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Glutathione S-Transferase Enzyme (*GSTT1* and *GSTM1*) Gene Polymorphisms and Oxidative Stress in Egyptian Patients with End Stage Renal Disease

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Glutathione S-transferase enzyme *(GSTT1 and GSTM1)* gene polymorphisms have been associated with the genetic susceptibility to end stage renal disease (ESRD) in different populations. We investigated the association between *GSTT1* and *GSTM1* genes, and ESRD in Egyptian population. The samples of 133 ESRD and 91 control subjects were collected, and their clinical characteristics were assayed. Glutathione S-transferase enzyme *(GSTT1 and GSTM1)* gene polymorphisms were detected by polymerase chain reaction (PCR). Serum level of malondialdehyde (MDA), the oxidative stress and lipid peroxidation biomarker, and plasma glutathione S-transferase enzyme *(GST)*, the antioxidant enzyme, were estimated in the ESRD patients as well as in the control subjects. We demonstrated the association of MDA and *GST* enzyme levels with *GSTT1* and *GSTM1* genotypes. We investigated the association between MDA and lipid parameters in the ESRD patients. Increased of the *GSTM1* deletion genotype, *(GSTT1/0)* and both deletion genotypes (0/0) in the ESRD patients when compared with the control subjects ($P < 0.0001$, OR = 3.786, 95% CI = 2.151-6.664), ($P = 0.001$, OR = 3.172, 95% CI = 1.595-6.308) and ($P = 0.045$, OR = 1.945, 95% CI = 1.009-3.749), respectively. Highly significant increase of MDA level in the ESRD patients as compared with the control

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subjects (P < 0.0001). Highly significant decrease of *GST* enzyme level in the ESRD patients as compared with the control subjects (P < 0.0001).The level of MDA is significantly increased in all *GST* genotypes in the ESRD patients as compared with the control group. Also, there were significant association between The genetic risk factors of *GSTT1* and *GSTM1* genes in the ESRD, and high level of MDA in the ESRD patients, while the level of *GST* enzyme is significantly decreased in *GST* genotypes except the both deletion (0/0) genotypes, in which the level of *GST* enzyme is not significantly decreased in the ESRD patients as compared with the control subjects. There are significant association between the genetic risk factors of *GSTT1* and *GSTM1* genes in the ESRD, (*GSTM1* null and *GSTT1/0* genotypes), and low level of *GST* enzyme in the ESRD patients. There were significant positive correlation between MDA and total cholesterol, triglyceride, and LDL-cholesterol, and significant negative correlation between MDA and HDL-cholesterol in the ESRD patients as compared with the control subjects. In conclusion, the *GSTM1* (null) genotype, *(GSTT1/0)* and (0/0) genotypes are independent risk factors for ESRD. The oxidative stress and lipid abnormalities are associated with ESRD in the studied Egyptian population.

Keywords: Glutathione S-transferase enzyme (GSTT1 and GSTM1) gene polymorphisms; end stage renal disease; malondialdehyde; glutathione S-transferase enzyme; oxidative stress.

1. INTRODUCTION

Over 1.1 million patients are estimated to have end stage renal disease (ESRD) world-wide, and an addition of 7% annually. In USA incidence and prevalence counts are expected to increase by 44 and 85%, respectively, from 2000 to 2015 and incidence and prevalence rates per million population by 32 and 70%. In the developing countries growth of ESRD population has similar trends. Average incidence of ESRD in Middle East countries with similar renal care systems is 93 per million population [1]. ESRD represents a clinical condition in which there has been an irreversible loss of endogenous renal function. Patients with ESRD must receive a kidney transplant or live on dialysis [2]. The main causes of ESRD in Egypt, other than diabetic nephropathy, included hypertensive kidney disease, chronic glomerulonephritis, unknown etiology, chronic pyelonephritis, schistosomal obstrctive uropathy and schistosomal nephropathy [3]. Abnormal calcium (Ca), phosphorus (P), and vitamin D metabolism are very common in patients with ESRD [4].

Free radicals are reactive species with unpaired electrons [5]. Free radicals are formed in both physiological and pathological conditions in mammalian tissues [6]. Lipid peroxidation is a complex process involving the interaction of oxygen-derived free radicals and polyunsaturated fatty acids, generating very reactive electrophilic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). These aldehydes modify and damage proteins, causing lysosomal dysfunction and increased lysosomal lipofuscin storage [7,8]. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals [9]. Excess free radicals can result from tissue damage and hypoxia, overexposure to environmental factors (smoking, ultraviolet radiation, and pollutants), a lack of antioxidants, or destruction of free radical scavengers. When the production of damaging free radicals exceeds the capacity of the body's antioxidant defenses to detoxify them, a condition known as oxidative stress occurs [10]. Oxidative stress has been defined as a loss of counterbalance between free radicals and/or reactive

oxygen species (ROS) production and antioxidant defense mechanisms. There is considerable disequilibrium between oxidants and antioxidants in patients with chronic renal insufficiency/failure (CRI) [11]. The occurrence of impaired oxidative status is highlighted by several biomarkers; among them, malondialdehyde (MDA), a terminal compound of lipid peroxidation, is commonly used as an index of oxidative stress [12].

Glutathione S-transferase *(GST)* is a multigene family of enzymes that detoxify reactive electrophiles, products of oxidative stress, and known or suspected carcinogenic compounds through conjugation with reduced glutathione (GSH) [13,14,15]. In humans, *GST* enzymes have been assigned to at least eight separate classes designated alpha, mu, kappa, omega, pi, sigma, theta and zeta, that are encoded by *GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT and GSTZ* genes, respectively. In addition, each class includes several genes and isoenzymes [16]. Genetic variations of *GST* may influence individual susceptibility to some diseases associated with the deleterious effects of oxidative metabolism [17]. *GSTM1* and *GSTT1* polymorphisms are the most common polymorphisms of *GST* enzymes in the human population with major ethnic differences and have been studied most extensively in many studies [18]. The *GSTM1* gene is located on chromosome 1 (1p13.3), while the *GSTT1* gene exists on chromosome 22 (22q11.2). Both of them are polymorphic. The *GSTM1*0* (*GSTM1* deficiency) and *GSTT1*0* (*GSTT1* deficiency) allele represent a deletion of the *GST*M1 and *GSTT1* gene and result in a loss of enzymatic activity [19]. This study aims to investigate the association between glutathione S-transferase enzyme (*GSTT1* and *GSTM1*) gene polymorphisms and ESRD, to evaluate the oxidative status of ESRD patients and its association with *GSTT1* and *GSTM1* gene polymorphisms, and to study the association between ESRD and lipid peroxidation in the Egyptian population.

2. SUBJECTS AND METHODS

2.1 Subjects

The present study was carried out on end stage renal disease (ESRD) patients (n = 133), with a mean age of 42.97 ± 15.99 years. All the patients were undergoing haemodialysis treatment following diagnosis of ESRD by nephrologists. Some causes of ESRD in the Egyptian patients in this study were: hypertension, vesicoureter reflux, obstructive uropathy, ischaemic heart diseases, drugs and glomerulonephritis, diabetic nephropathy, systemic lupus erythematosus and unknown causes. The control subjects (n = 91), with a mean age of 24.81 ± 13.44 years, they were collected from donor blood bank, and they were free from the diseases. Most of the cases in patients and control group were men, ESRD patients: 95 men / 38 women and the control subjects: 80 men / 11 women. This study was approved by Ethical Board of the Mansoura University. Informed written consent was obtained from all participants, (patients and control subjects), in this study.

Five milliliters (5 ml) of peripheral blood samples were divided into three (3) tubes: 1 ml of blood was collected in an ethylenediaminetetra-acetic acid (EDTA) tube as an anticoagulant for isolation of DNA and the polymerase chain reaction (PCR) technique on these DNA samples was applied, 2 ml of blood were collected in an EDTA tube and centrifuged to obtain plasma for evaluation of the glutathione S-transferase enzyme *(GST)* and 2 ml of blood were collected in a tube without EDTA then centrifuged to obtain serum for estimation of the malondialdehyde (MDA) and biochemical parameters in the ESRD patients and in the control subjects.

2.2 Malondialdehyde (MDA) Assay

MDA was determined by the double heating method [20]. The principle of the method was spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA. For this purpose 2.5 ml trichloroacetic acid (100 g/l) solution was added to 0.5 ml serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water the mixture was centrifuged at 1000 g for 10 min, and 2 ml supernatant was added to 1 ml TBA (6.7 g/l) solution in a test-tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer at 532 nm.

2.3 Glutathione S-Transferase (GST) Assay

The activity of glutathione S-transferase was measured with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione method [21]. The enzyme and both substrates were incubated in 1 ml of 0.1 M potassium phosphate, pH 6.5, containing 2.5% ethyl alcohol to dissolve 1-chloro-2,4-dinitrobenzene at 30^{o}C. After an addition of 3 ml of 0.33 N HCl to stop the reaction, absorbance at 340 nm was measured.

2.4 Determination of Genotypes

Genomic DNA was isolated and purified from whole blood using a DNA purification kit (Fermentas spin columns, Canada). Analysis for *GSTM1* and *GSTT1* gene polymorphisms was carried out by multiplex PCR method [22]. Genomic DNA (100-150 ng) was amplified in a total volume of 25 ml reaction mixture containing 20 pmol of each of the following primers: *GSTM1*; forward 5'- GAACTCCCTGAAAAGCTAAAGC-3' and reverse 5' GTTGGGCTCAAATATACGGTGG-3', *GSTT1*, forward: 5'-TTCCTTACTGGTCCTCACATCTC-3' and reverse 5'-TCACGGGATCAT GGCCAGCA-3'. Exon 7 of the CYP1A1 gene was coamplified and used as an internal control, using primers: forward: 5'-GAACTGCCACTTCAGCTGTCT-3' and reverse 5'-CAGCTGCATTTGGAAGTGCTC-3'. Each set of reactions included positive and negative controls. The multiplex PCR method was used to detect the presence or absence of the *GSTT1* and *GSTM1* genes in the genomic DNA samples, simultaneously in the same tube. The reaction mixture was subjected to initial denaturation at 94ºC for 2 min, followed by 35 cycles of 94ºC for 2 min, 59ºC for 1 min and 72ºC for 1 min. The final extension was done at 72ºC for 10 min. The PCR products were eletrophoresed in 2% agarose gels, and visualized by ethidium bromide staining. DNA from samples positive for *GSTM1* and *GSTT1* genotypes yielded bands of 215 bp and 480 bp, respectively, while the internal positive control (CYP1A1) PCR product corresponded to 312 bp, as shown in (Fig. 1).

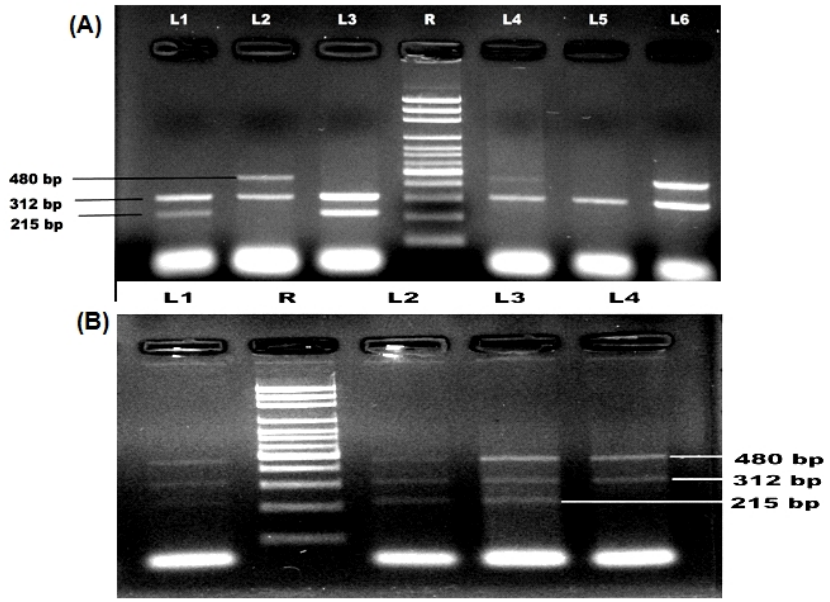

Fig. 1. Ethidium bromide-stained 2% agarose gel of representative multiplex PCR products of GSTT1 (480 bp) and GSTM1 (215 bp) genes with CYP1A1 (312 bp) as internal control. DNA Ladder (100 bp): Lane (R). (A): GSTT1 deletion genotype: Lanes (1 and 3). GSTM1 deletion genotype: Lanes (2, 4 and 6). Both deletion genotypes: Lane (5). (B): Both present genotypes: Lanes (1-3). GSTM1 deletion genotype: Lane (4)

2.5 Statistical Analysis

Data were obtained using SPSS version 15. Results were expressed as means ± standard deviation (SD). Comparisons between the ESRD patients and control subjects markers were made using chi-square 2x2 analysis and independent t-test. Correlation between two parameters was determined by Pearson's correlation coefficient (r). Chi square and odds ratio were calculated with 95% confidence interval. A *p* value less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the clinical characteristics of ESRD patients (n = 133) and control subjects (n = 91). The most important renal functional parameters i.e. serum creatinine, total protein and albumin levels showed significant differences ($P < 0.0001$) between two groups, confirming the absence of any renal disease in the control subjects. The lipid parameters also revealed highly significant differences between ESRD patients and control subjects ($P < 0.0001$).

Table 1. Clinical characteristics of ESRD patients and control subjects

Parameters	ESRD patients (N = 133) (Mean ± SD)	Control subjects (N = 91) (Mean ± SD)	*P*-value
Hemoglobin (g/dL)	9.7 ± 1.87	14.3 ± 0.95	< 0.0001*
Creatinine (mg/dL)	8.8 ± 3.54	0.6 ± 0.18	< 0.0001*
Sodium (mmol/L)	130.7 ± 5	142.2 ± 1.9	< 0.0001*
Potassium (mmol/L)	5.2 ± 0.85	4.4 ± 0.5	< 0.0001*
Calcium (mg/dL)	9.1 ± 1.08	10 + 0.09	< 0.0001*
Phosphorus (mg/dL)	5.7 ± 1.78	3.3 ± 0.67	< 0.0001*
Total Protein (g/dL)	7.28 ± 0.71	8.3 ± 0.1	< 0.0001*
Albumin (g/dL)	3.3 ± 0.37	4.6 ± 0.2	< 0.0001*
Total Bilirubin (mg/dL)	0.7 ± 0.25	0.6 ± 0.2	< 0.0001*
GPT (U/L)	20.2 + 12.26	14.3 ± 2	< 0.0001*
GOT (U/L)	21.3 ± 10.5	14.4 ± 5.35	< 0.0001*
Alkaline Phosphatase (U/L)	127.9 ± 107.8	81.9 ± 10.75	< 0.0001*
Total Cholesterol (mg/dL)	179.1 ± 71.14	114.6 ± 32.3	< 0.0001*
Triglyceride (mg/dL)	224 ± 104.76	101.4 ± 62.2	< 0.0001*
HDL (mg/dL)	39.6 ± 19.9	64.4 ± 17.38	< 0.0001*
LDL (mg/dL)	112.1 ± 66.19	72 ± 33.79	< 0.0001*
Systolic BP (mm Hg)	144.4 ± 22.1	115.3 ± 8.3	< 0.0001*
Diastolic BP (mm Hg)	89.5 ± 12.09	78.7 ± 3.4	< 0.0001*
Haemodialysis Duration (years)	7.9 ± 4.05	0	< 0.0001*

**Significant value (P < 0.05), Results were expressed as mean ± SD. SD: standard deviation. Data obtained using independent t-test. ESRD: end stage renal disease.*

Table 2 shows the distribution of *GSTT1* and *GSTM1* genotypes and their frequencies in the ESRD patients (n = 133) and in the control subjects (n = 91). The frequency of *GSTT1* (null or deletion) genotype was 63 (47.4%) in the ESRD patients and 39 (42.9%) in the control subjects, while the frequency of *GSTT1* (present or positive) genotype was 70 (52.6%) in the ESRD patients and 52 (57.1%) in the control subjects. The frequencies of *GSTT1* (deletion) and *GSTT1* (present) genotypes were not significantly different between ESRD patients and control subjects ($P > 0.05$). The frequency of *GSTM1* (deletion) genotype was significantly increased in the ESRD patients, 85 (63.9%), as compared with the control subjects, 29 (31.9%), ($P < 0.0001$, OR = 3.786, 95% CI = 2.151-6.664), while the frequency of *GSTM1* (present) genotype was significantly lower in the ESRD patients, 48 (36.1%), than in the control subjects, 62 (68.1%).

The frequency of both present genotypes *(GSTT1/GSTM1)* was significantly lower in the ESRD patients, 24 (18%), than in the control subjects, 39 (42.9%). The frequency of *GSTT1* present and *GSTM1* deletion genotypes *(GSTT1/0)* was significantly higher in the ESRD patients, 46 (34.6%), than in the control subjects, 13 (14.3%), ($P = 0.001$, OR = 3.172, 95% CI = 1.595-6.308). The frequency of *GSTT1* deletion and *GSTM1* present genotypes (0/*GSTM1*) in the ESRD patients, 24 (18%), was not significantly different from the control subjects, 23 (25.3%), ($P > 0.05$). The frequency of both deletion genotypes (0/0) was significantly higher in the ESRD patients, 39 (29.3%), than in the control subjects, 16 (17.6%), ($P = 0.045$, OR = 1.945, 95% CI = 1.009-3.749).

Table 2. Distribution of *GSTT1* and *GSTM1* genotypes in ESRD and control cases subjects

Marker: GSTT1	**ESRD patients (N = 133) N (%)**	**Control subjects (N = 91) N (%)**	***P*-value**	**OR**	**95% CI**
Null	63 (47.4%)	39 (42.9%)	0.505	1.200	(0.701-2.053)
Present	70 (52.6%)	52 (57.1%)			
Marker: GSTM1	**ESRD patients (N = 133) N (%)**	**Control subjects (N = 91) N (%)**	***P*-value**	**OR**	**95% CI**
Null	85 (63.9%)	29 (31.9%)	< 0.0001*	3.786	(2.151-6.664)
Present	48 (36.1%)	62 (68.1%)			
Genotype combination	**ESRD patients (N = 133) N (%)**	**Control subjects (N = 91) N (%)**	***P*-value**	**OR**	**95% CI**
GSTT1/GSTM1	24 (18%)	39 (42.9%)	< 0.0001*	0.294	(0.160-0.538)
GSTT1/0	46 (34.6%)	13 (14.3%)	0.001*	3.172	(1.595-6.308)
0/GSTM1	24 (18%)	23 (25.3%)	0.192	0.651	(0.341-1.243)
0/0	39 (29.3%)	16 (17.6%)	0.045*	1.945	(1.009-3.749)

**Significant value (P < 0.05). Results obtained using chi-square 2x2 analysis. OR: odds ratio. CI: confidence interval.*

Table 3 shows the level of serum malondialdehyde (MDA), the oxidative stress biomarker, was significantly higher in the ESRD patients than in the control subjects ($P < 0.0001$), while the level of plasma glutathione S-transferase enzyme, the antioxidant enzyme, was significantly decreased in the ESRD patients as compared with the control subjects ($P < 0.0001$). The oxidative stress was associated with the ESRD.

Table 3. MDA (oxidative stress biomarker) and *GST* (antioxidant enzyme) in ESRD patients and control subjects

Parameter	**ESRD patients (N = 133) (Mean ± SD)**	**Control subjects (N = 91) (Mean ± SD)**	***P*-value**
MDA (nmol/mL)	1.99 ± 1.18	0.97 ± 0.38	< 0.0001*
GST (mmol/mL)	0.09 ± 0.03	0.19 ± 0.07	< 0.0001*

*Data were expressed as mean ± SD. MDA: malondialdehyde. GST: glutathione S-transferase enzyme.*Significant value (P < 0.05) obtained using independent t-test.*

Table 4 shows the association between MDA and the different *GST* genotypes, and the association between *GST* enzyme and the different *GST* genotypes in the ESRD patients and control cases. The mean value of MDA was significantly higher in all *GST* genotypes in the ESRD patients than in the control subjects, ($P < 0.05$). While, the mean value of *GST* enzyme was significantly lower in the *GSTT1* (null), *GSTT1* (present), *GSTM1* (null), *GSTM1* (present), *GSTT1*/*GSTM1, GSTT1*/*0* and 0/*GSTM1* genotypes in the ESRD patients than in the control subjects, ($P < 0.05$), and there was nonsignificant decrease in level of *GST*

enzyme in the *GST* both deletion (0/0) genotypes in both groups (ESRD patients and control subjects), (*P* = 0.055).

Table 4. The Relationship between *GST* genotypes and (MDA and *GST* Enzyme) in ESRD patients and control subjects

Genotypes	**ESRD patients (N = 133) (Mean ± SD)**	**Control subjects (N = 91) (Mean ± SD)**	***P*-value**
	MDA (nmol/ml)		
GSTT1 (Null)	63 (1.97 ± 1.36)	39 (1.04 ± 0.44)	< 0.0001*
GSTT1 (Present)	70 (2.01 ± 0.99)	52 (0.91 ± 0.31)	< 0.0001*
GSTM1 (Null)	85 (2.10 ± 1.32)	29 (1.02 ± 0.36)	< 0.0001*
GSTM1 (Present)	48 (1.78 ± 0.84)	62 (0.95 ± 0.38)	< 0.0001*
GSTT1/GSTM1	24(1.70 ± 0.87)	39 (0.91 ± 0.31)	< 0.0001*
GSTT1/0	46 (2.16 ± 1.03)	13 (0.93 ± 0.31)	< 0.0001*
0/GSTM1	24 (1.87 ± 0.82)	23 (1.017 ± 0.47)	< 0.0001*
0/0	39 (2.03 ± 1.62)	16 (1.08 ± 0.39)	0.001*
Genotypes	**GST (mmol/ml)**		***P*-value**
GSTT1 (Null)	63 (0.096 ± 0.032)	39 (0.17 ± 0.081)	< 0.0001*
GSTT1 (Present)	70 (0.091 ± 0.032)	52 (0.21 ± 0.059)	< 0.0001*
GSTM1 (Null)	85 (0.094 ± 0.031)	29 (0.14 ± 0.062)	< 0.0001*
GSTM1 (Present)	48 (0.091 ± 0.033)	62 (0.22 ± 0.059)	< 0.0001*
GSTT1/GSTM1	24 (0.085 ± 0.032)	39 (0.22 ± 0.049)	< 0.0001*
GSTT1/0	46 (0.094 ± 0.032)	13 (0.17 ± 0.069)	0.002*
0/GSTM1	24 (0.097 ± 0.034)	23 (0.22 ± 0.073)	< 0.0001*
0/0	39 (0.095 ± 0.031)	16 (0.115 ± 0.042)	0.055

**Significant value (P < 0.05). Results obtained using independent t-test.*

Table 5 shows the Pearson's correlation between MDA and Lipid parameters. There were significant positive correlations between MDA, the oxidative stress biomarker, and total cholesterol, triglyceride and low density lipoprotein-cholesterol (LDL-cholesterol). There was a significant negative correlation between MDA and high density lipoprotein-cholesterol (HDL-cholesterol) in the studied subjects (ESRD patients and control subjects), as shown in (Fig. 2).

Table 5. Correlation between MDA and Lipid parameters among the studied subjects

MDA (nmol/mL)		
Parameter	**r-value**	***P*-value**
TC (mg/dL)	0.212	0.001*
TG (mg/dL)	0.268	< 0.0001*
HDL (mg/dL)	-0.152	0.022*
LDL (mg/dL)	0.333	< 0.0001*

**Significant value (P < 0.05). Results obtained by Pearson's correlation coefficient.*

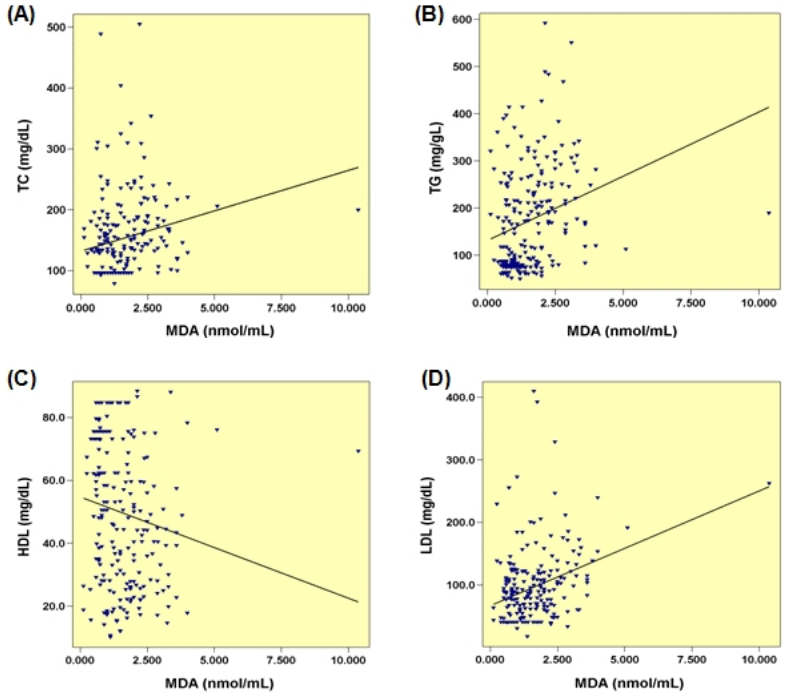

Fig. 2. Correlation between malondialdehyde (MDA) and lipid parameters among the studied subjects. (A): MDA, significant positive correlation, with total cholesterol (TC) (r = 0.212, *P* = 0.001). (B): MDA, significant positive correlation, with triglyceride (TG) (r = 0.268, *P* = 0.022). (C): MDA, significant negative correlation, with HDL-Cholesterol (r = -0.152, *P* < 0.0001). (D): MDA, significant positive correlation, with LDL-Cholesterol (r = 0.333, *P* < 0.0001). Significant value (*P* < 0.05)

3.2 Discussion

End stage renal disease (ESRD) is a multifactorial disease. Clinically, ESRD is an advanced form of chronic renal failure (CRF) where renal function has declined to ~10% of the normal prior to initiation of dialysis or transplantation [23]. Glutathione S-transferases (*GST*s) belong to a group of multigene and multifunctional detoxification enzymes, which defend cells against a wide variety of toxic insults from chemical, metabolites, and oxidative stress [18]. In this present study, we have observed that there were highly significant differences in the biochemical parameters between ESRD patients and control subjects.

The current study showed that the frequency of *GSTMI* (present) and *GSTTI/GSTM1* genotypes were significantly lower in the ESRD patients than in the control subjects, whereas the *GSTM1* (null), *GSTT1/0* and 0/0 genotypes were significantly higher in the ESRD patients as compared with the healthy subjects in the Egyptian population. These results were consistent with other studies, a study from India [24] reported that the (null) genotype of *GSTM1* and *GSTT1* were associated with the higher risk for CRF. Also, a study in China [25] showed that the *GSTT1* (null) genotype subjects may be at risk for CRF development among patients with diabetes, whereas there is no such risk for subject with hypertension. In contrast, the *GSTM1* and *GSTT1* null genotypes were not a risk factor for CRF development [26].

The present study showed a significant increase in serum MDA level in the Egyptian ESRD patients as compared with the control subjects. Our results were in agreement with several studies demonstrated that MDA is a good indicator for evaluating oxidative stress in degenerative disease like chronic kidney disease (CKD), and MDA level was significantly increased in the CKD patients as compared with the control subjects [27,28,29]. Also, the increased oxidative stress is a hallmark of ESRD [30] .

The present study showed that the *GST* enzyme levels, the antioxidant enzyme, were significantly decreased in the ESRD patients as compared with the control subjects. These results were in agreement with a study showed that decreased the levels of *GST* in CRF [31].

The findings of this present study investigated that the mean value of MDA was significantly higher in the *GSTT1* (null), *GSTT1* (present), *GSTM1* (null), *GSTM1* (present), *GSTT1*/*GSTM1*, *GSTT1/0*, *0/GSTM1* and both deletion (0/0) genotypes in the ESRD patients than in the control subjects, whereas the mean value of *GST* enzyme was significantly lower in the *GSTT1* (null), *GSTT1* (present), *GSTM1* (null), *GSTM1* (present), *GSTT1*/*GSTM1*, *GSTT1*/*0* and *0/GSTM1* genotypes, while the level of *GST* enzyme in the both deletion (0/0) was not significantly lower in the ESRD patients than the control subjects. These results were in agreement with a study reported that the *GST* levels were lower among subjects with deletion in one/both *GST* genes, whereas MDA levels were found to be correspondingly raised, in both diabetic and nondiabetic CKD [32].

MDA is a byproduct of lipid peroxidation. Increased lipid peroxidation has been observed in dialysis patients and in predialysis adults with advanced CRF [27,33]. In this study, by using Pearson's correlation, there were significant positive correlations between MDA and total cholesterol, triglyceride, and LDL-cholesterol. There was a significant negative correlation between MDA and HDL-cholesterol among the studied subjects. These results were in agreement with other results [29]. This present study demonstrated that there were lipid abnormalities and oxidative stress in the ESRD patients and these results were in agreement with other evidences [28,29,34].

4. CONCLUSION

The findings of this study demonstrates that: the *GSTM1* (null), *GSTT1/0* and both deletion (0/0) genotypes are significantly increased in the ESRD patients as compared with the control subjects, whereas the *GSTMI* (present) and *GSTTI/GSTM1* genotypes are significantly decreased in the ESRD patients as compared with the control subjects. There were highly significant genetic linkage between *GSTT1* and *GSTM1* genes, and end stage renal disease (ESRD). The *GSTM1* (null), *GSTT1/0* and both deletion (0/0) genotypes are considered as risk factors for ESRD and can be used as predicting and prognostic factors for ESRD, while the *GSTMI* (present) and *GSTTI/GSTM1* genotypes are considered as protective factors against the ESRD in the Egyptian population.

Also, this study evaluates the oxidative status of the studied groups (ESRD patients and control subjects) through measurement of serum level of malondialdehyde (MDA), the biomarker of oxidative stress and lipid peroxidation, and plasma level of glutathione S-transferase enzyme *(GST)*, the antioxidant enzyme, for both groups, then the results are compared and show that there are a significant increase in the level of MDA and a significant decrease in the level of *GST* enzyme in the ESRD patients as compared with the healthy group. According to these results, the oxidative stress is strongly associated with ESRD.

This present study investigates that the level of MDA is significantly increased in all *GST* genotypes in the ESRD patients as compared with the control group. So, there are significant association between the genetic risk factors of *GSTT1* and *GSTM1* genes in the ESRD, (*GSTM1* null, *GSTT1/0* and 0/0 genotypes), and high level of MDA in the ESRD patients, while the level of *GST* enzyme is significantly decreased in *GST* genotypes except the both deletion (0/0) genotypes, in which the level of *GST* enzyme is not significantly decreased in the ESRD patients as compared with the control subjects. There are significant association between the genetic risk factors of *GSTT1* and *GSTM1* genes in the ESRD, (*GSTM1* null and *GSTT1*/*0* genotypes), and low level of *GST* enzyme in the ESRD patients. This association between the oxidative stress and genetic risk factors of *GSTT1* and *GSTM1* genes in the ESRD increases the risk of these genetic factors in the ESRD patients.

In the studied subjects (ESRD patients and control subjects), there are significant positive correlations between serum MDA, the lipid peroxidation biomarker, and total cholesterol (TC), triglyceride (TG) and low density lipoprotein-cholesterol (LDL-cholesterol). There is a significant negative correlation between MDA and high density lipoprotein-cholesterol (HDL-cholesterol). This reflects that the lipid peroxidation is one of the most important complications of ESRD in the Egyptian population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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