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Glutathione, Glutathione S Transferase Levels and M1/T1 Null Genotype Association with Covid-19 Infection Combined with Diabetes Mellitus Type 2 Patients

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Abstract

Background: The companied of covid-19 infection with other disease has been reported to be more influences in infection progressing and development, this regarding the uncontrolling of some cellular processing like oxidative stress mechanisms,

Aim of the study: detection glutathione, glutathione S transferase levels and M1/T1 null genotype association with COVID-19 infection Combined with diabetes mellitus type 2 patients.

Materials and Method: a case control study was implemented to assessment biomarkers using classical lab work procedures and genetic markers using congenital PCR, then association between biomarker and genotyping were implemented.

Results: The results showed that there were significant differences among study groups in age, duration, FBG, HbA1c and IS, The GSTase level showed a non-significant elevation in DM and covid-19 infected patients, non-significant GSH level was decreased in DM and covid-19 infection groups than control groups, The correlation between GSH and GSTase with study parameters showed significant association in FBG, INS, IN with GSTase, in DM-covid-19 but not in other groups. The results indicate that GSTase affected by DM in covid-19 infection cases but GSH didn't impact. The correlation between GSH and GSTase level showed a significant positive relation in control and covid-19 groups while weak positive relation in DM2-covid-19 group. The GST genotyping including normal and null genotyping showed a non-significant association of null genotyping for GSTT and GSTM genes with study groups (p= 0.755, 0.1773) respectively, null genotyping of both genes (GSTT+GSTM) showed non-significant association with all other genotyping (p=0.6288) among study groups. The association between GSTT, GSTM genotyping and GSH, GSTase level showed non-significant differences in GSTase and GSH levels among study groups.

Conclusion: the present findings concluded that the DM2 has slightly influence in the covid-19 infection cases regarding GSTase, GSH levels and M1/T1 gene polymorphism.

Keywords
Glutathione, Glutathione S transferase, M1/T1 Null Genotypes, Covid-19, Diabetes Mellitus type 2 patients

INTRODUCTION

In the last years, diabetes mellitus type 2 (DM2) has reported as a chronic metabolic disorder, resulted from association between genetic and environmental factors interaction. There is increasing in the prevalence and incidence of DM2 in the worldwide, even reaching epidemic rate in some countries [1] . According to WHO, T2DM will be the 7th leading cause of death by 2030 worldwide [2]. These increasing were happened because high prevalence obesity and the unhealthy lifestyles. serious complications were resulted from Uncontrolled and prolonged DM2, several of them being life-threatening [3].

Diabetes mellitus type 2 is one of several risk factors have been related to covid-19 infection and complication. In addition to other factors including sex, age, deprivation and comorbidities like hypertension, cardiopathy, immunosuppression and chronic obstructive pulmonary disease [4, 5]. Richardson et al., [6] found about 33.8% of 5700 covid-19 patients attended to 12 hospitals had DM2, furthermore, about 18 different studies of meta -analysis demonstrated that the risk of severe disease was 2.4-fold higher in DM2 compared with others [7]. While another one observed about 2.6-fold higher risk by fasting blood glucose increased at admission [8].

Glutathione S-transferases (GSTase) enzymes are the primary defense mechanism in the cell against reactive oxygen species (ROS) as phase II detoxifying isoenzymes were showed to metabolize different electrophilic molecules, such as drugs, toxins, carcinogens, and DNA products generated by ROS. In humans Eight types of soluble GSTs have been detected, called Kappa (K), Mu (M), Alpha (A) and Pi, which formed 4% of the total proteins in the liver cells [9, 10]. The GST isoemzyme family is intensely studied in DM2 among the several candidate genes contributed in DM pathogenesis, this is because potential modulating roles in individual susceptibility to induced disorders. glutathione S transferase isoemzyme type T1 (GSTT1) genes and type M1 (GSTM1) and are extremely polymorphic and the null genotypes in humans lead to function losing and mutations are accompanied by lack of enzyme activity [11, 12].

Some reports investigated the possible association between GSTM1 and GSTT1 null alleles and DM2; some population like North Indian, Turkish and Southern Iran demonstrated an association between GSTM1 deletion and DM2 while Chinese, Egyptian, and Brazilian population reported significant relation between GSTM1/GSTT1 null genotypes and DM [13], and this might be led to effect in the redox regulation during covid-19 infection.

MATERIALS AND METHODS

Study design: case control study was included (DM2 patients infected with covid-19, patients with covid-19 and control group), all study contributors were attended to Hospital for medication; all cases were diagnosed by specialist physician. Blood samples and patients data were collected in sterile conditions with ethical approval and written consents from each individual and according to department of biology/university of Babylon number (B24 1001 15/1/2024).

Study biomarkers: study biomarkers included glycemic parameters (fasting blood parameters, insulin level, HbA1c%, insulin sensitivity and insulin resistance) by classical lab procedures, glutathione and glutathione S- transferase levels were detected using colorimetric methods.

Genotyping detection: whole DNA was extracted using FavorPrep™ Blood/ Genomic DNA Extraction Kit. Concentration was detected, then GSTT and GSTM null genotyping were detected using primers were reported in by Kiran et al., [14]. Results of null genotyping were detected by electrophoresis using 1% agaros, 0.5 TBX, 80V for 30 min.

Data analysis: results were demonstrated as mean±SE for study parameters, sex and genotyping of GSTT and GSTM was represented as a percentage%, significant was determined using X^2, ANOVA one way, and correlation among study parameters at p value less than 0.05 all analysis implemented using SPSS v 23.

RESULTS

This study was aimed to estimate the GST controlling state in DM2 patients during covid-19 infection, via detect GSH, GSTase levels and GSTT1, GSTM1 genes deletion mutation in study groups. The results showed that there were significant differences among study groups in age and duration (p=0.000) (Table 1).

Table 1 Mean differences of age, BMI and duration distribution in study groups

Categories	DM2+covid19	Covid19 only	Control group	P
Age (year)	57.58±3.08	41.19±3.56	32.73±3.07	0.00
BMI(kg/m^2)	27.43±0.822	26.72±1.045	26.83±1.332	0.637
Duration (day)	13.70±3.039	9.23±2.298		0.000

The study parameters showed significant changes in FBG, HbA1c and IS (P=0.000, 0.021) respectively, other parameters were non-significant changes (p>0.05). The GSTase level showed a non-significant elevation in DM and covid-19 infection patients than control group, while GSH level was decreased in DM and covid-19 infection groups than control groups in non-significant changes (table 2), from these results, there were changes in the study parameters by DM2 in the covid-19 patients.

Table 2 Mean differences of parameters in study groups

Categories	DM2+covid19	Covid19 only	Control group	P
FBG	248.79±22.10	118.31±7.20	94.91±3.926	0.000
HbA1c	9.025±0.33	5.37±0.126	5.15±0.52	0.000
Insulin	23.99±3.98	26.92±5.99	21.69±4.33	0.685
IR	16.66±4.67	7.28±1.59	5.162±1.028	0.239
IS	0.30±0.010	0.32±0.009	0.33±0.00	0.021
GSTase level	119.59±25.09	90.09±9.56	55.29±9.86	0.198
GSH level	9.304±1.016	13.67±1.89	14.64±1.76	0.078

(ANOVA one way, $p<0.05$)

The correlation between GSH and GSTase with study parameters are clarified in table (3). the results show changes in correlation between FBG, HbA1c, duration with GSH and GSTase in study groups, and several significant associations were observed in FBG, INS, IN with GSTase, in DM-covid-19 but not in other groups. The findings indicate that GSTase affected by DM in covid-19 infection cases but GSH didn't impact. The correlation between GSH and GSTase level showed a significant positive relation in control and covid-19 groups while weak positive relation in DM2 –covid-19 group.

Table 3 Correlation between GSTase, GSH with study parameters in study groups.

		Dm2-covid-19 group		Covid-19 group		Control group	
		GSH level	GSTase level	GSH level	GSTase level	GSH level	GSTase level
GSTase level	r	-0.229-	1	-0.512-*	1	-0.424-*	1
	p	0.223		0.012		0.024	
age	r	0.239	0.003	0.185	-0.151-	-0.375-*	0.300
	p	0.203	0.987	0.355	0.485	0.049	0.117
FBG	r	-0.116-	0.492**	0.016	-0.089-	-0.315-	0.345
	p	0.542	0.005	0.936	0.696	0.103	0.068
HBA1C	r	-0.078-	0.011	0.029	0.171	-0.345-	0.529**
	p	0.683	0.959	0.887	0.438	0.072	0.004
INR	r	-0.174-	0.799**	0.122	-0.056-	-0.010-	0.005
	p	0.357	0.000	0.544	0.799	0.959	0.982
INS	r	-0.061-	-0.061-	-0.048-	-0.090-	-0.040-	-0.057-
	p	0.750	0.737	0.814	0.682	0.840	0.770
IN	r	-0.116-	0.493**	0.136	-.017-	0.030	-0.035-
	p	0.540	0.005	0.498	0.950	0.880	0.861
DURATION	r	-0.129-	-0.125-	0.102	-0.069-	0.559	-0.586-
	p	0.497	0.505	0.614	0.76	0.248	0.224

(*$p<0.05$, and **$p<0.001$)

The amplification of GSTT and GSTM were clarified in figure (2), when deletion mutation (null genotyping) didn't give amplification products while normal genotyping give 312 and 215 bp for GSTT and GSTM respectively.

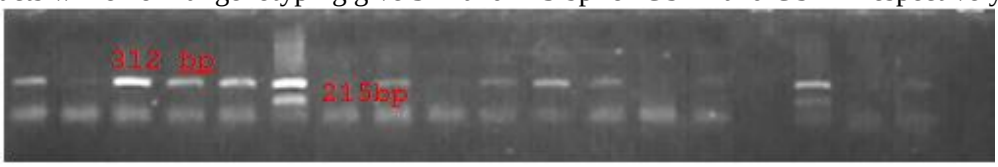

Fig. 1 amplification products of GSTT and GSTM using agaros gel and ethidum bromide staining (70 V, 0.5 TBE and 1% agaros for 40 min)

The GST genotyping including normal and null genotyping are explained in table (4), the results show non-significant association of null genotyping for both genes with study groups (p= 0.755, 0.1773) for GSTT1 and GSTM1 respectively, for null genotyping of both genes (GSTT1+GSTM1) there was non-significant association for all genotyping (p=0.6288) among study groups.

Table 4 GSTT1 and GSTM1 genotyping distribution in study groups

GSTase genotyping	DM2+covid19	Covid19 only	Control group	P
GSTT1 normal GSTT1-null	19 (61.29) 12(38.70)	21(70) 9(30)	21(67.74) 10(32.25)	0.7554
GSTM1 normal GSTM1 null	27(87.09) 4(12.90)	26(86.66) 4(13.33)	22(70.96) 9(29.03)	0.1773
GSTT1, GSTM1 normal GSTT1, null GSTM1 GSTM1, null GSTT1 Null GSTT1 and GSTM1	17(54.83) 2(6.45) 10(32.25) 2(6.45)	19(63.33) 2(6.66) 7(23.33) 2(6.66)	16 (51.61) 5(16.12) 6(19.35) 4(12.30)	0.62887

(X^2 at p value less than 0.05)

The association between GSTT1, GSTM1 genotyping and GSH, GSTase level were estimated in the current study, the results show non-significant differences in GSTase and GSH levels among study groups according to the GSTase genotyping although of several changes observed in some cases table (5).

Table 5 GSTase and GSH levels mean differences in study groups according to the GSTase genotyping

GSTase genotyping	DM2+covid19		Covid19 only		Control group	
	GSTase	GSH	GSTase	GSH	GSTase	GSH
GSTT1 normal	130.64±41.75	8.45±1.172	92.17±14.26	15.48±2.48	53.64±9.68	16.33±2.67
GSTT1-null	80.55±9.90	11.74±1.98	89.39±14.60	10.56±3.58	66.68±27.54	11.57±2.466
GSTM1 normal	101.25±27.82	10.07±1.179	92.10±12.25	14.39±2.37	63.16±18.54	13.92±2.155
GSTM1 null	178.77±72.63	7.77±2.824	86.18±10.08	11.91±2.82	52.14±11.09	16.08±3.919
GSTT1, GSTM1 normal	117.72±43.78	8.96±1.263	91.65±15.23	15.58±2.74	54.97±13.63	14.55±2.83
GSTT1, null GSTM1	240.4±154.82	4.41±0.55	100.0±0.09	14.68±5.425	51.51±14.49	19.18±5.49
GSTM1, null GSTT1	73.24±10.31	11.87±2.28	93.44±20.43	11.03±4.851	85.02±65.12	12.24±2.90
Null GSTT1 and GSTM1	117.09±8.91	11.13±5.00	79.27±12.73	9.15±1.80	52.93±19.70	10.91±4.63
P	0.529	0.362	0.988	0.762	0.820	0.529

(ANOVA one way at p value less than 0.05)

DISCUSSION

Statistical analysis found about 774 million infections and another 7 million deaths by covid-19 have been reported across the globe at the binging of 2024, The companied between DM2 and covid-19 infection have been reported to be a risk in numerous studies, several candidate genes have significant association to be DM2 patients was more predisposed to sever covid-19 infection and complications, in the present study, the results referred to non-significant association between GSH, GSTase and GSTase genotyping M1/T1 with covid-19 infection in DM2 patients, in spite of some changes which observed among study groups, the importance of GST system in the infection disease represented by oxidative stress control in the body, the role of GST system in ROS detoxification during covdi-19 or DM2 have been elucidated by numerous studies [15-17], the polymorphisms of oxidative stress genes are highly probable associated with susceptibility and covid-19 severity. glutathione S-transferase is one of the genetic factors that implicate to variant activity in response to oxidative stress products clearance among cases have gene polymorphisms. The GSTase genes polymorphisms M1/T1 typically included gene deletion, lead to protein product absence [18]. The present study found non-significant polymorphisms in different types (table 4) with study groups which agree with two ecological studies results that found a negative association between the mortality and case–fatality of covid-19 and the *GSTT1* null genotype frequency [19] and higher Val105 allelic frequency of GSTP1 in some population have higher prevalence and mortality rate [20]. Abbas et al. [21] observed that individuals with the null genotype of GSTT1 have a higher risk of mortality and lower overall survival during covid-19 infection. Furthermore, patients with (CC) allele in GSTM3 present higher odds of covid-19 developing [22]. Moreover, the combination between GSTP1 SNPs (rs1695 and rs1138272) and GSTPM3 genotype demonstrated cumulative risk for prevalence and severity of covid-19 [22]. Regarding to the assumption that oxidative stress have vital role in covid-19 infection, studies proposed that polymorphisms of GST gene might control cases susceptibility to covid-19 clinical manifestations developing.

The present work included GSH level detection belong to its role in oxidative stress regulation and association to some diseases such as thrombosis, pulmonary inflammation and covid-19 infection, glutathione peroxidase used GSH to eliminate ROS level that produced by platelets Activation [23], in spite of non-significant changes in GSH among study groups in present findings, slightly impacts were observed in group DM infection with covid-19 patients, the GSH impacts have been proved in ROS reduction in other investigations, the GSH level was lowered in covid-19 patients [24]. Another report found that all severe covid-19 patients have high mortality risk and low basal GSH levels [25]. GSH has role in immune response and inhibits viral replication, it has been found to reduce IL-6, and thus GSH supplement during covid-19 infection could be beneficial in patients with cytokine storm and redox imbalance [26, 27]. The association between GSH and GST levels also demonstrated changes in study groups, this change observed in DM2- covid-19 group in comparison with others, some studies explained this role of GSH in preventing DM2 prevention and management [28-31].

CONCLUSION

the present findings concluded that the DM2 has slightly influence in the covid-19 infection cases regarding to GSTase, GSH levels and M1/T1 gene polymorphism, the non-significant associations between GSH,GSTase and glycemic parameters didn't mean the absence role of oxidative stress in covid-19 infection companied with DM2, it may mean contributed other factors in redox regulation in these groups. Further studies might be benefit to explain their impact of GSH, GSTase in these factors.

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