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ASSOCIATION OF DELETION POLYMORPHISMS OF THE *GSTM1* AND *GSTT1* GENES WITH THE DEGREE OF LUNG DAMAGE IN ELDERLY PEOPLE AFTER COVID-19

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A feature of the SARS-CoV-2 virus, unlike other respiratory infections affecting the human body, is a rather high virulence and mortality. It has been established that severe forms of the disease are more common in elderly people with concomitant diseases. It has been established that free radical lipid oxidation plays an essential role in the pathogenesis of COVID-19. The body's antioxidant defense system prevents damage to cells and tissues from initiating free radical reactions. The family of enzymes glutathione-S-transferase (GST; EC 2.5.1.18) is interesting for research. The aim of this work was to analyze the association of polymorphisms of the *GSTM1* and *GSTT1* genes with the degree of lung damage in elderly people who had COVID-19. A survey of 51 elderly volunteers who had coronavirus infection aged 60 to 75 years (average age: 64.470± 0.602 years) was conducted. Informed consent to the study was obtained from all participants of the study (according to the protocol of the Ethics Committee of the YSC CMP No. 52 dated March 24, 2021, decision 1). Identification of samples by the GSTT1 and GSTM1 genes was carried out using polymerase chain reaction (PCR) according to the method described in the work of Zehra et al. (2018). According to the data obtained by us, 74.50% of all surveyed elderly people suffered a coronavirus infection with a mild degree of lung damage, and 25.49% with a severe degree. The results of our study show that the combination of zero deletion genotypes GSTM1 and GSTT1 are a risk factor for the development of severe lung lesions in elderly people in Yakutia.

Keywords: glutathione-S-transferase, *GSTM1* and *GSTT1* genes, deletion polymorphisms, COVID-19, SARS-CoV-2.

Introduction. The SARS-CoV-2 virus differs from other respiratory infections by its rather high virulence and mortality. Many researchers have found that severe forms of the disease are more common in elderly people with concomitant diseases: diabetes mellitus, cardiovascular, etc. [4].

It has been established that free radical lipid oxidation plays an essential role in the pathogenesis of COVID-19 [1]. The SARS-CoV-2 virus initiates the production of free radicals and inhibits antioxidant protection by suppressing the expression of the transcription factor Nrf2 (nuclear factor E2-related factor 2) [17]. Toxic products of lipid peroxidation are involved in damage to cells and tissues. Neutralization of toxic products of lipid peroxidation is carried out by the enzyme glutathione S-transferase, reducing the intensification of free radical oxidation of lipids [6,15].

Glutathione-S-transferases (GST; EC 2.5.1.18) are a large and widespread family of enzymes that are divided into three main groups: cytosolic; mitochondrial; microsomal. In humans, GST enzymes are mainly represented by the cytosolic family. There are 7 classes of cytosolic GST enzymes (α, μ, π, θ, σ, ω, ζ), which include 17 isoforms of the enzyme, each encoded by a separate gene or a group of genes located on different chromosomes [2]. The enzymes encoded by the GSTM1 and GSTT1 genes are the most studied, well expressed in human lung tissues, their genes are located on chromosomes 1p13.3 and 22q11.23, respectively [14]. A feature of these *GSTM1*

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and *GSTT1* genes is the presence of extended deletions in them, characterized by the absence of expression of the corresponding enzymes. Deletion polymorphisms of the *GSTM1* and *GSTT1* genes occur with high frequency in many human populations. Carriers of homozygous null deletion polymorphisms of the *GSTM1* and *GSTT1* genes have an increased risk of developing multifactorial diseases associated with oxidative stress, including respiratory, cardiovascular, oncological and other diseases [7,18,20].

The aim of this work was to analyze the association of polymorphisms of the *GSTM1* and *GSTT1* genes with the degree of lung damage in elderly people who have undergone COVID-19.

Material and methods of research. A survey of 51 elderly volunteers aged 60 to 75 years (average age: 64.470±0.602 years) was conducted. All the examined patients had a coronavirus infection, were discharged from the hospital in the period from August to September 2020, the examination and collection of the material was carried out in March 2021. Informed consent to the study was obtained from all participants of the study (according to the protocol of the Ethics Committee of the YSC CMP No. 52 dated March 24, 2021, decision 1).

Since coronavirus infection is associated with the development of pneumonia in patients, the diagnosis of lung damage (inflammation) was assessed by the percentage of destruction of lung tissue based on computed tomography. In our study, patients were divided into two groups: group 1 – patients with mild lung damage (CT1-2), group 2 – patients with severe lung damage (CT 3-4). Clinical indicators during the disease, such as the degree of damage assessed on CT, were taken from a medical record statement. All participants were personally interviewed, filled out a series of questionnaires. The general characteristics of the examined patients are presented in Table 1.

For genotyping, DNA was isolated from whole blood by the standard two-stage method of phenol-chloroform extraction. DNA samples were sampled by deletion polymorphisms of the biotransformation genes: *GSTT1* and *GSTM1*, which encode the glutathione S-transferase enzymes θ1 and μ1, respectively. Identification of samples by *GSTT1* and *GSTM1* genes was carried out using polymerase chain reaction (PCR) according to the method described in the work of Zehra et al. (2018).

The results were visualized electrophoretically in 3% agarose gel, with the addition of ethidium bromide. The PCR results were viewed in transmitted UV light on a transilluminator. The presence of deletion polymorphisms of the *GSTM1* and *GSTT1* genes was determined by the absence of the corresponding fragments: 219 bp – for *GSTM1* and 459 bp – for *GSTT1*. The presence of these fragments indicates the presence of at least one normal (without deletion) copy of the genes. β-globulin with a fragment of 268 bp was used as an internal control. Evidence of successful PCR analysis was the presence of an amplification of 268 bp, the β-globulin gene.

Statistical processing was carried out using the software package SPSS 11.5 for Windows and Microsoft Excel. The relationship between the degree of lung damage and genotypes in COVID-19 survivors was assessed by odds ratio (*OR*) with a 95% confidence interval (*95% CI*). Comparison of genotype frequencies in groups of sick and healthy individuals was carried out using the Fisher criterion. The differences were considered statistically significant at $p < 0.05$.

Results. According to the data obtained by us, 74.50% of all surveyed elderly people suffered a coronavirus infection with a mild degree of lung damage, and 25.49% with a severe degree (Table 1).

The distribution of deletion genotypes *GSTM1* and *GSTT1* in patients with mild and severe lung lesions is shown in Table 2. The frequency of $GSTM1^{-/-}$ and $GSTT1^{-/-}$ genotypes (null genotypes) was higher among volunteers with severe lung damage, compared with volunteers with a milder degree (76.92% vs. 55.26%; 69.23% vs. 44.73%, respectively), but the differences did not reach statistical significance.

Individuals who had COVID -19 with a combination of two null genotypes ($GSTM1^{-/-}$ / $GSTT1^{-/-}$) showed a sufficiently high risk of developing severe

Table 1

General characteristics of the examined patients who have had a coronavirus infection

Indicator:	Values
Number of examined	51
Men / Women	59/102
Disease severity status (CT stage):	
1-2	38 (74.50%)
Age, years:	63.947±0.673
3-4	13 (25.49%)
Age, years:	66.000±1.260

Table 2

The frequencies of *GSTM1* and *GSTT1* genotypes and their relationship with the degree of lung damage in elderly people who have had COVID-19

Genotypes		Group	n (%)	p	*OR (95% CI)*
GSTM1	$M^{+/+}$	1	17 (44.74)	0.20	2.69(0.63-11.38)
	$M^{+/+}$	2	3 (23.08)		
	$M^{-/-}$	1	21(55.26)		0.37(0.08-1.56)
	$M^{-/-}$	2	10(76.92)		
GSTT1	$T^{+/+}$	1	21 (55.26)	0.20	2.77(0.72-10.61)
	$T^{+/+}$	2	4 (30.77)		
	$T^{-/-}$	1	17 (44.73)		0.35(0.09-1.37)
	$T^{-/-}$	2	9 (69.23)		
GSTM1/GSTT1	$M^{+/+}/T^{+/+}$	1	9 (23.68)	0.70	1.70(0.31-9.17)
	$M^{+/+}/T^{+/+}$	2	2 (15.38)		0.58(0.10-3.15)
GSTM1/GSTT1	$M^{-/-}/T^{-/-}$	1	9 (23.68)	**0.02**	0.19(0.05-0.74)
	$M^{-/-}/T^{-/-}$	2	8 (61.54)		5.15(1.34-19.77)
GSTM1/GSTT1	$M^{-/+}/T^{-/+}$	1	12 (31.58)	0.47	2.53(0.48-13.27)
	$M^{-/+}/T^{-/+}$	2	2 (15.38)		0.39(0.07-2.06)
GSTM1/GSTT1	$M^{+/-}/T^{+/-}$	1	8 (21.05)	0.41	3.20(0.36-28.42)
	$M^{+/-}/T^{+/-}$	2	1 (7.69)		0.31(0.03-2.77)

lung damage by 5.15 times (61.54% vs. 23.68%), which is evidence of a reliable association of a combination of null genotypes with the development of more severe forms of lung damage in elderly people who had COVID-19 ($p< 0.05$).

Discussion. The pathogenesis of SARS-CoV-2 in COVID-19 disease is associated with the way the virus enters the human body. The SARS-CoV-2 virus enters the cell by interacting with the protein receptor - ACE2 (angiotensin converting enzyme 2). SARS-CoV-2 blocks the work of the ACE2 protein, stimulates its internalization. The loss of ACE2 receptor activity leads to a rapid drop in the production of angiotensin-1–7 (Ang 1-7), and consequently the accumulation of angiotensin II (Ang II). Imbalance between angiotensin II (hyperactivity) and angiotensin 1-7 (deficiency) may play a role in the occurrence of an acute increase in blood pressure [12].

In addition, the accumulation of Ang II octapeptide leads to an increase in the expression of transcription nuclear factor-kB (NF-kB). The results obtained in the work of a group of researchers Blanco-MeloD, Nilsson-PayantBE, LiuWC, (2020) in vitro on a model of human bronchial epithelial cells are an indirect confirmation of the expression of NF-kB. The results showed that when cells are infected with the SARS-CoV-2 virus, overexpression of proteins is observed: CCL20, CXCL1, IL-1B, IL-6, CXCL3, CXCL5, CXCL6, CXCL2, CXCL16 and TNF. These proteins can lead to chemotaxis of neutrophils into virus-affected tissues (lung tissues) and a strong inflammatory reaction. Neutrophils, in virus-infected tissues, intensively generate ROS, thereby shifting the prooxidant-actioxidant equilibrium towards the intensification of free radical processes [9]. The shift of the pro-oxidant-antioxidant equilibrium towards lipid peroxidation is evidenced by studies by other authors [10,13].

Authors Khomich O A, et.al . (2018) showed that a high level of ROS and a violation of the redox balance of the host is of great importance for the replication of viruses and the occurrence of the disease. Fuentes E, et.al. (2018) showed that the SARS-CoV-2 virus activates platelets [5]. Platelets, in turn, induce neutrophils to produce neutrophil extracellular traps, which play a key role in the development of thrombotic complications leading to acute respiratory failure in lung tissues, lead to the development of hypoxia, which further initiates free radical oxidative processes [8].

Glutation-S transferase enzymes neutralize the products of free radical oxidation, thereby they are inhibitors of the development of oxidative stress. The presence of deletion polymorphisms in the *GSTM1* and *GSTT1* genes leads to a higher risk of initiation of free radical reactions and the development of oxidative stress. In turn, oxidative stress plays an important role in susceptibility to SARS-CoV-2 infection and increases the risk of developing a large number of complications in COVID-19 [16].

Our study showed that patients carrying a combination of zero genotypes $GSTM1^{-/-}$ and $GSTT1^{-/-}$ who have had COVID-19 have a higher risk of developing severe lung lesions. In a study by Saadat (2020), it was shown that people with a zero genotype of the *GSTT1* gene had a higher risk of COVID-19 infection compared to people without deletion polymorphism.

The results of our study show that the combination of zero deletion genotypes *GSTM1* and *GSTT1* are a risk factor for the development of severe lung lesions in elderly people in Yakutia.

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