Interactions between the principal risk factors for reduction of the eGFR in unvaccinated COVID-19 survivors: Normal pre-COVID-19 eGFR, not having diabetes and being hospitalized

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Abstract. There are contradictory results regarding changes in estimated glomerular filtration rate (eGFR) in coronavirus disease 2019 (COVID-19) survivors. An analysis of eGFR changes and clinical characteristics associated with those changes was conducted among COVID-19 survivors. eGFR values were compared at different time points (before and 4-, 8- and 12-months after COVID-19 infection). A multivariate generalized linear mixed model (GENLINMIXED procedure) with a binary logistic regression link was used to determine factors associated with eGFR reduction of ≥10 ml/min/1.73 m^2. Being hospitalized (RR=2.90, 95% CI=1.10-7.68, P=0.032), treated with Ivermectin (RR=14.02, 95% CI=4.11-47.80, P<0.001) or anticoagulants (RR=6.51, 95% CI=2.69-15.73, P<0.001) are risk factors for a reduced eGFR. Having a low eGFR (<90 ml/min/1.73 m^2) before COVID-19 infection, having B-positive blood type, diabetes, taking vitamin C during the acute phase of COVID-19 or suffering from chronic COVID-19 symptoms, were identified as protective factors. Analysis involving a two-way interaction (A x B, where A and B are factors) demonstrated that the combination of patients with a normal eGFR value before COVID-19 infection without diabetes (RR=58.60, 95% CI=11.62-295.38, P<0.001), or a normal eGFR value with being hospitalized for COVID-19 (RR=38.07, 95% CI=8.68-167.00, P<0.001), increased the probability of a reduced eGFR. The changes in eGFR in COVID-19 survivors varied depending on patient characteristics. Furthermore, the principal risk factors for post-COVID-19 eGFR reduction were analyzed in separate models.

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Introduction

The coronavirus disease 2019 (COVID-19) mainly affects the respiratory system, although it affects every organ system (1). Extrapulmonary involvement includes dysregulation of the immune system, metabolic complications and adverse effects on various organs of the cardiovascular, renal, nervous, endocrine, musculoskeletal and other systems (1). The majority of infections are self-limiting, with patients returning to their usual state of health 12-14 days after receiving a positive test result, but 20% of symptomatic, infected, unvaccinated adults need hospitalization (2).

In the acute stage of COVID-19, kidney involvement is very common among patients that are hospitalized, with acute kidney injury (AKI) occurring in 15-28% of all intensive care unit admissions (3,4). COVID-19 has been hypothesized to affect the kidney by direct mechanisms, such as viral entry, local inflammation/complement activation and glomerulopathy (5). The kidney can also be affected indirectly, using

nephrotoxic drugs, sepsis, systemic inflammation and hypercoagulability with thromboembolic disease, among others (4). After the acute phase, a number of symptoms and effects on various organs prevail (such as fatigue, shortness of breath, chest pain and digestive problems among others), which is why the term 'chronic COVID-19' or 'long COVID-19' was coined, which describes the chronic impact of COVID-19 at all levels, although it is generally used to indicate symptomatology persisting for >12 weeks after infection (1). While AKI can lead to chronic kidney disease (CKD), little is known about the long-term effects of COVID-19 on kidney function (4).

The estimated glomerular filtration rate (eGFR) from serum creatinine is a common clinical indicator that physicians use as a pragmatic reference to kidney function (6). The longitudinal changes in eGFR after suffering from COVID-19 has been a subject of study with contradictory results, reporting that it did not cause changes (7), caused slight reduction (8), or that the changes were heterogeneous between patients (9). The aim of the present cohort study was to examine the changes in eGFR after one year post infection (4-, 8- and 12-months after), compared with eGFR before infection in a cohort of COVID-19 survivors (hospitalized or treated at home during the acute phase), and whether these changes are associated with any clinical characteristics of the patient.

Materials and methods

Study subjects. The study subjects consisted of patients with a mean age of 50.0±14.3 years, comprising 56.3% females and 43.7% males. Additional demographic data can be found in Table SI. A prospective cohort study was conducted with patients that sought medical consultation at the General Hospital [number 1 of the Mexican Institute of Social Security Institute (IMSS), (Colima, Mexico)] between 28th September 2020 and 30th December 2020. Patients had COVID-19-associated symptoms and had a positive diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from reverse transcription-PCR. Patients included in the present cohort received at-home ambulatory care or usual hospital treatment. Patients that were asymptomatic and paucisymptomatic were not included in the present study as previously defined (2). The inclusion criteria for the present study were: i) Patients were over the age of 18-years old; ii) patients were symptomatic during the acute phase of COVID-19; iii) patients were suffering from COVID-19 for the first time; and iv) patients were non-vaccinated for COVID-19. In addition, patients were required to have routine clinical laboratory tests to estimate the GFR from serum creatine within 2-months before COVID-19 infection. These tests were part of routine care unrelated to acute illness or pregnancy, ensuring a more accurate assessment of kidney function changes associated with COVID-19. The exclusion criteria of the present study were: i) Patients that were pregnant; and ii) patients that were undergoing renal replacement therapy treatment before infection with COVID-19. The present cohort study only included patients that survived the COVID-19 infection. Patients that did not survive during the acute stage of the disease or within the first 4-months after COVID-19 infection were not considered in the analysis. In addition, patient data were excluded after any of the following events: i) The patient decided to withdraw from the study voluntarily; ii) the patient was diagnosed with COVID-19 for the second time; or iii) the patient had a positive pregnancy result. The study was approved by the local health research committee of the IMSS-Colima, Mexico (approval no. R-2020-601-041). Inclusion in the study was voluntary, and each patient or their legal representative signed an informed consent letter in cases where the patient could not sign.

Measures and follow-up. Data were collected and entered into medical records when patients sought medical care due to COVID-19 infection. Universal variables (such as age, sex and education), pathological/non-pathological personal history (such as blood type and comorbidities) and signs and symptoms associated to COVID-19 were collected. Other collected data included treatment and hospital admissions. The patients overall self-assessment or symptom severity score was recorded for each patient routinely (part of the standard care protocol during the patients' interactions with the healthcare system) as previously described [0-10-point Visual Analog Scale; from 'very well' (a score of 0) to 'very poorly' (a score of 10)] (1,9). Patients were evaluated at 4-, 8-, and 12-months after COVID-19 (time periods following the initial COVID-19 infection/positive result). The primary aim of the present study was to determine the eGFR, according to the MDRD-4 formula or Levey equation (10), by establishing a baseline value for eGFR before COVID-19 infection (within 2 months before the onset of the illness). Subsequently, the study aimed to measure eGFR at 4, 8 and 12 months following COVID-19 infection. An eGFR of <90 ml/min/1.73 m^2 was considered a low eGFR value. The persistence of symptoms ('long COVID') was also evaluated according to the previous definition (continuation of COVID-19-related symptoms beyond the acute phase of the illness) (1).

Sample size. The sample size for the present study was calculated based on a previous report that followed the eGFR for 6-months in patients with a disease that affects renal function (diabetes), finding that 71.7% of patients had a reduction in the eGFR while the eGFR for 28.3% of patients remained unchanged (11). In total, 20 patients from each group (with and without reduction) were needed to reach the required power (0.8). Once the study was completed, the statistical power of detecting an eGFR reduction at 12-months post-COVID-19 (≥10 ml/min/1.73 m^2) in patients with normal vs. low eGFR pre-COVID-19 was calculated, resulting in 85.4%.

Statistical analysis. Data are expressed as percentages or mean ± standard deviation. The normal distribution of the data was verified using the Kolmogorov-Smirnov test. Fisher's exact tests and Cochran-Mantel-Haenszel chi-square tests were used to compare qualitative variables across multiple periods of time. Fisher's exact test was selected when the expected cell counts were anticipated to be ≤5 in >20% of the cells within a category (12). Two-way mixed ANOVAs, followed by the Bonferroni post hoc test, were used to compare the eGFRs across different evaluation periods and assess the differences between the pre-COVID-19 and 12-months post-COVID-19 periods. Univariate and multivariate binary logistic regression analyzes were used to determine the probability of developing low eGFR at the pre-COVID-19 time (binomial outcome: Yes

or no) with the presence of different general or clinical characteristics of the patients (transversal analyzes in pre-COVID-19). The data were summarized as odds ratios (ORs) with 95% confidence intervals (CIs) and P-values (cross-sectional analyzes in pre-COVID-19 data). For longitudinal analysis, the change from the baseline eGFR (pre-COVID-19) was used to examine the absolute differences between the post-COVID-19 evaluation periods. Pearson correlations were used for bivariate analyzes (eGFR of the baseline and the change in eGFR) in various strata of the patients studied. The area under the receiver operating characteristic (ROC) curve, CI, cut-off point, sensitivity and specificity of the eGFR in the pre-COVID-19 period was calculated to discriminate patients that would develop a decrease in the eGFR during the post-COVID-19 period.

For association analyzes, multivariate generalized linear mixed models (GENLINMIXED in SPSS (version 20; IBM Corp.) with separate random intercepts and a binary logistic regression link were used, as previously reported (13,14). The longitudinal nature of the data was accounted for through two random variables: i) The pandemic time points (pre-COVID-19 or post-COVID-19); and ii) the month of survey (months 1-12, ordinal scale), which is an indicator of time separation between the two different time-points. The target variable was the dichotomic reduction of eGFR (≥10 ml/min/1.73 m^2; yes or no). The fixed effects include continuous variables (age and body mass index) and dichotomous variables divided as yes or no for various pre-COVID-19 and COVID-19 clinical characteristics (high blood pressure and B-positive blood type). Analysis involving a two-way interaction (A x B, where A and B are factors) between the principal risk factors for the reduction of the eGFR post-COVID-19 were made in separate models. The aim was to obtain the marginal risk result from the aforementioned model, in which the binomial regression parameters of multivariate analysis were summarized as relative risk (RR) with a 95% CI and P-values. CinCalc version 1 (https://clincalc.com/stats/Power.aspx) (15) was used to calculate statistical power and sample size. The rest of the analyzes were performed with SPSS Statistics version 20 (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. In total, 311 patients were screened, of which 99 were included because the patients had an eGFR test 2-months before experiencing COVID-19 for the first time. During post-COVID-19 infection follow-ups, all patients remained for the first 4-months; at 8-months, 88 patients were analyzed; and 77 patients completed 12-months of follow-up. Not all patients completed the year of follow-up because, after 4-months, the patient either decided to voluntarily abandon the study and to not continue with the periodic evaluations (n=14), or the patient suffered from COVID-19 for the second time (n=8). As all patients had at least two measurements (pre-COVID-19 and 4-months post-COVID-19), the 99 patients were included in the analysis. Table SI presents the main personal history, clinical characteristics during and after COVID-19, and eGFR at different follow-up periods.

Table I. Relative risk from multivariate generalized linear mixed model with binary logistic regression link of various pre-COVID-19 and COVID-19 clinical characteristics to have a reduction of eGFR (≥10 ml/min/1.73 m^2) during the first year after acute COVID-19.

A, Pre-COVID-19 characteristics

		95% CI		
Covariate	AdRR	Lower	Upper	P-value
Low eGFR[a]	0.09	0.03	0.25	<0.001
Age, years	0.98	0.96	1.01	0.322
Female	0.59	0.27	1.27	0.178
High school[b]	0.57	0.30	1.08	0.086
B blood type[c]	0.29	0.10	0.85	0.024
BMI	0.96	0.91	1.02	0.191
Diabetes	0.06	0.02	0.17	<0.001
HBP	1.88	0.78	4.54	0.158
Smoking	0.59	0.24	1.41	0.232
Alcohol	2.17	0.97	4.87	0.059

B, COVID-19 disease characteristics

		95% CI		
Covariate	AdRR	Lower	Upper	P-value
During acute phase				
High symptoms[d]	0.81	0.37	1.75	0.589
Hospitalized	2.90	1.10	7.68	0.032
Para/NSAIDs	0.58	0.01	23.82	0.774
Antivirals	1.20	0.51	2.84	0.672
Antibiotics	0.49	0.19	1.26	0.139
Ivermectin	14.02	4.11	47.80	<0.001
Steroids	0.83	0.43	1.61	0.575
Anticoagulants	6.51	2.69	15.73	<0.001
Vitamins				
B complex	0.51	0.14	1.88	0.308
C	0.23	0.06	0.83	0.025
D	0.58	0.12	2.87	0.500
Long COVID[e]	0.24	0.09	0.65	0.005

[a]eGFR value <90 ml/min/1.73 m^2. [b]High school or higher education (reference; incomplete high school or less). [c]B-positive blood type; B and AB groups (reference; A and O groups). [d]≥8 symptom severity score using an analog scale from 0 to 10 points. [e]Symptoms of COVID-19 persisting for >12 weeks after infection. BMI, body mass index; HBP, high blood pressure; AdRR, adjusted relative risk; CI, confidence interval; Para/NSAIDs, paracetamol/non-steroidal anti-inflammatory drugs; COVID-19, coronavirus disease 2019; eGFR, estimated glomerular filtration rate.

Risk factors for a low eGFR pre-COVID-19. In the population analyzed, having a low eGFR (<90 ml/min/1.73 m^2) pre-COVID-19 was associated with age, arterial hypertension and smoking (Table SII). After multivariate analysis, it was

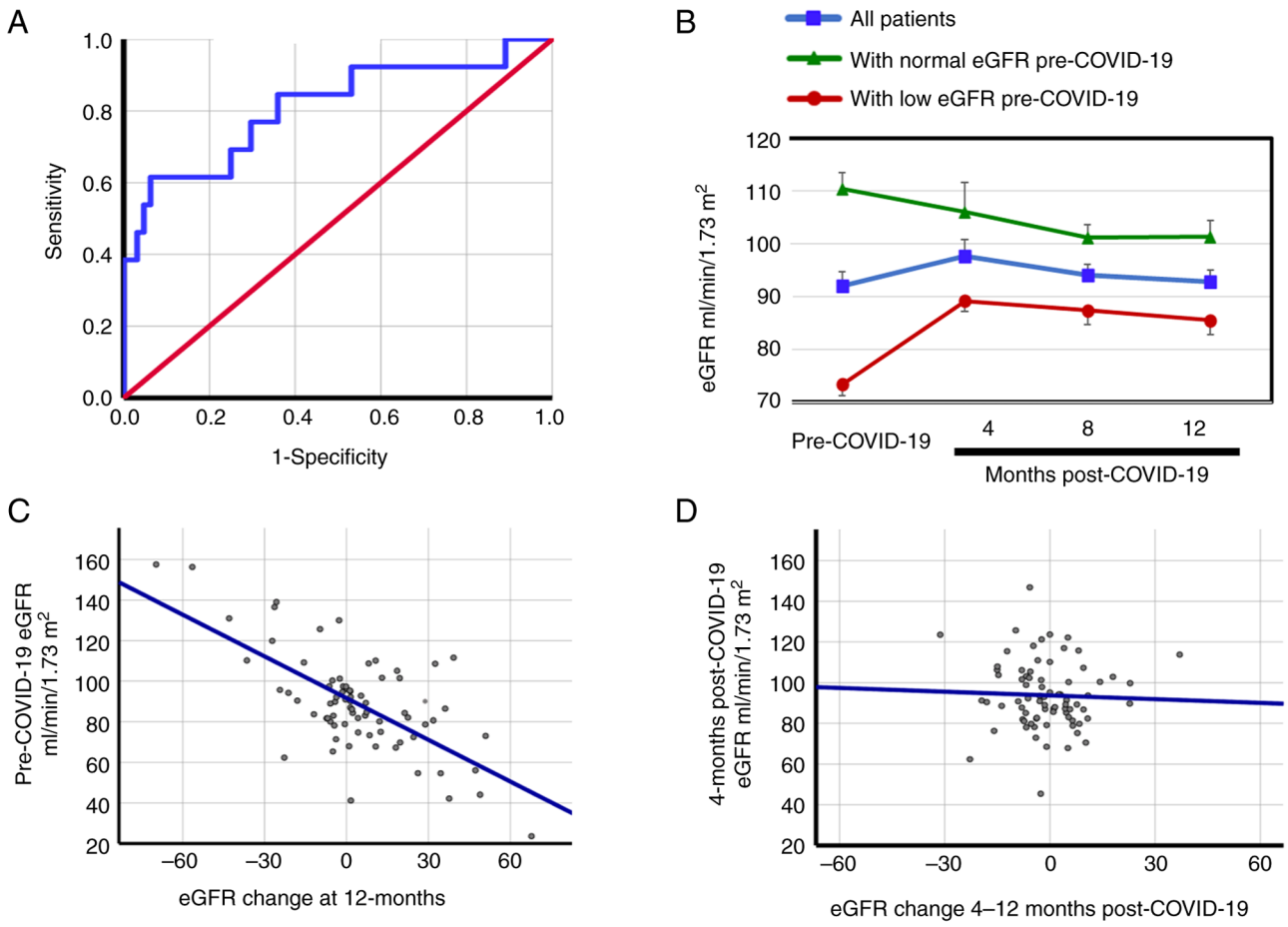

Figure 1. Impact of COVID-19 on eGFR and long-term implications. (A) Response receiver operating characteristic curve evaluating the predictive ability of pre-COVID-19 eGFR in subsequent eGFR reduction. (B) Follow-up of eGFR reduction over time among survivors. Patients with normal (≥90 ml/min/1.73 m²) vs. low eGFR (<90 ml/min/1.73 m²) pre-COVID-19 were different in their eGFR values throughout the follow-up period (P<0.001, linear mixed effects model test). Pre-COVID-19 (n=99); 4-months post-COVID-19 (n=99); 8-months post-COVID-19 (n=88); 12-months post-COVID-19 (n=77). (C) Correlation between eGFR reduction and the observed change at 12-months post-COVID-19. (D) Change generated in eGFR values between 4- and 12-months after COVID-19 infection. These subfigures provide insights into the impact of COVID-19 on renal function and its long-term implications. COVID-19, coronavirus disease 2019; eGFR, estimated glomerular filtration rate.

revealed that only hypertension (OR=2.91, 95% CI=1.02-8.32, P=0.047) and smoking (OR=3.52, 95% CI=1.27-9.72, P=0.015) increased the risk, by ~3x, of having a low eGFR (Table SII).

Risk factors for a reduction in the eGFR post-COVID-19. Table I presents the factors associated with a reduced eGFR of ≥10 ml/min/1.73 m² during the first year after acute COVID-19. The multivariate analysis indicated that being hospitalized (RR=2.90, 95% CI=1.10-7.68, P=0.032), being treated with Ivermectin (RR=14.02, 95% CI=4.11-47.80, P<0.001) or anticoagulants (RR=6.51, 95% CI=2.69-15.73, P<0.001) were factors that increased the risk of a reduced eGFR during the first year post-COVID-19. By contrast, a low eGFR (<90 ml/min/1.73 m²) before the disease (RR=0.09, 95% CI=0.03-0.25, P<0.001), having a B-positive blood type (RR=0.29, 95% CI=0.10-0.85, P=0.024), being diabetic (RR=0.06, 95% CI=0.02-0.17, P<0.001), taking vitamin C during the acute phase of COVID-19 (RR=0.23, 95% CI=0.06-0.83, P=0.025) or suffering from chronic symptoms of COVID-19 (RR=0.24, 95% CI=0.09-0.65, P=0.005), were protective factors that reduced the probability of a reduced eGFR post-COVID-19 (Table I).

The pre-COVID-19 eGFR (baseline eGFR value) was a significant predictor of the loss of renal function post-COVID-19. The area under the ROC curve for pre-COVID eGFR as a predictor of the reduction in eGFR (≥10 ml/min/1.73 m²) one year after COVID-19 was 0.81 (95% CI=0.66-0.96, P<0.001) with a pre-COVID-19 eGFR cut-off of 89.9 ml/min/1.73 m² (Fig. 1A), and sensitivity of 0.85 and a specificity of 0.39. This suggests that a negative result (an eGFR <89.9 ml/min/1.73 m²) can be an adequate 'rule-out' test for post-COVID-19 eGFR reduction (16).

In Table II the proportion of patients with a reduced eGFR (≥10 ml/min/1.73 m²) across three follow-up periods within the initial year post-acute COVID-19 infection, categorized by diverse clinical characteristics, is presented. A comprehensive statistical analysis was undertaken to understand these results, employing distinct tests to reveal differences within table cells. For each category, a careful selection of statistical tests was made to ensure the accuracy and relevance of the analysis. It was observed at 12-months post-COVID-19 (Table II), the largest difference between the proportion of patients with reduced eGFR was between patients with normal vs. low pre-COVID-19 eGFR (30.6 vs. 4.9%, respectively). Other

Table II. Number and proportion of patients with reduced eGFR values (≥10 ml/min/1.73 m^2), compared with pre-COVID-19 values, in three periods during the first year after acute COVID-19 according to diverse clinical characteristics.

	Month after COVID-19						
	4 (n=99) eGFR reduction, n (%)		8 (n=88) eGFR reduction, n (%)		12 (n=77) eGFR reduction, n (%)		
Clinical characteristics	No	Yes	No	Yes	No	Yes	P-value[a]
All	82 (82.8)	17 (17.2)	68 (77.3)	20 (22.7)	64 (83.1)	13 (16.9)	
Pre-COVID-19 low eGFR[b]							
No	34 (68.0)	16 (32.0)	26 (60.5)	17 (39.5)	25 (69.4)	11 (30.6)	<0.001[c]
Yes	48 (98.0)	1 (2.0)	42 (93.3)	3 (6.7)	39 (95.1)	2 (4.9)	
B blood type[d]							
No	75 (81.5)	17 (18.4)	62 (75.6)	20 (24.4)	59 (81.9)	13 (18.1)	0.049[c]
Yes	7 (100.0)	0 (0.0)	6 (100.0)	0 (0.0)	5 (100.0)	0 (0.0)	
Diabetes							
No	49 (77.8)	14 (22.2)	38 (69.1)	17 (30.9)	34 (75.6)	11 (24.4)	<0.001[c]
Yes	33 (91.7)	3 (8.3)	30 (90.9)	3 (9.1)	30 (93.8)	2 (6.3)	
Hospitalized							
No	41 (85.4)	7 (14.6)	33 (82.5)	7 (17.5)	32 (94.1)	2 (5.9)	0.039[e]
Yes	41 (80.4)	10 (19.6)	35 (72.9)	13 (27.1)	32 (74.4)	11 (25.6)	
Ivermectin							
No	70 (83.3)	14 (16.7)	60 (78.9)	16 (17.2)	54 (81.8)	12 (18.2)	0.824[c]
Yes	12 (80.0)	3 (20.0)	8 (66.7)	4 (33.3)	10 (90.9)	1 (9.1)	
Anticoagulant							
No	50 (87.7)	7 (12.3)	40 (85.1)	7 (14.9)	37 (90.2)	4 (9.8)	0.005[e]
Yes	32 (76.2)	10 (23.8)	28 (68.3)	13 (31.7)	27 (75.0)	9 (25.0)	
Vitamin C							
No	55 (84.6)	10 (15.4)	45 (77.6)	13 (22.4)	42 (80.8)	10 (19.2)	0.901[e]
Yes	27 (79.4)	7 (20.6)	23 (76.7)	7 (23.3)	22 (88.0)	3 (12.0)	
Long COVID[f]							
No	24 (80.0)	6 (20.0)	19 (79.2)	5 (20.8)	18 (90.0)	2 (10.0)	0.899[e]
Yes	58 (84.0)	11 (16.0)	49 (76.6)	15 (23.4)	46 (80.7)	11 (19.3)	

[a]P-value that resulted from comparing the groups of patients that had, or did not have, a decreased eGFR compared with their pre-COVID-19 value, according to the presence or absence of a certain factor (pre-COVID-19 low eGFR, B blood type, diabetes, hospitalized, long COVID, or the use during the acute stage of COVID-19 of ivermectin, anticoagulant, or vitamin C). All time periods (4-, 8- and 12-months after COVID-19 infection) were simultaneously analyzed and statistical tests were performed on 2x6 contingency tables. [b]eGFR <90 ml/min/1.73 m^2. [c]Fisher's exact test. [d]B-positive blood type; B and AB groups (in contrast to A and O groups). [e]Cochran-Mantel-Haenszel chi-square test analysis. [f]Symptoms of COVID-19 persisting for >12 weeks after infection. Each stratum (with or without a clinical characteristic), in each time period, was considered as 100%, in order to make comparisons of the proportion of patients that reduced their eGFR values between those that did or did not have a clinical characteristic in particular. COVID-19, coronavirus disease 2019; eGFR, estimated glomerular filtration rate.

variables with apparent differences in the proportion of patients with a reduced eGFR were: i) The presence or absence of a B positive blood type; ii) diabetes; iii) hospitalization; and iv) the use of anticoagulants during acute COVID-19 (Table II).

In Table III shows the differences between the pre-COVID-19 and 12-months post-COVID-19 periods. Using the absolute values of the eGFRs before and 12-months after COVID-19 infection, no changes were observed when all patients were considered [92.1±26.7 vs. 92.9±19.3 ml/min/1.73 m^2, respectively (P=1.000)] (Table III), which could suggest that COVID-19 does not influence this variable. However, in patients with a normal pre-COVID-19 eGFR (>90 ml/min/1.73 m^2), the eGFR was significantly reduced 12-months post-COVID-19 compared with pre-COVID-19 [101.4±18.3 vs. 110.4±22.4 ml/min/1.73 m^2, respectively (P<0.001)]. By contrast, in patients with a low pre-COVID-19 eGFR, the eGFR increased from 73.4±15.5 to 85.5±17.2 ml/min/1.73 m^2 pre-COVID-19 and 12-months post-COVID-19, respectively (P<0.001). The presence or absence of other variables did not seem to influence the eGFR levels between the pre-COVID-19 and 12-months post-COVID-19 periods.

Table III. eGFR (ml/min/1.73 m^2) before and 12-months after COVID-19 according to diverse clinical characteristics.

Clinical characteristic	Pre-COVID-19	12-months after COVID-19 infection	P-value[a]
All	92.1±26.7	92.9±19.3	1.000
Pre COVID-19 low eGFR[b]			
No	110.4±22.4	101.4±18.3	<0.001
Yes	73.4±15.5	85.5±17.2	<0.001
B blood type[c]			
No	92.3±27.6	94.4±18.3	0.998
Yes	87.1±23.2	83.2±10.6	1.000
Diabetes			
No	97.7±28.0	93.9±18.1	1.000
Yes	82.2±21.4	91.4±21.1	0.999
Hospitalized			
No	95.2±17.1	95.6±18.3	0.998
Yes	89.5±33.1	90.7±19.9	0.993
Ivermectin			
No	92.9±28.2	94.0±19.5	1.000
Yes	87.5±17.7	86.6±18.1	1.000
Anticoagulants			
No	94.3±26.4	95.3±19.9	0.836
Yes	89.1±27.5	90.3±18.7	0.897
Vitamin C			
No	92.8±27.8	91.6±20.1	1.000
Yes	90.7±25.4	95.8±17.8	0.998
Long COVID[d]			
No	93.8±23.2	92.3±15.4	1.000
Yes	90.9±28.4	92.1±20.1	1.000

[a]Two-way mixed ANOVA followed by the Bonferroni's post hoc test were used for the comparisons (pre-COVID-19 vs. 12-months after COVID-19 infection). [b]eGFR <90 ml/min/1.73 m^2. [c]B-positive blood type; B and AB groups (reference; A and O groups). [d]Symptoms of COVID-19 persisting for >12 weeks after infection. COVID-19, coronavirus disease 2019; eGFR, estimated glomerular filtration rate.

Significance of considering the eGFR value before COVID-19 as the baseline value. Fig. 1B indicates that patients with low eGFRs pre-COVID-19 have increased eGFRs 12-months post-COVID-19 infection compared with pre-COVID-19 [85.5±17.2 vs. 73.4±15.5 ml/min/1.73 m^2, respectively (P<0.001)], while the eGFRs were reduced in patients with normal eGFR pre-COVID-19 (110.4±22.4 ml/min/1.73 m^2 pre-COVID-19 vs. 101.4±18.3 ml/min/1.73 m^2 12-months post-COVID-19, P<0.001). However, when the changes in the eGFRs only in the post-COVID-19 stages were considered, taking the 4-months post-COVID-19 eGFRs as the baseline, the changes in eGFRs were not significant in any of the subgroups of patients. For example, the 4- and 12-months post-COVID-19 eGFRs in the patients with normal eGFRs pre-COVID-19 were 106.1±16.2 vs. 101.4±18.3 ml/min/1.73 m^2, respectively (P=0.207), and in the patients with low eGFRs pre-COVID-19 the 4- and 12-months post-COVID-19 eGFRs were 89.2±14.8 vs. 85.5±17.2 ml/min/1.73 m^2, respectively (P=0.126). Additionally, Fig. 1C indicates that there is a high correlation between pre-COVID-19 eGFR and the change in eGFR at 12-months post-COVID-19 (r=-0.664; P<0.001). This lost significance when the eGFR at 4-months post-COVID-19 was correlated with the change generated between 4- and 12-months post-COVID-19 (r=-0.039; P=0.369) (Fig. 1D). The previous results indicated variations in the effects of the COVID-19 infection on the eGFR value among survivors. The eGFR changes depended on the value used as the baseline for the comparison (such as the pre-COVID-19 or the 4-months post-COVID-19 eGFR) and subsequently, its clinical implications.

Interactions between variables that affect changes in the eGFR. The high correlation between the pre-COVID-19 eGFR and the change in the eGFR 12-months post-COVID-19 may be influenced by diabetes or whether the patient was hospitalized during COVID-19. Fig. 2 presents that the correlations between the eGFR pre-COVID-19 with the eGFR change at 12-months are maintained in patients without diabetes (Fig. 2A; r=-0.754; P<0.001) and in patients that were hospitalized (Fig. 2B; r=-0.764; P<0.001). This was not observed in patients with

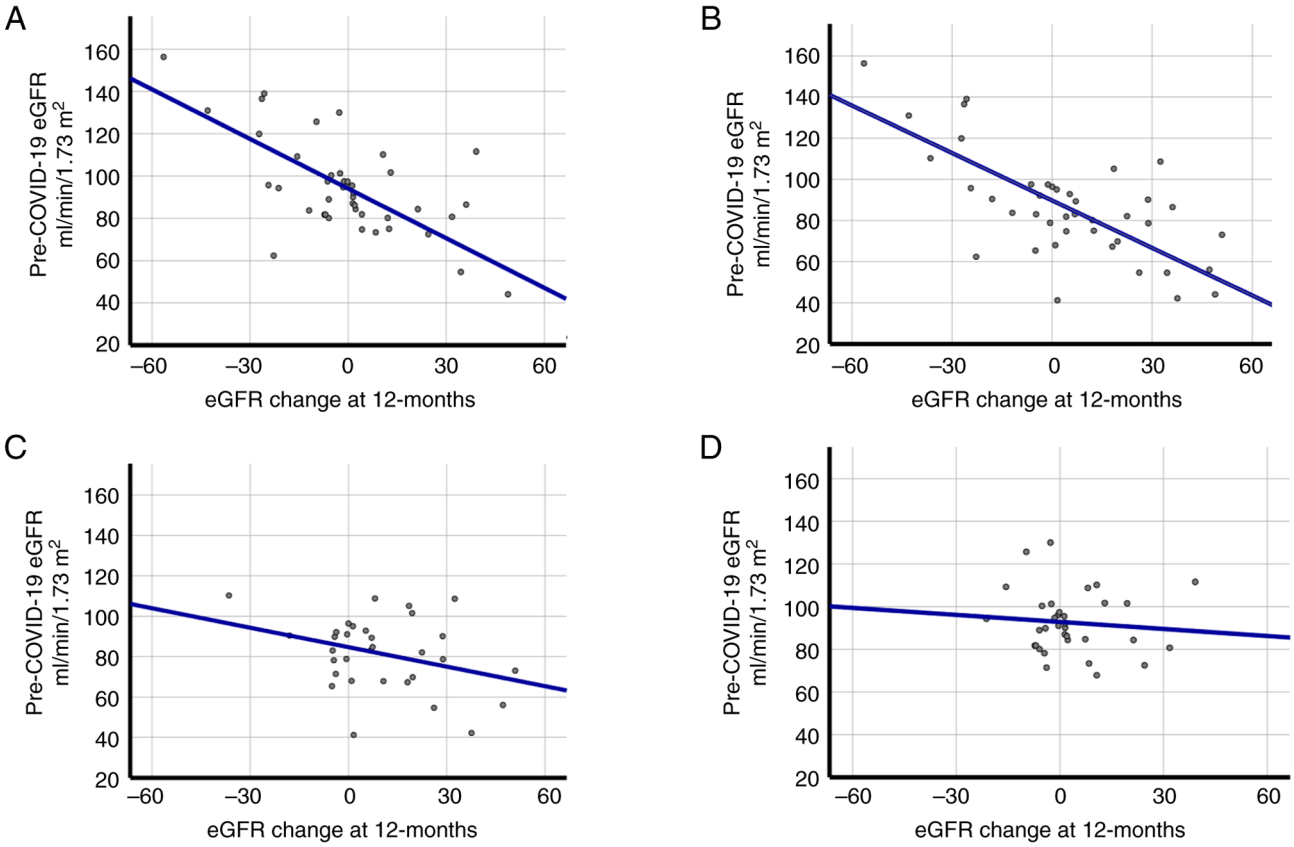

Figure 2. Correlation between pre-COVID-19 eGFR and eGFR change at 12-months post-COVID-19. (A) Correlation between pre-COVID-19 eGFR and the eGFR change at 12-months post-COVID-19 in patients without diabetes (r=-0.754; P<0.001). (B) Correlation between pre-COVID-19 eGFR and the eGFR change at 12-months post-COVID-19 in patients that were hospitalized (r=-0.764; P<0.001). (C) Correlation between pre-COVID-19 eGFR and the eGFR change at 12-months post-COVID-19 in patients with diabetes (r=-0.330; P=0.065). (D) Correlation between pre-COVID-19 eGFR and the eGFR change at 12-months post-COVID-19 in patients that were not hospitalized (r=-0.096; P=0.589). COVID-19, coronavirus disease 2019; eGFR, estimated glomerular filtration rate.

diabetes (Fig. 2C; r=-0.330; P=0.065) and in patients that were not hospitalized (Fig. 2D; r=-0.096; P=0.589).

Additionally, a multivariate generalized linear mixed model with a binary logistic regression link was performed to confirm the interactions between diverse characteristics and reduced eGFR (≥10 ml/min/1.73 m^2) during the first year after acute COVID-19 (Table IV). Analysis involving a two-way interaction (A x B, where A and B are factors) indicated that the combination of patients with a normal pre-COVID-19 eGFR without diabetes (RR=58.60, 95% CI=11.62-295.38, P<0.001) or with being hospitalized for COVID-19 (RR=38.07, 95% CI=8.68-167.00, P<0.001), or patients without diabetes combined with being hospitalized (RR=11.17, 95% CI=1.95-64.05, P=0.007), had a higher risk compared with the outcome of the variables separately. Fig. 2 and Table IV suggested a strong interaction between the principal risk factors (normal pre-COVID eGFR, without diabetes and hospitalization in acute COVID-19).

Discussion

The present cohort study revealed that the changes in the eGFR among unvaccinated COVID-19 patients 1 year after the infection vary depending on the clinical characteristics of the patient. The main risk factors associated with a decreased eGFR of ≥10 ml/min/1.73 m^2 were: i) Having a normal eGFR value before COVID-19; ii) not having diabetes; and iii) being hospitalized. While an increased eGFR was observed in patients with: i) Low pre-COVID-19 eGFR; and ii) diabetes and no hospitalization. Due to the observed variation in the longitudinal changes in the eGFR during the first year of COVID-19 infection and its potential clinical implications for kidney health, changes in the eGFR should probably be evaluated using a pre-COVID-19 eGFR value instead of only using values post-infection which may falsely indicate no changes or improvements in kidney function.

The present study revealed that, 1 year after the initial COVID-19 infection, the majority of patients with a significantly reduced eGFR had a normal eGFR pre-COVID-19 (30.6%), compared with the 4.9% of patients with a low eGFR pre-COVID-19. By contrast, patients with a low eGFR before COVID-19 infection (73.4±15.5 ml/min/1.73 m^2) had a significantly (P<0.001) increased eGFR 12-months after COVID-19 infection (85.5±17.2 ml/min/1.73 m^2). Additionally, having diabetes, was a protective factor against a reduced eGFR value while not having diabetes was a risk factor.

These results, which initially seemed to contradict what is currently known about traditional risk factors for changes in eGFR and kidney disease, could be explained by variations in the expression of angiotensin-converting enzyme 2 (ACE2)

Table IV. Relative risk from multivariate generalized linear mixed model with binary logistic regression link (models without or involving a two-way interaction) of principal risk factors to have a reduction of eGFR (≥10 ml/min/1.73 m^2) during the first year after acute COVID-19.

A, Principal risk factors in a model without interactions

Risk factors to reduction of eGFR				95% CI		
Pre-COVID-19 eGFR	Diabetes	Hospitalized	AdRR	Lower	Upper	P-value
Normal[a]	-	-	11.55	4.53	29.45	<0.001
-	No	-	6.78	2.48	18.53	<0.001
-	-	Yes	6.17	2.39	15.96	<0.001

B, Model involving a two-way interaction (pre-COVID-19 eGFR and diabetes)

Risk factors to reduction of eGFR				95% CI		
Pre-COVID-19 eGFR	Diabetes	Hospitalized	AdRR	Lower	Upper	P-value
Normal[a]	No	-	58.60	11.62	295.38	<0.001
Normal[a]	Yes	-	6.34	1.22	32.91	0.028
Low[b]	No	-	3.93	0.78	19.67	0.097

C, Model involving a two-way interaction (pre-COVID-19 eGFR and hospitalization)

Risk factors to reduction of eGFR				95% CI		
Pre-COVID-19 eGFR	Diabetes	Hospitalized	AdRR	Lower	Upper	P-value
Normal[a]	-	Yes	38.07	8.68	167.00	<0.001
Low[b]	-	Yes	1.70	0.32	9.16	0.533
Normal[a]	-	No	4.14	1.06	16.31	0.042

D, Model involving a two-way interaction (diabetes and hospitalization)

Risk factors to reduction of eGFR				95% CI		
Pre-COVID-19 eGFR	Diabetes	Hospitalized	AdRR	Lower	Upper	P-value
-	No	Yes	11.17	1.95	64.05	0.007
-	Yes	Yes	1.03	0.17	6.34	0.968
-	No	No	1.22	0.21	6.83	0.818

Analysis involving a two-way interaction (A x B, where A and B are factors). All models were adjusted by age, sex, body mass index, high blood pressure, diabetes, normal/low pre-COVID-19 eGFR, hospitalization during COVID-19 in acute phase and long COVID (symptoms of COVID-19 persisting for >12 weeks after infection). [a]eGFR ≥90 ml/min/1.73 m^2. [b]eGFR <90 ml/min/1.73 m^2. AdRR, adjusted relative risk; CI, confidence interval; COVID-19, coronavirus disease 2019; eGFR, estimated glomerular filtration rate.

in patients with diabetes and CKD. ACE2 is an enzyme attached to the membranes of cells in the lungs, arteries, heart, kidney and intestines, which serves as a cell-surface receptor and is the entry point into cells for coronaviruses, including SARS-CoV-2 (17). Higher expression levels of ACE2 in patients with comorbidities such as cardiovascular disease, chronic obstructive pulmonary disease and diabetic pancreatic islets increase the susceptibility of contracting SARS-CoV-2 infection and subsequent COVID-19 severity (18). However, it has been demonstrated that the expression levels of ACE2 in the kidney is reduced in its glomerular and tubular region in patients with diabetes, as well as in various nephropathies (diabetes, hypertension and lupus), as well as in chronic kidney disease (18). This could explain why patients living with

diabetes and CKD before COVID-19 infection have a protective factor for reduced kidney function once they survived COVID-19. This is due to the lower number of receptors for the virus (ACE2) that they have in their kidneys. However, kidney damage and diabetes are factors for greater disease severity and mortality in COVID-19 (2), which was not analyzed in the present study.

The increased eGFR post-COVID-19 in the patients with a low eGFR before COVID-19 should be taken with caution since it may not necessarily reflect an improvement in renal function since it is known that an increase in eGFR could be a mechanism for kidney damage in several clinical conditions (2) (AKI, dehydration and hyperfiltration in diabetes). Therefore, only evaluating eGFR after COVID-19 infection may not be the most appropriate way to assess kidney function in surviving patients as it could increase after COVID-19 and be interpreted as a normal or improved function, instead the pre-COVID-19 eGFR (baseline) should also be evaluated. The findings of the present study demonstrated that patients that were hospitalized had a significantly increased reduction of eGFR compared with patients that were not hospitalized, which is consistent with previous reports (19,20). Furthermore, the present study revealed that the use of anticoagulants and Ivermectin during the acute illness increased the risk of reducing renal filtration during the first year after suffering from COVID-19 (6 and 14 times, respectively) (Table I). Coagulopathy with COVID-19 disease is widely reported, and the use of anticoagulants has been established to combat this disorder (17,21). It is probable that in the present study, the use of anticoagulants was associated with the reduction of post-COVID-19 eGFR, not because of the drug itself, but because of the probable hypercoagulability present in the patients that conditioned the use of anticoagulants. Ivermectin has been proposed and used to treat COVID-19 in different demographic groups. Clinical trials have not demonstrated significant beneficial effects (22), although its usefulness is still under discussion (23). However, although Ivermectin has been postulated to be safe for COVID-19 treatment, there is debate since it is well-known that it can cause adverse effects (24). Studies in rats demonstrated that Ivermectin can compromise kidney and liver integrity (25,26).

The present study demonstrated for the first time that Ivermectin can cause affectation at the glomerular filtration level in patients with COVID-19. However, studies with a larger number of patients are needed to confirm this finding.

By contrast, the use of vitamin C was a protective factor, which reduced the probability of lowering eGFR post-COVID-19 by >4 times. Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. It is an antioxidant and acts as a scavenger of free radicals, giving it anti-inflammatory properties. Vitamin C serves a crucial role in modulating cellular immunity and maintaining vascular integrity (27,28). Animal trials have demonstrated that vitamin C can prevent kidney damage caused by Ivermectin administration (27,29). In experiments with rabbits treated with Ivermectin and vitamin C, there was a decrease in serum urea, reducing a number of the adverse effects of Ivermectin (30). As a result, a number of studies strongly recommend coadministration of vitamin C when prescribing Ivermectin (27,28).

Vitamin C has been recommended for patients with COVID-19 as it may act as a protective factor against glomerular filtration loss caused by COVID-19 or associated pathophysiological processes. It could also reduce the toxicity of drugs such as Ivermectin (31,32). Additionally, vitamin C stimulates endothelial cell proliferation, prevents apoptosis and preserves endothelial function while enhancing nitric oxide generation (33).

In addition to other factors, blood type was also analyzed in the present study. Recent research indicates that individuals with blood type B may have different immune responses and susceptibility patterns to viral infections compared with other blood groups (34). In the present study, it was observed that having blood type B (B and AB groups) was correlated with being a protective factor against the loss of eGFR. This is consistent with previous studies that demonstrate that this blood group is protective for long-COVID-19 (1,35). It has also been demonstrated that patients with blood type B/AB exhibited a longer median time to end-stage renal disease compared with patients with blood type O/A (36), which suggests that the B blood group antigen may have a protective effect against the progression of IgA nephropathy. This association could potentially be associated with its influence on the inflammatory status of the patient (36). However, further research is necessary to fully understand the effects of blood type B on kidney function.

A strength of the present study was that the pre-COVID-19 data was considered as the baseline to analyze the longitudinal changes of eGFR post-illness, which helped to assess those changes with more certainty. As presented in Fig. 2, taking only post-COVID-19 data, as has been performed, to the best of our knowledge, in the majority of studies analyzing renal function (7,8), could lead to incorrect interpretations. Failure to stratify the population would also lead to inaccurate results. For example, if the data of the entire population was used and compared before and after a year of COVID-19, no changes would be observed (92.1±26.7 vs. 92.9±19.3 ml/min/1.73 m^2, P=1.000) in the eGFR, which is not correct for all subgroups of patients. The present study also had limitations, mainly the number of patients, a lack of urinary protein/albumin detection before and after COVID-19, and other additional markers of inflammation or coagulopathy that would have enriched the work. These aspects would be desirable to consider in future research.

In summary, the changes in the eGFR associated to COVID-19 infection in unvaccinated patients were highly variable and depended on the characteristics of the patient. Considering an eGFR value before COVID-19 as a baseline for the comparison appeared to be crucial for the interpretation of the results. Other factors were also identified as increasing the chance of reducing the eGFR (such as the use of Ivermectin or anticoagulants), or as protective factors (such as vitamin C treatment or B blood type). These factors interact with each other to further increase risk. Renal function in COVID-19 survivors is a relevant topic that requires further investigation. Identifying characteristics of those patients with changes in eGFR after COVID-19 may help prioritize which patients need close outpatient follow-up post-pandemic. An eGFR <90 ml/min/1.73 m^2 cannot diagnose CKD, especially for patients with an eGFR between 60 and 90 ml/min/1.73 m^2 in the absence of albuminuria; however, patients with the

preclinical manifestation of kidney damage should not be overlooked as albumin in urine was not measured. Future studies are required to answer these questions.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JGE and IDE participated in the conception of the study. KILA, JAGS and MICR participated in the acquisition of the data for the work. JDM, JGOO, EMZ, VM, VGCM, HRTL JAGS, MROC, GAHF, FRL, MICR and IDE participated in the design of the study, and HRTL, JAGS, MROC, GAHF, KILA and IDE participated in the analysis/interpretation of the data. JGE, JDM, KILA, MROC and IDE drafted and revised the article. JGE, VGCM, KILA, JAGS, MICR and IDE provided intellectual content of critical importance to the work described. All authors read and approved the final version of the manuscript and confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study was approved by the local health research committee of the IMSS-Colima, Mexico (approval no. R-2020-601-041) on the 24th September 2020. Inclusion in the study was voluntary, and each patient or their legal representative signed an informed consent letter in cases where the patient could not sign.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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