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Association between AXIN1 gene polymorphisms (wnt signaling pathway gene) and nephropathy induced by diabetes and hypertension in the Egyptian population

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ABSTRACT

Background: Chronic renal disease is a public health concern worldwide. The epidemiology of this disease can help in the protection strategy. The detection of the polymorphism of the CKD candidate gene can assist in the early detection Chronic. And thus, early detection and treatment can often avoid or postpone the kidney disease. ***Objectives:*** Our research aimed to investigate how polymorphisms of the AXIN-1 gene and CKD incidence are associated with Egyptians. ***Methods:*** This study included 3 groups; control group, a pre-nephropathy group including (diabetes 2 and hypertension) subgroups, and a nephropathy group including (diabetic nephropathy, hypertensive nephropathy, and diabetic hypertensive nephropathy) subgroups. All the volunteers are subjected to complete clinical examination and routine laboratory analysis. In this research, we performed genotyping for two SNPs (rs9921222 and rs1805105) throughout the Egyptian population (PCR-RFLP). **Results**: our results showed that in both (rs9921222 and rs1805105) SNPs, there was a significant difference in genotypes distribution (P<0.001) ***Conclusion:*** The AXIN-1 SNPs (rs9921222 and rs1805105) may be used as one of the Egyptian population's CKD susceptibility factors and larger studies can confirm our results.

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Introduction

High morbidity and mortality are associated with chronic kidney disease (CKD); this is a growing health concern. CKD is characterized as either kidney damage or kidney injury, with the presence over three months of at least one marker of renal impairment. [1].

CKD is classified based on the six GFR stages and the three albuminuria stage. Earlier signs can be detected by routine laboratory testing of chronic kidney disease such that early detection can sometimes eliminate or postpone them. [2].

Renal disorder in the end-stage (ESRD) emerges as one of Egypt's big health problems. As the cost of dialysis and transplantation is rising, the number of patients with ESRD puts tremendous pressure on Egypt's health care services.

The number of patients receiving RRTs (renal replacement therapy) is estimated to reach 1,4 million worldwide, with the annual rate of occurrences rising to 8%.[3].

ESRD has several different causes among patients. The main risk factors for chronic kidney disease (CKD) include age, diabetes mellitus and hypertension and drugs, such as long-term use of analgesics, leading to analgesic nephropathy and kidney damage. [4].

The key effects, regardless of cause, of chronic renal disease include progress to kidney failure, reduced renal function complications, and heart disease. More and more research suggests that early identification and diagnosis will avoid or postpone some of these adverse outcomes. Chronic kidney disease is, regrettably, underdiagnosed and undertreated and has missed prevention opportunities. [5, 6].

Recognizing and evaluating mutations causing diseases is one of the biggest obstacles in medical genomics. [7].

AXIN1 is a major gene located at 16p13.3 chromosome and plays a crucial role in the development of embryos and postnatal diseases and in pathogenesis. [8]. It is a multi-domain scaffolding protein that integrates with several proteins and acts as the main negative controlling protein of the wnt signal pathway [9]. This pathway, by regulating cellular proliferation and differentiation via the ß catenin destruction, is important in human tumor genesis. [10]. Impaired control of the target genes of the Wnt was shown to include many other human diseases, including nephropathy. [11–13].

SNPs are the most common forms of human genome genetic variability. A SNP can change gene expression leading to a particular cell phénotype [14, 15]. Polymorphisms in the AXIN1 gene, for example, have been shown to be associated with disease and cancer growth [16–18]. AXIN1 protein integrates with various protein molecules that control wnt, TGF-β, SAPK / JNK, and p53 signals [19, 20].

A lot of studies showed that several human diseases are associated with AXIN1 [16–18, 21–23]. Nevertheless, no research suggested that AXIN1 directly associates with the progression of CKD. In this study, we have examined possible connections in the

Egyptian population between different AXIN1 genes SNPs and CKD risk.

The goal of this study is to evaluate the association between nephropathy induced by diabetes or hypertension in the Egyptian population with two novel gene polymorphisms of the AXIN-1 gene which is an important member of the wnt signaling pathway proteins.

2-Patients and Methods

Subjects

Our study is composed of 3 groups. The first group is the control group including 46 of healthy volunteers, a second group is a pre-nephropathy group including (16 Diabetic patients, 14 Hypertensive patients), and nephropathy group including (25 Diabetic Nephropathy patients, 28 Hypertensive Nephropathy patients,14 Diabetic Hypertensive Nephropathy patients, all the patients are age-matched with healthy volunteers. They are all subjected to the same measurements including routine laboratory analysis, Creatinine, Urea, ALT, AST, Calcium, Phosphorus, Sodium, and Potassium.

All The healthy volunteers, Diabetic and Hypertensive groups are chosen from patients out clinics in El Ahrar hospital and nephropathy groups were selected from those admitted at dialysis unit, El Ahrar hospital in the period of October 2017 till March 2018.

All the volunteers are subjected to complete full history about their living standards, family history of disease and Clinical Pathological Features. The methodology of research was approved by Zagazig University's ethics committees. Informed written consent was obtained from each individual.

Sample Collection

Blood samples were collected under complete aseptic conditions by clean venipuncture using sterile disposable syringes. We divided the blood sample into 2 portions: 1 ml of whole blood was collected into tubes containing EDTA for hemoglobin. About 5 ml of blood were withdrawn from each patient as well as controls. Blood was delivered into clean dry test tubes and allowed to clot at room temperature. 5 ml of whole blood was centrifuged at 1600 rpm for 5 min and the serum was separated into 1.7 ml Eppendorf tubes. Serum samples were stored in tightly closed vials at -80oC until used for biochemical analysis.

Routine laboratory analysis

Creatinine, Urea, ALT, AST, Calcium, Phosphorus, Sodium, Potassium were measured in serum by routine enzymatic methods. They were measured by colorimetric methods using a spectrophotometer.

DNA Extraction

DNA was isolated using the Genomic DNA spin column purification kit (Fermentas, Germany) according to a previous study protocol [24]. Genomic DNA of each individual was extracted from 200ml of EDTA-anticoagulated peripheral blood samples by a DNA isolation kit. The

procedure was performed according to the instruction manual.

Genotyping of AXIN-1 C>T (r9921222s)

Isolated DNA was genotyped for the detection of rs9921222 (C/T) polymorphism allele for each volunteer. The genotyping step has been made in ZSMRC using the RFLP-PCR technique [25]**.** In this method, the amplification for the region containing the desired SNP is done by a PCR reaction followed by digestion with a suitable restriction enzyme which gives digested products that can be identified by the gel electrophoresis technique.

Each PCR reaction is done in a total volume of 20 µl containing 5 µl of template DNA with a concentration of 1 µg, 10 µl of the master mix, 3 µl of nuclease-free water, and 1 µl of each primer of the two primers used in the reaction. The primers were designed by BLAST software in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
The sequence of forward primer was 5’ CCCAGTCCAGACACAAACCT 3’ and that of the reverse primer was 5 GGGCCAGTTGTCAGTATTGC’3'. The thermal cycling conditions were 95^0c/5min for activation followed by 35 cycles of 95^0c for 30 seconds for denaturation then 54^0c for 30 seconds for annealing, then 72^0c for elongation and a final cycle 72^0c for 10 min for final elongation. The choosing of annealing temperature was done by making gradient PCR.

The expected amplicon size was 475 bp. The PCR product was digested using suitable restriction enzyme “BspHI” (10 µ PCR product + 1 µ enzyme +2 µ buffer of enzyme + 15 µ free nuclease water), mix and then incubated at 37 C for 24 h. the enzyme makes cutting at the "C" allele and not the "T” allele. This enzyme was chosen by using NEB restriction enzyme finder and cutter (https://enzymefinder.neb.com/#!#nebheader) (http://nc2.neb.com/NEBcutter2/).

The digested amplicon was subjected to gel electrophoresis with 2% agarose stained with ethidium bromide under ultraviolet light. Three genotypes were given; CC genotype with two bands with size 229,246; CT genotype with three bands with size 475,229,246; and TT genotype with one band with size 475

Genotyping of AXIN-1 T>C rs1805105.

Similarly, the genotyping of rs1805105 (T>C) was done using the RFLP-PCR method with the above-mentioned steps [25]. The sequence of forward primer was 5’ ATCTGGATACCTGCCGACCT 3’and that of the reverse primer was 5’ AGGACATCCGGTGTGGGTTA 3'. The thermal cycling conditions were 95^0c/5min for activation followed by 35 cycles of 95^0c for 30 seconds for denaturation then 58^0c for 35 seconds for annealing, then 72^0c for elongation and a final cycle 72^0c for 10 min for final elongation. The choosing of annealing temperature was done by making gradient PCR.

The expected amplicon size was 284 bp. The PCR product was digested using “BtsCI” which makes cutting at the "T” allele and not “C” allele. The digested amplicon was subjected to gel electrophoresis with 2% agarose stained with ethidium bromide under ultraviolet light. Three genotypes were given; TT genotype with four bands with 62-222-282-2bp, TC genotype with five 62-222-282-2-284bp, and CC genotype with one band with size 284bp.

Statistical analysis

Data have been analyzed with the Social Science Statistical System (SPSS version 20.0). Qualitative data were defined by number and percentage, quantitative data by mean ± SD and the following tests were used to assess the mean differences between different groups for the mean. Chi-square test (X^2) was used for estimating the significance of difference and association of the qualitative variable. Odds ratio (OR) was used for estimating significant association and to find the strength of association or risk. Differences between quantitative independent groups were tested by t-test or Mann Whitney. Pearson correlation or Spearman's correlation between parameters was done. For significant results P value was set at < 0.05 and < 0.001 for high significant results.

Results

Anthropometric parameters

Our data using statistical analysis showed a variety of results. Some of the parameters were associated with Nephropathy groups while others were not.

For age and sex, there was no significant difference between the 3 general groups in our study ($P > 0.05$) **(Table 1, 2)**

Routine laboratory measurements:

There was a significant decrease in the blood HB level in all the nephropathy subgroups than both control and pre-nephropathy subgroups ($P < 0.001$) (**Table 3).**

There was a significant increase in both blood urea and creatinine level in all the nephropathy subgroups than both control and pre-nephropathy subgroups ($P < 0.001$) (**Table 4).**

Similarly, There was a significant increase in the blood potassium level in all the nephropathy subgroups and hypertension subgroup than both control and other pre-nephropathy subgroups ($P < 0.001$) (**Table 7).**

There was no significant difference among all the groups in the calcium or phosphorous or ALT or AST or sodium. **(Table 5, 6,7).**

Distribution of genotypes and alleles

Our results showed that in both (rs9921222 and rs1805105) SNPs, there was a significant difference in genotypes distribution among 6 subgroups included in 3 general groups (control, pre-nephropathy-nephropathy) ($P<0.001$) **(Table 8).** But, for allele distribution, our statistical analysis showed significant difference in alleles distribution in only **(**rs1805105) ($P = 0.13$) but not for (rs9921222) ($P =0.005$) **(Table 9)**

In AXIN-1 (C/T) (rs9921222) SNP, for CC genotype, the highest frequency was for the diabetic group (87.5%) followed by nephropathy subgroups (72.0% for Diabetic Nephropathy, 71.4% for mixed nephropathy, and 67.9% for Hypertension nephropathy) with a significant value P<0.001. However, for TT genotype, the highest frequency was for hypertension nephropathy and diabetic nephropathy (28.6% and 24%) respectively **(Table 8).**

The comparison between genotype distribution in the control group and each other group individually showed significant differences only in diabetic, hypertension nephropathy, and diabetic nephropathy

subgroups (*P* = 0.011, < 0.001, 0.001) respectively **(Table 8).**

The comparison between genotype distribution in hypertension and hypertension nephropathy subgroups showed a significant difference (*P*= 0.012). But no significant difference in genotype distribution between diabetic and diabetic nephropathy subgroups (*P*= 0.33) **(Table 8)**

In AXIN-1 (T/C) (rs1805105) SNP, TT genotype showed highest frequency for hypertension nephropathy group (82.1%) followed by control group (71.7%). However, for CC genotype, the highest frequency for mixed nephropathy (42.9%) **(Table 8)**

The comparison between genotype distribution in the control group and each other group individually showed a significant difference only in mixed nephropathy (*P*= 0.003) **(Table 8)**

The comparison between genotype distribution in hypertension and hypertension nephropathy subgroups showed a significant difference (*P*= 0.019). But no significant difference in genotype distribution between diabetic and diabetic nephropathy subgroups (*P*= 0.82) **(Table 8)**

Regarding C >T (rs9921222) CC was sig associated with diabetic subgroup (OR 5.7 CI 1.1-15.62*) and nephropathy (OR 1.8 CI 0.75-3.58) subgroups while TT associated with hypertension nephropathy and diabetic nephropathy (OR 3.37 CI 1.12-10.5*), but regard T >C (rs1805105) TT with control (OR 2.31 CI 0.85-5.66)and HTN nephropathy group (OR 3.35 CI 1.08-10.7*) and CC associated with mixed nephropathy(OR 4.2 CI 1.3-25.6*) **(data not shown in the tables)**

Discussion

Till now there is no cure for neither diabetic nor hypertensive nephropathy, but the treatments may stop or just delay the rapid progression for this fatal disease.

Most of the recent studies changed their direction in the research point to find a new strategy for the early detection of nephropathy.

Single nucleotide variants are good genetic markers for several diseases [26]. They can be used as a useful tool for the early detection of many diseases including nephropathy [27–30].

Actually, Prioritizing and identifying mutations that cause disease is one of the major challenges in medical genomics.

AXIN1 gene encodes a protein that acts as a negative regulator for the Wnt pathway which is an important cellular pathway responsible for many biological processes [31]. The deregulation of the Wnt pathway is associated with several diseases [32–38] and health problems including both diabetic nephropathy and hypertension [39].

For both type 1 and type 2 models, the Wnt pathway is activated in the renal models [40]. Wnt deregulation in diabetes represents a new pathogenic mechanism for diabetic nephropathy and a new therapeutic goal [41].

Different procedures to inhibit the activated Wnt/β-catenin signal has been reported to

improve the condition and side effects of diabetic nephropathy. [42, 43].

However, another study explores the double role of Wnt / beta-catenin in diabetic nephropathy pathogenesis. [12].

The high association between the AXIN1 gene and the Wnt pathway is the reason for suggesting AXIN1 polymorphisms as genetic markers for the early detection of nephropathy [44]. However, no research associated AXIN1 directly with the progression of CKD. In this study, we examined the possible relationship between different AXIN1 gene SNPs and CKD risk in the Egyptian population.

Our study suggested the association between two SNPs (rs9921222 and rs1805105) in the AXIN1 gene with the susceptibility of nephropathy incidence in diabetic or hypertensive patients in Egypt.

The tagged SNPs in this study are different in nature as the first (rs9921222) is an intron variant. But the second (rs1805105) is a synonymous coding sequence variant.
Our suggestion was achieved by our results that showed a significant difference (P<0.001) in genotypes distribution in both (rs9921222 and rs1805105) SNPs among the six subgroups used in this study.

The mechanism by which these two polymorphisms participated in the nephropathy incidence in the Egyptian population may be explained by the variation nature for each polymorphism.
Most of the intronic variants have unknown functional significance. However, the linking between a specific intron variant and a specific disease can give useful information about the possible effect of this variant [45].

Regarding rs9921222 SNP, it is an intronic variant with low information about its function in the gene. It is one of the loci that had been proved to have an association with low bone mineral density and bone fracture [46]. A few studies discussed this locus with bone mineral density and osteoporosis [47–49]. However, one another study discussed this locus with colorectal cancer [50].
The impact of this intron variant may be due to the change in the mRNA splicing mechanism. The **mutated introns** are not recognized well by spliceosomes or frame reading which may lead to different isoforms of protein or can affect gene expression and protein level [45].

We are well aware that introns should be eliminated while the exons are expressed. Mutations in this sequence might cause mRNA to retain large parts of intronic DNA or to separate whole exons from the mRNA**.** These modifications may lead to nonfunctional protein development [51, 52]**.**

On the contrast, (rs1805105) is a synonymous variant in which GA**T** is substituted with GA**C**. The substitution from T to C is a synonymous variation (has no effect on the produced amino acid which is aspartic acid). The synonymous variation is the most common in all the type of single nucleotide variation [53]. More than 50 human diseases are connected with synonymous mutations [54].This locus was

discussed with some diseases including colorectal cancer [50], bladder cancer [18], ovarian [17], renal cell carcinoma [23], hepatocellular carcinoma [55], cardiomyopathy [16], atrial septic defect [56], diabetic nephropathy [57].

While synonymous genetic variations have long been thought of to have no phenotypic effects, there is increasing evidence that they can affect the expression of genes, mRNA secondary configuration, mRNA stability, protein folding, and eventually organism fitness. [58].

Several codons are encoded with the same amino acid but not used in the same frequency which is known as codon bias. Translation machinery uses Codon Bias to control the speed and accuracy of the translation. [59].

The substrate characteristics of tRNA for a rare codon that impact on the translation timing and the co-translation of the protein. This is reflected in the use of codon bias in many organisms[60].

The disturbance in the polymorphism of the AXIN1 gene could affect its function as a negative regulator to wnt pathway which will help in over-activation which participated in nephropathy incidence.

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Table (1): Age distribution among studied group

		Mean	Std. Deviation	Minimum	Maximum	F	*P*
Age (year)	Control Group	49.8478	12.24557	21.00	62.00	2.042	0.087
	Hypertension Group	51.3571	8.17696	28.00	62.00		
	Diabetes Group	49.7500	12.56715	7.00	62.00		
	mixed nephropathy Group	49.6429	11.41259	17.00	62.00		
	Hypertension nephropathy Group	52.6786	8.52471	32.00	66.00		
	Diabetic nephropathy Group	52.2800	11.58490	22.00	68.00		

F =ANOVA test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

Table (2): Sex distribution among studied groups

			Group						X^2	*P*
			Control Group	Hypertension Group	Diabetes Group	mixed nephropathy Group	Hypertension nephropathy Group	Diabetic nephropathy Group		
Sex	Male	N	27	9	8	9	12	15	3.21	0.65
		%	58.7%	64.3%	50.0%	64.3%	42.9%	60.0%		
	Female	N	19	5	8	5	16	10		
		%	41.3%	35.7%	50.0%	35.7%	57.1%	40.0%		
Total		N	46	14	16	14	28	25		
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%		

X2 =Chi square test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

Table (3): Hb distribution among studied groups

		Mean	Std. Deviation	Minimum	Maximum	F	*P*
Hb (gm/dl)	Control Group	13.2565	0.88233	11.30	14.80	66.891	0.00**
	Hypertension Group	12.8500	1.62753	8.10	15.20		
	Diabetes Group	12.7500	1.81365	7.60	14.90		
	mixed nephropathy Group	8.5214	0.94964	6.90	9.90		
	Hypertension nephropathy Group	9.1357	1.35489	6.40	11.50		
	Diabetic nephropathy Group	9.4600	1.28582	7.40	11.80		

F =ANOVA test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

Table (4): Urea and Creatinine distribution among studied groups

		Mean	Std. Deviation	Minimum	Maximum	F	*P*
Urea (mg/dl)	**Control Group**	**26.4783**	**7.24719**	**12.00**	**42.00**	**217.18**	**0.00****
	Hypertension Group	**35.8571**	**7.39914**	**22.00**	**50.00**		
	Diabetes Group	**27.5625**	**9.26620**	**12.00**	**42.00**		
	mixed nephropathy Group	**130.2857**	**31.29863**	**92.00**	**188.00**		
	Hypertension nephropathy Group	**125.4643**	**23.99457**	**79.00**	**167.00**		
	Diabetic nephropathy Group	**129.3600**	**22.34554**	**85.00**	**167.00**		
Creatinine (mg/dl)	**Control Group**	**0.7848**	**.15912**	**.40**	**1.10**	**201.79**	**0.00****
	Hypertension Group	**0.9357**	**.14991**	**.70**	**1.20**		
	Diabetes Group	**0.7750**	**.24358**	**.40**	**1.40**		
	Mixed nephropathy Group	**10.4071**	**2.38181**	**6.70**	**16.70**		
	Hypertension nephropathy Group	**8.7179**	**1.49470**	**5.90**	**12.20**		
	Diabetic nephropathy Group	**8.6280**	**2.89317**	**4.50**	**16.60**		

F =ANOVA test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

Table (5): Calcium and phosphorus distribution among studied groups

		N	Mean	Std. Deviation	Minimum	Maximum	F	*P*
Calcium (mg/dl)	**Control Group**	**46**	**8.3674**	**.42534**	**7.40**	**9.30**	**2.170**	**0.075**
	Hypertension Group	**14**	**8.2357**	**.56242**	**7.80**	**10.10**		
	Diabetes Group	**16**	**8.2687**	**.67697**	**7.20**	**9.90**		
	mixed nephropathy Group	**14**	**8.2929**	**.89224**	**6.90**	**9.50**		
	Hypertension nephropathy Group	**28**	**8.2821**	**.79352**	**6.30**	**10.00**		
	Diabetic nephropathy Group	**25**	**7.9480**	**.63319**	**6.30**	**8.80**		
Phosphorous (mg/dl)	**Control Group**	**46**	**4.3174**	**.70026**	**2.50**	**6.50**	**2.191**	**0.062**
	Hypertension Group	**14**	**4.4214**	**.56864**	**3.20**	**5.20**		
	Diabetes Group	**16**	**4.5750**	**.67971**	**4.00**	**6.50**		
	mixed nephropathy Group	**14**	**4.4643**	**.44654**	**4.10**	**5.70**		
	Hypertension nephropathy Group	**28**	**4.6143**	**.48665**	**4.00**	**5.60**		
	Diabetic nephropathy Group	**25**	**4.5080**	**.61774**	**3.20**	**5.70**		

F =ANOVA test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

Table (6): ALT and AST distribution among studied groups

		Mean	Std. Deviation	Minimum	Maximum	F	*P*
ALT (U/I)	**Control Group**	**28.5435**	**9.65827**	**12.00**	**122.00**	**2.123**	**0.078**
	Hypertension Group	**33.5000**	**11.23045**	**15.00**	**102.00**		
	Diabetes Group	**27.8750**	**9.40833**	**15.00**	**42.00**		
	mixed nephropathy Group	**29.7857**	**3.66225**	**13.00**	**25.00**		
	Hypertension nephropathy Group	**28.4643**	**9.74768**	**12.00**	**76.00**		
	Diabetic nephropathy Group	**34.4400**	**24.94674**	**14.00**	**114.00**		
AST (U/I)	**Control Group**	**28.4348**	**9.48760**	**14.00**	**81.00**	**2.211**	**0.061**
	Hypertension Group	**31.6429**	**10.29371**	**15.00**	**84.00**		
	Diabetes Group	**29.3750**	**8.29357**	**17.00**	**48.00**		
	mixed nephropathy Group	**28.1429**	**3.71809**	**11.00**	**22.00**		
	Hypertension nephropathy Group	**28.2143**	**9.09095**	**10.00**	**91.00**		
	Diabetic nephropathy Group	**31.2000**	**10.70261**	**13.00**	**67.00**		

F =ANOVA test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

Table (7): Sodium and Potassium distribution among studied groups

		Mean	Std. Deviation	Minimum	Maximum	F	*P*
Sodium (mEq/L)	**Control Group**	**148.1522**	**4.60419**	**130.00**	**150.00**	**2.087**	**0.081**
	Hypertension Group	**154.9286**	**12.35042**	**137.00**	**172.00**		
	Diabetes Group	**152.4375**	**5.59725**	**135.00**	**152.00**		
	mixed nephropathy Group	**150.3571**	**8.14869**	**138.00**	**162.00**		
	Hypertension nephropathy Group	**152.8571**	**10.23688**	**129.00**	**166.00**		
	Diabetic nephropathy Group	**150.2800**	**9.53730**	**138.00**	**168.00**		
Potassium (mEq/L)	**Control Group**	**3.9826**	**.37077**	**2.80**	**4.50**	**36.76**	**0.00****
	Hypertension Group	**6.0714**	**.88876**	**4.70**	**7.40**		
	Diabetes Group	**4.9125**	**.79990**	**3.80**	**6.80**		
	mixed nephropathy Group	**6.0357**	**1.01041**	**4.70**	**7.70**		
	Hypertension nephropathy Group	**6.3500**	**1.02180**	**4.30**	**8.50**		
	Diabetic nephropathy Group	**6.0700**	**1.24867**	**3.90**	**7.80**		

F =ANOVA test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

Table (8): Genotypes distribution among studied groups

			Group						X^2	*P*
			Control Group n= 46	Hypertention Group n=14	Diabetes Group n=16	Mixed nephropathy Group n=14	Hypertension nephropathy Group n=28	Diapetic nepgropathy Group n=25		
(rs9921222) C_T	CC	N	21	8	14	10	19	18		
		%	45.7%	57.1%	87.5%	71.4%	67.9%	72.0%		
	CT	N	21	5	1	3	1	1	34.8	<0.001
		%	45.7%	35.7%	6.2%	21.4%	3.6%	4.0%		
	TT	N	4	1	1	1	8	6		
		%	8.7%	7.1%	6.2%	7.1%	28.6%	24.0%		
			X^2	0.56[a]	8.9[b]	2.9[c]	16.2[d]	13.8[e]		
			P	0.75	0.011	0.225	000	0.001		
			X^2				8.9[f]	2.2[g]		
			P				0.012	0.33		
(rs1805105) T_C	TT	N	33	8	8	5	23	13		
		%	71.7%	57.1%	50.0%	35.7%	82.1%	52.0%		
	TC	N	10	5	5	3	1	9	23.77	<0.001
		%	21.7%	35.7%	31.2%	21.4%	3.6%	36.0%		
	CC	N	3	1	3	6	4	3		
		%	6.5%	7.1%	18.8%	42.9%	14.3%	12.0%		
			X^2	1.2 [a]	3.12 [b]	11.6 [c]	5.2 [d]	2.7 [e]		
			P	0.55	0.2	0.003	0.07	0.24		
			X^2				7.9 [f]	0.37 [g]		
			P				0.019	0.82		

X^2 chi-square test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

[a] Comparison between control and hypertension groups

[b] Comparison between control and diabetes groups

[c] Comparison between control and mixed nephropathy groups

[d] Comparison between control and hypertension nephropathy groups

[e] Comparison between control and diabetic nephropathy groups

[f] Comparison between hypertension and hypertension nephropathy groups

[g] Comparison between diabetes and diabetic nephropathy groups

Table (9): Alleles distribution among studied groups

			Group						X^2	P
			Control Group n= 46	Hypertention Group n=14	Diabetes Group n=16	mixed nephropathy Group n=14	Hypertension nephropathy Group n=28	Diapetic nepgropathy Group n=25		
(rs9921222) C/T	C	N	63	21	29	23	39	37		
		%	68.4%	75.0%	90.6%	82.1%	69.6%	74.0%	7.6	0.13
	T	N	29	7	3	5	17	13		
		%	31.6%	25.0%	9.4%	17.9%	30.4%	26.0%		
			X	0.43	6.08	1.9	0.02	0.47		
			P	0.51	0.018	0.16	0.88	0.49		
			X				2.6	3.4		
			p				0.6	0.06		
(rs1805105) T/C	T	N	76	21	21	13	47	35		
		%	82.6%	75%	65.6%	46.4%	83.9%	70%	16.4	0.005
	C	N	16	7	11	15	9	15		
		%	17.4%	25%	34.4%	53.6%	16.1%	30%		
			X^2	0.8 [a]	4 [b]	14 [c]	0.04 [d]	3 [e]		
			P	0.4	0.045	000	0.8	0.08		
			X^2				0.9 [f]	0.16 [g]		
			P				0.3	0.67		

X^2 chi-square test, P>0.05 Not Significant, P<0.05 Significant, P<0.001 High significant

[a] Comparison between control and hypertension groups

[b] Comparison between control and diabetes groups

[c] Comparison between control and mixed nephropathy groups

[d] Comparison between control and hypertension nephropathy groups

[e] Comparison between control and diabetic nephropathy groups

[f] Comparison between hypertension and hypertension nephropathy groups

[g] Comparison between diabetes and diabetic nephropathy groups

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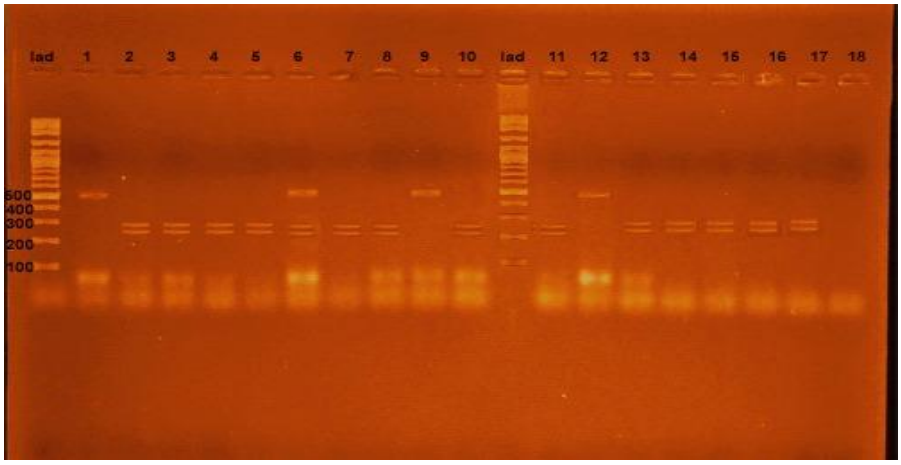
Genotyping of AXIN-1 (rs9921222) polymorphism

(Diabetic nephropathy)



Fig(1): Genotyping of AXIN-1 (rs9921222) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker (100pb).wells no 1,9,12 are (TT) genotype (one band at 475). Wells no 2,3,4,5,7,8,10, 11,13, 14,15,16,17 are (CC) genotype (two band at 229 and 246). Well no 6 are (CT) genotype (three band at 475,229,246).

(Hypertension nephropathy)

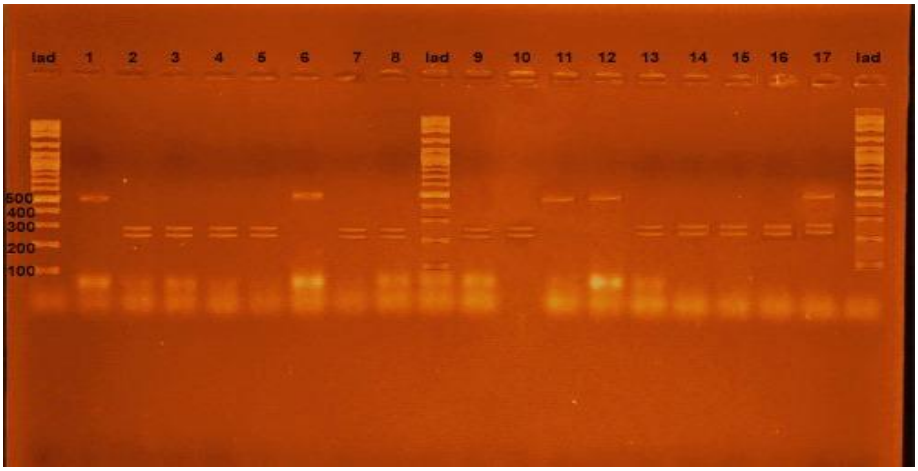


Fig (2):Genotyping of AXIN-1 (rs9921222) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb).wells no1,6,11,12 are (TT) genotyp (one band at 475). Wells no 2,3,4,5,7,8,9,10,13,14,15,16 are (CC) genotype (two band at **229** and **246)**.well no17 three band CT at 475,229 and 246.

(Diabetic hypertensive nephropathy group)

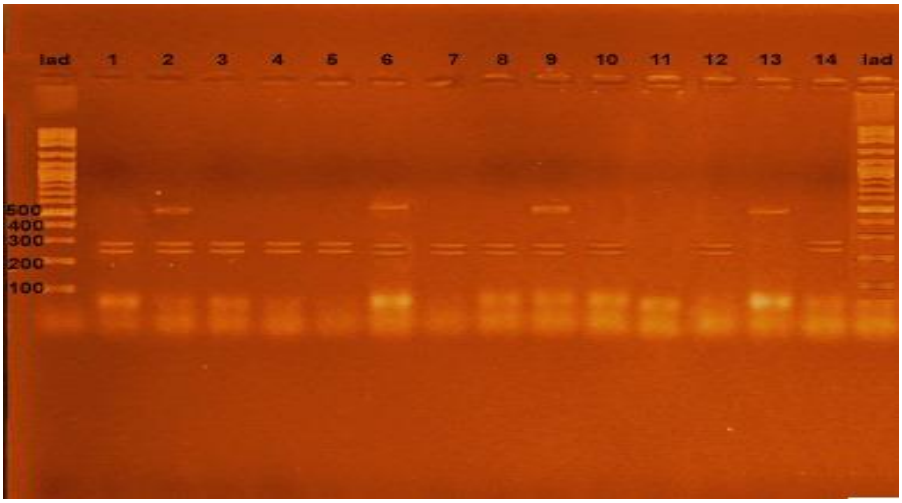


Fig (3): Genotyping of AXIN-1 (rs9921222) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb).well no 13 are (TT) genotype (one band at 475). Wells no1,3,4,5,7,8,10,12 ,14 are (CC) genotype (two bands at 229 and 246). Wells no 2,6 and 9 are (CT) genotype (three band at 475,229 and 246).

(Diabetic group)

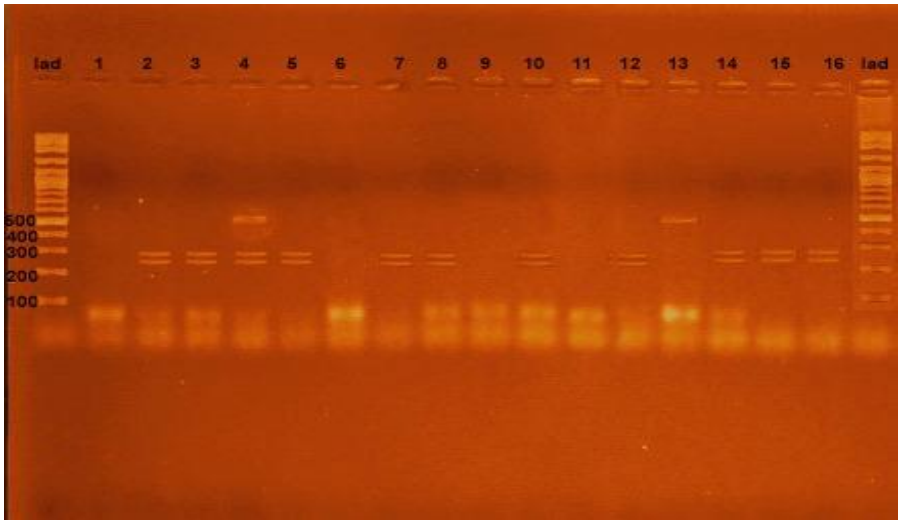


Fig (4): Genotyping of AXIN-1 (rs9921222) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker (100pb). Well no 13 are (TT) genotype (one band at 475). Wells no 2,3,5,7,8,11,12,14,15,16 are (CC) genotype (two band at 229 and 246). Well no 4 are (CT) genotype (three bands at 475,229 and 246).

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(Hypertension)

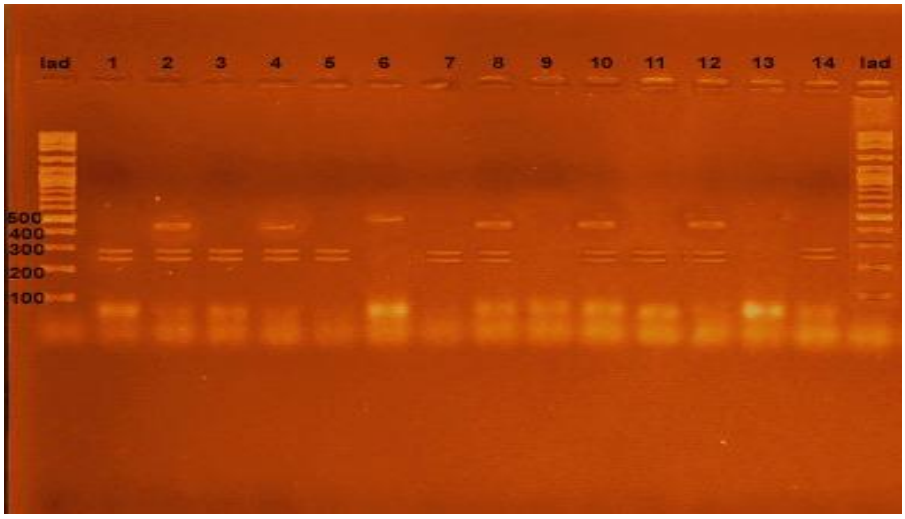

Fig (5): Genotyping of AXIN-1 (rs9921222) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb). Well no 6 are (TT) genotype(one band at 475). Wells no 1,3,5,7,11 14 are (CC) genotype (two bands at 229 and 246). Wells no 2,4,8,10, 12 are (CT) genotype (three bands at 475,229 and 246).

(Control group)

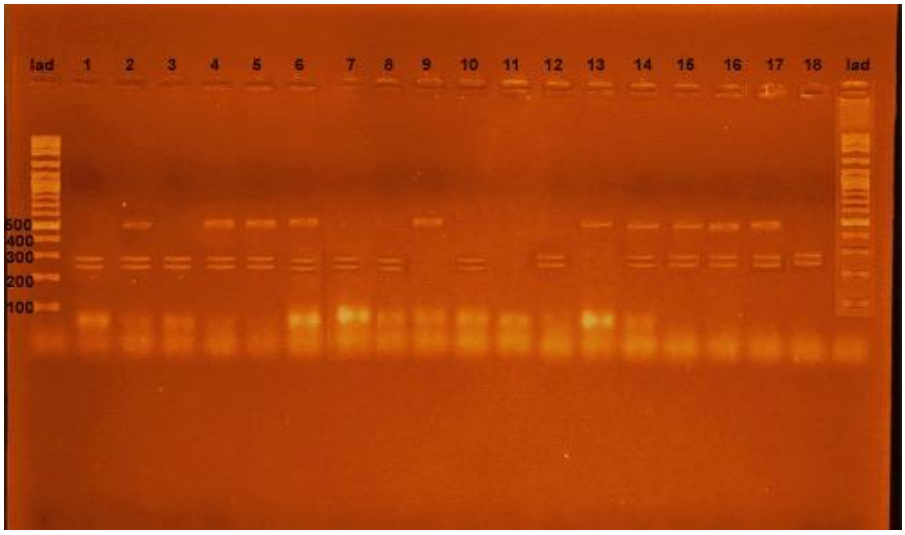

Fig (6): Genotyping of AXIN-1 (rs9921222) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker (100pb). Wells no 9 and 13 are (TT) genotype (one band at 475). Wells no 1,3,7,8,10,12, 18 are (CC) genotype (two bands at 229 and 246). Wells no 2,4,5,6,14,15,16, 17 are (CT) genotype (three bands at 475,229 and 246).

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Genotyping of AXIN-1 (rs1805105) polymorphism

(Diabetic nephropathy group)

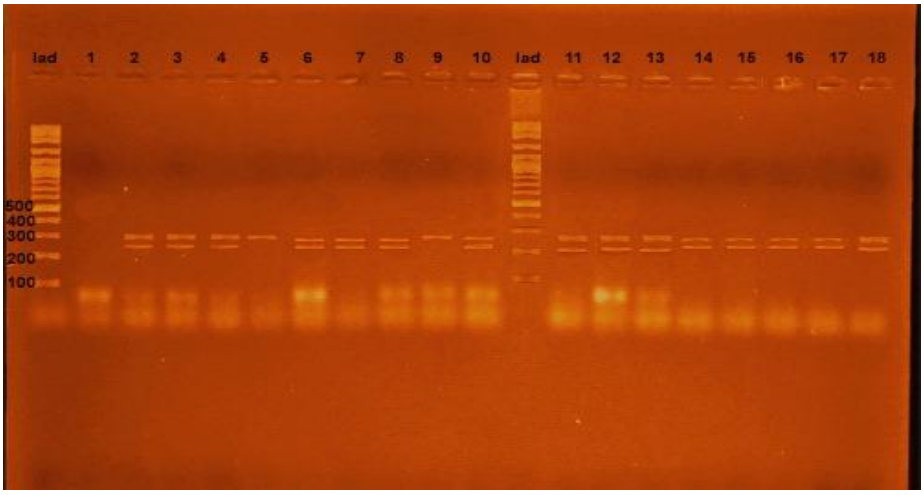

Fig (7): Genotyping of AXIN-1 (rs1805105) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb). Wells no 5 and 9 are (CC) genotype (one band at 284). Wells no 6, 7,8,10,14,15,16, 17 are (TT) genotype (two bands at **222** and **282)**. Wells no 2, 3, 4, 11, 12, 13, are (TC) genotype (three bands at 284,282 and 222).

(Hypertension nephropathy group)

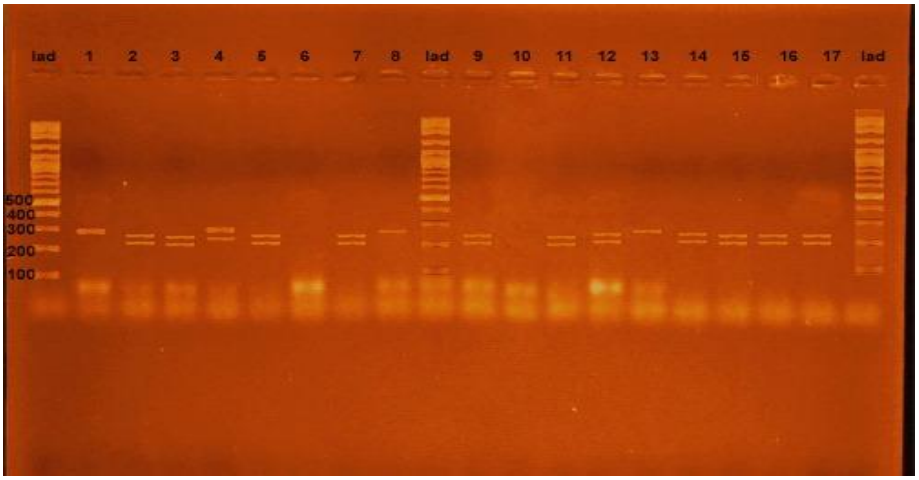

Fig (8): Genotyping of AXIN-1 (rs1805105) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb). Wells no 1,8 13 are (CC) genotype (one band at 284). Wells no 2,3,5,7,9,11,12,13,14,15,16, 17 are (TT) genotype (two bands at 222 and 282). Well no 4 are (TC) genotype (three bands at 284,282 and 222).

(Diabetic Hypertensive nephropathy group)

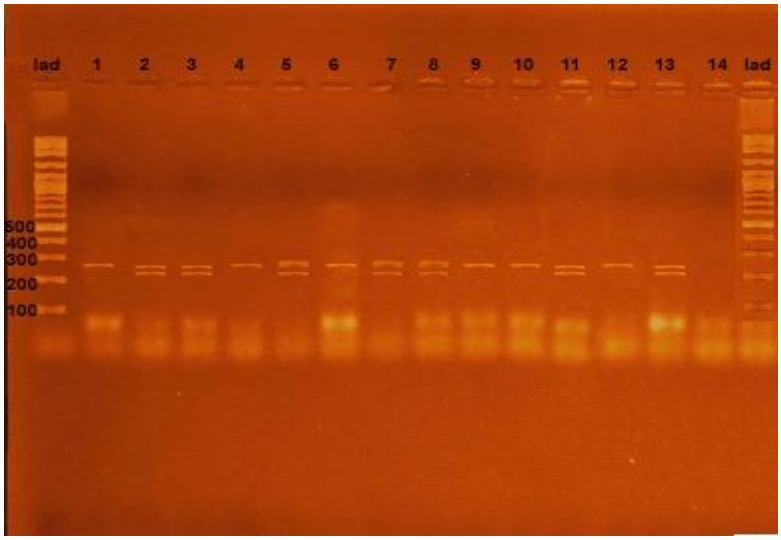

Fig (9): Genotyping of AXIN-1 (rs1805105) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb). Wells no 1, 4, 6,9,10, 12 are (CC) genotype (one band at 284). Wells no 2, 3, 11, 13, 14 are (TT) genotype (two bands at **222** and **282)**. Wells no 5,7 8 are (TC) genotype (three bands at 284,282 and 222).

(Diabetic group)

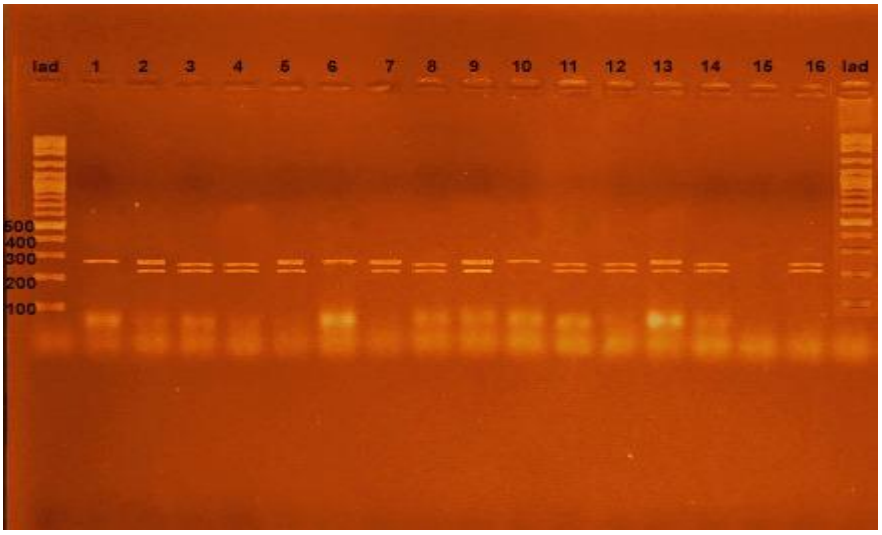

Fig (10): Genotyping of AXIN-1 (rs1805105) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker (100pb). Wells no 1,6,10 are (CC) genotype (one band at 284). Wells 3, 4, 6,11,12,14, 16 are (TT) genotype (two bands at 222 and 282**)**. Wells no 2,5,7,9, 13 are (TC) genotype (three bands at 284,282 and 222).

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(Hypertensive group)

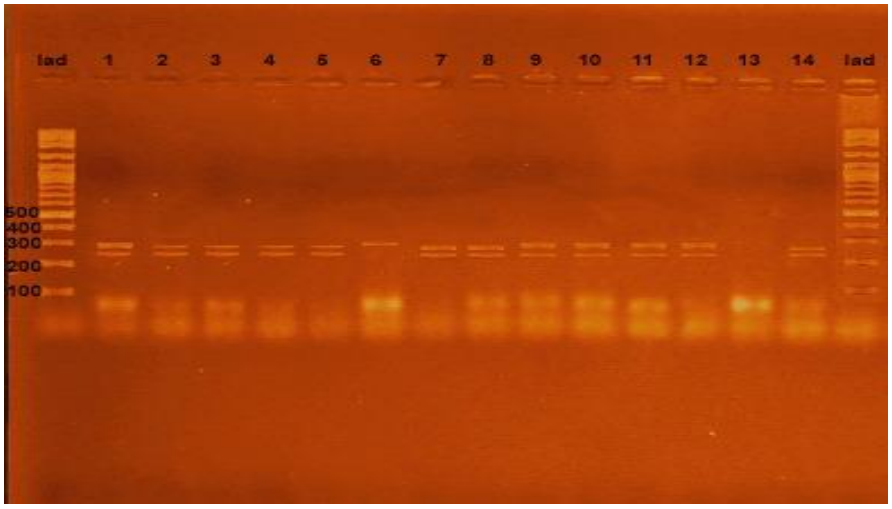

Fig (11): Genotyping of AXIN-1 (rs1805105) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb). Well no 6 (CC) genotype (one band at 284). Wells no 2,3,4,5,7,8, 14 are (TT) genotype (two bands at **222** and **282)**. Wells no 1,9,10,11, 12 are (TC) genotype (three bands at 284,282 and 222).

(Control group)

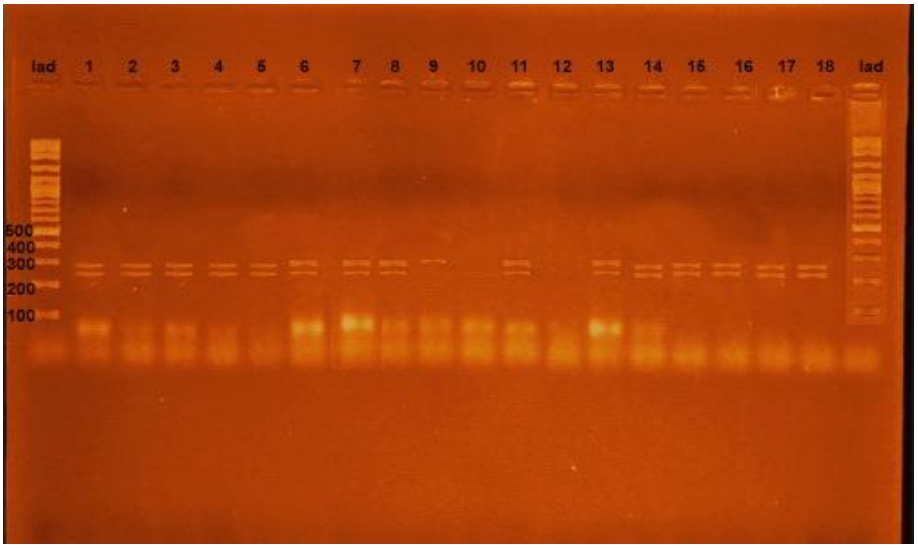

Fig (12): Genotyping of AXIN-1 (rs1805105) polymorphism on gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Molecular weight DNA standard marker(100pb). Well no 9 are (CC) genotype (one band at 284). Wells no 1,2,3,4,5,14,15,16,17, 18 are (TT) genotype (two bands at 222 and 282). Wells no 6,7,8,11,13 are (TC) genotype (three bands at 284,282 and 222).