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Glutathione S-transferase Theta (GSTT1) and Mu (GSTM1) Genes Polymorphisms in Patients with Coronary Heart Disease in Thi Qar Province

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Abstract

Coronary heart disease (CHD) is the common type of heart diseases. This study aimed to investigate the relationship between the GSTTI, GSTM1 null genotype and coronary heart diseases disease using multiplex PCR. The mean age of CHD was 40.72±13.44years and 38.10±13.55years for patients and control group, respectively. 70% of the patients were less than 50 years old and the rest were over 50 years old, with no significant difference between study groups according to their ages (P=0.85). The current study did not find significant differences between two study groups depending on their gender (P=0.90). Out of 30 cases, 17 (56.57 %) were males while 13 (43.33 %) were females. 66.67 % of patients group were smoker, while 33.33 % were non-smoker with significant difference between patients and control according to smoking (P=0.02). The GSTT1 (-) genotype showed no significant deference with CHD susceptibility (OR=1.31; 95%, CI=0.41-4.13). The current study showed that the majority of patients (60%) have null GSTM1 genotype (OR=2.78; 95%, CI=0.86-9.00). The analysis of GSTT1 (-) /GSTM1 (-) null genotypes were found that the susceptibility of coronary heart disease increases by 1.66 in null\null genotype (OR=1.66; 95%, CI=0.27-5.70).

Key words:

Coronary heart disease, GSTs, polymorphism, PCR.

Coronary heart disease (CHD) is a major health problem which develops from the interaction between genetic and environmental factors. CHD can causes a heart attack or death [1,2]. It is complicated by many comorbidity factors which present in patients (diabetes mellitus, obesity and overweight, etc) [3,4]. the development and installation of this disease is related with metabolic changes which cause an oxidative stress, lead to the over production of reactive nitrogen species (RNS) and reactive oxygen species (ROS) [5,6]. In humans, the molecules deficiency of antioxidant is due to a reduction in their synthesis. A food enriched with the precursors of glutathione (glycine and cysteine) , synthesis of cellular glutathione and glutathione concentration prevents damage associated with the oxidative stress [7].

Oxidative stress resulting from an imbalance between reactive oxygen species and antioxidant molecules, is related with lipids damage, proteins destruction and nucleic acids changing, which could causes several

diseases including atherosclerosis, cancer and diabetes mellitus [8].

In the pathology of cardiovascular diseases, the oxidative stress cause an impairment in the cases conditions related with generation of RNS and ROS. The common reactive species include superoxide anion (O2 • –) , hydroxyl radical (• OH) , nitric oxide (NO •) , and other non -free radical species such as hydrogen peroxide (H2O2) [9]. CytochromeP450 (CYP450) , lipoxygenase, NADPH oxidase (NOX) , xanthine oxidase (XO) , and mitochondrial respiration are the common enzymatic causes of reactive oxygen species in cardiovascular disease[10]. The most popular enzymatic antioxidants include glutathione peroxidase (GPx) , catalase (CAT) and superoxide dismutase (SOD) which reduce the harmful oxidants [11]. A significant contribution to the total antioxidants comes from antioxidant system in blood serum. Insufficiency of the antioxidant system can lead to a reduction antioxidant status of an individual [12].

The glutathioneS-transferases (GSTs) are a super family of genes. GSTs produce enzymes which activate the coupling of glutathione molecule to electrophilic substrates. These enzymes are detoxifying the exogenous and endogenous electrophiles which interact with biological molecules such as DNA [13]. The family is possessed of several isoforms of genes including: mu (GSTM), theta (GSTT), pi (GSTP) , alpha (GSTA) tau (GSTZ) sigma (GSTS) omicron (GSTO) and kappa (GSTK) [14]. The common various of GSTT1 and GSTM1 genes is null genotype, which has been correlated with increased cytogenetic damage and lack of enzyme activity [15]. The GSTM1 is the genes located on chromosome 1p13.3 which coding the mu class of enzymes [16]. The GSTT1 genes codes of the theta class of enzymes and located on chromosome 22q11.23 [17]. Many studies suggested the association between the null genotype of GSTM1 and GSTT1 with the development various types of cardiovascular diseases [18,19,20]. In Iraq, few studies have dealt with the correlated between GSTT1, GSTM1 null genotype and the development of coronary heart disease. This study aimed to reveal the effect of GSTM1 and GSTT1 null genotype on the risk of coronary heart diseases in Iraqi patients.

Materials and Method

The present study was conducted in the biotechnology unit of Mazaya university college. 50 samples (30 patients and 20 control) were collected. The study groups were distributed according to their age, gender and smoking. 2.5 ml of venous blood specimens were collected into EDTA tubes and they placed in refrigerator untill extraction of DNA.

Amplification of GSTT1 and GSTM1 genes

Genomic DNA was isolated from whole blood, using gSYNC^{TM}DNA mini kit. M1 and T1 genes were amplification by a multiplex PCR technique, in which an experience albumin gene used as an internal control. The primers (10pm) used in this study mentioned in table (1)

Table (1) : primer sequence

Name of gene	Primer sequence	Product size (pb)	ref.
GSTM1	F 5\-GAGGAACTCCCTGAAAAGCTAAAG-3 R5\-CTCAAATATACGGTGGAGGTCAAG-3	216	Okcu et al., 2004
GSTT1	F5/-TTCCTTACTGGTCCTCACATCTC-3 R5\-TCACCGGATCATGGCCAGCA-3	480	
albumin	F5/-GCCCTCTGCTAACAAGTCCTAC-3 R5/GCCCTAAAAAGAAAATCGCCAATC-3	350	

Table 2. PCR condition for amplification of studied genes

No. of steps	Steps	Temperature	Time	No.of cycle
1	Initial denaturation	95 °C	10 min.	1 Cycle
2	Denaturation	95 °C	1 min.	30 Cycles
	Annealing	57°C	1 min.	
	Extension	72°C	1 min.	
3	Final Extension	72°C	10 min.	1 Cycle

Statistical Analysis

The statistical analysis was done using the SPSS 17. Mean ± SD, chi-square and odd ratio (OR) with 95% confidence interval 95% CI were used in this study. P values ⩽ 0.05 means significant differences. OR⩽ 1.5 means significant differences.

Results

The mean age of coronary heart disease patients and control group was 40.72±13.44years and 38.10±13.55years, respectively. 70% of the patients were less than 50 years old and the rest (30%) were over 50 years old. As for the control group, 60% of them were aged less than 50 years, compared to 40% aged more than 50 years, with no significant difference between study groups according to their ages (P=0.85) (table 1). The current study did not find significant differences between the two study groups depending on gender (P=0.90). Out of 30 cases, 17 (56.57 %) were males while 13 (43.33 %) were females (table 2). 66.67 % of patients group were smoker, while 33.33 % were nonsmoker with significant difference between patients and control group based to smoking (P=0.02) (table 3).

Table (1) CHD patients and control group according to ages

Age (years)	Patients (N =30)		Controls (N=20)		P value
	N	%	N	%	
< 50	21	70 %	12	60 %	0.85
≥ 50	9	30 %	8	40 %	
Total	30	100 %	30	100 %	
Mean age±SD	40.72±13.44		38.10±13.55		

Table (2) CHD patients and control groups according to gender

Gender	Patients (N =30)		Control (N=20)		P value
	N	%	N	%	
Male	17	56.57 %	11	55 %	0.90
Female	13	43.33 %	9	45 %	
Total	30	100 %	20	100 %	
$\chi 2 = 0.014$, df = 1, P.value ≤ 0.05 mean significant					

Table (3) CHD patients and control groups according to smoking

Smoking	Patients (N =30)		Control (N=20)		P value
	N	%	N	%	
Non-smokers	10	33.33 %	13	65 %	0.02
Smokers	20	66.67 %	7	35 %	
Total	30	100 %	20	100 %	
$\chi 2 = 4.84$, df = 1, P.value ≤ 0.05 mean significant					

The GSTT1 and GSTM1 polymorphic in coronary heart diseases patients and control are shown in table 4. The current study found that the null of GSTT1 genotype was slightly higher in coronary heart diseases patients (46.67%) compared to the control group (40%) without significant differences between the two study groups (OR=1.31; 95%, CI=0.41-4.13). On the other hand, the differences between the patients and control group were significantly (OR=2.78; 95%, CI=0.86-9.00). The current study showed that the majority of patients (60%) have null GSTM1 genotype, while only 35% of the control group have null GSTM1 genotype. The combined effect of GSTT1 and GSTM1 null genotypes were showed that the susceptibility of coronary heart disease increases by 1.66 in null\null genotype (OR=1.66; 95%, CI=0.27-5.70).

Table 4. GSTT1 and GSTM1 genotype among patients and control group

Polymorphism	Controls (N=20)		Patients (N =30)		OR	95%CI
	N	%	N	%		
GSTT1 (+)	12	60%	16	53.33%	1.00	—
GSTT1 (-)	8	40%	14	46.67%	1.31	0.41 - 4.13
Total	20	100 %	30	100 %		
GSTM1 (+)	13	65%	12	40%	1.00	—

GSTM1 (-)	7	35%	18	60%	2.78	0.86 - 9.00
Total	20	100 %	30	100 %		

OR= Odds Ratio, 95 % CI= Confidence Interval

Table5. Combination of GSTT1 and GSTM1 genotype among patients and control group

Polymorphism	Controls (N=20)		Patients (N =30)		OR	95%CI
	N	%	N	%		
GSTT1\GSTM1						
Present \Present	10	50 %	12	40%	1.00	—
Null \ Null	6	30 %	12	40%	1.66	0.45 - 6.05
Present \ Null	4	10 %	6	20 %	1.25	0.27 - 5.70
Total	20	100 %	30	100 %		

OR= Odds Ratio , 95 % CI= Confidence Interval

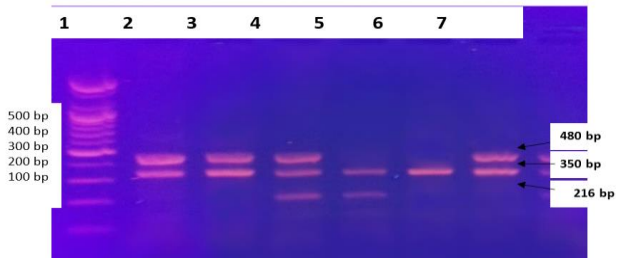

Fig.1 PCR product of studied gene fragment electrolytic on 1.5% agarose gel.

Lane 1

: DNA Ladder (2000 bp) ; **Lane 2,3,7** : GSTM1 null genotype; **Lane 5**: GSTT1 null genotype; **Lane 6** null both GSTM1 & GSTT1 genes; **Lane 4,8**: Normal (contain both genes)

Discussion

There are paucity genetic studies on of coronary heart disease in our country. In this study, The mean age was 40.72±13.44years and 38.10±13.55years in the CHD patients and control group, respectively (P = 0.85), which is less compared to study of [21,22]. Although coronary heart disease was common in male (56.57 %), but this study suggested no significant differences between the CHD patients and the control group according to gender (P = 0.90). The similar finding was reported by Doney et al. [18] whom found that males had more cardiovascular disease (52.5%) than females (47.5%). Also, [22] was found that the percentage of male patients is higher (71.3%) than females, with significant differences between patients and control according gender (0.015). The majority of patient with coronary heart disease (66.67%) were smoker with significant differences between patients and control group (P=0.02). in similar finding [22] found that the smoking is significantly associated with the development of coronary heart disease (p=0.000). On the contrary, the results of [18, 21] who found that the percentage of non-smoking patients was higher than that of smoking patients.

The relationship between GSTT1 and GSTM1 polymorphisms and coronary heart disease has not been proven in detail. In this paper, we studied the relationship between polymorphisms of GSTs and coronary heart disease. This study found that the GSTT1 null genotype was higher in patients (46.67%) compared to control group (40%) ; but, it was not related with an increased risk to pathogenesis of coronary heart disease (OR=1.31; 95%, CI=0.41-4.13) . This result is similar to study of [22, 23], who studied the relationships between GSTT1 and GSTM1 genes with coronary artery disease and did not find a relationship between the null of GSTT1 gene and coronary artery disease. The null GSTM1 genotype related significantly with coronary heart disease susceptibility (OR=2.78; 95%, CI=0.86-9.00) . this result disagreement with [22, 23], who found no relationship between coronary artery disease and GSTM1 null genotype. On the other hand, the effect of GSTT1 / GSTM1 null genotype was found increased risk of coronary heart disease susceptibility (OR=1.66; 95%, CI=0.27-5.70). This result is identical to study of [22], which found that the GSTT1 / GSTM1 null genotype was significantly associated with the development of coronary heart disease (OR=2.1; 95%, CI=0.88-5.03).

Conclusion

The present study investigated the correlation between the GSTT1,GSTM1 null genotypes and development of coronary heart disease. This study showed that the GSTM1 null genotype and GSTT1/GSTM1 null genotype are associated with the development of coronary heart disease.

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Ethics

The volunteers signed a written consent form and all research steps had the approval of the ethics committee of Mazaya college.

Conflict

There is no conflict of interest.

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