103 end stage renal diseases (ESRD), 104 primary nephrotic (P.N) patients and 102 normal healthy controls from Mansoura city in Egypt to deduce the association between ACE gene polymorphism and ESRD, P.N. The selected samples were assayed for genotyping of ACE I/D by (PCR) based DNA amplification using specific flanking primers. The results revealed that there was a significance distribution in DD genotype between ESRD and control group ($p < 0.05$), with risk value ($OR > 1$) which resulting in increasing the risk for ESRD. There was significance distribution in ID genotype between ESRD and control group ($p < 0.05$), without disease risk ($OR < 1$). Based on these observations we conclude that ACE DD genotype implicate a strong possible role in the in renal damage among Egyptians. The study will help in predetermining the timing, type and doses of therapy for ESRD patients.

Key words: Angiotensin converting enzyme-1; end stage renal diseases; primary nephrotic; gene polymorphism.

Angiotensin-converting enzyme (ACE) is one of the limiting enzymes in the renin-angiotensin-aldosterone system (RAAS). An elevated angiotensin II level causes deleterious effects on renal hemodynamics and induces the expression of different growth factors, leading to glomerulosclerosis⁽¹⁾. ACE inhibitors and angiotensin receptor blockers reduce proteinuria in patients with nephrotic syndrome,⁽²⁾ stressing the role of the RAAS in the pathogenesis of nephritic syndrome. A polymorphism of the ACE gene, consisting of a 287-bp fragment within intron 16 defined by insertion (I) or deletion (D), has been shown to influence the circulating and cellular ACE concentration^(3,4).

The ACE gene I/D polymorphism is reportedly associated with the progression of several renal diseases, including diabetic nephropathy, IgA

nephropathy, autosomal dominant polycystic kidney disease, and graft failure in renal transplant recipients. The D allele has a dominant effect and is associated with higher plasma ACE and angiotensin II levels⁽⁵⁾.

The ACE DD genotype is associated with increased circulating ACE levels, which are generally two times as high as those found for the II genotype; ID heterozygotes are associated with intermediate ACE levels⁽⁶⁾. The ACE gene I/D polymorphism are reportedly associated with the progression of several renal diseases, including diabetic nephropathy, IgA nephropathy, autosomal dominant polycystic kidney disease, and graft failure in renal transplant recipients. The D allele has a dominant effect and is associated with higher plasma ACE and angiotensin II levels.

Our study aims to determine the ACE I/D genotype distribution in adult primary nephrotic syndrome and end stage renal disease patients and evaluate its effect on clinical parameters.

I. EXPERIMENTAL PROCEDURES:

Patients and controls: Our subjects divided in to three different groups, *group (1)*: 104 primary nephrotic patients, 58 males (55.8%) and 46 females (44.2%), and their age ranged from 19-46 years with a mean of 30.63 ± 6.99 years. *group (2)*: 103 ESRD patients 63 males (61.2%) and 40 females (38.8%), and their age ranged from 19-55 years with a mean of 38.99 ± 8.2 years. And, *group (3)*: 102 unrelated healthy adults with no renal diseases as a control group; 59 males (57.8%) and 43 female (42.2%), and their age ranged from 20-45 years with a mean of 27.89 ± 5.39 years.

Genotyping of the ACE gene I/D polymorphism:

Genomic DNA was extracted from 300 μ l of whole blood, DNA extraction kit (Promega Company, USA). I/D polymorphism of the ACE gene was determined according to the method of **Rigatet al⁽³⁾**. The sequences of the sense and antisense primers were (5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'), respectively. PCR was performed in a final volume of 50 μ l that contained 25 μ l master mixes, ≈ 500 ng of genomic DNA, 12.5 pmol of each primer and 5% dimethylsulphoxide (DMSO). Amplification was performed using a Gene Amp PCR system G-storm. Samples were denatured for 1 minute at 94°C and then cycled 30 times through the following steps: 45 seconds at 94°C, 1 minute at 62°C, and 1 minute at 72°C. PCR products were electrophoresed in 1.6% agarose gel and visualized directly with Ethidium Bromide staining. I allele was detected as a 490-bp band, and the D allele was detected as a 190-bp band (figure 1). 1st PCR was confirmed by using the second PCR (9). A second PCR amplification was performed for each DD type with a primer pair that recognizes an insertion-specific sequence (5'- TGG GAC CAC AGC GCC CGC CAC TAC-3'; 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3'), with identical PCR conditions except for an annealing temperature of 67°C and the absence of 5% DMSO.

Biochemical parameters:

Total cholesterol, Triglyceride & LDL, Serum creatinine & albumin were measured for all study population according to methods of; **Young⁽⁷⁾**; **Stein⁽⁸⁾**; **Henry⁽⁹⁾**; **Doumas et al.⁽¹⁰⁾**; respectively.

Statistical analysis:

The allele distribution of the ACE gene I/D polymorphism was tested for Hardy-Weinberg equilibrium in patient and control groups. A computer software package (SPSS), version 14 ⁽¹¹⁾, was used

in the analysis. For quantitative variables, mean and median (as a measure of central tendency), standard deviation. Frequency and percentage are presented for qualitative variables. Chi square test used to estimate differences in qualitative variables. P value < 0.05 was considered to be statistically significant.

II. RESULTS

The genotype frequencies 1st PCR (II, ID and DD-genotypes) in nephrotic group were 10, 43 and 51; respectively. In addition the incidences of II, ID and DD in ESRD group were 0, 21 and 82; respectively. Where, in control group the incidences of II, ID and DD were 12, 66 and 24; respectively. The genotype frequencies 2nd PCR (II, ID and DD-genotypes) in nephrotic group were to be found 10, 66 and 28; respectively. In addition the incidences of II, ID and DD in ESRD were 0, 45 and 58; respectively. Where, in control group the incidences of II, ID and DD were 12, 74 and 16 respectively. Out of 51 patients with P.N showed initially the DD genotype, 23 patients were proven to have the ID.

This was confirmed by the second PCR. Out of 82 patients with ESRD showed initially the DD genotype 24 patients were proven to have the ID genotype, also out of 24 control showed initially the DD genotype 8 control were proven to have the ID this was confirmed by using the second PCR. The frequencies of the II, ID, and DD genotypes in controls were found to be 12, 74, and 16, respectively, which statistically non-significant compared to those of P.N and significant compared to ESRD patients.

There was a significance in distribution in DD genotype between primary nephrotic and control group ($p=0.049$), with risk value equal $1.98 > 1$ which resulting in increasing the risk for disease. While there was no significance distribution in both ID & II genotype between both P.N and control group ($p > 0.05$), without disease risk ($OR < 1$).

There was a significance in distribution in DD genotype between ESRD and control group ($p=0.000$), with risk value equal $6.92 > 1$ which resulting in increasing the risk for ESRD. There was significance distribution in ID genotype between ESRD and control group ($p=0.000$), without disease risk ($OR < 1$).

III. DISCUSSION

The genetic origin of kidney diseases has been a focus of research in the past few years. Various reports suggest that susceptibility to develop ESRD has a significant genetic Component. The renin-angiotensin System (RAS) has been considered to be responsible for the pathogenesis and progression of kidney diseases. Genetic polymorphisms of the various components of the RAS system have been associated with differences in the clinical course of chronic kidney disease ⁽¹²⁾. There is significant evidence showing that the RAAS is involved in the

pathogenesis of progressive renal disorders. As a matter of fact, in recent studies the association between disease progression and the ID/DD genotype of the ACE gene has been well described⁽²⁾. Interestingly, it has been found that patients with the DD genotype experienced a more severe clinical course⁽¹³⁾. Angiotensin II is the most active product of the RAAS and it has a strong influence on local and systemic hemodynamic regulation. Angiotensin II is a potent vaso-active peptide that causes blood vessels to constrict, resulting in increased blood pressure. Angiotensin II is also a renal growth factor that modulates key elements of renal disease progression, including renal mesangial cell growth, extracellular matrix synthesis, and degradation and inflammatory processes⁽¹⁴⁾. The effects of angiotensin II are mediated by the release of several factors, including transforming growth factor-B (TGF-B), plasminogen activator inhibitor-1, monocyte chemo-attractant protein-1, and the activation of various nuclear transcription factors, including activator protein-1 and nuclear factor kappa B (NF-κB)⁽¹⁵⁾. If the renin-angiotensin-aldosterone system is abnormally active, blood pressure will be too high. The ACE DD genotype is associated with increased circulating ACE levels, which are generally two times as high as those found for the II genotype; ID heterozygotes are associated with intermediate ACE levels⁽⁶⁾. A polymorphism of the ACE gene, consisting of a 287-base pair fragment within intron 16 defined by insertion (I) or deletion (D), has been shown to influence the circulating and cellular ACE concentration. The ACE gene I/D polymorphism are reportedly associated with the progression of several renal diseases, including diabetic nephropathy, IgA nephropathy, autosomal dominant polycystic kidney disease, and graft failure in renal transplant recipients. The D allele has a dominant effect and is associated with higher plasma ACE and angiotensin II levels⁽³⁾.

Our study aims to estimate the frequencies of different polymorphisms of ACE gene in ESRD, P.N. And Study the effect of gene polymorphism on biochemical parameters including (Total Cholesterol, Triglyceride (TG), and LDL Cholesterol, serum creatinine, albumin & uric acid) in P.N., ESRD and control groups. In the current study, we tried to increase the specificity of ACE I/D genotyping by using DMSO in the first PCR. In addition, a second PCR was performed for the samples that showed DD genotype in the first step. Genotype frequencies 1st PCR (II, ID) were decreased significantly by 16.6% & 34% in P.N. While, frequencies 1st PCR (DD) were increased significantly by 112.5% in P.N compared to control group, but; genotype frequencies 1st PCR (II, ID) were decreased significantly by 100%, and 68.1% in ESRD. While, the frequencies 1st PCR (DD) were increased significantly by 241.6% compared to

control group. As a matter of fact, in recent studies the association between disease progression and the ID/DD genotype of the ACE gene has been well described by **Bagga and Srivastava**,⁽¹⁶⁾. This agrees with the study of **Sasongkoet al.**⁽¹⁷⁾ who reported that the differences in the distribution of the ACE gene polymorphism between INS patients and controls were not statistically significant. This is, however, disagree with most other studies in which the DD genotype was significantly more common in nephrotic patients compared to normal individuals^(2, 4, and 18). Our study revealed a highly significant difference in the presence of DD genotype and D allele of ACE gene among ESRD patients and normal controls validating that the ACE gene polymorphism is an important genetic determinant of non-diabetic nephropathies. Overall findings were demarcating that D allele of ACE gene confers a high risk of developing renal diseases (**OR = 3.30**) and this association was highly compounded when D allele was present in homozygous state (**OR = 6.92**). Even inclusion of the heterozygous ID state known to have low levels of ACE production along with the DD genotype depicted a high risk of renal failures (**OR = 0.29**). It has been found that patients with the DD genotype experienced a more severe clinical course **Mahaet al.**,⁽¹⁹⁾. We confirmed our results by measuring different clinical parameters and their relation to polymorphism. ID polymorphism showed increase in cholesterol, triglyceride, LDL, and creatinine by (29%, 151%, 35%, 188%) in P.N patients; while (37%, 254%, 105%, 105%) in ESRD compared to control. On the other hand; DD showed increase in cholesterol, triglyceride, LDL, and creatinine by (33%, 275%, 60%, 20%) in P.N patients; while (38%, 250%, 112%, 723%) in ESRD compared to control. Moreover, ID and DD polymorphism showed decrease in albumin by 25% and 23% in P.N and ESRD patients; respectively compared to control. Kidney disease is clinically characterized by increasing rates of urinary albumin excretion, starting from normoalbuminuria, which progresses to microalbuminuria, macroalbuminuria/over nephropathy, and eventually to ESRD **Choudhry et al.**,⁽²⁰⁾. Therefore; ACE gene polymorphism appears to be an important genetic determinant in causation and progression of renal diseases and ACE DD genotype was found to be strongly associated with ESRD.

IV. CONCLUSION

Conclusively, ACE gene polymorphism appears to be an important genetic determinant in progression of renal diseases and ACE DD genotype was found to be strongly associated with ESRD.

V. ACKNOWLEDGEMENT

The authors would like to thank members of Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University for assistance.

REFERENCES

- [1] **Oktem, F.; Sirin, A.; Bilge, I.; Emre, S.; Agachan, B.; Ispir T., (2004):** ACE I/D gene polymorphism in primary FSGS steroid-sensitive nephrotic syndrome. *Pediatr Nephrol*; **19:384-9.**
- [2] **White, CT.; Macpherson, CF.; Hurlet, RM.; Matsell, DG. (2003):** Antiproteinuric effects of enalapril and losartan: a pilot study. *Pediatr Nephrol*; **18:1038-43.**
- [3] **Rigat, B.; Hubert, C.; Alhenc-Gelas, F.; Cambien, F.; Corvol P.; Soubrier, F. (1990):** An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest*; **86:1343-6.**
- [4] **Costerousse, O.; Alledgrini, J.; Lopez, M., Alhenc-Gelas, F. (1993):** Angiotensin I converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. *Biochem J*; **290:33-40.**
- [5] **Bagga, A.; Srivastava, RN. (2002):** Nephrotic syndrome and proteinuria. *Indian J Pediatr*; **69:1053-4.**
- [6] **Ribichini, F.; Steffenino, G.; Dellavalle, A. (1998):** Plasma activity and insertion/deletion polymorphism of angiotensin I-converting enzyme, a major risk factor and a marker of risk for coronary stent restenosis. *Circulation*; **97:147-54.**
- [7] **Young DS., (2001):** Effects of disease on Clinical Lab. Tests, 4th ed. AACC.
- [8] **Stein, EA., (1987):** Lipids, lipoproteins, and apolipoproteins. In: Tietz NW, ed. *Fundamentals of Clinical Chemistry*. 3rd ed. Philadelphia: WB Saunders 448-481.
- [9] **Henry, RJ., (1974):** Clinical chemistry. Principles and Techniques. Second Edition. Harper and Row. P 525.
- [10] **Doumas, BT., Watson, WA., and Biggs, HG., (1971):** Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim.* **31: 87 – 96.**
- [11] **Lvesque R. SPSS., (2007):** programming and Data Management: a guide for SPSS and SAS users, Fourth Edition, SPSS.inc., Chicago III.
- [12] **Agrawal S., Agarwal S.S., Naik S., (2010):** Genetic contribution and associated pathophysiology in end-stage renal disease. *The Application of Clinical Genetics*, **3**, pp. 65 – 84.
- [13] **Frishberg, Y.; Becker-Cohen, R.; Halle, D., (1998):** Genetic polymorphisms of the renin-angiotensin system: the outcome of focal segmental glomerulosclerosis in children. *Kidney Int*; **54:1843-9.**
- [14] **Paul M., Poyan M.A., Kreutz R., (2006):** "Physiology of local renin-angiotensin systems". *Physiol. Rev.* **86 (3): 747–803.**
- [15] **Serdaroglu, E.; Mir, S.; Berdeli, A. (2005)** ACE gene insertion/deletion polymorphism in childhood idiopathic nephrotic syndrome. *Pediatr Nephrol*; **20:1738-43.**
- [16] **Bagga A, and Srivastava RN., (2002):** Nephrotic syndrome and proteinuria. *Indian J Pediatr*; **69:1053-4.**
- [17] **Sasongko, T.; Sadewa, AH.; Kusuma, PA.; et al. (2005):** ACE gene polymorphism in children with nephrotic syndrome in the Indonesian population. *Kobe J Med Sci*; **51:41-7.**
- [18] **Al-Eisa, A.; Haider, MS.; Srivastava, BS. (2001):** Angiotensin converting enzyme gene insertion/deletion polymorphism in idiopathic nephrotic syndrome in Kuwaiti Arab children. *Scand J Urol Nephrol*; **35:239-42.**
- [19] **Maha S.A., Samar S., Inas Abdel-L., Hanaa N., Soheir A.A. and Sanaa Abdel-S., (2010):** Effect of angiotensin-converting enzyme gene insertion/deletion polymorphism on steroid resistance in Egyptian children with idiopathic nephrotic syndrome. *Journal of the Renin-Angiotensin-Aldosterone System*. **11(2); 111-118.**
- [20] **Choudhry N., Nagra S.A., Shafi T., Mujtaba G., Abiodullah M. and Rashid N., (2012):** Lack of association of insertion/deletion polymorphism in angiotensin converting enzyme gene with nephropathy in type 2 diabetic patients in Punjabi population of Pakistan. *African Journal of Biotechnology*. **11(6), pp. 1484-1489.**

Table (1): represents Chi-Square Test I/D allele in ESRD, P.N and control groups.

genotype |

groups	II	ID	DD	I allele	D allele	p-value
control	12	74	16	98	106	----
P.N	10	66	28	86	122	0.172
ESRD	0	45	58	45	161	0.000

P value Chi-Square Test <0.05

Table (2):represents Odds Ratio (OR) for ESRD, P.N and control groups.

Alleles	Control	Primary Nephrotic	OR	(95% CI)	Risk	P-value for chi square test
D	106	122	1.312	(0.889-1.93)	+	P>0.05
I	98	86	0.762	(0.517-1.125)	-	P>0.05

Alleles	Control	ESRD	OR	(95% CI)	Risk	P-value for chi square test
D	106	161	3.30	(2.15-5.08)	+	P<0.05
I	98	45	0.302	(0.19-0.46)	-	P<0.05

P Chi-square Test <0.05 significant; P OR <1 Risk disease; P OR >1 Non Risk disease

Table (3):shows The prevalence of ACE (I) and (D) polymorphism genotype 1st PCR in patients and control groups.

groups	genotype	II	ID	DD	p-value
		Control	12	66	
P.N		10	43	51	
Control		12	66	24	0.000
ESRD		0	21	82	

P Chi-square Test <0.05 significant

Table (4): Prevalence of ACE I and D polymorphism genotype 2nd PCR in patients and control groups.

groups	genotype	II	ID	DD	p-value
		Control	12	74	
P.N		10	66	28	0.143
ESRD		0	45	58	0.000

P Chi-square Test <0.05 significant

Table (5): Comparison of lipid profile, creatinine& albumin in Control, P.N & ESRD if ACE ID/DD.

Group	Cholesterol mg/dL mean±S.d	Triglyceride mg/dL mean±S.d	LDL mg/dL mean±S.d	Creatinine mg/dL mean±S.d	Albumin g/dL mean±S.d
Control ID	170.75 ±18.3	70.09 ±16.7	73.3 ±10.9	0.95 ±0.97	4.19 ±0.44
Control DD	171.3 ± 17.95	73.25 ±18.7	70.8 ± 12.6	1.3 ± 2.06	3.99 ± 0.83
P.Nephrotic ID	220.12 ±89.7	176.2 ±9.8	99.64 ±34.1	2.6 ±2.2	3.13 ±0.99
P.Nephrotic DD	228.4 ± 93.7	174.5 ± 10.6	112.0 ±0.80	3.10 ± 2.4	3.1 ± 0.89
ESRD ID	234.76 ±35.1	248.9 ±42.3	150.5 ±12.3	10.49 ±2.76	3.02 ±0.44
ESRD DD	236.45 ± 42.1	256.4 ± 43.1	149.7 ±12.7	10.71 ±2.99	3.03 ±0.42

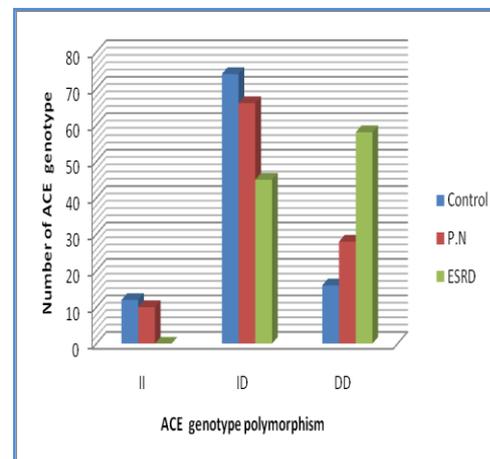
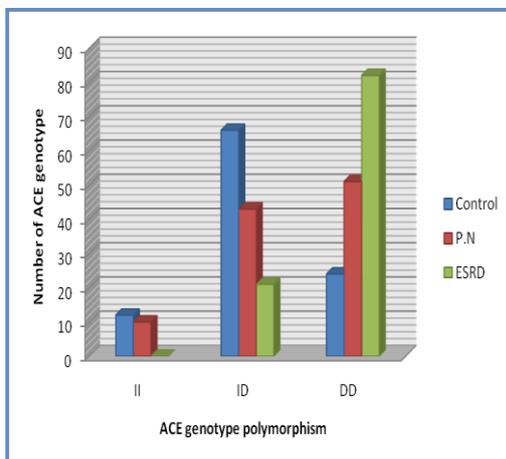


Fig (1) shows 1st PCR genotyping Fig (2) shows 2st PCR genotyping

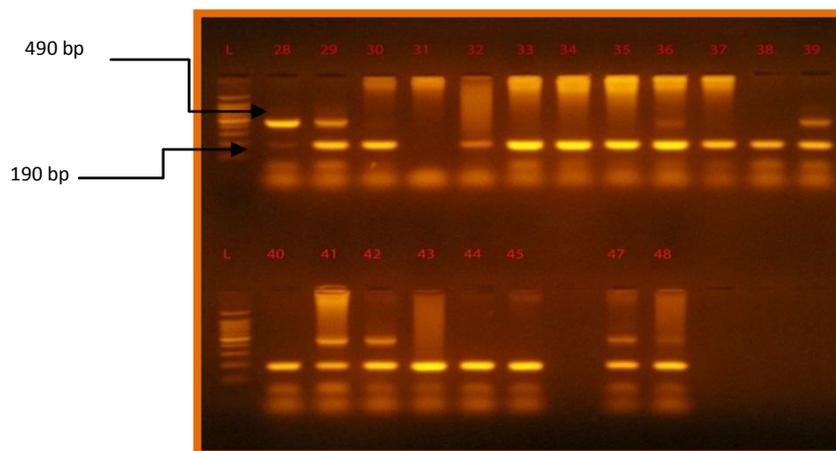


Figure (3): Agarose gel electrophoresis of 1st PCR products of ACE gene. Lines 29, 39, 41, 42, 47 heterozygous ID, 30, 32-38, 40, 43, 44, 45, 48 homozygous DD cases, 28 homozygous II cases. (I allele 490 bp, D allele 190bp).

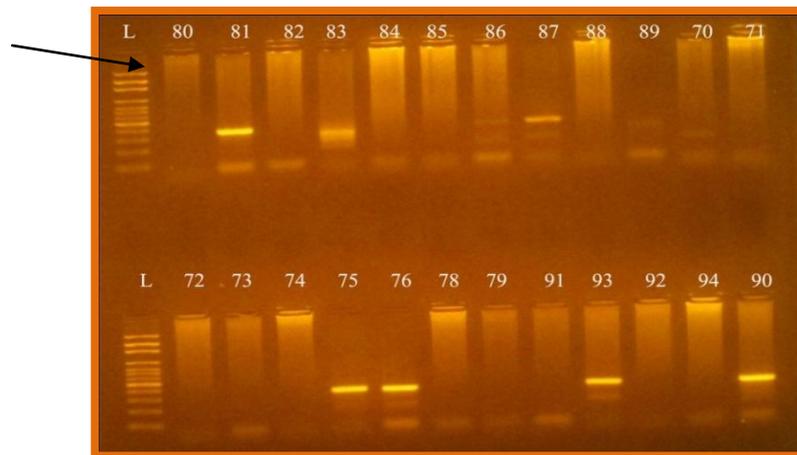


Figure (4):Agarose gel electrophoresis of 2nd PCR products of ACE gene. Lines 81, 87, 75, 76, 93, 90 heterozygous ID, the other Lines represent DD homozygous. (ID 330 bp)

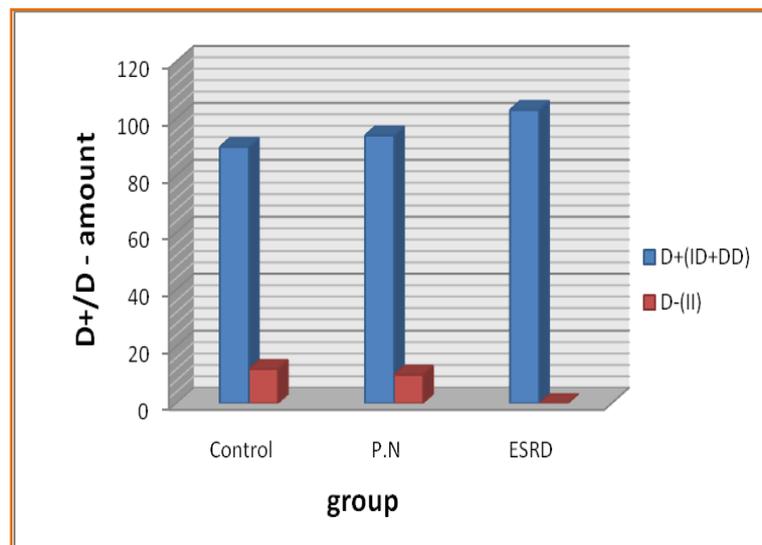


Figure (5): D⁺/D⁻ in P.N, ESRD & control groups.