ACE2 levels are altered in comorbidities linked to severe outcome in COVID-19

Subtitle:

ACE2 serum levels are increased in metabolic disease

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Key Points

Question: Severity of outcome in COVID-19 is disproportionately higher among the obese,

males, smokers, those suffering from hypertension, kidney disease, coronary heart disease

(CHD) and/or type 2 diabetes (T2D). Thus, we asked if the coronavirus SARS-CoV-2 receptor

ACE2 was altered in the sera from these high-risk groups?

Findings: In a single center population-based study of 5457 Icelanders, the Age,

Gene/Environment Susceptibility Reykjavik Study (AGES-RS), we find that ACE2 levels are

significantly elevated in serum from smokers, obese and diabetic individuals, while reduced in

males.

Meaning: These results demonstrate that individuals with comorbidities associated with

severe outcome in COVID-19 have altered serum levels of ACE2 that may influence productive

infection of SARS-CoV-2 in these individuals.

Abstract

Aims: Severity of outcome in COVID-19 is disproportionately higher among the obese, males, smokers, those suffering from hypertension, kidney disease, coronary heart disease (CHD) and/or type 2 diabetes (T2D). We examined if serum levels of ACE2, the cellular entry point for the coronavirus SARS-CoV-2, were altered in these high-risk groups.

Methods: Associations of serum ACE2 levels to hypertension, T2D, obesity, CHD, smokers and males in a single center population-based study of 5457 Icelanders from the Age, Gene/Environment Susceptibility Reykjavik Study (AGES-RS) of the elderly (mean age 75±6 years).

Results: Smokers, males, and individuals with T2D or obesity have altered serum levels of ACE2 that may influence productive infection of SARS-CoV-2 in these high-risk groups.

Conclusion: ACE2 levels are upregulated in some patient groups with comorbidities linked to COVID-19 and as such may have an emerging role as a circulating biomarker for severity of outcome in COVID-19.

Introduction

The current coronavirus disease 2019 (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. SARS-CoV-2 and other SARS coronaviruses interact with the receptor ACE2[2], to enter the host cell. SARS-CoV-2 binds ACE2 with a 10- to 20-fold higher affinity than other SARS coronaviruses do[3]. Furthermore, when the SARS-CoV-2 spike protein S binds ACE2, concomitant shedding of the receptor promotes cellular entry of the virus[4]. COVID-19 is associated with major respiratory failure with highest rates of severe outcome among the elderly, males and/or those with an underlying chronic disease including but not limited to coronary heart disease (CHD), hypertension, kidney disease, type 2 diabetes (T2D) and obesity ($kg/m^2 > 30$)[5-7]. This is particularly concerning for the more economically developed countries which have high prevalence of obesity and T2D[8].

While lower survival in COVID-19 might be attributed to the frailty and vulnerabilities of the high-risk patient groups mentioned above, other underlying factors could play role. For instance, variable levels and/or activity of the membrane bound receptor ACE2[2], and its soluble counterpart may contribute to this susceptibility. Just how the balance between the levels of soluble ACE2 and its membrane-bound receptor counterpart is maintained, has still to be fully defined. We postulate that productivity of the SARS-CoV-2 infection may vary among individuals depending on their endogenous levels of circulating ACE2. To address this, we examined ACE2 levels in serum from persons with hypertension, obesity, T2D or CHD and other relevant phenotypes, in a population of 5457 individuals aged 65 and above.

Methods

Study population

Participants aged 66 through 96 were from the Age, Gene/Environment Susceptibility Reykjavik Study (AGES-RS) cohort[9], a single-center prospective population-based study aimed to understand aging in the context of gene-environment interactions. The AGES-RS was approved by the NBC in Iceland (approval number VSN-00-063), and by the National Institute on Aging Intramural Institutional Review Board (U.S.) and the Data Protection Authority in Iceland.

Study design and statistical analysis

The following co-morbidities and characteristics were investigated in multiple regression analysis for an effect on ACE2 levels: age, sex, and body mass index (BMI) (kg/m^2) categories. Type 2 diabetes was defined as fasting serum glucose >7.0 mmol/L or self-reported history of diabetes or the use of insulin or oral glucose-lowering drugs. Hypertension was investigated using four different variables; systolic blood pressure >140 or diastolic blood pressure >90, use of ARB medications and ACE inhibitors as well as any other hypertension medications. Information on current smoking was obtained from questionnaires. CHD was ascertained using hospital records and/or cause of death information. A CHD event was defined as any occurrence of myocardial infarction, ICD-10 codes: I21-I25, coronary revascularization (either CABG-surgery or PTCA intervention) or death from CHD. Chronic obstructive pulmonary disease (COPD) was defined according to hospital records ICD-10 code: J44, medications and questionnaire. Estimated glomerular filtration rage (eGFR) was calculated from age, sex, and serum creatinine. Means and standard deviations were calculated for continuous characteristics and numbers and percentages for categorical characteristics. The Framingham Risk Score is a multivariable risk function that estimates an individuals 10-year CVD risk[10].

Protein measurements

Serum protein levels of ACE2 was measured in 5457 individuals from the AGES-RS using the

aptamer-based protein profiling platform[11]. Various metrics related to the performance of the

proteomic platform including aptamer specificity, assay variability and reproducibility have

already been described[11]. ACE2 was investigated on a log2 scale, where a one-unit increase

represents a doubling in levels on the RFU scale. A multiple linear regression with log2-ACE2

levels as the dependent variable was performed with adjustments for the clinical covariates

mentioned above. Observed levels of ACE2 were graphically presented by BMI-categories using

violin plots. A P-value <0.05 was considered statistically significant in hypothesis testing.

Results

Results from multiple linear regression analysis with ACE2 levels (log2) as the outcome are

highlighted in **Table 1**. ACE2 serum levels were reduced in serum from males compared to

females (β = -0.0385, P < 0.001). In contrast, serum ACE2 levels were increased in overweight

(β = 0.0259, P = 0.004) and obese individuals (β = 0.0246, P = 0.036) when compared to lean

(BMI < 25) individuals. ACE2 levels measured higher in severely obese (β = 0.0331, P = 0.087),

but did not reach significance most likely because few individuals are severely obese in this

cohort of old people. The violin plots in **Figure 1** demonstrate elevated levels (log2 scale) of

ACE2 in serum in response to increasing adiposity presented by different BMI categories. Both

individuals with impaired fasting glucose levels (β = 0.0295, P < 0.001) and those with

established T2D (β = 0.0377, P = 0.003) showed higher ACE2 levels compared to those with no

T2D or with normal glucose levels (**Table 1**). Serum levels were significantly increased in

current smokers (β = 0.0299, P = 0.018), but were not associated with CHD, COPD or estimated

glomerular filtration rate (eGFR) (**Table 1**). Given many of the clinical traits and covariates listed in **Table 1** are components of the Framingham risk score (FRS), we examined the association of the FRS with ACE2 levels. Here, ACE2 serum levels were positively associated with the FRS ($\beta = 0.0726$, $P = 0.008$).

Discussion

The severity of outcome in COVID-19 is greatly influenced by various comorbidities, unhealthy lifestyle and being a male[5-7]. We observed an increase in circulating levels of ACE2 in some of these groups including smokers, obese and diabetics and found that ACE2 levels were positively correlated with the FRS. In contrast, ACE2 levels were decreased in serum from males compared to females, thus showing an opposite effect from that of the comorbidities associated with COVID-19. Intriguingly, studies have shown that estrogen levels may induce ACE2 activity and levels in females[12]. However, these results do not agree with a recent study showing higher plasma levels of ACE2 in male patients suffering from heart failure[13]. Our observation that ACE2 levels were elevated in smokers agrees well with a study showing upregulation of ACE2 mRNA in the respiratory tract of smokers as well as rodents exposed to cigarette smoke[14].

ACE2 is a membrane-bound ectoenzyme that is released into the circulation *via* ectodomain shedding[15]. Variable levels of ACE2 in serum is likely a result of genetic factors, differential gene expression and/or ectodomain shedding influenced by disease or administration of drugs *via* negative or positive feedback loops of the renin-angiotensin system[16]. As proteins in circulation emanate from virtually every tissue of the body[11], we cannot easily determine how much each tissue contributes to ACE2 release into blood. For instance, high serum ACE2 levels may not mirror higher: activity or abundances of its membrane bound counterpart in pulmonary tissues.

However, we show that serum levels of ACE2 are altered in some frequent comorbidities and other related phenotypes associated with severity of outcome in COVID-19.

Limitations

The findings showing higher ACE2 levels in the serum of smokers, diabetic and obese individuals warrant further validation in independent study populations. This work does not show that altered ACE2 serum levels in these patient groups causes worse clinical outcomes in COVID-19, though that is a possibility. The results presented may be limited to that of serum and may not reflect the effect of these comorbidities on ACE2 levels in pulmonary tissues, but lung injury is the key component linked to outcome severity in COVID-19. Furthermore, the measured circulating levels of ACE2 may not reflect its cell surface receptor levels in solid tissues. Finally, all participants of the AGES-RS are white (Caucasians) which may limit the transferability and generalizability of the results as ACE2 levels differ across races and ethnicities[17].

Conclusions

Individuals that are males, smokers, manifest with diabetes or obesity are at high risk of developing severe outcome from COVID-19. In case of the smokers, obese and diabetics, the increased risk could partly be explained by the vulnerability of these patient groups. We find that serum levels of ACE2 are altered in all these groups. However, while serum ACE2 levels were increased in smokers, diabetic and obese individuals, males had reduced ACE2 serum levels. These results encourage further efforts in defining the relationship between soluble ACE2 levels and the membrane bound ACE2 form in solid tissues.

Acknowledgment

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Figure Legends

Figure 1. The y-axis represents log2-transformed distribution of ACE2 levels in the serum while

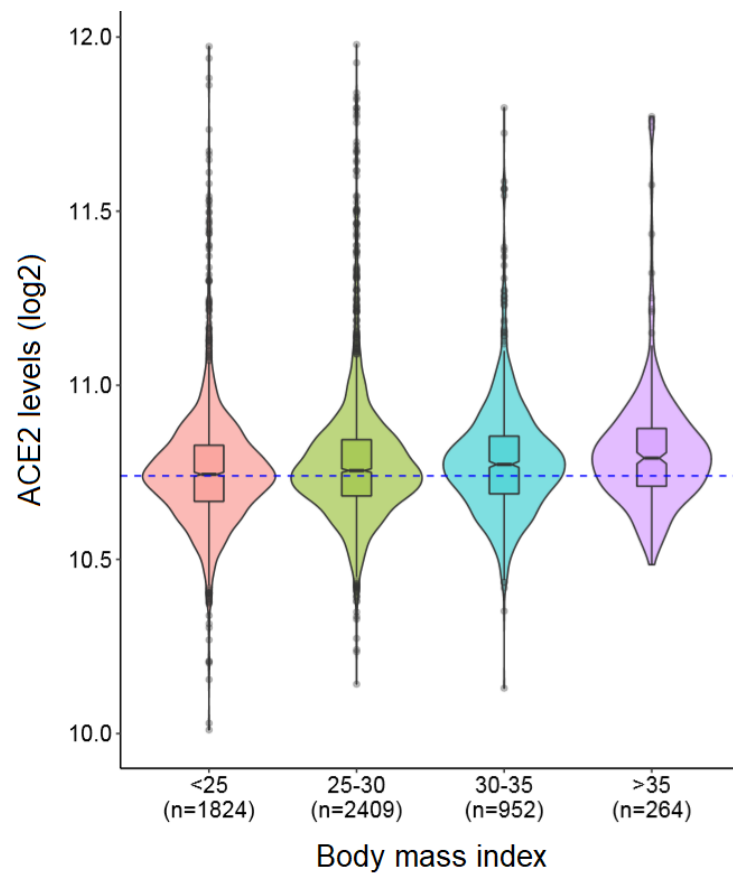the x-axis shows different categories of BMI (kg/m^2), also expressed in Table 1.

Table 1. Results from a multiple linear regression analysis with ACE2 levels (log2) as the dependent variable

Characteristic[a]	β coefficient	Std. Error	t value	95% CI		P -value	
Age (years)	-0,0014	0,0008	-1,90	-0,0029	0,0000	0,057	
Sex (females ref.)	0,0385	0,0084	4,60	0,0221	0,0550	<0.001	***
BMI (kg/m^2) (<25 reference)						0.022 [b]	*
Overweight, 25-30	0,0259	0,0090	2,88	0,0083	0,0435	0,004	**
Obese, 30-35	0,0246	0,0118	2,10	0,0016	0,0477	0,036	*
Severly obese, >35	0,0331	0,0193	1,71	-0,0048	0,0710	0,087	
T2D status (glucose<5.6 mmol/L reference)						<0.001 [b]	***
Impaired fasting glucose 5.6-6.9 mmol/L	0,0295	0,0085	3,45	0,0127	0,0462	<0.001	***
T2D	0,0377	0,0128	2,94	0,0126	0,0629	0,003	**
Hypertension							
Systolic bp. >140 or diastolic bp.>90	-0,0085	0,0078	-1,10	-0,0237	0,0067	0,273	
ARBs	-0,0344	0,0124	-2,78	-0,0586	-0,0102	0,005	**
ACE inhibitors	-0,0156	0,0127	-1,22	-0,0405	0,0093	0,221	
Other hypertension medication	-0,0089	0,0094	-0,94	-0,0274	0,0096	0,346	
Smoking status (never reference)						0.044 [b]	*
Former	0,0137	0,0086	1,59	-0,0032	0,0305	0,111	
Current	0,0299	0,0127	2,36	0,0051	0,0549	0,018	*
CHD	-0,0040	0,0098	-0,41	-0,0232	0,0152	0,685	
COPD	-0,0186	0,0121	-1,54	-0,0422	0,0051	0,123	
eGFR (per 10 mL/min/1.73m^2)	0,0024	0,0024	1,00	-0,0023	0,0071	0,315	

[a] Phenotypes definitions are provided in Methods. Age in years 76.6(5.6); 57.3% females; Impaired fasting glucose 5.6-6.9 (n=1983; Mean BMI 27.1(4.4), T2D (N=660); Hypertension (n=2739); ARBs users (n=753), ACEIs users (n=699); Other antihypertensive users (n=1991); Never smoker (n=2253), former smoker (n=2405), current smoker (n=655); CHD (n=1217); COPD (n=644); eGFR mL/min/1.73m2 = 75.0(17.3)

[b] P- value from a F-test

* P-value<0.05, ** P-value < 0.01, *** P-value <0.001

Figure 1