Original Article

Is diabetes a real susceptibility for SARS-CoV-2 oral manifestation?

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Abstract

Background: Furin, a polybasic cleavage enzyme, is increasingly recognized in the pathogenesis of metabolic syndromes like diabetes. Its cleavage action is an essential activation step for the SARS-CoV-2 attachment site at the junction of S1 and S2, the two subunits of the spike. This allows effective cleavage by furin and has a role in determining viral infectivity and host range. The increased expression of the furin enzyme in the saliva is remarkable enough to be noted as a susceptibility factor for diabetic patients.

Aim of the Study: The present study focuses on the qualitative assessment of the furin enzyme through an immunological ELISA test.

Materials and Methods Used: The study consisted of three groups, each of whom was a COVID-19 recovered patient ($n = 20$), a diabetic patient ($n = 20$), and a healthy patient ($n = 20$).

Result: The study assessed significantly increased levels of the furin enzyme generally in diabetic patients and COVID-19 recovered patients as compared to the healthy control group.

Conclusion: The estimation of furin in saliva still holds the possibility of being a prognostic marker in many COVID-19 infected patients. Further evidence-based studies are required to establish the same.

Keywords: Diabetic patients, ELISA, Furin, saliva

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BACKGROUND

The oral manifestation of COVID-19 is highly prevalent and requires special focus to study the disease process, as the oral cavity is considered an important portal for the virus entry and initiation process. Airborne transmission is the most dominant route for COVID-19 infection.[1] Similarly, many research groups have reported gastrointestinal manifestations of COVID-19, purposing the oral–fecal route as an alternative route of SARS-CoV-2 transmission.[2-5]

Epidemiological surveys show that diabetic patients are more likely to contract the COVID-19 infection.[24-26] The mortality risk due to SARS-CoV-2 infection is fourfold higher in diabetic patients than in nondiabetic individuals.[27] In India, more than 70% of COVID-19-associated mortalities are due to comorbidities including diabetes, hypertension, CVD, and pulmonary problems.[28,29] In diabetic patients, elevated plasma furin level acts as an independent predictor for disease onset, progression, and premature mortality; this provides information about increased CVD risk in these patients.[30,31] The diabetic population has now crossed

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more than 120 million worldwide, and this in turn poses an increased threat for further COVID-19 infection and related mortalities in these patients. Hence, understanding the disease process and its pathobiology behind the increased mortality in COVID-19-affected diabetic patients is highly essential to develop prominent prognostic biomarkers and accurate therapies that save their lives.

Earlier pandemic episodes with SARS-CoV-1 and MERS manifested respiratory associated symptoms, unlike SARS-CoV-2, which had mild to severe oral–nasal manifestations. The oral manifestations of SARS-CoV-2 included oral pain, gingivitis, and ulcers, especially in severe cases.[6] Dry mouth, mouth ulcers, and amblygeustia were experienced by a relatively high proportion of COVID-19 patients, especially patients with co-morbidities like diabetes.[7,8]

The present study focuses on the estimation of furin levels by ELISA in COVID-19 recovered (n = 20) and diabetic patients (n = 20) in comparison with normal individuals (n = 20). The study was pioneered as a pilot study to assess the level of furin enzyme in different cross sections of the Bangalore population over a period of time.

SARS-CoV-2 and furin enzyme

The SARS-CoV-2, a member of the Coronaviridae family, has spike projections of glycoproteins (S protein) on their viral envelope that facilitate the entry of viruses into the host receptors and fuse with the cell membrane. This molecular event is the gateway for viral entry into the host cells. The S proteins are type I transmembrane proteins composed of two subunits S1 and S2. The S1 subunit is responsible for receptor binding, while the S2 subunit is involved in membrane fusion. The S1 domain contains an N-terminal domain (NTD) and a receptor-binding domain (RBD) used for binding the SARS-CoV-2 receptor: the angiotensin converting enzyme 2 (ACE2) and heparan sulfate.[9] To facilitate fusion, the S protein that is synthesized as an immature protein precursor is proteolytically cleaved at the S1/S2 boundary, and at the S2′ site located close to the S2 fusion peptide (FP), it is also cleaved by serine proteases, leading to S2 fusion peptide generation that allows viral–host membrane fusion initiation. The study results demonstrate that SARS-CoV-2 has a superior affinity for the ACE2 receptor compared to SARS-CoV[10,11] and was associated with S protein cleavage by furin, a proprotein convertase member (also known as dibasic-processing enzyme) that is ubiquitously expressed. The cell entry of SARS-CoV-2 depends on the binding of the viral S protein to the cellular receptor ACE2 and on S protein priming by furin and TMPRSS2.[12]

The present short study is based on the estimation of furin, indicating the oral cavity as a platform for COVID-19 invasion via the respiratory route and possibly the fecal–oral route.

MATERIALS AND METHODS

The present study included a sample size of 60 individuals. It comprises subjects representing different study variables like age, sex, vaccination status, other co-morbidities, and history of COVID-19 infection.

The furin levels of samples were obtained from the subjects as unstimulated saliva and were collected as per WHO saliva collection protocol.[32] They were assessed using an enzyme-linked immunosorbent assay (ELISA) test kit. The plate had been pre-coated with a human furin antibody. Furin present in the sample is added and binds to antibodies coated on the wells. The biotinylated human furin antibody is added and binds to furin in the sample. The furin levels were estimated and compared in the three groups of samples.

RESULTS

The statistical analysis performed on various parameters of the present study includes age, sex, vaccination status, history of COVID-19 infection, co-morbidities like diabetes, hypertension, etc., Correlation tests were done to determine the significance between the three study groups of normal, COVID-19 recovered, and diabetic patients. All statistical procedures were performed using Statistical Package for Social Sciences (SPSS) 20.0. All quantitative variables, like age and gender, were expressed in mean and standard deviation. Qualitative variables were expressed in percentages, especially furin expression. Chi-square was used for the association between variables and correlation between different groups. The probability value ($P < 0.05$) was considered statistically significant.

Table 1 demonstrates that the mean age distribution of diabetic patients was about 58.75, that of healthy subjects was about 22.9, and that of COVID-19 recovered patients was about 27.9. The age range was from 23 to 70 years of age.

Table 2 illustrates that the majority of patients were vaccinated; around 76.7% of the subjects were fully vaccinated, and about 23.3% were partially vaccinated.

Table 3 demonstrates the average value of furin levels estimated through ELISA. The mean value is greater in diabetic and COVID-19 recovered subjects as compared to

Table 1: Mean age distribution of the subjects

Groups	*n*	Minimum	Maximum	Mean	SD
DM	20	38	97	58.75	12.871
Healthy	20	21	28	22.9	1.553
COVID-19 recovered	20	21	52	27.3	7.630

Table 2: Distribution of the subjects based on vaccination status

Vaccination status	Groups			Total
	DM	Healthy	COVID-19 recovered	
Fully				
Count	18	15	13	46
%	90.0%	75.0%	65.0%	76.7%
Partial				
Count	2	5	7	14
%	10.0%	25.0%	35.0%	23.3%
Total				
Count	20	20	20	60
%	100.0%	100.0%	100.0%	100.0%

Chi-square value -3.54, *P* -0.17

Table 3: Comparison of the ELISA readings among the groups

Groups	*n*	Minimum	Maximum	Mean	SD	*P*
DM	20	0.136	0.637	0.399	0.141	0.075
Healthy	20	0.169	0.747	0.322	0.126	
COVID-19 recovered	18	0.154	0.749	0.425	0.158	

normal healthy subjects. The mean ELISA readings of the three groups of diabetic, healthy, and COVID-19 recovered subjects were 0.399, 0.126, and 0.158, respectively.

DISCUSSION

Furin is a 794 amino acid, calcium-dependent multidomain enzyme. It has catalytic and *P* domains presented in the experimental 3D structures. On the N-terminal side, the protein also contains a so-called prodomain that acts as an intramolecular chaperone and assists in the proper folding of the enzyme. This prodomain was reported to regulate furin activity *in vitro* and *in vivo*.[13,14]

The role of furin in various viral infections like HIV-1, the Ebola virus, and the Hong Kong influenza virus is well established.[15,16] The acquisition of the infectious capacity and/or cell-cell spread of various viruses requires the maturation of the viral cell surface glycoproteins by furin.[17-19]

Furin could cleave the SARS-CoV-2 S protein. The binding of the S protein to the ACE2 receptor requires several proteolytic cleavages and conformational changes, like any other enzyme-receptor interaction. The cleavage of the S protein (cleavage motif PRRAR) is performed by the enzyme furin at the S1/S2 site. This cleavage is expected to promote disordering of the S protein and then exposure of a domain (the RBD domain) critical for ACE2 binding. S protein processing can occur independently of furin, although the presence of this protease significantly increases cleavage.[20-22]

The present study on the estimation of furin levels present in saliva was conducted on 60 subjects of various age groups. The age range of the subject population is wide, and statistical analysis is largely affected by the smallest change. However, the expression of furin has not.

Furin mRNA expression has been described mainly in oral epithelial cells through the scRNA-Seq technique by Zhong M *et al.*[23] The spinous layer in all examined tissues turned out to have large numbers of furin-positive cells. The percentage of furin-positive cells in the lip, tongue, and gingiva was higher than that of buccal and palatal mucosa. gastrointestinal tract manifestations. In the context of COVID-19, it was suggested that furin could cleave the SARS-CoV-2 S protein. The expression of ACE2 and furin in oral epithelial cells is present at both mRNA and protein levels. As furin is responsible for the maturation of the insulin pro-receptor, one could speculate that more furin in the circulation reflects a compensatory mechanism to increase the synthesis of active insulin receptors.[30,31] Another possible mechanism of action of furin in DM development may be via pancreatic beta cells, as furin has been demonstrated to control the proliferation and differentiation of pancreatic beta cell lines, as well as involvement in the maturation of insulin secretory granules.[32]

The present study demonstrates the age distribution across a wide range. There is only little-known data about furin upregulation in aging. The study conducted by AbdelMassih AF *et al.*[33] proved that furin is implicated in vascular aging and that the serum levels of furin are positively correlated with age dependent atherosclerosis. The above relationship might explain the increased deaths from COVID-19 among the elderly. Serum furin levels might serve as an independent predictor of COVID-19 complications in at-risk groups.

This study clearly presents evidence of increased levels of furin in salivary secretion and the susceptibility of diabetic patients to oral mucosal lesions and associated symptoms.[34] The acquisition of a polybasic cleavage site by the new SARS-CoV-2 seems to be an important feature for the endothelial penetrability and pathogenicity of COVID-19.

Screening salivary and serum levels of furin early in the course of COVID-19 complications might serve as an

important strategy to anticipate poor outcomes and prevent them.

CONCLUSION

The present study on the estimation of the furin enzyme in unstimulated saliva was the first ever to be attempted. Furthermore, significant expression of furin was discovered in saliva, and its increased expression in diabetic patients suggests the possibility of COVID-19 transmission through the oral mucosa and increased susceptibility. This provides a new insight into future prevention strategies and clinical care. More evidence is still needed to reinforce the current findings from the study and its application strategies.

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Conflicts of interest
There are no conflicts of interest.

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