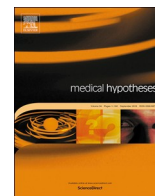




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## Letter to Editors

## A role of glycation and methylation for SARS-CoV-2 infection in diabetes?



## ARTICLE INFO

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## ABSTRACT

Type-2 diabetes (T2D) is a major comorbidity of COVID-19, and poorly controlled diabetes is associated with high mortality rate, emphasizing the necessity to improve glycemic control. Angiotensin-converting enzyme 2 (ACE2) is the receptor responsible for SARS-CoV-2 access to human cells, and ACE2 expression is increased in patients with diabetes and hypertension treated with ACE-inhibitors or angiotensin II receptor blockers. We hypothesize that an upregulation of ACE2 due to its non-enzymatic glycation could be considered, as well as a change of the protein tertiary structure in terms of amino acid (mostly lysine) available to be glycated. In fact, in a single ACE2 molecule, 34 lysine residues are present in the extracellular portion, and at least one of these is co-involved in a fundamental hydrogen-bond interaction with the SARS-CoV-2 receptor binding domain (RBD). The worse outcome of COVID-19 in people with diabetes could be related to the non-enzymatic glycation that triggers the activity of ACE2. Moreover, DNA methylation of genes regulating islet beta-cell function, as well as in insulin resistance of peripheral tissues such as liver, muscle, and adipose tissue may be involved, as already demonstrated for cancer conditions. DNA methylation, besides being considered as a biomarker to predict the risk of obesity and T2D, has been suggested also as a target for dietary and pharmacological treatments. The present observations may suggest further interventions in order to improve the outcome of COVID-19 in people affected by diabetes.

Type-2 diabetes (T2D) is a major comorbidity of COVID-19. In a retrospective multi-center study on 7337 cases of COVID-19 in Hubei (China), among which 952 had pre-existing type-2 diabetes, it has been shown that people with diabetes required more medical intervention, presented a higher multiorgan impairment and mortality rate with respect to patients without diabetes. Furthermore, poorly controlled diabetes, in terms of glycemic variability, was associated with the high mortality rate, emphasizing the necessity to improve glycemic control [1].

Angiotensin-converting enzyme 2 (ACE2) is the receptor believed responsible for SARS-CoV-2 access to human cells [2,3]. ACE2 has also been proposed as a target to identify potential drugs against SARS-CoV-2 infection [4]. Fang et al. [5] in a commentary suggested that people affected by diabetes and hypertension, often treated with drugs such as ACE inhibitors and angiotensin II receptor blockers (ARBs) that upregulate ACE2 receptor expression, may present a higher risk of developing severe and fatal COVID-19. Drugs used for diabetes treatment, such as pioglitazone and liraglutide, could induce increase of ACE2 activity, thus facilitating the entry of SARS-CoV-2 into lung cells, conditioning a more severe and often fatal disease [5,6]. Debate on the topic is still open, and opposing opinions have been discussed, in particular that SARS-CoV-2 infection induces a down-regulation of ACE2 [4], which could be detrimental, because ACE2 represents not only the receptor used by the virus to enter the host's pneumocytes, but has a role also in lung protection [4]. ACE2 expression is increased in patients with diabetes and hypertension treated with ACE-inhibitors or ARBs, as an adaptive reaction to counteract the high levels of AngII and AngI. Recent studies have evidenced that severely ill patients with COVID-19 present a greater prevalence of hypokalemia, as result of renal potassium wasting [7].

Alternatively, an upregulation of ACE2 due to its non-enzymatic

glycation could be considered. The non-enzymatic glycation of proteins is a process that links chronic hyperglycemia to a series of pathophysiological changes considered important for the development of the chronic complications of diabetes [8]. Glucose can react with a free amino group of several molecules, including proteins, nucleic acids and lipids, to form an unstable aldimine compound, the Schiff base. Following rearrangement, this base produces a stable ketoamine, the Amadori product. Since this reaction does not involve enzymes, the influencing variables are the glucose and protein concentrations; therefore, in diabetes, the presence of elevated levels of glucose makes this reaction very active, leading to irreversible accumulation of altered proteins.

In previous studies on non-enzymatic glycation, using an accurate methodology such as mass spectrometry, we have confirmed the *in vitro* and *in vivo* non-enzymatic glycation of albumin, showing that the number of glucose molecules that react with the lysine residues of albumin are directly related to the *in vitro* or *in vivo* (plasma) glucose levels [9]. Considering now the occurrence of a non-enzymatic glycation of ACE2, its possible pathogenetic role in changing the protein tertiary structure in terms of amino acid (mostly lysine) available to be glycated, needs to be taken into consideration. Looking at the molecular structure (RCSB PDB protein Data Bank id: 6LGZ, DOI: <https://doi.org/10.2210/pdb6LGZ/pdb>), in a single ACE2 molecule, 34 lysine residues are present in the extracellular portion, and at least one of these is co-involved in a fundamental hydrogen-bond interaction with the SARS-CoV-2 receptor binding domain (RBD) [10].

In diabetes disease also immunoglobulins (IGG) are exposed to high glucose concentrations; utilizing a MALDI/MASS Spectrometry approach we have demonstrated that the number of glucose molecules condensed on IGG are related to the glucose levels and that in the case of poorly controlled diabetes, 20 glucose molecules react non-

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enzymatically with the lysine residues of IGG [11]. Further experiments on IGG with mass spectrometry and computer-molecular modelling have evidenced that the Fab fragments are more prone to attack by glucose, particularly in the light and heavy variable regions [11]. So, in persons with diabetes the immune deficiencies observed could be due to an extensive glycation of Fab fragment, thus inhibiting the process of molecular recognition between antibody and antigen; this mechanism is consistent with the severe inflammatory state of patients with diabetes affected by COVID-19.

Taking into consideration other clinical findings regarding co-morbidities during the epidemic diffusion in China, surveys of cancer patients affected by COVID-19 indicated that lung adenocarcinoma (LUAD) was the most frequent tumour type, and such patients presented more severe symptoms [12,13]. Chai et al. [13] in a bioinformatic evaluation of genetic alteration, RNA expression, and DNA methylation of ACE2 across over 30 tumours, have suggested a link between variations of ACE2 receptors and propensity to SARS-CoV-2 infection and COVID-19 severity. Regarding the specific mechanisms involved, the study highlighted the fact that although ACE2 expression is upregulated in several cancers, including LUAD, the upregulation is not the result of a genetic variations, but rather is due to epigenetic disorders involving methylation [13]. Moreover, the authors suggest that other mechanisms, including glycosylation [14] could be involved.

We here hypothesize that the worse outcome of COVID-19 in people with diabetes could be related, at least in part, to the two mechanisms already suggested for cancer patients, specifically methylation and glycosylation, and in addition to the non-enzymatic glycation as we have discussed above, that could trigger the activity of ACE2. These considerations arise also from the knowledge that diabetes has a strict link with DNA methylation of genes involved in islet beta-cell function, as well as in insulin resistance of peripheral tissues such as liver, muscle, and adipose tissue [15]; moreover the DNA methylation appears as a non-permanent condition, linked also to degree of diabetes control, and a potential target for diabetes control and therapy evaluation [16,17]. DNA methylation, besides being considered as a biomarker to predict the risk of obesity and T2D, has been suggested also as a target for dietary and pharmacological treatments [18]. On the other side, glycation processes represent one of the most investigated milestones in pathogenesis, evolution and therapeutic target of diabetes [19,20]. The present observations may suggest further interventions in order to improve the outcome of COVID-19 in people affected by diabetes.

#### 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.

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