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ASSOCIATION BETWEEN MYOSIN HEAVY CHAIN POLYMORPHISM AND PROGRESSION OF RENAL FAILURE

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ABSTRACT : "*MYH9*gene" is responsible for encoding non muscle myosin heavy chain protein that "expressed" in the "kidney, platelets and liver and in smaller amounts in the thymus, spleen, and intestine". The expression of that protein in the podocytes which are highly specialized cell, because of capacity to ultrafilter blood and support "glomerular capillary pressures". Unbalanced and irregular in *MYH9* geneexpression, or change in its positioning, or task.these transformationcause "cytoskeleton damage, causing proteinuria:, hematuria, or "renal failure". Renal failure is the result of a squally of different illnesses and accidents that affect directly or indirectly on the renal system. Kidney which has an vitalcharisma in regulating many body functions, so its deterioration leads to the deterioration of the whole human body, renal failure is considered incurable and need hard-hitting ways to avoid it or to compensate the function of the kidney. To evaluate the role of *MYH9* SNP on developing of renal failure. The study depending on "methodology of Case-control study", hundred subjects involved; 50 "patients"complainig renal failure who attended Marjan Medical City in Hilla,Iraq and 50 apparently healthy controls. DNA was extracted venous blood. The "*MYH9* gene polymorphisms" were recognizedby applying the procedure of "polymerase chain reaction restriction fragment length polymorphism" (PCR-RFLP). Data analyses statically Statistical using SPSS. Genotype at rs4821480in patients with RF: genotyping were TT (59%), GT (34%) and GG (6.0%) and for control TT(45%)GT(40%), GG(15%). This analysis of data indicated the TT genotype homozygote at rs4821480 convenesin dependently a threating of RF than does the GT and GG genotypes. There is no significant correlation between alleles distribution and age, sex, resident, jobs, smoking habit, family history, body mass index (BMI), medical history (P>0.05). The genotyping of the *MYH9* SNP rs4821480 help to identify individuals at risk of developing progression in renal function and increase the susceptibility for developing renal function deterioration.

Key words : MYH9 SNP, renal failure, allele distribution and genotyping.

INTRODUCTION

The elucidation of chronic kidney disease in the last period had been simplified for assessing its proof of identity and final explanation of chronic renal failure as it iscorrelated with drop of glomerular filtration rate over a period of 3 months. Deterioration in filteration of kidney of "less than 60 mL/min/1.73 m^2" in adult establish the diagnosis of renal failure and for rate less than 60 mL/min/1.73 m^2 can considered renal failure with some other markers indicate renal defective as urine sediment or abnormal in x ray or renal biopsy beside other finding (Simons *et al*, 1991).

For the progression of different renal diseases with aid of final attitude of recent researches indicates that intact "actin cytoskeleton" is a essential to maintain normal the typical purpose of podocyte building and filtration (Lalwani *et al*, 2000). The "nonmuscle myosinprotein", that translated from of the 'nonmuscle myosin heavy chain 9 (MYH9) gene", which expressed in body cells and binds to "actin cytoskeleton" to carry out specific "intracellular motor functions" (Sellers, 2000).

Former workings on MYH9 mutations that linking with a number of kidney diseases "(May-Hegglin, Epstein, Fechtner and Sebastian syndromes)"approached to identifying the link between "MYH9 mutations and podocyte injury", which suggested that any fluctuations of protein due to mutation in gene will lead to impairmentof the "glomerular filtration barrier", this defect can lead to developing "proteinuria and/or haematuria" and even "renal failure" in advanced deterioration of kidney function (Herrema *et al*, 2006; Perry *et al*, 2006). And the effort of newly works of a "genome-wide association study

(GWAS)" was recognized "MYH9" as a major susceptibility gene for ESRD, in different types of nephropathy as 'idiopathic focal segmental glomerulosclerosis, HIV-associated nephropathy and hypertension" in different ethnicities '(African-Americans, Europeans and Hispanic Americans)" these previouse work suggested a link between the MYS gene and glomerular function (Saleem *et al*, 2008; Cheng *et al*, 2011; Kopp *et al*, 2006; Kao *et al*, 2008).

MYH9 gene, which is has a locus on "chromosome 22 q12.3-13.2", has 40 "exons" that translate a protein of approximately "224,000 kDa",this"non-muscle myosin heavy chain protein" that dimerizes to form specific"motor domain of non-muscle myosin IIA", an important motor protein that distributed in various cells (Behar *et al*, 2010) and this protein is synthesized according to codon of messenger RNA, which translated by transfer RNA that lead to synthesis of this specific regulatory protein. The gene mainly expressed n "fibroblasts, erythroblasts, and kidney cells" (Ghiggeri *et al*, 2003). Irregularity in "expression, positioning, or function" in squally give rise to "cytoskeleton"impairment,and in result of this causing "proteinuria, haematuria, or renal failure"as registered in in various circumstances (Althaus *et al*, 2009).

Anomalous in "MYH9" expression considered major predisposingfactor for developing and progression in thefunction of kidney according to the documentations of "genome-wide association studies (GWAS)" that established onnumerous nephropathies, including" idiopathic focal segmental glomerulosclerosis", "human immunodeficiency virus" (HIV)-. The previous link demonsetrated in different societyas "African-Americans and Hispanic Americans to Europeans" (Diez-Ojea Beatriz *et al*, 2014; Pattaro *et al*, 2009; Kopp *et al*, 2008; Klag *et al*, 2008).

Study of Kidney Disease and Hypertension revealed that vascular changes, arteriolar nephrosclerosis, participants with low-level proteinuria and elevated blood pressures that lead to extensive focal global glomerulosclerosis, these event are related to mutation in "MYH9" (Barry *et al*, 2010; Freedman *et al*, 2010).

METHODS

This type of methodology applying the steps of case-control study included hundred subjects, 50 patients with renal failure, who attended Marjan Medical City and 50 apparently healthy controls.

All information about patient and control group registrated regarding "Age, sex, resident, jobs, smoking habit, family history, body mass index (BMI), medical history, hemoglobin, platelets, serum creatinine level, urea, potassium and sodium" were recorded. Informed consent was obtained from all participants.

The extraction of DNA from venous blood according to protocol of kit (Miller method, 2014).

DNA yield was assessed using different methods: nanodrop instrument, which is highly sensitive and directly provides us with the concentration of DNA, A260/A280 ratio and A260/A230 ratio and agarose gel electrophoresis (Lee *et al*, 2012).

The *MYH9* gene polymorphisms detection depending on amplification the segment that containg polymorphic region using the polymerase chain reaction and then for purpose of genotyping we depend on "PCR-RFLP" technique by using special "restrictive endonuclease".

The rs4821480, polymorphis in the MYH9 gene was amplified by using the sequences of primers used for polymerase chain reaction (PCR) amplification and restriction enzymes as listed in Table 1.

Genomic DNA was amplified in a final volume of 25µl.

PCR conditions that give best result summarized in Table 3.

The amplicon obtaing from run of PCR was incubated with DraI for genotyping process. Digestion conditions that give best result were summarized in Table 3.

Those mentioned reagents were mixed and incubated at 37°C for overnight. After digestion with RsaI, products of digestion were run by electrophoresis on 2.5% agarose gels 2 h at 100 V and the bands visualized with uses of 'ethidium bromide UV light. A 100 base-pair ladder (Bioneer, Inc. - Korea) was used as a size marker

RESULTS

The amplification of polymorphycic segment revealed the product of about537 bp, which are represented in Fig. 1.

Table 1 : forward and reverse primers of rs4821480, polymorphism.

Tm	Primers Sequences	genes
60	CCGCTGGGCAGGGGTGTT F TCTTCTGTGAGGTTGGT GGTG R	rs4821480, polymorphism MYH9

Table 2 : Reagents used for preparation of PCR reaction.

Reagent	Volume
Genomic DNA	7µl
F- "primer"	0.7 µl
R- "primer"	0.7 µl
Promega master mix	12.5 µl
DDW	4.0 µl

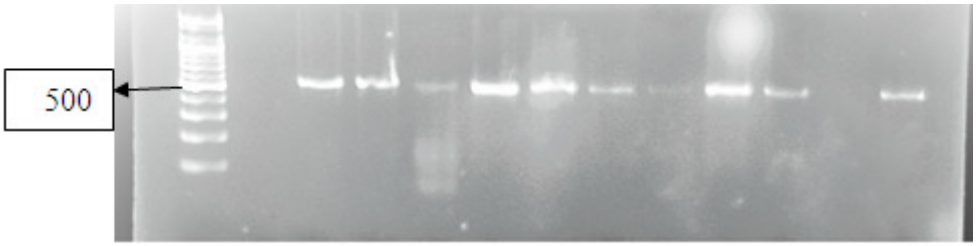

Fig. 1 : PCR product.

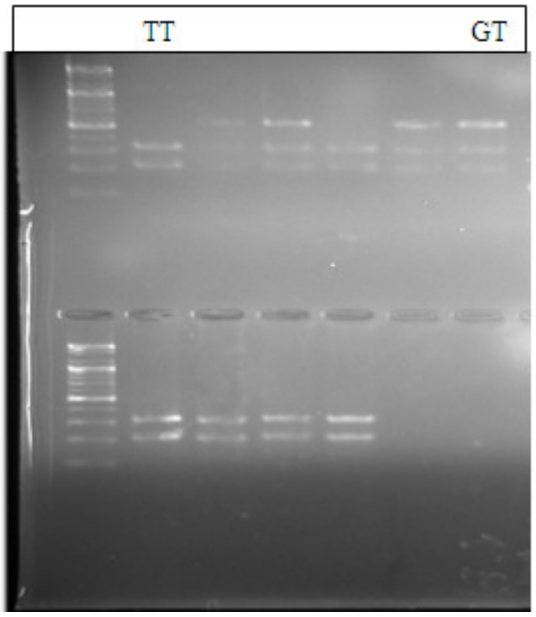

Fig. 2 : Restriction digestion of PCR products demonstrating the patterns of different genotypes of MYH9 on 2% agarose, 100V and for 60 minutes; bands (TT), (GT) and (GG).

The product of PCR after incubated with restrictive endonuclase revealed fragment sizes of different allells, which are recognizedas band of different size and accordingly the allell are classified as follow:

322+215 bp (TT allele)

537 322, 215bp (GT allele)

537 (G allele)

1- Two bands (322+215 bp) are homozygote (TT).

2- Three bands (537, 322, 215bp) are heterozygote (GT).

3- One band (537) (G G)

Genotyping of MYH9 Polymorphism (rs4821480) are representing in Table 5.

The evaluation of TT versus GT genotype as independent hazard for developing and deterioration of kidney function are representing in Table 6.

Analysis of data by Fisher exact probability test are representing in Table 7.

DISCUSSION

In recent years from the effort of scientist work

Table 3 : PCR reaction program steps.

Type of cycle	Temperature	Time	No. of Cycles
Initial Denaturation	94°c	3 minutes	1 cycle
Denaturation	94 °c	35 seconds	30 cycles
Annealing	55 °c	45 seconds	
Extension	72 °c	55 seconds	
Final Extension	72 °c	5 minutes	1 cycle
Hold	4 °c	∞	

Table 4 : Reagents used for preparation of digestion reaction.

Reagents	Volumes for 1x
Water	9.5 µl
NEB Buffer	2 µl
PCR product	8 µl
Enzyme	0.5 µl

increasing evidence involved the importance of predisposing of genetic factors on development and progression a disease as widely considered to be a "polygenic" disorder. The conclusion about developing of many diseases related to environmental and genetic cooperated these factor lead to risk of developing the disease.

This report is aimed to investigate one of genetic predisposing factor associated with of CKD so we choose MYH9 SNP and its role on developing and progression of renal failureas these problem are predisposing to increasing in morbidity and mortality and considered a health problem in society.

MYH9 gene, which codifies the "myosin-IIA protein" that contain an "IQdomain" which responsible on its biological function these related to role of catalytic action of enzyme present in the podocyte foot that contribute to filament movement. In animal studies, mutations in MYH9 are related to "phenotypic kidney abnormalities including albuminuria and FSGS" (Zhang *et al*, 2012; Johnstone *et al*, 2011) as well as defects in morphogenesis (Müller *et al*, 2011). The "pathogenesis" of MYH9-related kidney disease is not fully assumed. In spite of establishing the role of MYH9-related disorders transformation the "podocyte cytoskeleton" and as a result lead to "glomerular filtration barrier damage that basis for

Table 5 : Comparison of Alleles and Genotype Frequency of (rs4821480) of MYH9 Polymorphism in renal disease and control groups.

Genotype	CKD no. (%)	Control no. (%)
TT	**32 61.5%**	**16 38%**
GT	**17 32.6%**	**22 52.4**
GG	**3 5.7%**	**4 9.5%**
Allele		
T	81 77.8%	**54 65%**
G	20 22.2%	**30 35%**

Table 6 : TT versus GT genotype in MYH gene polymorphysim as risk factor.

(rs4821480) TT vs. GT	Odd ratio	CI	P value
	1.8	1.46-4.66	<0.05

Table 7 : Fisher exact probability test for TT vs GT.

P value	Two tailed	Peasrson
(rs4821480) TT vs.GT	3.7	0.025
	4.66	0.03

developing : proteinuria and hematuria, and finally to renal failure" (Singh *et al*, 2009). So the study try to find the relation between Genotype and "allele distributions of the MYH9 polymorphisms" at rs4821480 and development of CKD to consider it as risk factor.

The result of genotyping of *MYH9 polymorphisms* (rs4821480) as documented in Table 4 there are two main phenotyping TT and GT.

We study the possibility of TT homozygous phenotype against GT heterozygous by calculating the Odd ratio and result was 1.8 meaning that the persons with TT genotype have risk factor that assessed on developing renal failure more than GT phenotype and the T allele considered as risk factor. The assosation between MYH9 polymorphysim and developing renal failure confirmed with other study (Beatriz Taviraa *et al*, 2013; Wenrong Cheng *et al*, 2011; Cooke *et al*, 2012).

In conclusion, The genotyping SNP rs4821480 of "MYH9" assessed in identifying the categories at risk of developing deterioration in renal function.

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