Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Clinica Chimica Acta 508 (2020) 213–216

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Letter to the editor

An evidence for correlation between the glutathione S-transferase T1 (*GSTT1*) polymorphism and outcome of COVID-19

ARTICLE INFO

Keywords:
GSTT1
GSTM1
COVID-19
Polymorphism

To the editor,

Oxidative stress is associated with the pathogenesis of several multifactorial diseases. In human, numerous gene families are involved in the cellular detoxification with high level of genetic polymorphisms. Members of the glutathione S-transferases (GSTs) superfamily are involved in catalyzing the conjugation reactions of reactive intermediates of electrophilic compounds with cytosolic glutathione. The glutathione S-transferase T1 (*GSTT1*, MIM: 600436) and M1 (*GSTM1*, MIM: 138350) belong to class mu and theta, respectively. Both genes display a deletion polymorphism, null allele. Homozygosity for the null alleles, namely the null genotypes, results in the absence of corresponding enzyme activity [1].

The *GSTT1* and *GSTM1* null genotypes increase the risk of several oxidative stress associated multifactorial diseases [1]. It is well known that oxidative stress is an important issue in viral respiratory infections [2,3]. Pulmonary fibrosis is one of the most important complications of COVID-19 disease, which is associated with oxidative stress [4]. It is suggested that melatonin can reduce COVID-19 infection-associated oxidative stress [4,5]. On the other hand, GST activity is significantly increased in melatonin treated experimental animals [6,7]. Very recently, a study was published on association between *ACE* insertion/deletion polymorphism and the prevalence of COVID-19 [8]. However, there are no data on the *GSTT1* and *GSTM1* polymorphisms and COVID-19. Taken together, these facts sufficiently provide us with a theoretical hypothesis to perform the present ecologic study.

Considering that the prevalence (per 10^6 people), case-fatality (per 100 infected cases) and mortality (per 10^6 people) of the COVID-19 may be affected by various social factors, we considered the life expectancy at birth (LE), density of medical doctors per 10,000 population, density of nursing and midwifery personnel per 10,000 population, age-standardized prevalence of tobacco smoking among persons aged 15 years and older (%) and the gross national income (GNI) per capita (PPP international $) as the indices for economic situation and health services in different countries as the potential confounding variables. The latest data available for countries were obtained from the World Health Organization website www.who.int/countries/en/. Based on the nature of the present data, the number of COVID-19 diagnostic tests performed per one million population in each country was also used as a confounding variable. Data for the prevalence, fatality, mortality, and level of performed diagnostic test of COVID-19 on April 30, 2020 were obtained from the website www.worldometers.info/coronavirus/countries. The frequencies of the *GSTT1* and *GSTM1* null genotypes of different countries were obtained from previous reports (Table S1 in supplement file).

Data from Algeria, Argentina, Australia, Austria, Bahrain, Belgium, Brazil, Bulgaria, Burkina Faso, Cameroon, Canada, Chile, China, Colombia, Croatia, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Estonia, Finland, France, Germany, Ghana, Greece, Hungary, Iceland, Indonesia, Iran, Italy, Japan, Kazakhstan, Lebanon, Mexico, Moldova, Morocco, Netherlands, Norway, Philippines, Poland, Portugal, Romania, Russia, Saudi Arabia, Serbia, Singapore, Slovakia, Slovenia, South Korea, Spain, Sweden, Thailand, Tunisia, Turkey, UAE, Ukraine, United Kingdom, and United States were included in the analysis, of which 28, 15, 7, 8, and 1 were European, Asian, American, African, and Oceanian countries, respectively.

Normal distribution of variables was checked using one-sample Kolmogrov-Smirnov test and non-normally distributed variables, comprising epidemiologic measures of disease (prevalence, mortality and case-fatality rates), GNI and the number of tests performed per one million population, were log-transformed. Correlation analysis and multivariable linear regression analysis were used. Variables with $P < 0.1$ in the univariable analysis were introduced into the multivariable models (see Tables S2 and S3 in the supplement file). Six different models were fitted for combination of each epidemiologic parameter with each polymorphism. Epidemiologic measures (prevalence, mortality and fatality) were considered as outcome variables and frequencies of the null genotypes as well as the above-mentioned potential confounders were introduced into the model as explanatory variables. A backward removal method was used for each model construction and a two-tailed $P < 0.05$ was considered significant in the final model.

In univariate analysis, there was a significant correlation between the frequency of *GSTT1* null genotype and three epidemiological parameters of the COVID-19. However, the correlation between the frequency of the null genotype of *GSTM1* and the above-mentioned variables was not significant (Table 1). Based on multivariate linear

https://doi.org/10.1016/j.cca.2020.05.041
Received 1 May 2020; Received in revised form 18 May 2020; Accepted 21 May 2020
Available online 23 May 2020

Table 1
Univariable analysis for association of COVID-19 epidemiologic parameters with the *GSTT1* and *GSTM1* polymorphisms in the various countries around the world.

Polymorphisms	Prevalence		Fatality		Mortality	
	r	P	r	P	r	P
GSTT1	−0.348	0.007	−0.317	0.014	−0.488	< 0.001
GSTM1	0.202	0.125	0.068	0.609	0.207	0.116

regression analyses, the partial correlation coefficients of the *GSTT1* null genotype frequency showed negative associations with the log-fatality (partial r = −0.424, P = 0.001) and log-mortality of the COVID-19 (partial r = −0.389, P = 0.005). The log-prevalence of the COVID-19 was not associated with the frequency of the *GSTT1* null genotype after adjusting for possible confounders (Table 2). Fig. 1 shows the normality of the distribution of the linear regression model.

Some countries such as Australia, USA, and Canada are immigrant-friendly countries, i.e., they have very mixed population. Therefore, estimation of allelic frequencies for genetic polymorphisms is not easy. On the other hand, in some countries, COVID-19 is showing very large differences among ethnic minorities or different provinces. For example, African-Americans are disproportionally affected by COVID-19; in China the majority of the patients with COVID-19 were inhabitants of the Hubei province, and about 50% of infected cases were related to “Shincheonji Church” in South Korea. “Sensitivity analysis” may play an important role in testing the robustness of the output in a model. The “sensitivity test” was performed by removing the data of the above-mentioned countries and reanalyzing the remained data (Tables S3–S5). After removing these countries, the similar results were obtained. The partial correlation coefficients of the frequency of null-*GSTT1* showed negative associations with the log-fatality (partial r = −0.445, P = 0.001) and log-mortality of COVID-19 (partial r = −0.353, P = 0.016).

It means that the countries with lower frequency of the *GSTT1* null genotype, showed higher mortality and case-fatality due to COVID-19 infection. Overall, the *GSTT1* polymorphism presents distinct distribution in Caucasian and Asian populations. European and East-Asian countries show low and high frequency of the null genotype, respectively. It should be noted that with the present findings, it is possible to explain, at least in part, some of the remarkably observed differences in mortality and case-fatality due to COVID-19 between East Asian and European countries by the *GSTT1* polymorphism.

The present findings revealed that the *GSTT1* and *GSTM1* null genotypes have differential behavior versus COVID-19 fatality/mortality. It should be noted that these genes, although belonging to a gene superfamily, are completely independent loci with different tissue distribution patterns. *GSTM1* was not detected in the lung tissue, whereas the *GSTT1* was expressed in the lung tissue [9,10]. It has already been reported that these polymorphisms have different relationships with numerous multifactorial diseases. This may explain, at least in part, our present findings. On the other hand, as the outcome of COVID-19 is associated with oxidative stress and GSTT1 is involved in the cellular detoxification process, it can be explained why the *GSTT1* null genotype is associated with COVID-19 fatality/mortality but does not have correlation with the prevalence of COVID-19.

Viral respiratory infections including infection with COVID-19 are associated with oxidative stress in infected persons [2,3]. The *GSTT1* null genotype increase the risk of numerous multifactorial traits associated with oxidative stress or inflammation [1]. Therefore, it was postulated that at the country level, there is a positive relationship between the frequency of *GSTT1* null genotype and the mortality and

Table 2
Multivariable linear regression analyses for association of COVID-19 epidemiologic measures with the *GSTT1* polymorphism in the various countries around the world.

Parameters	Confounders included	Confounders remained	Presence of Null-genotype	Partial r for the genotype	P	Adjusted r^2 for model
Prevalence	Log-test, log-GNI, LE, Density of medical doctors, Density of nurses, Prevalence of tobacco smoking, *GSTT1* polymorphism	Log-test, LE	No	–	NS	0.642
Fatality	Log-test, *GSTT1* polymorphism	Log-test, *GSTT1* polymorphism	Yes	−0.424	0.001	0.244
Mortality	Log-test, log-GNI, LE, Density of medical doctors, Density of nurses, Prevalence of tobacco smoking, *GSTT1* polymorphism	LE, *GSTT1* polymorphism	Yes	−0.389	0.005	0.414

Note: Log-test (log-number of performed COVID-19 test per 10^6 population), log-GNI (log-GNI per capita), LE (life expectancy at birth), density of medical doctors, density of nurses and prevalence of tobacco smoking (age-standardized prevalence of tobacco smoking among persons aged 15 years and older) as possible conformers were used for multivariable linear regression models.

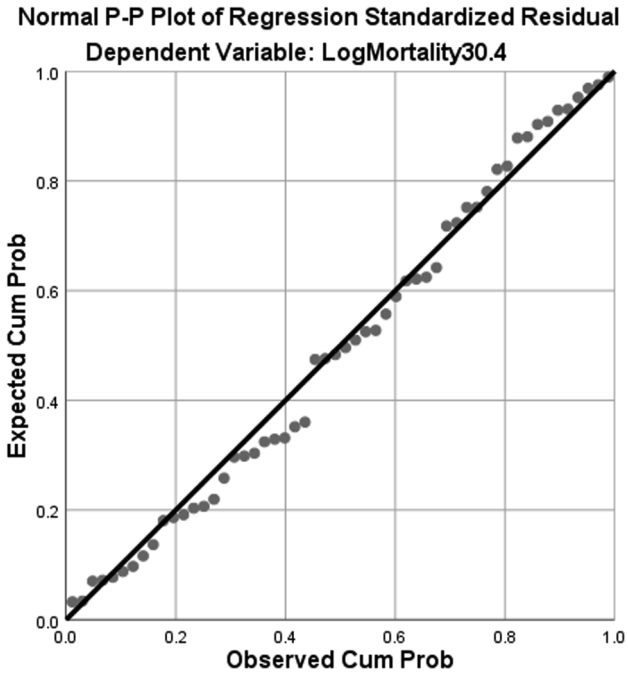

Fig. 1. Normal P-P plot of regression standardized residual. Dependent variable is log-transformed of mortality, explanatory variables are the life expectancy at birth and frequency of the null genotypes of *GSTT1*. "Sensitivity analysis" was performed after Australia, USA, and Canada, South Korea and China were removed from the data set.

case-fatality of COVID-19. However, the observed associations are contrary to the primary hypothesis.

Although the null genotypes of *GSTT1* and *GSTM1* are usually associated with the increased risk of numerous multifactorial traits, there are several conflicting reports. For example, epidemiologic evidence suggests that cruciferous vegetables, that are rich in isothiocyanates, have anticancer properties. Isothiocyanates may inhibit the bioactivation of procarcinogens found in tobacco smoke. Since GSTM1 and GSTT1 play an important role in isothiocyanate metabolism, the inverse association between the intake of these vegetables and the lung cancer risk is stronger in those who are null for both *GSTM1* and *GSTT1* [11]. In this manner, the protective roles of the *GSTT1*- and *GSTM1*-null genotypes could be explained.

The present findings suggest that the null genotype of *GSTT1* may be regarded as a predictor for mortality and case-fatality of COVID-19 in the current pandemic in various countries. It should be noted that the present study has some limitations. An ecologic study does not compare persons, but large groups of people even countries with each other. As the results may incorrectly be used for interpretation at the individual level, the findings of these studies are prone to fallacy. It should be declared that such studies do allow an initial investigation of the health status and needs of publics. The present study, like other studies which have used a similar design, should be considered as a means of producing hypotheses rather than developing definitive information about the nature of associations between predicted factors and health outcomes. Hence, the above-mentioned correlations do not mean a causal relationship between the *GSTT1* polymorphism and outcome of the COVID-19. Several case-control, cohort and experimental studies should be done to confirm the present findings. Second, in addition to the above-mentioned potential confounders, there may be other confounding variables (such as other genetic polymorphisms and some environmental pollutants,) which should be taken into account in the future studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The author is indebted to Dr. Maryam Ansari-Lari (Shiraz University) for her assistance in statistical analysis and critical reading of the manuscript. This study was supported by Shiraz University, Iran.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2020.05.041.

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Mostafa Saadat
Department of Biology, College of Sciences, Shiraz University, Shiraz 71467-13565, Iran