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THE STUDY OF THE PREVALENCE OF EXPRESSION OF ANGIOTENSIN II TYPE 1 RECEPTOR POLYMORPHISMS AND SUSCEPTIBILITY TO HYPERTENSION AMONG RENAL FAILURE AND CORONARY HEART DISEASE PATIENTS

Mohamed Amer[a&b], Amr E. Ahmed[b], Doaa ahmed[c] and Abdou K.A.[d]

[a.]Laboratory department in Applied medical Science in October 6 University (O6U), Giza, Egypt.
[b.]Biotechnology department, Faculty of Postgraduate Studies for Advanced Sciences (PSAS), Beni-Suef University (BSU), Egypt.
[c.]Misr University for Science & Technology (MUST), Giza, Egypt.
[d.]Environmental toxicology department, Faculty of veterinary medicine, Beni-Suef University (BSU), Egypt.

Abstract

The main objective of the current study is to assess the relation of polymorphisms of AGTR1 with hypertension, and perform a meta-analysis of association of the rs5186 SNP and hypertension to both understand the relation between genetic variant and hypertension across multiple populations. The renin-angiotensin system (RAS) plays a fundamental role in blood pressure maintenance and is implicated as a likely etiologic factor in the development of hypertension, gene expression and protein expression of the angiotensin II type I receptor (AT1R) (SNP ID: rs5186) and its association with essential hypertension in a Northern Indian population, AGTR1 encodes the type 1 receptor, which mediates the main cardiovascular impact of angiotensin II including vasoconstriction, stimulation of Na+ reabsorption and aldosterone secretion. This gene may play a role in the generation of reperfusion arrhythmias following rebuilding of blood flow to the ischemic or infracted myocardium.

Expression of AT1R gene and the A1166C polymorphism are analyzed in 60 hypertensive patients; the first 30 patients taken from AKU disease, and the other 30 patients taken from cardiology, CCU. Identification and Detection of Polymorphisms of AT1R gene by Total DNA extraction, PCR and routine analysis.

The distribution of genotypes in the patients (with heart & kidney), and control groups accordance with the Hardy-Weinberg equilibrium, showed that heterozygous genotypic pattern (CC) is more frequent in patients with kidney and heart cases, than (AC) while in controls the most frequent genotype was AA. There was a significant association found in the AT1R genotypes (AC+CC) with essential hypertension (X^2 =64.765, p = 0.000) compared with control, also a significant association of hypertension (AT1R occurrence) in alleles (A+C) (X^2 =22.947, p = 0.000) compared with control .By comparing genotyping distributions of (AT1R) (A1166C) gene between group 3 & group 4 (kidney & heart patients respectively) significant difference occurred, (x2 =22.947, p = 0.000), but there was no significant difference when genotyping distributions of (AT1R) (A1166C) gene compared between alleles groups (X^2 =0.178, p = 0.673).Our study also showed that by comparing the means values of biochemical parameters between control group and kidney patients, the results showed that, There were significant differences between control group (group 1) and Kidney patients group (group 3), according to urea, creatinine, cholesterol and TG, (p<0.001), While there was no significant difference between control group (group 1) and Kidney patients (group 3), with respect to Hb where (p=.077). Also comparing the biochemical parameters between control and heart cases groups , the results showed that, There were significant differences between control group (group 1) and heart patients group (group 3), according to Ck, Ck-mb, cholesterol, TG, and TG LDH, (p<0.001). While there was no significant difference between control group (group 1) and heart patients (group 3), with respect to Hb where (p=.085).

Our study reveals that A allele and AA genotype of AT1R A1166C gene polymorphism are associated with a protective effect against essential hypertension, while C allele and AC genotype of AT1R A1166C are correlated with the raised danger of basic hypertension in kidney and heart diseases.

***Keywords*:** Receptors, angiotensin II _ genetics _ polymorphism _ cardiovascular diseases _ hypertension.

Introduction

The renin-angiotensin system (RAS) assumes a fundamental role in blood pressure and is considered as a causative factor in the progression of hypertension (Laragh and Pickering, 1991). Hypertension causes high levels of both systolic and diastolic blood pressure and is, therefore, a predisposing factor for cardiovascular disease, stroke, and renal disease (Messerli *et al.*, 2007). By the year 2025, it is estimated that incidence rates of hypertension will increase by 60% when contrasted with year 2000 and more than 1.5 billion persons around the world will suffer from hypertension (Kearney *et al.*, 2005).The blood pressure, and is reported to be involved in essential hypertension controlled by The renin-angiotensin aldosterone system (RAAS) (Watt *et al.*, 1992). Angiotensin II type 1 receptor (AT1R) is a G protein-coupled receptor that intercedes a large portion of the biological actions of the RAS. Type 1 receptor and type 2 receptor are two subtypes of angiotensin II receptors (Zhu *et al.*, 2003). The human AGTR1 gene contain five exons and maps to chromosome 3q24. AGTR1 encodes a membrane protein with 359 amino acids, which comprised well-conserved seven-transmembrane domains (Hongju *et al.*, 2015). AGTR1 encodes the type 1 receptor, which mediates the principle cardiovascular impacts of angiotensin II including vasoconstriction, stimulation of Na^+ reabsorption and aldosterone secretion. This gene may be involved in the development of reperfusion arrhythmias leads to reclamation of blood flow to the ischemic or infarcted myocardium (Schieffer *et al.*, 1995). It is a significant effector controlling BP and volume in the cardiovascular system. The conversion of angiotensinogen (AGT) to angiotensin I is catalyzed by renin (REN), secreted from the juxtaglomerular equipment in response to decreased renal perfusion pressure. (Remuzzi *et al.*, 2005). Angiotensin I converting enzyme (ACE) then cleaves angiotensin I to produce angiotensin II, which controls heart and kidney function by engaged to and

activating angiotensin II receptors (type I and type II) (Nishiyama and Kobori, 2018). Numerous biological actions of RAAS including vasoconstriction and sodium reabsorption performed by the angiotensin II type I receptors (Zhang *et al*., 2017). Development of Various causes CKD, especially diabetic nephropathy is possibly related to increase RAAS activation (DN) (Bermejo *et al*., 2018), and is mediated by hypertensive injury (Yamout *et al*., 2014) and accelerated renal fibrosis (Mezzano *et al*., 2001). The physiological importance of this pathway in the advanced of CKD is depend on RAAS components including ACE, ACE2, AGT, angiotensin II receptor (type I and type II) and renin (REN) being candidate genes for different CKD-related phenotypes. Numerous studies have demonstrated RAAS gene variants in the development of CKD (Ramanathan *et al*., 2016).

The principle goal of the present study is to survey the relationship of polymorphisms of AGTR1 with hypertension, and play out a meta-investigation of relationship of the rs5186 SNP and hypertension to comprehend the connection between genetic variations and hypertension across various populaces.

Materials and Methods

The study was performed in correspondence with the ethical guidelines of the Declaration of Helsinki on biomedical research on people, and was consented by the Institutional Human Research Ethical

About 80 samples were collected from 30 samples who suffered from coronary heart disease in the National Heart Institute (NHI). About samples were collected from 30 samples who suffered from renal failure in the Hospital - O6U University and 20 samples were control. The age of the patients enrolled in this study ranged from 19 – 69 years and the male – to – female ratio of the cases was 2: 1.

Ethical Consideration

The study was performed in conformity with the proposal submitted and granted ethics approval, including any alterations mad to the proposal required by the PSA – BSU –REC. Approval number: 3/020.

Total DNA Extraction

About 50 µl whole blood in EDTA was used for the purification of DNA. About 200 µl of Genomic Lysis Buffer was added to 50 µl of EDTA blood and a sample was mixed from 4 - 6 seconds, at that point let stand 5-10 minutes at RT. Move the mixture to a Zymo-Spin™ Column in an Collection Tube. Centrifuged at 10k xg for 60 Seconds. Throw away the Collection Tube with the flow through. The Column was transferred to a new clean Collection Tube. Add 0.1 ml of DNA Pre-Wash Buffer to the spin column. Centrifuged at 10k xg for one minute. Add 0.5 ml of g-DNA Wash Buffer to the spin column. Centrifuge at 10k x g for one minute. Transfer the spin column to a new micro-centrifuge tube. About 0.05 ml DNA Elution Buffer was added to the spin column. Incubate 2-5 minutes at RT and then centrifuge at top speed for 30 seconds to elute the DNA. The eluted DNA can be utilized for molecular based applications or stored -20ºC for future use

DNA concentration

NanoDrop Microvolume Spectrophotometers was used in DNA concentration. Total DNA extraction from each blood sample was processed. The concentration of DNA in each sample was achieved by measuring O.D of each one at 260 nm and the concentration was calculated according to the equation (Glasel, 1994).

$$\text{DNA}\ \mu\text{g}/\mu\text{l} = \frac{\text{O.D260} \times 50 \times \text{Dilution Factor}}{1000}$$

Where :(O.D 260) is the absorbance, the optical density, at 260 nm (A 260). (50) is average extinction coefficient of DNA (50 µg /OD 260).

The purity of RNA was calculated by measuring O.D of each sample at 260 nm and 280 nm then the ratio was calculated by the following equation

$$\text{Purity} = \frac{\text{O.D 260}}{\text{O.D 280}}$$

Where: (O.D260) is the absorbance, the optical density, at 260 nm (A 260). (O.D280) is the absorbance, the optical density, at 280 nm (A 280).

Basic PCR Protocol:

Tubes were incubated in a thermal cycler at 94°C for 5 minutes to completely denature the template. 25–35 cycles of PCR amplification were performed as follows: Denaturation at 94°C for 45 s, annealing at 55°C for 30 s and extending at 72°C for one and a half minutes. Another round of incubation was carried out for an additional 10 min at 72°C and the reaction was maintained at 4°C. Then stored at –20 °C.

Polymerase Chain Reaction (PCR) for AGTR1

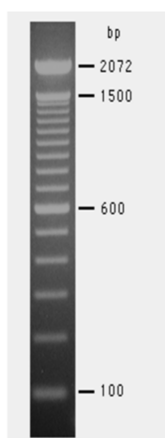
PCR was performed by adding 20µl of PCR Master Mix (2X), 0.5µl Forward primer (10mM), 0.5µl 10mM Reverse primer(10mM), µg 1µl DNA template and the final volume was completed to 20 µl using the deionized water. The final concentration of Master Mix components became 1X Green Go Taq Flexi Buffer, 0.2mM dNTPs Mix each, 1.5mM $MgCl_2$, 0.1mM of each primer and 1.0 U Taq DNA polymerase. The PCR was performed in a thermal cycler according to the following program: Initial denaturation step for 5 minutes at 95°C for polymerase activation; denaturation step for 40 seconds at 95°C; annealing step for 30 seconds at For AGTR1 extension step for 30 seconds at 72°C and final extension for 5 minutes at 72°C.The number of cycles (denaturation, annealing and extension) was 30cycles. After the program was performed the PCR product was electrophoresed in 2% agarose gel against 100bp DNA ladder as a marker using 1X TBE as a running buffer.

Agarose A gel electrophoresis

2.0 % Agarose A was prepared as following: About 2.0 gm from Agarose A powder was dissolved in TBE (1X) buffer and heated in microwave for 2.0 min. then cooled to 55 ºC and 2.5 ul Ethidium bromide was added and poured to gel tray. About 10 ul PCR product was added to 2 ul 6.0 X loading DNA dye then loaded to agrose gel well. Gel was running in 90.0 volt for 90 minutes and Image was captured in BioRad gel imager then data analysis was observed.

DNA marker 100 bp

0.5 µg/lane 2% agarose gel stained with ethidium bromide. Cat. No. 15628-019.



Clinical analysis

All clinical parameters urea, creatinine, cholesterol, triglycerides and LDH were measured using Spectrum Company kits, and CBC analysis performed using CELL-DYN Sapphire analyzer, Abbott laboratory.

Statistical analysis

All statistical analysis was performed utilizing SPSS-24; Chi-square (Pearson Chi square) was utilized to affirm the understanding of predestine genotype frequencies with those expected. The statistical t-test was utilized to determined the contrast among genotype groups. An odds proportion at [95%] certainty intervals (CI) was determined as list of the relationship of the gene with the disease. Results for protein and gene expression analysis are expressed as mean ± SD. Statistical significance was realized as a p value <0.05.In the (ROC) curve the true positive rate is plotted in function of the false positive rate (100-Specificity) for various cut-off focuses, The Receiver Operating Characteristic (ROC) curve was used. Each point on the ROC curve performs a sensitivity/specificity pair comparing to a specific decision threshold. A test with impeccable separation (no overlap in the 2 distributions) has a ROC curve that goes through the upper left corner (100 % sensitivity, 100 % specificity). Hence the closer the ROC curve is to the upper left corner, the higher the comprehensive precision of the test (Zweig & Campbell, 1993). The region under the ROC curve (AUC) is a measure of how well a parameter can recognize among two diagnostic groups (diseased/normal).

Results

Baseline characteristics of the study subjects

This study has been directed to evaluate the expression of Angiotensin II type 1 receptor polymorphisms and susceptibility to hypertension among renal failure and Coronary heart disease patients, in which patients with basic hypertension (N =60) and normal healthy controls (N = 20) were enrolled. The lipid profile for example Total cholesterol, Triglyceride, High Density Lipoprotein and Low Density Lipoprotein, urea, creatinine, LDH, CK, and CKMB were comparable in patients and control.

- **Distributions of allele and genotype Angiotensin II Type 1 Receptor (AT1R) (A1166C) Gene Polymorphism between control (group 1) and kidney and heart cases (group2).**

The distribution of genotypes in the patients (with heart & kidney), and control groups conformity with the Hardy-Weinberg balance, showed that heterozygous genotypic type (CC) is more common in patients with kidney and heart cases, than (AC) while in controls the most frequent genotype was AA (Table 1).There was a significant correlation found in the AT1R genotypes (AC+CC) with basic hypertension (x^2 =64.765, p = 0.000) compared with controls, also a significant association of hypertension (AT1R occurrence) in alleles (A+C) (x^2 =22.947, p = 0.000) compared with control.

- **Distributions of allele and genotype Angiotensin II Type 1 Receptor (AT1R) (A1166C) Gene Polymorphism between kidney cases (group 3) and heart cases (group 4).**

By comparing genotyping distributions of (AT1R) (A1166C) gene between group 3 & group 4 (kidney & heart patients respectively) significant difference occurred, (x^2 =22.947, p = 0.000), but there was no significant difference when genotyping distributions of (AT1R) (A1166C) gene compared between alleles groups (x^2 =0.178, p = 0.673) (Table 2).

Table 1 : Represents compared frequency distributions of allele and genotype Angiotensin II Type 1 Receptor (AT1R) (A1166C) Gene Polymorphism between control (group 1) and kidney and heart cases (group2).

Genotype and related allele	**Group 1 Control Group N=20 , %**	**Group 2 Kidney & heart cases N=60 , %**	**X^2**	**P - value**
A1166C (AT1R) (SNP ID: rs5186)	AA = 17 (85%) CC=2 (10%) AC=1 (5%)	AA=0 CC=38 (63.3%) AC=22 (36.7%)	64.765	.000
	A=35 (87.5%) C=5 (12.5%)	A=22 (18.3%) C=98 (81.7%)	22.947	.000

Table 2: represents compared frequency distributions of allele and genotype Angiotensin II Type 1 Receptor (AT1R) (A1166C) Gene Polymorphism between kidney cases (group 3) and heart cases (group 4).

Genotype and related allele	**Group 3 Kidney cases N=30 , %**	**Group 4 heart cases N=30 , %**	**X^2**	**P - value**
A1166C (AT1R) (SNP ID: rs5186)	AA = 0 (0%) CC=16 (53.3%) AC=14 (46.7%)	AA=8 (26.7%) CC=22 (73.3%) AC=0 (0%)	22.947	.000
	A=14 (23.3%) C= 46 (76.6%)	A=16 (26.7%) C=44 (73.3%)	0.178	0.673

- **Biochemical parameters between control and kidney cases :**

Comparing the mean values of biochemical parameters between control group (group 1) and kidney patients group (group 3) showed that: There were significant differences between control group (group 1) and Kidney patients group (group 3), according to urea, creatinine, cholesterol and TG, (p<0.001). While there was no significant difference between control group (group 1) and Kidney patients (group 3), with respect to Hb where (p=.077).

Table 3: represents comparing the biochemical parameters between control and kidney cases

Parameter	biochemical analysis results for control and kidney cases				
	Mean µ±SD		DF	t- test	P value
	Control (n=20)	Kidney (n=30)			
Urea	29.25 ± 7.886	120.6 ± 26.31	48	15.038	.000
creatinine	0.708 ± 0.140	8.616 ± 2.429	48	14.494	.000
cholesterol	145 ± 20.85	200.13 ± 42.31	48	5.394	.000
TG	83.6 ± 19.98	148.1 ± 38.4	48	6.899	.000
Hb	21.94 ± 33.69	10.88 ± 1.107	48	1.806	0.0772

Table 4: Comparison between AA and CC according to kidney function

Kidney Parameters	Mean ± SD.		df	Test statistic t	p value
	Control (n = 20)	CC (n = 16)			
Urea	29.2500±7.8865	± 30.2489 112.93	34	11.916	000
Creatinine	0.7080 ± 0.1406	7.8000±2.5581	34	20.204	000

From table (4) a significant difference occurs between AA and CC with respect to urea P< 0.001 and creatinine P< 0.001.

Table 5 : Comparison between AA and AC according to kidney function

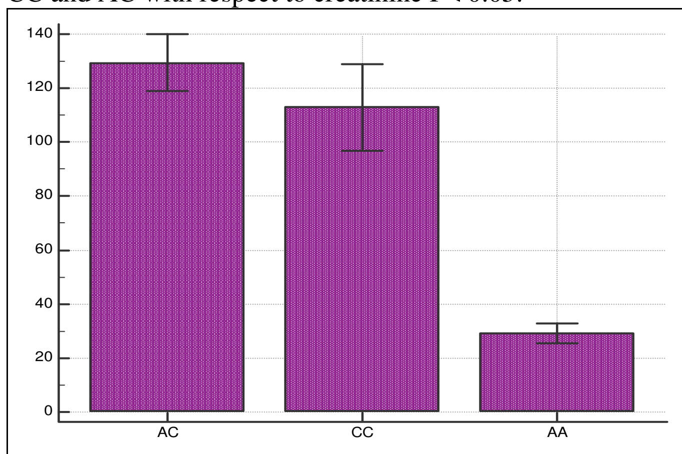
Kidney Parameters	Mean ± SD.		df	Test statistic t	p value
	Control (n = 20)	AC (n = 14)			
Urea	29.2500±7.8865	129.35±18.2950	32	21.847	000
Creatinine	0.7080± 0.1406	9.5500± 1.9630	32	12.420	000

From table (5) a significant difference occurs between AA and AC with respect to urea P< 0.001 and creatinine P< 0.001.

Table 6 : Comparison between AC and CC according to kidney function

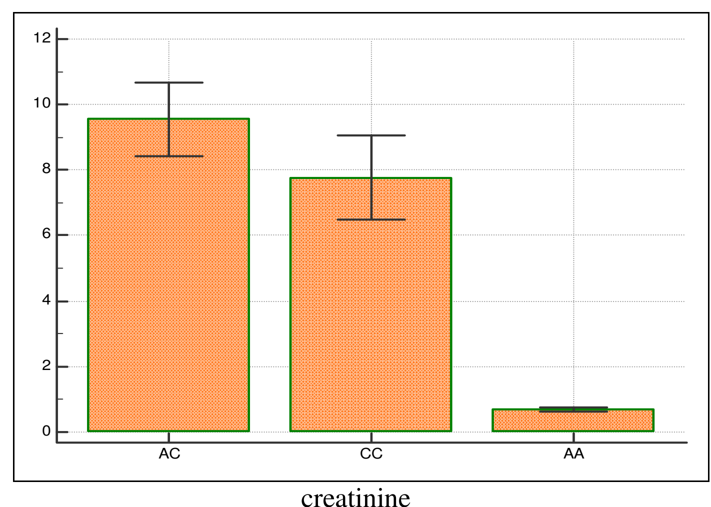
Kidney Parameters	Mean ± SD.		df	Test statistic t	p value
	CC (n = 16)	AC (n = 14)			
Urea	112.9375 ± 30.25	± 18.295 129.357	28	1.766	0.0883
Creatinine	7.8000 ± 2.5581	9.5500 ± 1.9630	28	2.078	0.047

By comparing the mean values between CC and AC according to kidney functions the results showed that, there is no significant difference between CC and AC with respect to urea at the 0.05 level -There is significant difference between CC and AC with respect to creatinine P< 0.05.



Urea

Fig. 1: Comparison between AC, CC & AA with respect to urea



creatinine

Fig. 2: Comparison between AC, CC & AA with respect to urea

ROC curve analysis shows, agreement (sensitivity, specificity) for urea to diagnose kidney patients from control. Urea can distinguish between each of AC & CC genotypes in patient group and control group at the level of significant P <0001and p=0.01 respectively. table (7) & table (8).

Table 7: Agreement (sensitivity, specificity) for urea to diagnose (kidney Ac) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Urea	1.000	<0.0001	0.897	1.000	>43	100.00	100.00	100	95

Table 8: Agreement (sensitivity, specificity) for urea to diagnose (kidney cc) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Urea	1.000	<0.0001	0.903	1.000	>43	100.00	100.00	100	85

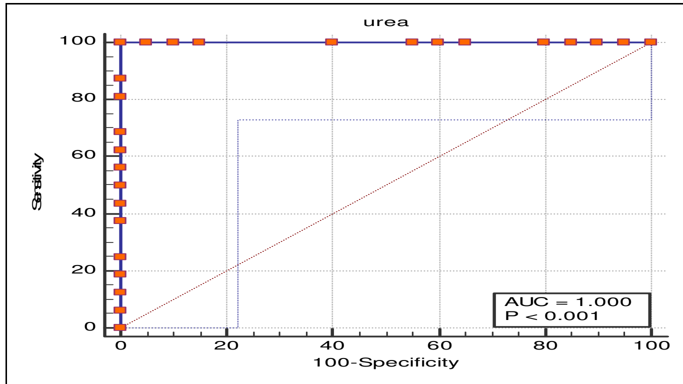


Fig. 3: ROC curve for urea to diagnose (kidney Ac) from control

In case of creatinine the analysis shows, the agreement (sensitivity, specificity) for creatinine to diagnose kidney from control which mean a significant difference occurs between creatinine and control in kidney patients of AC & CC genotypes (in which P <0001) table (9)and (10) respectively.

Table 9: Agreement (sensitivity, specificity) for creatinine to diagnose (kidney Ac) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
creat.	1.000	<0.0001	0.897	1.000	>0.91	100.00	100.00	100	100

Table 10 : Agreement (sensitivity, specificity) for creatinine to diagnose (kidney cc) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Creatinine	1.000	<0.0001	0.903	1.000	>0.91	100.00	100.00	100	100

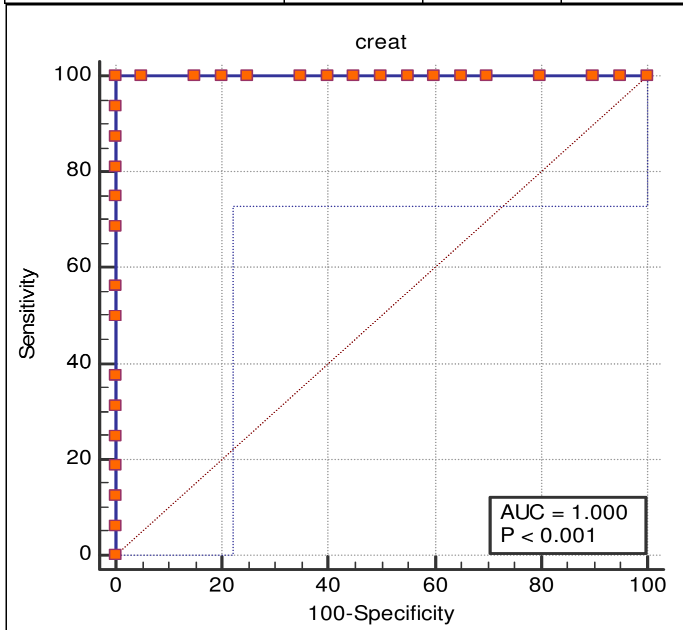


Fig. 4 : ROC curve for creatinine to diagnose (kidney Ac) from control

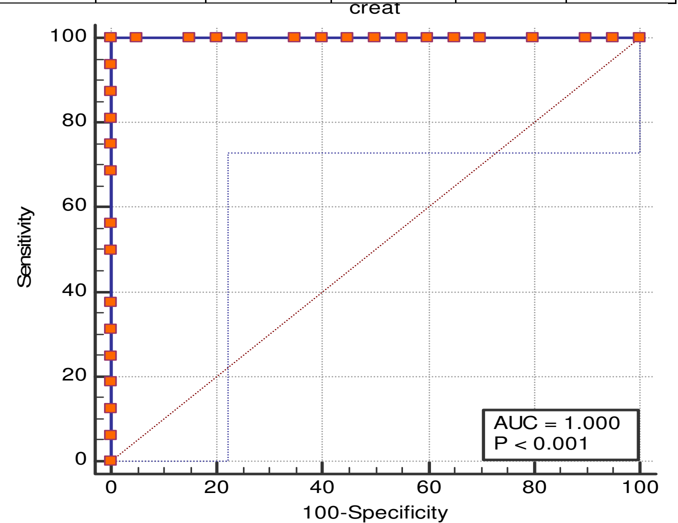


Fig. 5: ROC curve for creatinine to diagnose (kidney cc) from control

For cholesterol analysis the data showing the agreement (sensitivity, specificity) for cholesterol to diagnose kidney from control which mean a significant difference occurs between cholesterol and control in kidney patients of AC & CC genotypes (in which P <0001 & P>168) table (11)and (12) respectively.

Table 11: Agreement (sensitivity, specificity) for cholesterol to diagnose (kidney Ac) from control

	AUC	P	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Cholesterol	0.850	<0.0001	0.686	0.949	>189	64.29	100.00	78	98

Table 12 : Agreement (sensitivity, specificity) for cholesterol to diagnose (kidney cc) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Cholesterol	0.917	**<0.0001**	0.776	0.983	>168	81.25	95.00	86	92

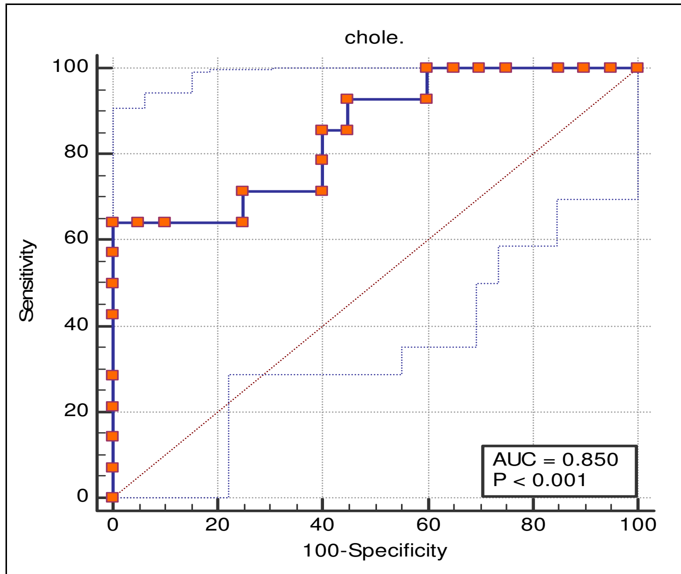


Fig. 6: ROC curve for cholesterol to diagnose (kidney Ac) from control

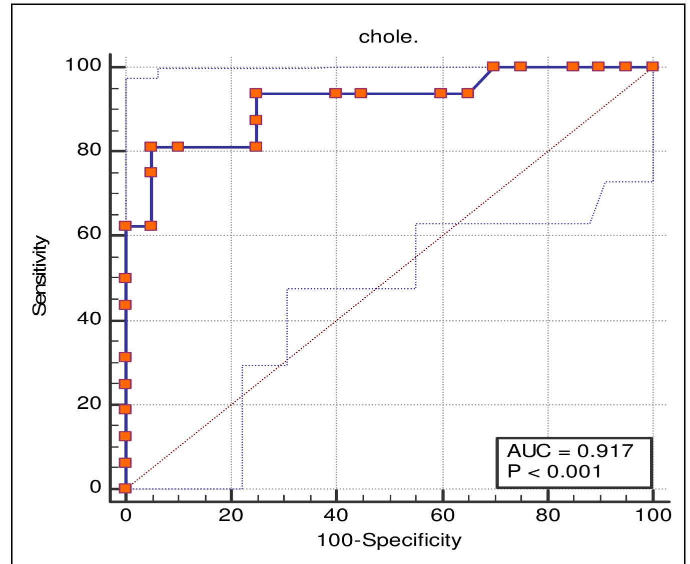


Fig. 7: ROC curve for chole. to diagnose (kidney cc) from control

Also Triglyceride analysis showing the agreement (sensitivity, specificity) for triglycerides to diagnose kidney from control which mean a significant difference occurs between triglyceride and control in kidney patients of AC & CC genotypes (in which P <0001 & P>168)table (13)and (14) respectively .

Table 13: Agreement (sensitivity, specificity) for TG to diagnose (kidney Ac) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
TG	0.904	<0.0001	0.753	0.978	>97	85.71	85.00	93	77

Table 14: Agreement (sensitivity, specificity) for TG to diagnose (kidney cc) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
TG	0.978	<0.0001	0.864	1.000	>123	87.50	100.00	88	98

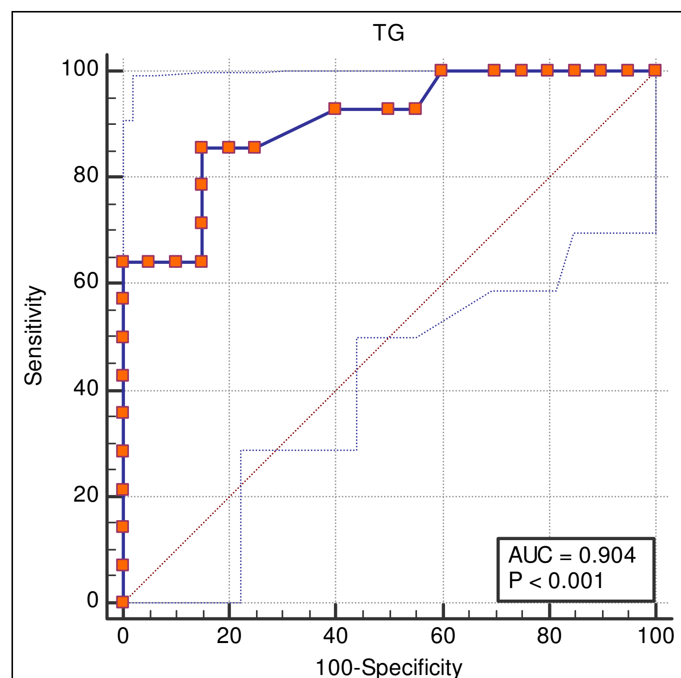


Fig. 8: ROC curve for TG to diagnose (kidney Ac) from control

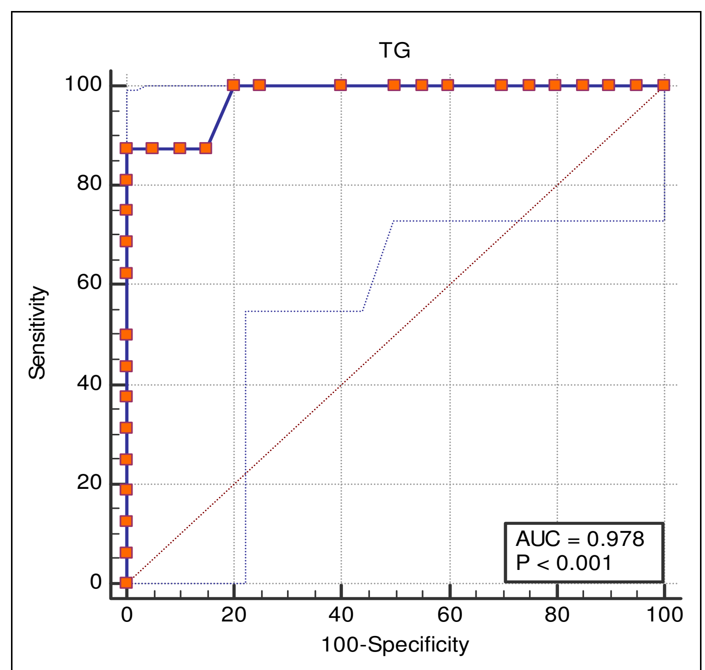


Fig. 9: ROC curve for TG to diagnose (kidney cc) from control

➢ Biochemical parameters between control and heart cases :

Comparing the mean values of biochemical parameters between control group (group 1) and *heart* patients group (group 3) showed that:

There were significant differences between control group (group 1) and *heart* patients group (group 3), according to CK, CK-MB, cholesterol, TG, and TG LDH, ($p<0.001$).

While there was no significant difference between control group (group 1) and ***heart*** patients (group 3), with respect to Hb where ($p=.085$).

Table 15: represents show comparing the Biochemical parameters between control and heart cases

	biochemical analysis results for control and heart cases				
Parameter	**Mean µ±SD**		**DF**	**t- test**	**P value**
	Control (n=20)	**Heart (30)**			
Ck	68.25 ± 23.79	93.33 ± 31.30	48	3.042	0.0038
Ck-mb	16.05 ± 5.27	57.6 ± 20.27	48	3.042	0.0038
cholesterol	145 ± 20.85	248.76 ± 354.78	48	8.94	0.0001
TG	83.6 ± 19.98	108.53 ± 33.8	48	2.965	0.0047
LDH	111.5 ± 31.228	237.5 ± 26.23	48	15.416	.000
Hb	21.94 ± 33.69	11.13 ± 2.107	48	1.761	0.0845

Table 16: Comparison between AA and CC according to heart

Heart Parameters	**Mean ± SD.**		**df**	**Test statistic t**	**p value**
	Control (n = 20)	**CC (n = 22)**			
Ck	68.25 ± 23.793	± 32.09 91.136	40	2.603	0.0129
Ckmb	16.05±5.2763	53.091±18.938	40	8.445	000
LDH	111.5±31.2284	234.409±29.296	40	13.160	000

Our results from table (16) showing a significant difference between AA and CC with respect to Ck $P< 0.05$, Ckmb $P< 0.001$,and LDH $P< 0.001$

Table 17: Comparison between AA and AC according to heart

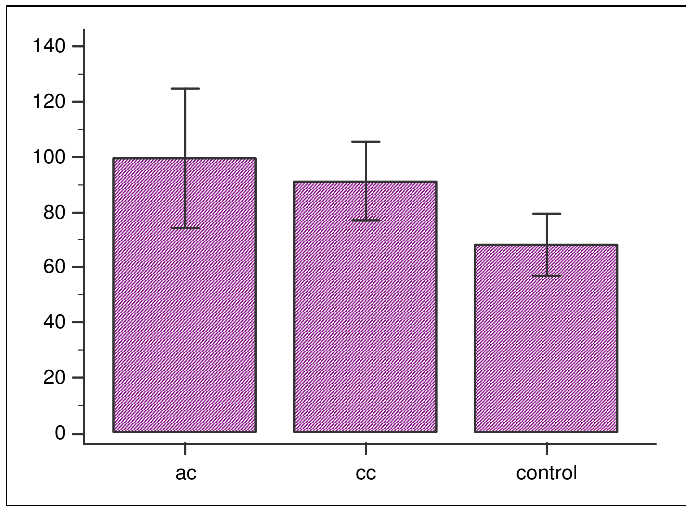
Heart Parameters	**Mean ± SD.**		**df**	**Test statistic t**	**p value**
	Control (n = 20)	**AC (n = 8)**			
Ck	68.25± 23.793	± 30.227 99.375	26	2.897	0.0075
Ckmb	16.05 ±5.2763	70. ± 19.6759	26	11.555	000
LDH	111.5±31.2284	246.125±12.7216	26	11.703	000

Statistical analysis for AC genotypes showing a significant difference between AA and AC with respect to Ck $P< 0.01$, Ckmb $P< 0.001$ and LDH $P< 0.001$. Table (17)

Table 18 : Comparison between CC and AC according to heart

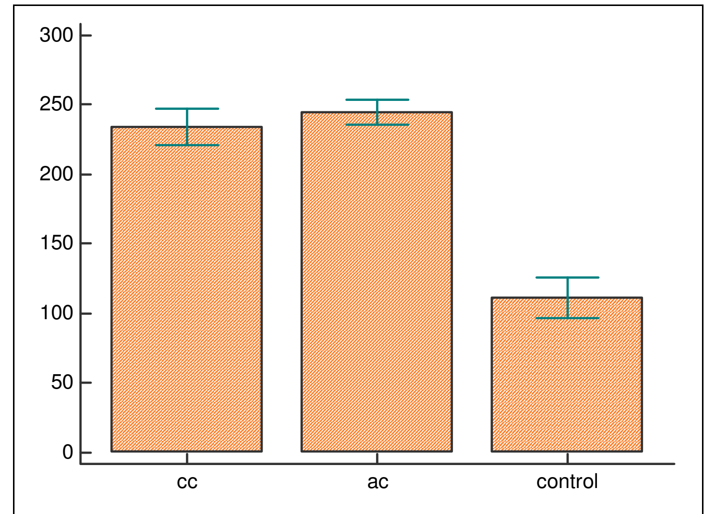
Heart Parameters	**Mean ± SD.**		**df**	**Test statistic t**	**p value**
	CC (n = 22)	**AC (n = 8)**			
Ck	91.136± 32.09	± 30.227 99.3750	28	0.631	0.5333
Ckmb	53.0909±18.9383	70. ± 19.6759	28	2.141	0.0411
LDH	234.409±29.296	246.125±12.7216	28	1.085	0.2872

The comparison data between CC and AC according to heart showed that no significant difference between CC and AC with respect to Ck at the 0.05 level and LDH at the 0.05 level while there is a significant difference between CC and AC with respect to Ckmb $P< 0.005$. Table (18)



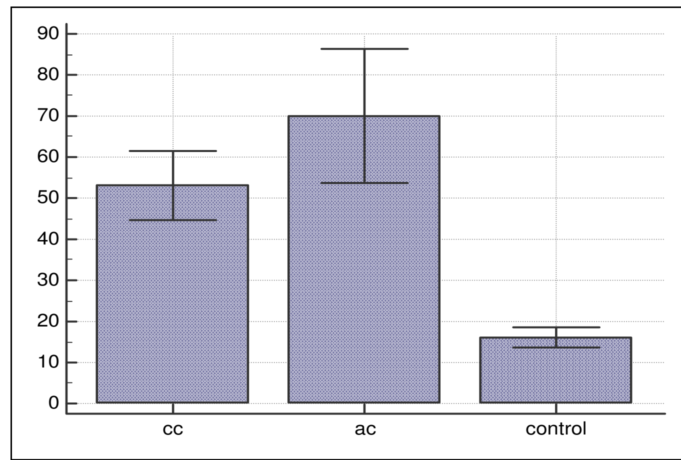
CK

Fig. 8: Comparison between AC, CC & AA with respect to CK



LDH

Fig. 10 : Comparison between AC, CC & AA with respect to LDH



CKMB

Fig. 9 : Comparison between AC, CC & AA with respect to CKMB

- ROC curve analysis showing that, Ck can distinguish between each of AC & CC genotypes in patient group and control groups at the level of significant P=0.0030 and p=0.01 respectively. table (19) & table (20)

Table 19: Agreement (sensitivity, specificity) for ck to diagnose (heart CC) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Ck	0.707	0.0100	0.546	0.837	>89	54.55	80.00	75.0	61.5

Table 20: Agreement (sensitivity, specificity) for ck to diagnose (heart AC) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Ck	0.791	0.0030	0.596	0.920	>78	75.00	75.00	75	67

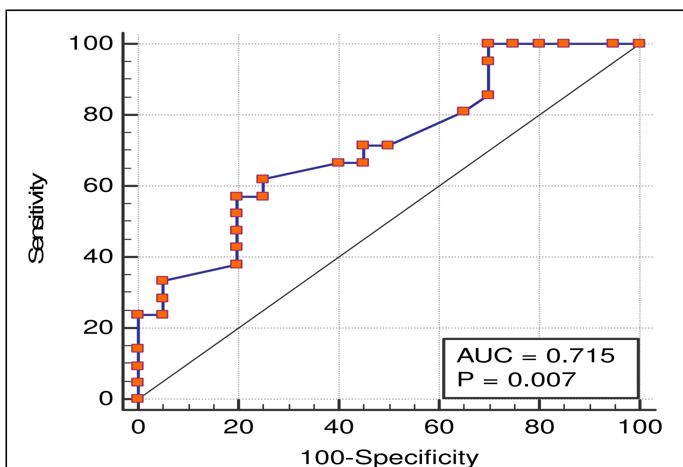


Fig. 11: ROC curve for ck to diagnose (heart cc) from control

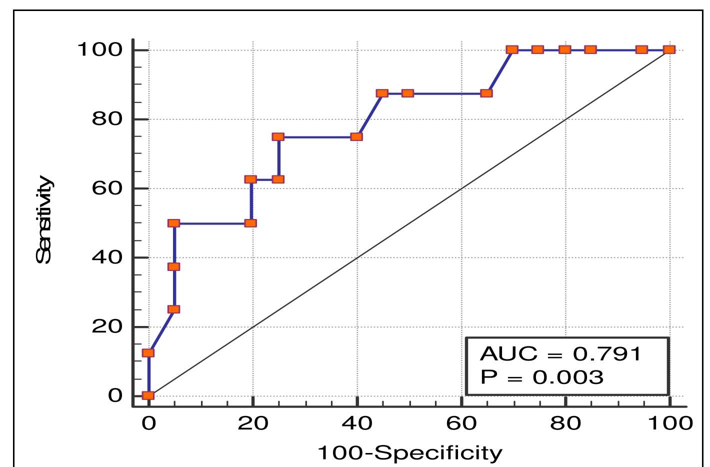


Fig. 12: ROC curve for ck to diagnose (heart Ac) from control

- In case of CKMB the analysis showing that , CKMB can distinguish between each of AC & CC genotypes in patient group and control groups at the level of significant p <0.001 table (21) & table (22)

Table 21 : Agreement (sensitivity, specificity) for ckmb to diagnose (heart CC) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
CKMB	0.999	<0.0001	0.914	1.000	≤25	100.00	95.45	95.2	100.0

Table 22: Agreement (sensitivity, specificity) for ckmb to diagnose (heart AC) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
CKMB	1.000	<0.0001	0.877	1.000	≤ 25	100.00	100.00	100	100.0

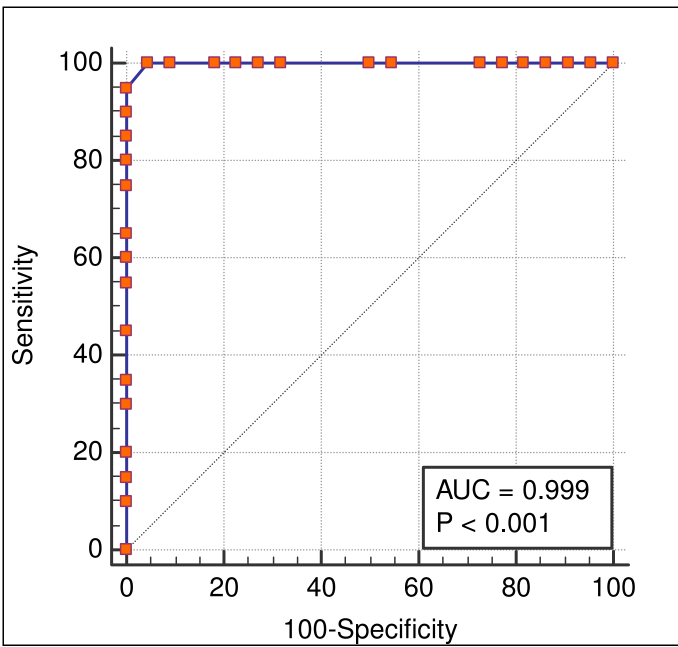


Fig. 13: ROC curve for ckmb to diagnose (heart cc) from control

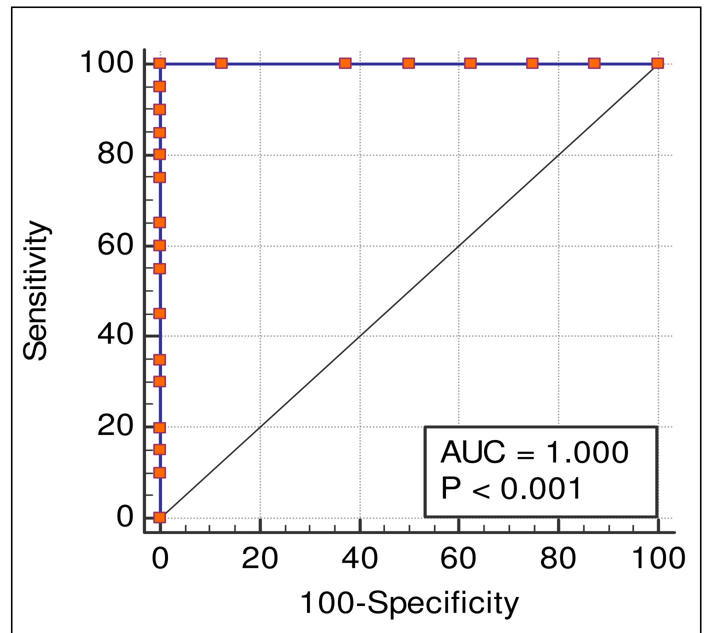


Fig. 14: ROC curve for ckmb to diagnose (heart ac) from control

- When we compared between AC& CC genotypes group in heart patients the analysis conclude that CKMB can distinguish between the patients with CC and the patients with AC groups at the level of significant 0.05. table (23)

Table 23: Agreement (sensitivity, specificity) for ckmb to diagnose (heart AC) from cc

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
ckmb	0.744	0.0234	0.553	0.885	>67	62.50	81.82	55.6	85.7

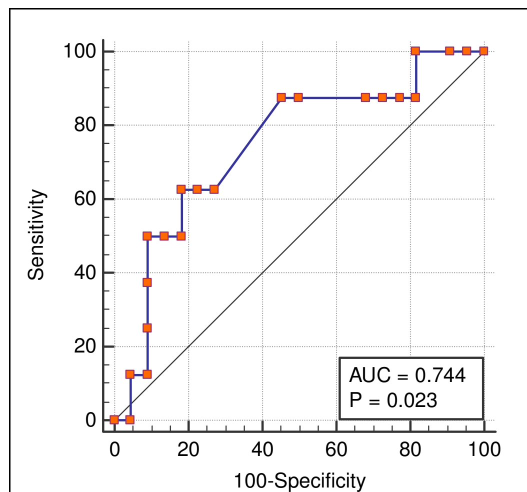


Fig. 15: ROC curve for ckmb to diagnose (heart Ac) from cc

- ROC curve analysis showing that, LDH can distinguish between each of AC & CC genotypes in patient group and control groups at the level of significant p<0.0001. table (24) & table (25)

Table 24 : Agreement (sensitivity, specificity) for LDH to diagnose (heart cc) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
LDH	0.989	<0.0001	0.895	1.000	≤179	100.00	95.45	95.2	100.0

Table 25 : Agreement (sensitivity, specificity) for LDH to diagnose (heart AC) from control

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
LDH	1.000	<0.0001	0.877	1.000	≤179	100.00	100.00	100.	100.0

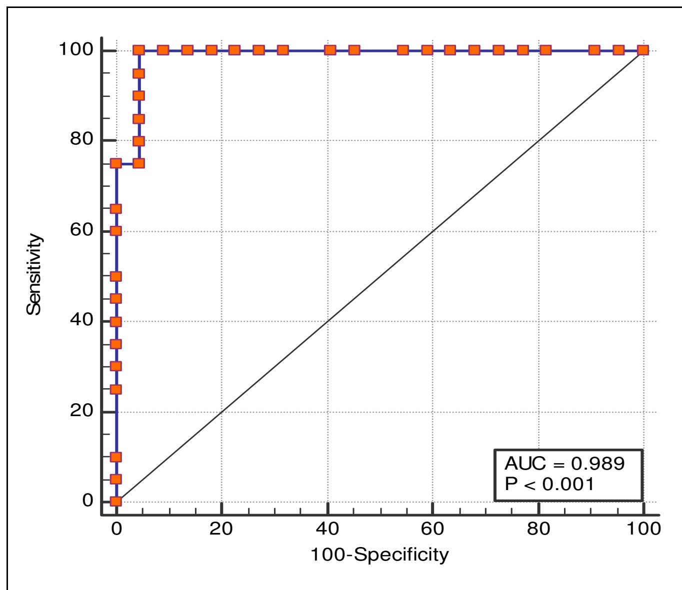


Fig. 16 : ROC curve for LDH to diagnose (heart cc) from control

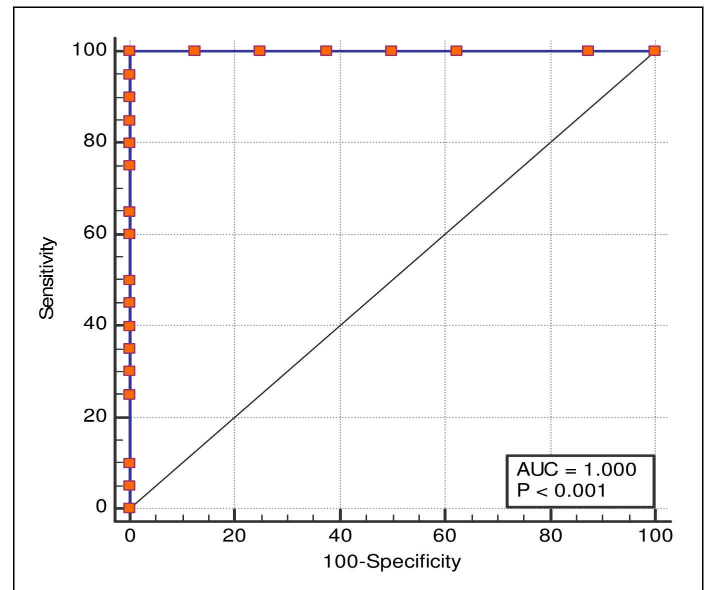


Fig. 17: ROC curve for LDH to diagnose (heart AC) from control

- LDH cannot distinguish between the patients with AC genotype and the patients with CC genotype groups at the level of significant 0.05, (p=0.679), table(20)

Table 26 : Agreement (sensitivity, specificity) for LDH to diagnose (heart CC) from control heart AC

	AUC	p	95% C.I		#Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
LDH	0.679	0.679	0.484	0.837	>237	75.00	63.64	42.9.	87.5

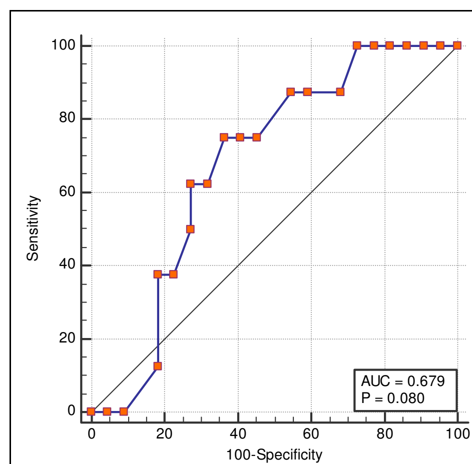


Fig. 18 : ROC curve for LDH to diagnose (heart CC) from (heart AC)

Discussion

The principle pathway having significant obligation regarding the improvement of fundamental hypertension is RAAS (Yim HE, Yoo KH, 2008). One of the RAAS antecedents, angiotensin II, perform axial job to trigger antagonistic impacts in hypertension through AT1R (Singh and Karnik, 2016). A1166C is one of AT1R genes more reported. However, of the reports blunder was. The AT1R is an individual from G- protein coupled receptor and superfamily that is communicated in many tissues. Human AT1R gene is located on chromosome 3q21–25. The AT1R activation leads to vasoconstriction and water retention (Farrag *et al.*, 2011). It might likewise control cell

proliferation and vascular extra cellular protein synthesis, with impacts on renal vasculature, glomerular fiborsis (Lee *et al*., 2009). To locate the polymorphism, gene and protein expression of angiotensin II type 1 receptor is the present study primary of its kind. These findings are in contrast to other studies where in A allele (Farrag *et al*., 2011), C allele (Mehri *et al*., 2012) have been reported to be predisposing factors for basic hypertension. Regarding to other papers which investigate the relationship between AT1R A1166C gene polymorphism and the danger of significant hypertension. A portion of those, studies (Parchwani *et al*., 2018) was indicated that AT1R A1166C gene polymorphism was related with the danger of significant hypertension, while different results (Soualmia *et al*., 2014) unsuccessful to affirm the relationship. In our computation we found that A allele and AA genotype of AT1R A1166C gene polymorphism were related with decreased hazard of basic hypertension, while C allele and AC and CC genotypes were, respectively, associated with increased odds of essential hypertension (Yang *et al*,, 2017). Our meta-analysis of association of the rs5186 SNP on multiple populations recognized that the C allele and AC/CC genotypes had a measurably critical raised in kidney and heart patients. It was reported that the c-allele of AT1RA1166C gene polymorphism may be related with a quiker reject in renal functions (Lovati *et al*., 2001). It has been recommended that the renal and systemic Angiotensin II activity would be increased in subjects with c-allele of AT1RA1166C gene polymorphism. Conflicting results were reported about the connection between the presence of AT1R C-1166 allele and kidney harm (Buraczynska *et al*., 2002) and in 2006 (Buraczynska *et al*., 2006) found an relationship between this allele and the movement to ESRD. Others (Zsom *et al*., 2011) reported that the basic determination may modify the relationship of genetic polymorphism and dialysis dependent ESRD. On the other hand, (Coll *et al*., 2003) in China and (Lee *et al*., 2009) in Spain found that the quicker progression of renal damage was related with AA genotype. In basic hypertension, the C allele of A1166C was uncovered to have a vital job in impacting AT1R activities through influencing mRNA stability and transcription or alternatively be connected to different SNPs (Liu *et al*., 2015). Another study likewise found that C allele of AT1R A1166C was related with higher expression of AT1R gene and raised plasma level of AT1R (Chandra *et al.*, 2014). Gene-gene interaction study likewise upheld our points of view, they found that AT1R A1166C connected to ACE I/D (Wang and Staessen, 2000) the genotype distribution of AT1RA 1166C between controls (AA Genotype), kidney and heart patients (AC& CC genotypes) was in agreement with Chi-square (Pearson Chi square) analysis in which (P = .000, X^2 =64.765 respectively) Table (1), In the current study. In addition alleles distributions of (AT1R) (A1166C) gene between control cases and heart & kidney cases were in agreement with Chi-square (Pearson Chi square) analysis in which (P =.000, X^2 =22.947 respectively) Table (2). Also the study showed that there was agreement with Chi-square (Pearson Chi square) analysis gene between kidney cases and heart cases for genotyping distributions of (AT1R) (A1166C) gene in which (P =.000, X^2 =22.947, respectively), But in case of alleles distributions of (AT1R) (A1166C) gene no significant difference occurs (P =0.673 , X^2 =0.178, respectively) table (2) .In coronary artery disease, post meta-examination found a high danger of coronary artery disease in C allele (Zhang *et al.*, 2013), while in the patient cardiovascular disease, it was reported that found of C allele was related with raised degrees of oxidative pressure markers in cardiovascular breakdown patients, for example, protein carbonyl and myeloperoxidase (Cameron *et al.*, 2006). This pathway may clarify our outcomes demonstrating that C allele of AT1R A1166C gene polymorphism was related with higher odds of having basic hypertension. However, further studies are required to decisively explain the exact mechanism of how AT1R A1166C gene polymorphism influences fundamental hypertension. Our study also showed that by comparing the means values of biochemical parameters between control group and kidney patients, the results showed that, There were significant differences between control group (group 1) and Kidney patients group (group 3), according to urea, creatinine, cholesterol and TG, (p<0.001). There was no significant difference between control group (group 1) and kidney patients (group 3), with respect to Hb where (p=.077) table (3). Also, comparing the biochemical parameters between control and heart cases groups, the results showed that, There were significant differences between control group (group 1) and heart patients group (group 3), according to Ck, Ck-mb, cholesterol, TG, and TG LDH, (p<0.001) table (15). There was no significant difference between control group (group 1) and heart patients (group 3), with respect to Hb where (p=.085) table (15).We found that A allele and AA genotype were fundamentally connected with a reduced hazard of basic hypertension, while C allele, AC genotype, and CC genotype were related with raised hazard (Ben Abda *et al.*, 2011). Since found, PCR-RFLP was generally utilized for genotyping in different SNPs. Although both genotyping procedures were demonstrated having a similar efficacy, in any case, PCR-RFLP was accounted for giving a simple form scheme of insulates (Tanahashi *et al.*, 2000). Our study had a few critical impediments. To start with, a few components which may pivotally affect basic hypertension, for example, age, physical inertia, and body weight (Olack *et al.*, 2015) were not analyzed. Second, in the sub-group examination, false positive discoveries may happen due to a few specimen size.

Conclusions

Our results uncovers that A allele and AA genotype of AT1R A1166C gene polymorphism are related with a protective impact against basic hypertension, while C allele and AC genotype of AT1R A1166C are associated with the elevated risk of basic hypertension in kidney and heart diseases. Our study may add to better understanding concerning gene-disease association between AT1R gene polymorphism and the risk of hypertension.

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