

Glutathione S-Transferase enzyme (GSTT1 and GSTM1) Gene Polymorphisms in End Stage Renal Disease among Egyptian Population

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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. 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 to 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 to the control subjects ($P < 0.0001$). Highly significant decrease of GST enzyme level in the ESRD patients as compared to the control subjects ($P < 0.0001$). MDA level was significantly increased in the (GSTT1/0) and (0/0) genotypes as compared to the both present genotypes (GSTT1/GSTM1), while GST enzyme level was significantly decreased in the (GSTT1/0) and (0/0) genotypes as compared to the (GSTT1/GSTM1) and (0/GSTM1) genotypes in the studied subjects. 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 to 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 the genetic risk factors of 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 (Malekmakan et al., 2009).

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 (Zhou et al., 2012). The main causes of ESRD in Egypt, other than diabetic nephropathy, included hypertensive kidney disease, chronic glomerulonephritis, unknown etiology, chronic pyelonephritis, schistosomal obstructive uropathy and schistosomal nephropathy (El-Minshawy, 2011). Abnormal calcium (Ca), phosphorus (P), and vitamin D metabolism are very common in patients with ESRD (Sisman et al., 2012).

Free radicals are reactive species with unpaired electrons (Halliwell and Gutteridge, 2007). Free radicals are formed in both physiological and pathological conditions in mammalian tissues (Mohan and Priya, 2009). 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 (Schutt et al., 2003; Krohne et al., 2010).

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals (Hamid et al., 2010). 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 (Scheibmeir et al., 2005).

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) (Romeu et al., 2010). 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 (De Vecchi et al., 2009).

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) (Hayes et al., 2005; Sharma et al., 2004; Richard et al., 2000). 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 (Hayes and Strange, 2000). Genetic variations of GST may influence individual susceptibility to some diseases associated with the deleterious effects of oxidative metabolism (De Alvarenga et al., 2007).

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 (Nowier et al., 2009). 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 GSTM1 and GSTT1 gene and result in a loss of enzymatic activity (Ye et al., 2004).

This study aims to investigate the association between glutathione S-transferase enzyme (GSTT1 and GSTM1) gene polymorphisms and ESRD, and to evaluate the oxidative status of ESRD patients and its association with GSTT1 and GSTM1 gene polymorphisms in the Egyptian ESRD 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 dialysis treatment following diagnosis of ESRD by nephrologists. The control subjects (n = 91), with a mean age of 24.81 ± 13.44 years, they were collected from donor blood bank free from the diseases.

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 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 of Draper and Hadley, (1990). 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 by a method based on the description of Habig et al. (1974). 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°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 using the method described by Abdel-Rahman et al. (1998). 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 94°C for 2 min, followed

by 35 cycles of 94°C for 2 min, 59°C for 1 min and 72°C for 1 min. The final extension was done at 72°C for 10 min. The PCR products were electrophoresed 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 (Figure 1).

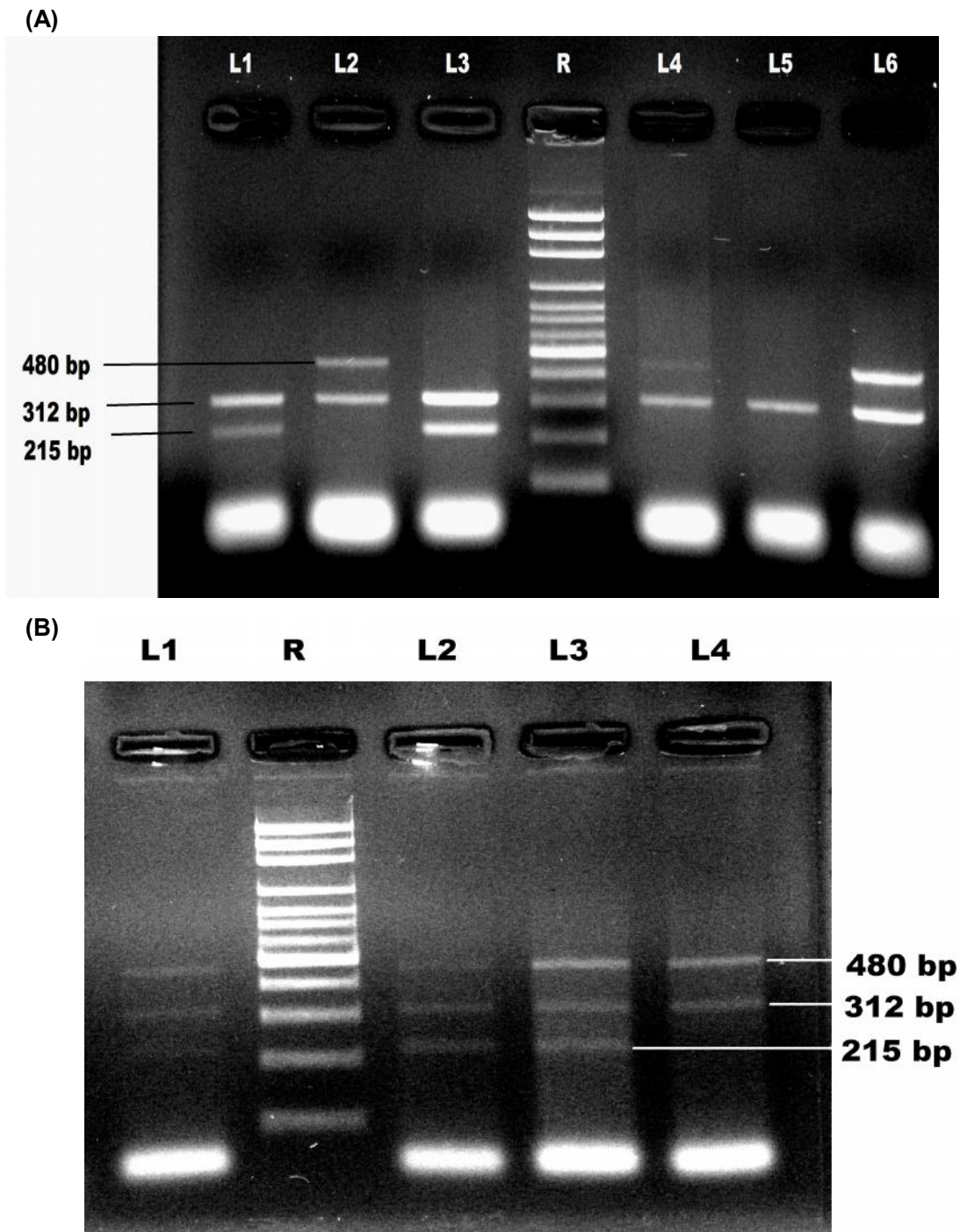


Fig. (1): Ethidium bromide-stained 2% agarose gel of representative multiplex PCR products of GSTT1 (480 bp) and GSTM1 (215 bp) genes with CYP1A1 (312 bp) as internal control. DNA Ladder (100 bp): Lane (R). (A):

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 $\pm$ standard deviation (SD). Comparisons between the ESRD patients and control subjects markers were made using chi-square 2x2 analysis, independent t-test and one-way (ANOVA) with Tukey SD as post-hoc 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 $\pm$ SD)	Control Subjects (N = 91) (Mean $\pm$ SD)	P-value
Hemoglobin (g/dL)	9.74 $\pm$ 1.87	14.39 $\pm$ 0.95	< 0.0001*
Creatinine (mg/dL)	8.84 $\pm$ 3.54	0.6 $\pm$ 0.18	< 0.0001*
Sodium (mmol/L)	130.7 $\pm$ 5	142.18 $\pm$ 1.9	< 0.0001*
Potassium (mmol/L)	5.2 $\pm$ 0.85	4.37 $\pm$ 0.5	< 0.0001*
Calcium (mg/dL)	9.05 $\pm$ 1.08	10 $\pm$ 0.09	< 0.0001*
Phosphorus (mg/dL)	5.65 $\pm$ 1.78	3.3 $\pm$ 0.67	< 0.0001*
Total Protein (g/dL)	7.28 $\pm$ 0.71	8.3 $\pm$ 0.1	< 0.0001*
Albumin (g/dL)	3.25 $\pm$ 0.37	4.57 $\pm$ 0.2	< 0.0001*
Total Bilirubin (mg/dL)	0.7 $\pm$ 0.25	0.6 $\pm$ 0.2	< 0.0001*
GPT (U/L)	20.23 $\pm$ 12.26	14.25 $\pm$ 2	< 0.0001*
GOT (U/L)	21.26 $\pm$ 10.5	14.4 $\pm$ 5.35	< 0.0001*
Alkaline Phosphatase (U/L)	127.99 $\pm$ 107.8	81.96 $\pm$ 10.75	< 0.0001*
Total Cholesterol (mg/dL)	179.06 $\pm$ 71.14	114.65 $\pm$ 32.3	< 0.0001*
Triglyceride (mg/dL)	224 $\pm$ 104.76	101.4 $\pm$ 62.2	< 0.0001*
HDL (mg/dL)	39.56 $\pm$ 19.9	64.37 $\pm$ 17.38	< 0.0001*
LDL (mg/dL)	112.1 $\pm$ 66.19	72 $\pm$ 33.79	< 0.0001*
Systolic BP (mm Hg)	144.37 $\pm$ 22.1	115.27 $\pm$ 8.3	< 0.0001*
Diastolic BP (mm Hg)	89.47 $\pm$ 12.09	78.68 $\pm$ 3.4	< 0.0001*
Dialysis Duration (years)	7.94 $\pm$ 4.05	0	< 0.0001*
Age	42.97 $\pm$ 15.99	24.81 $\pm$ 13.4	< 0.0001*

*Significant value (*P* < 0.05), Results were expressed as mean $\pm$ 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 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 to 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 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 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), see (Figure 2).

Table (2): Distribution of GSTT1 and GSTM1 genotypes in ESRD patients and control 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 (NS)	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 (NS)	0.651	(0.341-1.243)
0/0	39 (29.3%)	16 (17.6%)	0.045*	1.945	(1.009-3.749)

NS: not significant. *Significant value ($P < 0.05$). Results obtained using chi-square 2x2 analysis. OR: odds ratio. CI: confidence interval.

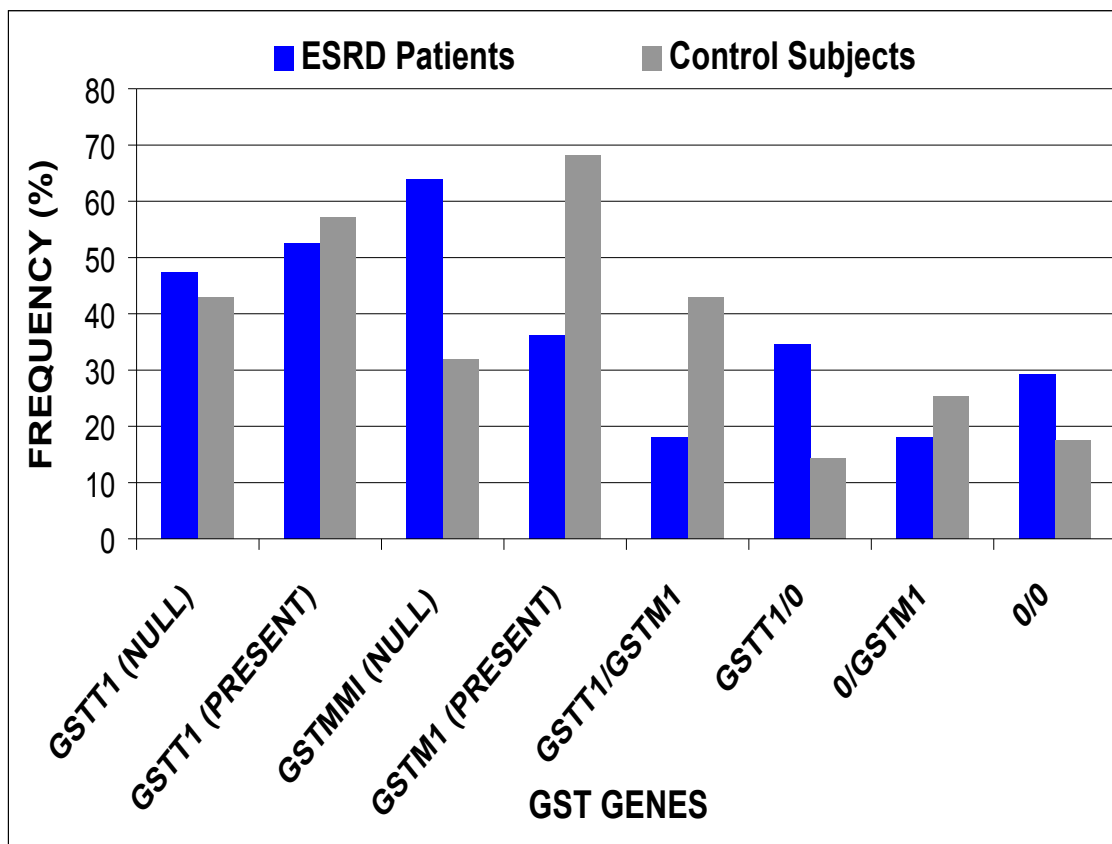


Fig. (2): Distribution of GST genotypes in ESRD patients and control subjects.

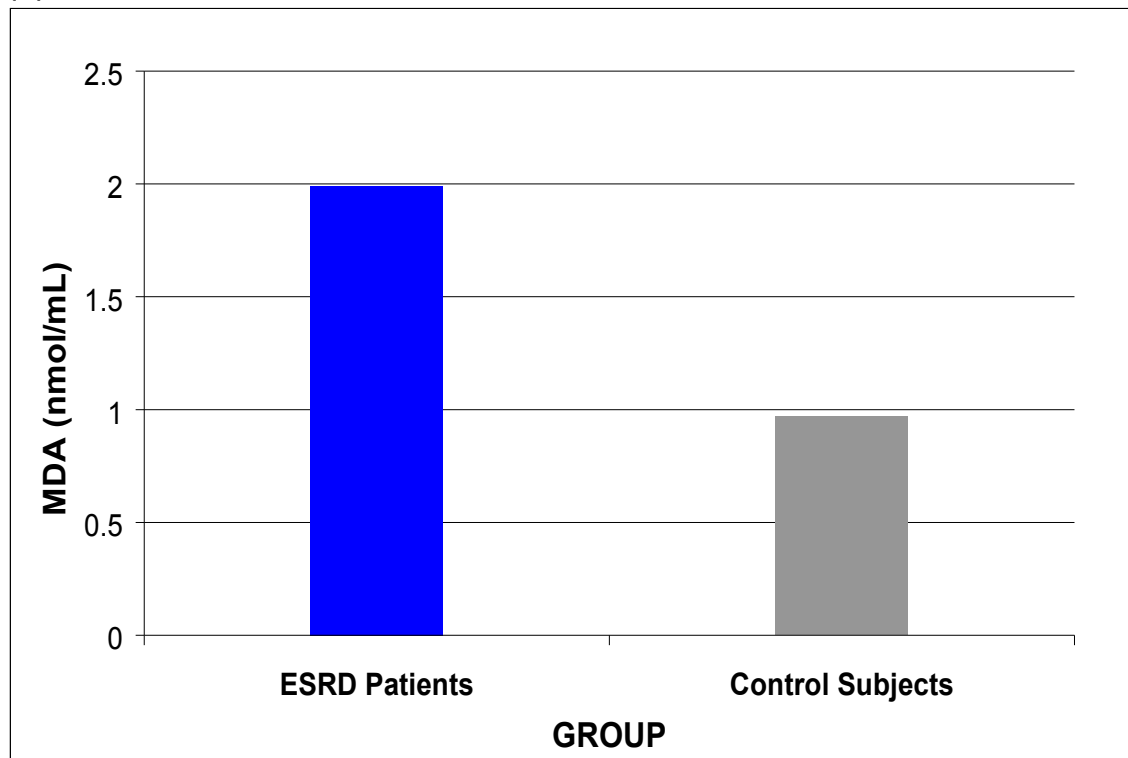
Table 3 shows the level of serum malondialdehyde (MDA), the oxidative stress biomarker, was significantly higher in the ESRD patients than in the control subjects ($P < 0.0001$), while the level of plasma glutathione S-transferase enzyme, the antioxidant enzyme, was significantly decreased in the ESRD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

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

Parameter	ESRD Patients (N = 133) (Mean $\pm$ SD)	Control Subjects (N = 91) (Mean $\pm$ SD)	P-value
MDA (nmol/mL)	1.99 $\pm$ 1.18	0.97 $\pm$ 0.378	< 0.0001*
GST (mmol/mL)	0.093 $\pm$ 0.032	0.199 $\pm$ 0.0719	< 0.0001*

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

(A)



(B)

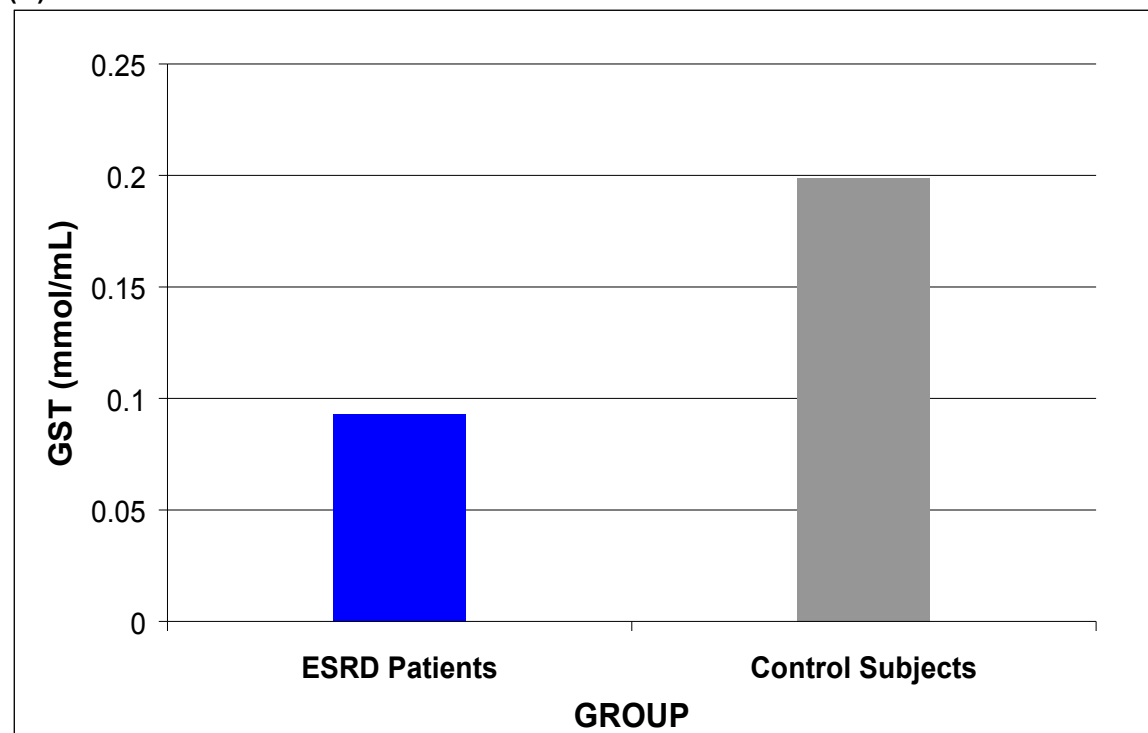


Fig. (3): (A): MDA (oxidative stress biomarker) and (B): GST (antioxidant enzyme) in ESRD patients and in controls.

Table 4 shows the association between MDA and GST enzyme, and the different GST genotypes in the studied subjects (n=224), (ESRD patients and control cases). The mean value of MDA was significantly elevated in the GSTT1/0 and both deletion (0/0) genotypes more than in the both present genotypes (GSTT1/GSTM1), ($P < 0.05$).

In contrast, the mean value of GST enzyme was significantly decreased in the GSTT1/0 and both deletion (0/0) genotypes than in the 0/GSTM1 and both positive (GSTT1/GSTM1) genotypes ($P < 0.05$). The oxidative stress was highly associated with GSTM1 (null genotype) and both null genotypes (0/0) in the studied subjects, see (Figure 4).

Table (4): oxidative stress parameters in different genotypes among the studied subjects

Studied Subjects (N = 224)			
Genotypes		N (%)	MDA (nmol/ml)
GSTT1/GSTM1		63/224 (28.12%)	1.21 ± 0. 702
GSTT1/0		59/224 (26.33%)	1.89 ± 1.049
0/GSTM1		55/224 (24.55%)	1.45 ± 0.796
0/0		47/224 (20.98%)	1.75 ± 1.442
Mean Difference between Genotypes			P-value
GSTT1/0	GSTT1/GSTM1	0.687	0.002 ^a
	0/GSTM1	0.444	0.128 (Ns)
	0/0	0.141	0.885 (Ns)
0/0	GSTT1/GSTM1	0.545	0.024 ^a
	0/GSTM1	0.302	0.457 (Ns)
0/GSTM1	GSTT1/GSTM1	0.243	0.616 (Ns)
Genotypes		N (%)	GST (mmol/ml)
GSTT1/GSTM1		63/224 (28.12%)	0.174 ± 0.082
GSTT1/0		59/224 (26.33%)	0.111 ± 0.053
0/GSTM1		55/224 (24.55%)	0.158 ± 0.082
0/0		47/224 (20.98%)	0.101 ± 0.036
Mean Difference between Genotypes			P-value
GSTT1/0	GSTT1/GSTM1	-0.062	< 0.0001 ^a
	0/GSTM1	-0.047	0.002 ^b
	0/0	0.0102	0.846 (Ns)
0/0	GSTT1/GSTM1	-0.073	< 0.0001 ^a
	0/GSTM1	-0.057	< 0.0001 ^b
0/GSTM1	GSTT1/GSTM1	-0.015	0.631 (Ns)

NS: not significant. ^{a,b} Significant value ($p < 0.05$). ^aSignificantly different from (GSTT1/GSTM1). ^bSignificantly different from (0/GSTM1). Results obtained using one-way (ANOVA) with Tukey SD as post-hoc test.

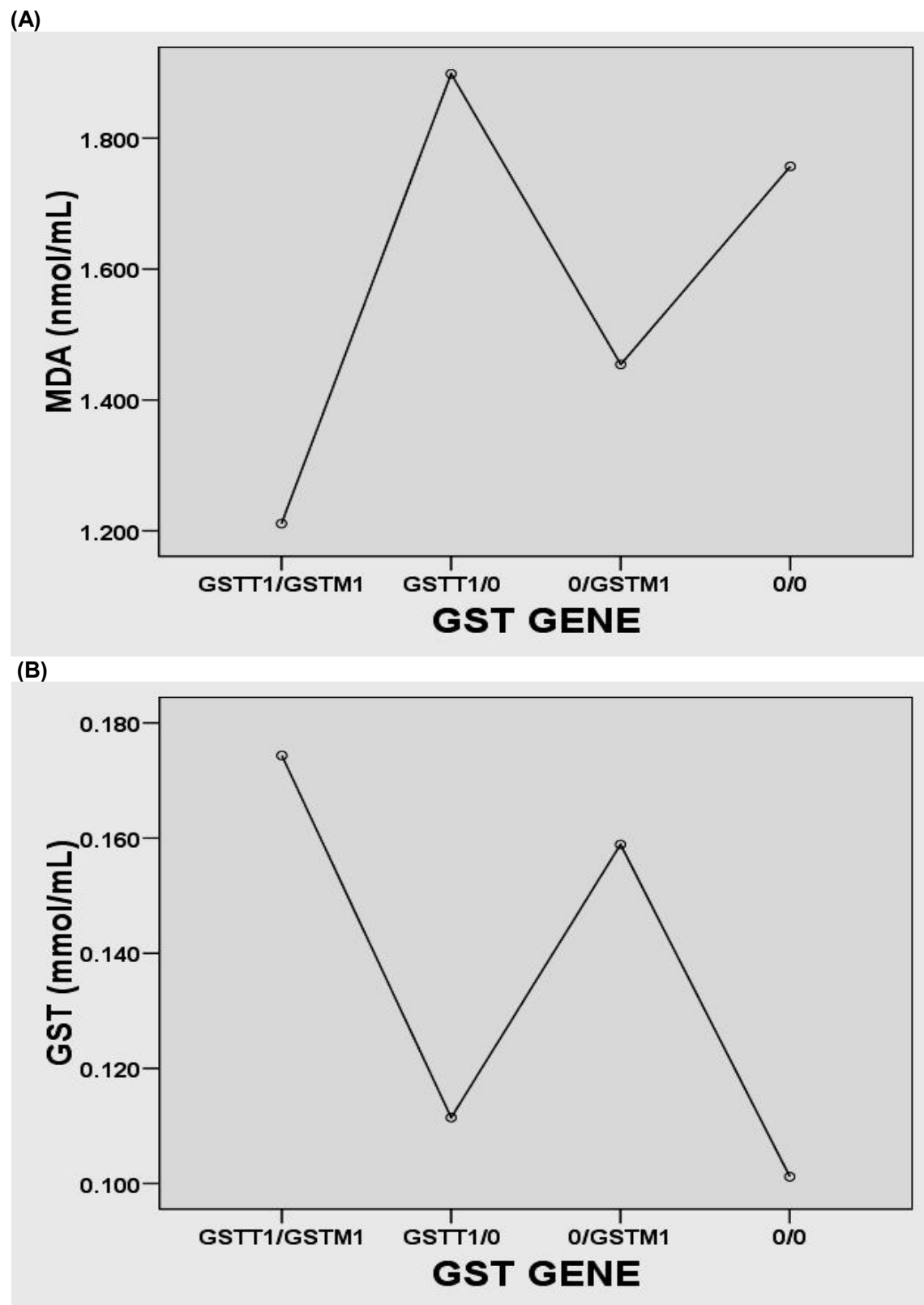


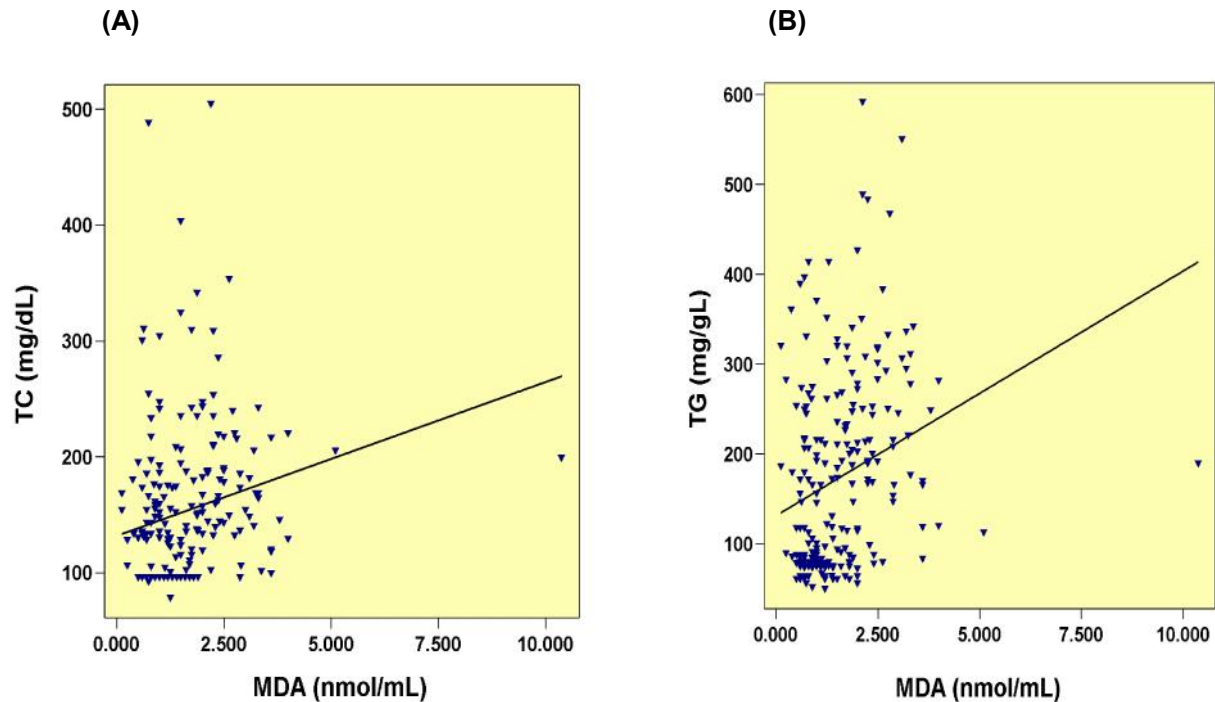
Fig. (4): (A): MDA (oxidative stress biomarker) and **(B):** GST (antioxidant enzyme) with GSTT1 and GSTM1 genotypes among the studied subjects.

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 (Figure 5).

Table (5): Correlation between MDA and Lipid parameters among the studied subjects

Parameter	MDA (nmol/mL)	
	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.



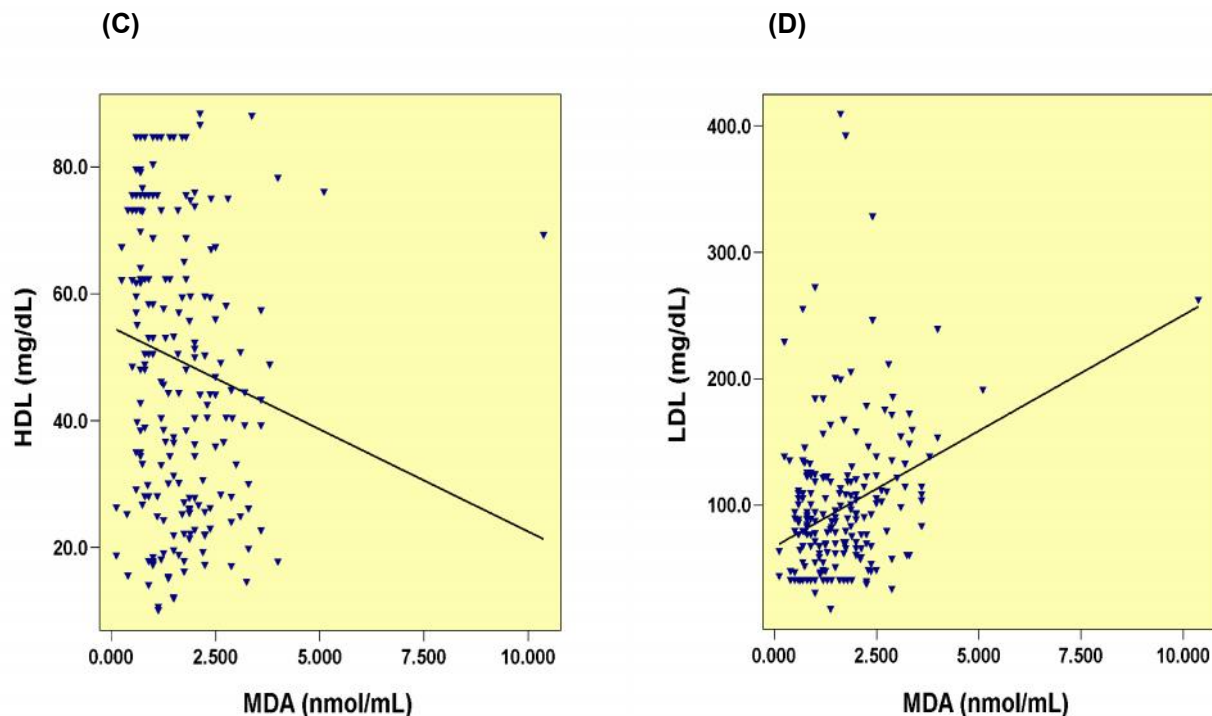


Fig. (5): Correlation between malondialdehyde (MDA) and lipid parameters among the studied subjects.

(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 (Tripathi et al., 2008). Glutathione S-transferases (GSTs) 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 (Nowier et al., 2009).

In this present study, we have observed that there were highly significant differences in the biochemical parameters between ESRD patients and control subjects ($P < 0.0001$).

Our study showed that the frequency of GSTM1null/deletion, GSTT1/0 and 0/0 genotypes were significantly higher in the ESRD patients as compared to the healthy subjects in the Egyptian population ($P < 0.05$). These results were consistent with other studies. In a study from India done by Agrawal et al. (2007) who reported that the null genotype of GSTM1 and GSTT1 were associated with the higher risk for CRF. According to their study in China, Yang et al. (2004) noted 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, another study done by Akgul et al. (2012) who suggested that the GSTM1 and GSTT1 null genotypes were not a risk factor for CRF development. The cause of the disparity with those of Agrawal et al. (2007) may be polymorphic differences between populations.

Increased oxidative stress is a hallmark of ESRD (Suvakov et al., 2013). 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 to the control subjects done by Suresh et al. (2008), Padalkar et al. (2012) and Patel et al. (2012). Our results were in agreement with these studies, and showed a significant increase in serum MDA level in the Egyptian ESRD patients as compared to the control subjects.

In This present study, the GST enzyme levels, the antioxidant enzyme, were significantly decreased in the ESRD patients as compared to the control subjects. These results confirmed those of Pagliuso et al. (2008) who found that decreased the GST levels in CRF.

The findings of this present study investigated that the mean value of MDA was significantly increased in the GSTT1/0 and both deletion (0/0) genotypes more than in the both positive (GSTT1/GSTM1) genotypes ($p < 0.05$), whereas the mean value of GST enzyme was significantly lower in the GSTT1/0 and both deletion (0/0) genotypes than in the 0/GSTM1 and both positive (GSTT1/GSTM1) genotypes, ($p < 0.05$), in the studied subjects (ESRD patients and control subjects). These results were in agreement with Datta et al. (2010) who 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.

MDA is a byproduct of lipid peroxidation. Increased lipid peroxidation has been observed in dialysis patients and in predialysis adults with advanced CRF (Zwolinska et al., 2004; Suresh et al., 2008). 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 done by Patel et al. (2012).

This present study demonstrated that there were lipid abnormalities and oxidative stress in the ESRD patients and these results were in agreement with Abrass (2004), Padalkar et al. (2012) and Patel et al. (2012).

4. CONCLUSION

This study shows a significant association between GSTT1 and GSTM1 genes, and end stage renal disease (ESRD). The GSTM1 deletion genotype, (GSTT1/0) and both deletion (0/0) genotypes are independent risk factors for ESRD. This study also demonstrates that a significant increase in the level of malondialdehyde (MDA), the biomarker of oxidative stress and lipid peroxidation, and a significant decrease in the level of glutathione S-transferase enzyme (GST), the antioxidant enzyme, in ESRD. This reflects that the oxidative stress and lipid abnormalities are strongly associated with ESRD; also the high levels of MDA and low levels of GST enzyme are significantly associated with the risk factors of GSTT1 and GSTM1 genotypes in ESRD of the Egyptian population.

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