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Metallothionein gene polymorphism is considered to be a risk factor for chronic diseases

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Abstract---Metallothioneins are considered the main proteins to protect the cell from toxins like heavy metals and oxidative stress encoded by the Metallothionein gene. Any mutation in this gene leads to a defect in the function of Metallothionein proteins, leading to an increase in the effect of heavy metals. Reactive oxygen species, the SNP A-G (rs28366003) in promotor of the metallothionein gene converts the A allele to G allele, and therefore decreases the transcription qualification of the Metallothionein gene; the aim of the study is correlation between A-G (rs28366003) polymorphism in promotor of Metallothionein gene and the risk factor as well as detecting the levels of heavy metals in patients with Kidney failure, this study includes 60 persons with Kidney failure and 20 persons as a control group with age gape (30-55) years, the blood sample collects from the subject and separate into two-tube, EDTA tube to extraction the DNA and Gel tube to biochemical test. The result showed that there is a relationship between polymorphism in the promotor of the Metallothionein gene in the site A-G (rs28366003). The distribution of different genotypes is wild A.A. 22 %, hetero AG 25%, and mutant 53% in the patients. In comparison, the distribution of genotypes in the healthy people are A.A. 45%, AG 30%, and G.G. 25%. Also, the value of O.R was 2.0769, considered a major risk factor in the patients. The result shows an increase in the levels of some heavy metals and biochemical tests in the patient's group compared to the healthy group. Conclusion: In this research, we define the relationships between the mutation of the metallothionein gene in the site

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A-G (rs28366003) in kidney failure patients, and the increase in the level of some heavy metals in patients compared to the healthy people.

Keywords**----**Mutation, ARMS-PCR, Metalothionen, Kidney failure.

Introduction

The metallothioneins are groups of proteins that play a major role in protecting the cell from harmful substances like reactive oxygen species and heavy metals, and protect the DNA from damage. Also, these proteins organize the physiological metals of (za and cu). These proteins featuring by containing a high level of cysteine, nearly 30 percent, and these proteins don't have any aromatic amino acids; the metallothionein proteins specialized by binding to heavy metals like Pb, cu, and zn (Kimura et al.,2016 ; Riken et al., 2016 and Kesson et al., 2014).

Also, the previous study confirmed that metallothionein proteins are important in the metabolism regulation of trace elements in the cell. The Lucas of metallothionein gene in the chromosome 16, and the family of metallothionein proteins contain four isoforms, MTI, MTII, MTIII, and MTIV. All of them are expressed in specialized cells in different body parts, and all of them have a role in protecting the cells from oxidative stress and heavy metals (Bonomini et al., 2015; Cruz et al., 2015 and Ferraro et al., 2010). M.T. proteins have an important role in cellular defenses against oxidative stress, as well as the negative switch between oxidative stress-mediated apoptosis and apoptosis that is not triggered by oxidative stress (Ganasyam et al., 2012 and Jha et al., 2013).

The location of SNP A-G (rs28366003) in the promotor of a metallothionein gene, this polymorphism exists away of five bases upstream of initiation site; this polymorphism includes change the A nucleotide to G nucleotide in promotor of M.T. gene, subsequently causes reduce of metallothionein, proteins production and then decrease in the activity of metallothioneins to protect the cell from harmful substances (Kato et al., 2013 and Lederer et al., 2016).

High uric acid levels characterize kidney failure, creatinine, and urea in the patients' blood; many reasons cause kidney failures like diabetes millets, genetics, hypertension, and unhealthy foods (Rattanatham et al., 2014). Cadmium is considered the heaviest metal toxicity to the body ita accumulates in the kidney and cuses kidney failure over time, the role of metalothionen in removing cadmium toxicity through the bile. And the M.T. protein has a low molecular weight protein that has been shown to remove the free radicals in animals and possesses anti-apoptotic effects (Raudenska et al., 2014).

The researcher can demonstrate that heavy metals as it is essential elements for human health and include Cu, Fe, Zn, Cr, I, Se, Co, and Mo, and also found many types of heavy metals as Non-Essential for human health like Pb, As, Hg and Cd. The vital role of heavy metals is there present in the animal tissues in very low concentrations (Ruttkay-Nedecky et al., 2013 and Teramoto et al., 2013), some of which are very important for biological functions like metabolism and growth. Still, if the concentration of heavy metals increases above the normal

range, it becomes harmful to the health of the organism; the pollution has an essential role in increasing the concentration of heavy metals in the environment, and, therefore, increasing its in the body of an organism, and which are considered the main causes for many health problems (Tajima et al., 2015; Tamay-Cach et al., 2015 and Rutter et al., 2015).

The study aims to:

1- to expose the genetic variation of the metalothionen gene
2- the negative effect of heavy metals in patients with kidney failure in Mosul city

Materials and Methods

Collection of blood sample

This study embodies 55 patients of both sexes with kidney failure from ibn sena hospital \ Mosul city with age group (35-55) years and 20 healthy people considered as control with same age groups, the blood collected from venous and separated into two tubes, gel tube to get serum for biochemical parameters and EDTA tube for DNA extraction.

Extraction of DNA

The DNA was extracted from the blood of all samples in this study by using a manual protocol submitted by (Iranpour et al., 2010). It measured the purity and concentration of DNA by drop technique, and then the DNA was stored at -80 for genotyping test (Krizkova et al., 2016).

Genotyping test

By PCR-**AR**MS technique, we detected the SNP A-G (rs28366003) in the promotor of a metallothionein gene, was used four primers for wild and mutant alleles described in table 1(Yuta et al., 2016).

Table 1: shows the primers sequence to detect SNP A-G (rs28366003) in promotor of metallothionein gene

Primer	sequence	tm	Band size
F1	5-CGC.CTG.GAG.CCG.CAA.GTG.AC-3	61	198 bp
R1	5-ATC.CAT.GGC.GAG.CTG.AAG.A-3		
F2	5-ACT.GCT.TGC.CGC.GCT.GCA-3		135 bp
R2	5-TGG.AGG.AGG.CGT.GGT.GGA.GG-3		100 bp

And we added 100 ng of DNA template (4 µl), ten picomols from each primer, and (12.5 µl) of master mix, depending on the described program in the table 2, and the PCR reaction perform with a 25 µl final volume(Yuta et al., 2016).

Table 2: show the steps of PCR reaction to detect SNP A-G (rs28366003) in the promoter of the metallothionein gene

No.	Stage	Temperature	Time	Cycle number
1..	Initial-denaturation.	.95.0	5.0 min..	1
2..	Denaturation.	.95.0	0.45 sec..	35
3..	Annealing.	.61.0	1.0 min..	
4..	Extension.	.72.0	1.0 min..	
5..	Final-extension.	.720	7.0 min..	1
6..	Stop-reaction.	.4.0	5.0 min..	1

Biochemical Test

The biochemical tests in this study, like creatinine, uric acid, and urea, have been done by chemo test technique. Some heavy metals like cd, zn, and cu were measured by atomic absorption technique.

Result and Discussion

The result of this research exposes the relation among polymorphism in A-G (rs28366003) in promotor of a metallothionein gene; when observing the fig. 1, the PCR product includes three bands, 100 bp, 153 bp, and 198 bp, which proves present three different genotype A.A., AG and G.G. in the patients with different present table 1.

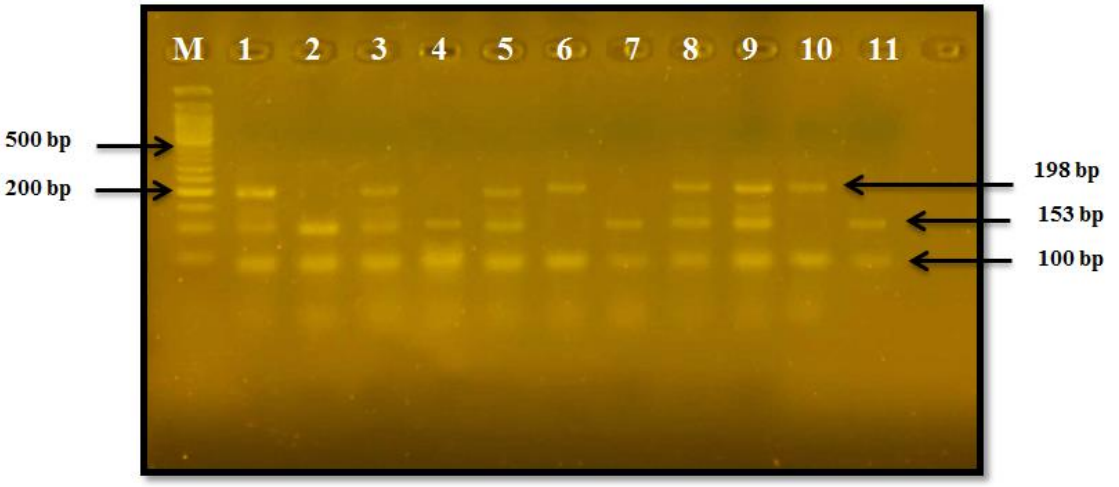

Fig 1: Show the PCR product for A-G (rs28366003) in the promotor of a metallothionein gene, which is separated by 2% across gel electrophoresis. M ladder, 198 bp and 153 bp A.A. genotype, 198 bp and 100 bp G.G. genotype, 198 bp, 135 bp, and 100 bp A.G. genotype.

Table 1: Distribution of the genotype of metallothionein gene and allele frequency in a sample of this study A normal allele and G mutant allele

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
AA	13	22	9	45	P = 0.2784	2.0769	0.5539 to 7.7877
AG	15	25	6	30			
GG	32	53	5	25			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
A	58	44	24	54	P = 0.2030	1.6034	0.7751 to 3.3169
G	62	56	16	46			

The result of this study showed in the table 1 the genotype and allele frequency in the promotor of metallothionein gene in the site A-G (rs28366003), the patient with kidney failure have higher redistribution of Mutant genotype G.G. (53%) compared with a healthy group (25%). Still, the wild genotype A.A. in the control group has high distribution (45 %) compared with patients (22 %). Still, the distribution of hetero genotype A.G. the percentage was similar between two groups (25 %) in patients and (30 %) in healthy people. Also, the results in this study showed the value of the O.D ratio of mutant genotype was more than one (2.0769). The value of the O.D ratio of the mutant allele (1.6034), and this ratio is considered a risk factor for complications of kidney failure in patients due to the mutation in the promotor of metallothionein gene.

As is known, this mutation in the promotor of the metallothionein gene in the site A-G (rs28366003) lead to a defect in the function of the main protein that coding by metallothionein gene, this protein have able to combined the different type of heavy metals together by thiol group, and then remove it's from the body by filtration in the kidney and secreted with the urine, and because this mutation the heavy metals accumulate in the body of the patient and causes many health problems (Krężel et al., 2017). And as a result of, oxidative stress and environmental pollution cause an increase in levels of heavy metals in the organism. Due to this mutation in the promotor of the metallothionein gene, the protein can not remove all the amount of heavy metals from the body and consequently increase the toxicity of heavy metals (Tajima et al., 2015 ; Tamay-Cach et al., 2015 and Rutter et al., 2015).

Table (2): The heavy metals levels in all cases

Parameter	Patient	Control
parameters	Con./ ng/dl ± SE	Con./ ng/dl ± SE
Zinc (Zn)	13.2 ± 0.9	± 0.1 2.8
Copper (Cu)	0.54 ± 0.06	0. 93 ± 0.012
Lead (pb)	3. 98 ± 0.63	1. 86 ± 0.37

Also, the result of this study showed in table 2 an increase in the levels of heavy metals in the patients, Zn (13.2 ± 0.9), Pb (3. 98 ± 0.63), compared with healthy group Zn (2.8 ± 0.1), Pb (1. 86 ± 0.37) and depression in the level of Cu (0.54 ±

0.06) in patients compared with healthy Cu (0.54 ± 0.06), sometime the heavy metals cause toxicity in the human through the direct effect on the metabolic process in the body and then increase in production of free radicals [10,23] (Ankica et al., 2020 and Landrigan et al., 2018).

Table (3): shows The levels parameters that related to kidney function

Groups	Uric acid mg/dl	Urea mg/dl	creatinine µ mol/l
Control	6.3 ± 0.64	17 ±1.1	58 ± 0.02
patients	5.5 ± 0.54	32 ± 2.1	88 ±0.02

In the table 3, the search showed a rise in the levels of uric acid (5.5 ± 0.54), urea (32 ± 2.1), and creatinine (88 ±0.02) in the patients compared with healthy groups (6.3 ± 0.64), (17 ±1.1), (58 ± 0.02) respectively, and this increase in the levels of this parameters due to lack the function of the kidney in the patients, in this cases the kidney cannot filtrate the body fluid properly. Also the result gives the correlation between the mutation in the promotor of metalothionen gene with an increase in the levels of parameters with kidney function (Bonomini et al., 2015; Cruz et al., 2015 and Ferraro et al., 2010).

Conclusion

In this study we detect the relation among the mutation of promotor of metallothionein gene in the site A-G (rs28366003) with kidney failure patients, and increase the level of some heavy metals in patients compare with healthy peoples.

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