

ABSTRACT

The first cases of patients with pneumonia which grew into an acute respiratory distress syndrome and caused breathing problems began to appear in December 2019. Coronavirus disease 2019 (COVID-19) is the cause of a global pandemic and it is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A complex interplay of factors is responsible for the progression of the disease. Some studies suggest that it promotes oxidative stress and thus may lead to oxidative damage to cells and DNA. The purpose of this study was to observe the relationship between oxidative DNA damage and a critical condition caused by COVID-19 using a comet assay technique.

The basic principle of the used method consists in fixation of lymphocytes in an agarose gel, removal of the membrane and cytoplasm of cells, incubation with specific enzymes and electrophoresis. In the process of electrophoresis, negatively charged DNA fragments migrates towards the anode and the cell thus acquires the typical shape of a comet. Comets are visualized using the DNA intercalation dye ethidium bromide.

We quantified single - strand breaks and oxidized pyrimidines and purines by using specific enzymes (modification of the method for detecting specific lesions). Results are reported as % tail DNA, thus the percentage of DNA in the comet's tail.

Determination of DNA damage was performed in the semi-automated software LUCIA Comet Assay (Laboratory Imaging, Czech Republic) for image analysis. Damage in lymphocytes of critically ill patients during their hospitalization was assessed. Statistical analysis was performed in GraphPad Prism and the results of the observed parameters show a statistically insignificant damage caused by SARS-CoV-2.

Key words: comet assay, critical condition, DNA damage, repair, SARS-CoV-2