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ORIGINAL ARTICLE

GrimAge is elevated in older adults with mild COVID-19 an exploratory analysis

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Abstract COVID-19 has been contained; however, the side effects associated with its infection continue to be a challenge for public health, particularly for older adults. On the other hand, epigenetic status contributes to the inter-individual health status and is associated with COVID-19 severity. Nevertheless, current studies focus only on severe COVID-19. Considering that most of the worldwide population developed mild COVID-19 infection. In the present exploratory study, we aim to analyze the association of mild COVID-19 with epigenetic ages (HorvathAge, HannumAge, GrimAge, PhenoAge, SkinAge, and DNAmTL) and clinical variables obtained from a Mexican cohort of older adults. We found that all epigenetic ages significantly differ from the chronological age, but only GrimAge is elevated. Additionally, both the intrinsic epigenetic age acceleration (IEAA) and the extrinsic epigenetic age acceleration (EEAA) are accelerated in all patients. Moreover, we found that immunological estimators and DNA damage were associated with PhenoAge, SkinBloodHorvathAge, and HorvathAge, suggesting that the effects of mild COVID-19 on the epigenetic clocks are mainly associated with inflammation and immunology changes. In

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conclusion, our results show that the effects of mild COVID-19 on the epigenetic clock are mainly associated with the immune system and an increase in GrimAge, IEAA, and EEAA.

Keywords Epigenetic clocks · Aging · COVID-19 · DNA damage · Epigenetic age acceleration · Immune system

Introduction

The COVID-19 pandemic caused by SARS-CoV-2 (a positive-stranded RNA virus belonging to the *Coronaviridae* family) had severe repercussions at different levels, including socioeconomic and public health [1, 2]. It was first observed when unexplained pneumonia cases were noted in Wuhan, China [3]. We now know that the virus can spread from an infected person through liquid particles from aerial vias such as mouth or nose when they cough, sneeze, speak, sing, or breathe—clinical manifestations of COVID-19 range from a minor illness to a severe infection, including long COVID-19 symptoms. Older adults (> 60 years) with comorbidities were the most affected population, with a significant risk of developing a severe illness and dying [4], so much so that the World Health Organization (WHO) recommended home lockdown and avoidance of contact with others for this age group [1].

Despite the recommended restrictions, the WHO reported that lower–middle–income countries accounted for 52% of excess deaths worldwide among persons aged ≥ 60 years, with an annual excess mortality rate of 1039 per 100,000 people. This means that older adults in low- and middle-income countries were disproportionately affected by COVID-19 [5]. An important number of risk factors and biological pathways contribute to COVID-19 severity in older adults; among them stands out age-related changes in the immune system, increased risk of underlying health chronic conditions (heart disease, diabetes, hypertension, and cancer) as well as reduced access to healthcare, just to mention few examples [5].

On the other hand, various studies have shown a link between epigenetics and aging. Epigenetic mechanisms (DNA methylation (MeDNA), acetylation, ncRNA's, histone modifications, and 3D genome architecture) represent the connection between external stimuli (environment and genetic mechanisms) without changing genome sequence [6]. Nevertheless, most of the current longevity epigenetic epidemiologic studies focus on the analysis of MeDNA patterns due to its stability, robustness, and technology available for its analysis. Moreover, analysis of the MeDNA patterns in the genome has shown a correlation with mortality. Research groups around the world have developed the so-called *epigenetic clocks* [7–9] that show to be adequate biomarkers for aging outcomes, with interesting associations between diseases such as lung, prostate, and breast cancer [10–12], Alzheimer's disease [13, 14], diabetes and metabolic syndrome [9, 15, 16], and cardiovascular disease [17–19] as well as, with several social variables such as education [20–22], income [20, 23, 24], and occupation [24, 25].

As previously mentioned, epigenetic status contributes not only to the inter-individual health status during aging but is also associated with the unexplained risk factors of COVID-19 severity. In this sense, the epigenetic landscape of COVID-19 patients has been studied by several groups worldwide, finding different epigenetic signatures associated with the infection [26]. Nevertheless, to date, there are few studies with epigenetic ages (HorvathAge, HannumAge, PhenoAge, GrimAge, among others), and some of them have shown that SARS-CoV-2 affected the normal pattern of MeDNA on immune cells and that severe COVID-19 was associated with an increased epigenetic age acceleration and mortality risk of GrimAge [27]. These results were confirmed by Cao et al. [28], who compared five epigenetic clocks using whole blood of COVID-19 patients and healthy individuals, showing an interesting stratification between GrimAge and the severity of COVID-19. Moreover, Calzari L. et al. found a significantly increased acceleration of GrimAge in patients with severe infection compared with mild prognosis in an Italian cohort study of patients with different comorbidities [29].

However, till today, results are quite controversial since Franzen et al. [30] reported that COVID-19 did not alter epigenetic ages. In addition, Wenchang X et al. reported on a Mendelian randomization analysis that COVID-19 can slow the acceleration of GrimAge [31]. In this context, the primary limitations of the previously mentioned studies are small samples, bias due to training statistical analysis, heterogeneity in ages between participants, and the fact that only people from developed countries were studied. As well, these studies mostly consider severe and non-COVID-19. Hence, to give light on the matter

and considering that most of the worldwide population developed a mild infection of COVID-19, in the present exploratory study, we aim to understand the association of mild COVID-19 with epigenetic ages and clinical variables obtained from a Mexican cohort of older adults.

Methods

Study design and participants

Cohort description

This is a cross-sectional study with information from the sixth wave (2022) of the "Cohort of Obesity, Sarcopenia, and Frailty of Older Mexican Adults" (COSFOMA). COSFOMA procedures and methods have previously been described in detail by Sánchez-García et al. [41]. Briefly, COSFOMA is a population-based prospective study that began in 2014, where participants were randomly selected from a Mexican public healthcare institute administrative record. In the first round, 1252 adults aged 60 years or more who were beneficiaries of the Mexican Institute of Social Security (IMSS, by its Spanish acronym) in Mexico City were included in the COSFOMA. A refreshment of the sample in 2019, where 1192 participants were added to the cohort. Finally, in 2022, 751 participants of COSFOMA showed up for follow-up. This cohort collected clinical and sociodemographic information annually through interviews conducted by trained health staff.

Sample collection

Two blood samples were obtained from all participants by venipuncture of the median cubital vein using the vacutainer system (EDTA). The samples were divided and classified according to use for DNA damage analysis and methylome analysis, and the second for clinical biochemical profile measurements. We collected a total of 47 blood samples for this study (two from each participant).

Clinical variables

Sociodemographic variables included sex, age, years of education, and living arrangements. Health-related variables included current smoking, current alcohol consumption, multimorbidity, number of medications currently used, use of antidepressant medications, and frailty (Linda Fried score index). Multimorbidity was defined as having two or more diseases from a predefined list of conditions, namely [32, 33] hypertension, diabetes, heart disease, cancer, kidney failure, cerebrovascular disease, arthritis, chronic liver disease, and chronic pulmonary disease. Insomnia was assessed by using Athena's scale according to [34].

Biochemical profile

We performed the following standard hematological tests and performed the following standard metabolic tests for glucose (mg/dL), according to the World Health Organization (WHO) recommendations [35], and cholesterol (mg/dL), creatinine (mg/dL), and albumin (mg/dL) levels, according to [36].

COVID-19 severity status assessment

Infection by COVID-19 was assessed by a telephone interview with the following questions:

- B1. Did the participant get sick with COVID-19? Yes/no
- B2. If the patient already had COVID-19, was he/she infected for a second time? Yes/no

The mexican clinical guide: "*Guía clínica para el tratamiento de la COVID-19 en México*" was used to determine the severity of infection [37].

DNA damage (comet assay)

Whole blood (4 mL) was diluted 1:1 with sterile phosphate-buffered saline (PBS 1X) supplemented with fetal bovine serum (FBS) and carefully poured onto 3 mL of Ficoll-Paque (GE Healthcare Bio-Sciences, Uppsala, Sweden). Then, tubes were centrifuged for 30 min at 500×g (without brake). PMBCs were collected and washed with 5 mL PBS 1X supplemented with FBS and centrifuged again for 10 min at 200×g, twice. Then, the PMBCs were stored in 100 mL of cell culture medium (DMEM) supplemented with FBS (10% v/v) at −80 °C for further use, as previously reported [38].

DNA damage was evaluated by single-cell electrophoresis (comet assay) following the manufacturer's instructions (Abcam, # ab238544, Cambridge, UK)

with few modifications. Briefly, PBMC samples were thawed at RT and centrifuged at 700 × g for 5 min; the pellet was washed once in PBS 1X and then centrifuged; and the pellet was resuspended 1:10 (v/v) with agarose. Samples were transferred into the slide previously prepared with an agarose base layer. The slides were then incubated in a lysis buffer for 30 min at 4 °C in the dark. Samples were incubated in an alkaline solution for 30 min at 4 °C in the dark. Electrophoresis was performed in ice-cold TBE electrophoresis solution; parameters were set as follows (15 V for 15 min on ice). Slides were incubated with Vista Green DNA dye (488-/526-nm excitation/emission) at RT for 15 min and stored at 4 °C in a wet chamber in the dark. For visualization, slides were analyzed by confocal microscopy using a FITC filter at the Unidad de Imagenología at the Instituto de Fisiología Celular-UNAM. We analyzed a total of 100 cells from each sample. Images were analyzed using Open Comet plugin v.1.3.1 [39] from Fiji v.2.9.0 software [40], which estimates the DNA damage by the head (%HeadDNA) and tail length (%TailDNA) using the fluorescence intensity from each cell analyzed in the samples.

DNA methylation analysis

DNA isolation

The Genomic DNA of all participants was isolated from peripheral blood using the AutoGenFlex STAR (Auto-Gen, Holliston, MA, USA), and purity and integrity were verified by 260-/280-nm measurements (BioTek Instruments, Winooski, VT, USA) and by electrophoresis in 0.8% agarose gels stained with ethidium bromide.

Methylation analysis

According to the manufacturer's protocols, we used the Illumina Infinium MethylationEPIC BeadChip Array for subsequent hybridization. We used standard methods and then processed using the Illumina DNAm array platform and scanning device (iScan, Illumina) located at the Instituto Nacional de Medicina Genomica (INMEGEN) in Mexico City, Mexico. The EPIC array contains 866,836 CpG sites (> 90% of CpG sites found in the 27 K and 450 K arrays), respectively [41], particularly enriched for promoter and enhancer regulatory regions [42]. The ratio of intensities between methylated (signal A) and unmethylated (signal B) sites was calculated according to the intensity of the methylated (M corresponding to signal A) and unmethylated (U corresponding to signal B) sites, as the ratio of fluorescent signals according to [43]. All samples passed GenomeStudio quality control steps based on built-in control probes for staining, hybridization, extension, and specificity, and the bisulfite conversion efficiency was high (intensity signal > 4000). We followed the quality control procedure using the Partek Genomics Suite and Minfi package according to normal-exponential out-of-band (NOOB) [44], and we excluded low-quality probes (mean detection *P*-value ≥ 0.01), probes with overlapping single-nucleotide polymorphisms (SNPs) (minor allele frequency cutoff = 0.05), cross-hybridizing probes [44, 45], and probes mapping sexual chromosomes so that all subjects could be more easily compared leaving 765,808 CpG's positions for the current analysis. By slide and array position, batch effects were removed by the function champ.runCombat from Combat [46].

Epigenetic age estimation

For the estimation of the epigenetic age, we used five measures of epigenetic age (HorvathAge, HannumAge, PhenoAge, skinHorvath, and GrimAge) for each sample; we used methods and algorithms available through an online DNA methylation calculator [8, 47] (https://labs.genetics.ucla.edu/horvath/dnamage/). Additionally, we performed more estimations and accelerations with the *methylclock* R package from Bioconductor [48]. Briefly, Horvath's clock uses 353 CpGs from different tissues, as described in Horvath (2013). Alongside Horvath's clock, three other age-related biomarkers are computed: AgeAcDiff, which gives the difference between DNAmAge and chronological age; intrinsic epigenetic age acceleration (IEAA) captures the intrinsic biological age of immune cells, independent of age-related changes in immune cell populations in the blood obtained after regressing DNAmAge and chronological age by cell counts, extrinsic epigenetic age acceleration (EEAA) captures immune cell biological aging due to both intrinsic immune cell age and age-driven changes in immune cell populations obtained after regressing DNAmAge and chronological age. Hannum's clock is

estimated using 71 CpGs described in [7] from blood samples. Levine's clock (PhenoAge) is estimated using 513 CpGs described in [49] from blood samples. SkinHorvath's (also known as Horvath's skin and blood clock) clock is estimated using 391 CpGs described in [50] from skin and blood cells. Finally, GrimAge's clock is estimated using 1030 CpGs from blood plasma proteins related to morbidity and mortality and cigarette smoking, estimated by packs per year [51].

Statistical methods

The correlation among DNAmAges and clinical variables of the samples was calculated with the Pearson correlation coefficient. A linear regression model was applied to determine the relationship between the epigenetic ages, chronological age, and both age + sex as cofounding variables (Supplementary Table 1S). Statistical analyses were performed using R (version 3.6.2). P values < 0.05 were considered statistically significant. Multiple linear regression analysis was performed using the function "lm" in R (Package stats version 4.2.2). This function is used to fit linear models, including multivariate ones. Linear correlation was performed with Pearson's Product Moment correlation coefficient (Package *stats* version 4.2.2). The test statistic is based on Pearson's product moment correlation coefficient cor(x, y) and follows a t distribution with length(x)-2 degrees of freedom if the samples follow independent normal distributions. If there are at least four complete pairs of observations, an asymptotic confidence interval is given based on Fisher's Z transform.

Ethical statement

All participants and/or their legal guardians were informed of the research procedures and signed a letter of consent before participating. This project was approved by the National Committee for Scientific Research and the Ethics Committee on Health Research (CONBIOÉTICA-09-CEI-009-20160601) of IMSS (Registration No. 2020-785-108). The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) as well as guidelines from the Ley General de Salud of Mexico. The manuscript is in line with the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals and aimed for the inclusion of representative human populations (sex, age, and ethnicity) as per those recommendations. The data are available upon express request addressed to the corresponding author and are currently in safekeeping by IMSS.

Results

Characteristics of the cohort

As mentioned in the Materials and Methods section, a total of 47 samples were included in this study. The main results from the clinical, sociodemographic, biochemical, and molecular variables are summarized in Table 1.

Epigenetic ages are significantly different, but only GrimAge, EEAA, and IEAA are accelerated in older adults with mild COVID-19 symptoms

First, we aimed to summarize the effect of mild COVID-19 symptoms on the epigenetic ages of older adults and concomitantly to understand the correlation between the chronological age and the epigenetic ages (Fig. 1). Our results demonstrate that all the epigenetic ages (skinHorvathAge, 61.09 ± 3.76 y.o.; PhenoAge, 59.26 ± 5.69 y.o.; HorvathAge, 64.56 ± 4.68 y.o.; HannumAge, 59.65 ± 4.92 y.o.; and GrimAge, 74.94 ± 4.98 y.o., n = 47) are significantly different as compared with the chronological ages (69.04 ± 4.52, n = 47) (Fig. 1A). Regarding the correlation analysis, the results from the corrplot show a robust positive correlation between the chronological age and all the epigenetic ages, suggesting that when the chronological age increases, the epigenetic ages also increase. However, the PhenoAge (Levine) correlates less with the HorvathAge (Horvath). On the other hand, the PhenoAge is poorly correlated with the HannumAge, as are the GrimAge vs. PhenoAge (Fig. 1B), suggesting that the differences found in the first analysis may be related to the different domains that each epigenetic clock captures. Later, we performed an analysis to see whether mild COVID-19 symptoms accelerated

Table 1 Population description

Population description		
Variable	Mean ± SD	% (*n*)
Sociodemographics (*n* = 47)		
Sex		46.8 (22) M 53.2 (25) F
Age	69.02 ± 4.47	
Living arrangements		4.3 (2) alone 95.7 (45) with someone
Health disparities (*n* = 47)		
Smoking		44.7 (21) yes 55.3 (26) no
Alcoholism		100 (47) no
Multimorbidity		37 (17) no diseases 30.4 (15) one 10.9 (5) two 8.7 (4) three 4.3 (2) four 2.2 (1) five 4.3 (2) seven 2.2 (1) eight
Frailty phenotype		10.6 (5) not frail 74.5 (35) prefrail 14.9 (7) frail
Hypertension (HTN)		36.2 (17) yes 63.8 (30) no
Type 2 diabetes (T2D)		6.4 (3) yes 93.6 (44) no
Obesity		55.3 (26) yes
Insomnia		21.3 (10)
Biochemical markers (*n* = 47)		
Glucose	104.67 ± 23.08	
Total cholesterol	205.09 ± 49.07	
Albumin (units)	4.79 ± 0.43	
Creatinin (units)	3.91 ± 20.14	
DNA damage (*n* = 33)		
DNA damage (% tail DNA, X ± SD)	39.62 ± 19.89	
Epigenetic ages (*n* = 47)		
Horvath (HorvathAge)	64.56 ± 4.68	
Hannum (HannumAge)	59.65 ± 4.92	
Levine (PhenoAge)	59.26 ± 5.69	
SkinHorvathAge	61.09 ± 3.76	
GrimAge	74.94 ± 4.98	
DNAmTL	6.89 ± 0.17	

the epigenetic ages in our population. As can be seen in Fig. 1C, only three epigenetic ages (GrimAge, EEAA, and IEAA) are accelerated as compared to the chronological age, in comparison with the other epigenetic ages (skinHorvathAge, PhenoAge, HorvathAge, HannumAge).

Epigenetic ages are significantly correlated with the chronological age, but only the telomere length estimation is anticorrelated

Once we demonstrated that epigenetic ages and accelerations are significantly different, we attempted to

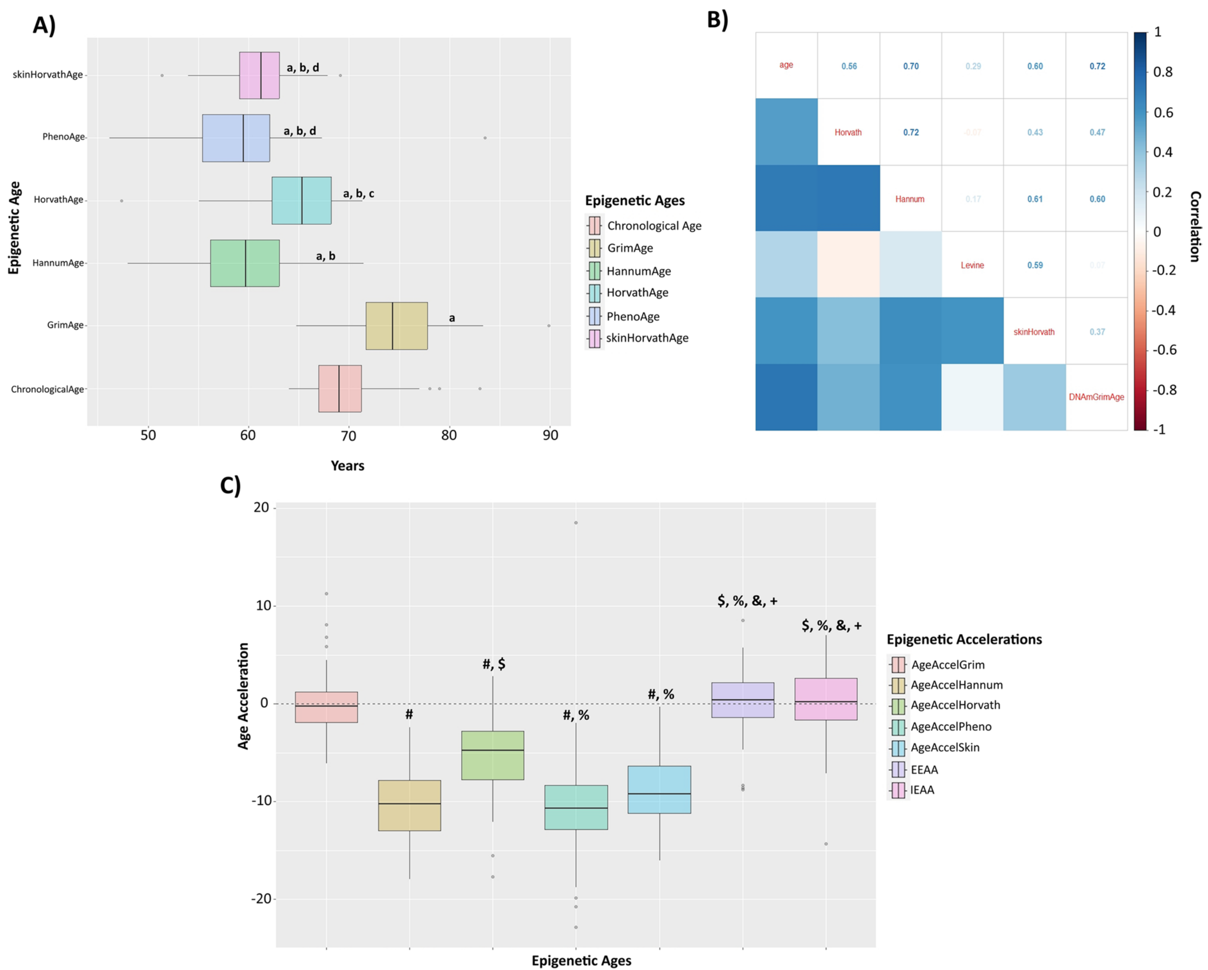

Fig. 1 Epigenetic ages and accelerations comparison of older adults with mild COVID-19 symptoms. **A** Epigenetic ages comparison (GrimAge, HannumAge, HorvathAge, PhenoAge, SkinHorvathAge) and chronological age. Lower-case letters indicate significant differences according to (a) significance vs Chronological Age, (b) significance vs GrimAge, (c) significance vs HannumAge, and (d) significance vs HorvathAge ($P < 0.05$. Kruskal–Wallis test with respective BH correction for multiple comparisons). **B** Pearson correlation for Epigenetic Ages: the color range is red to blue (−1 to 1), and the upper right side of the graph indicates the value for each comparison. **C** Epigenetic age accelerations comparison (GrimAge–AgeAccelGrim, HannumAge–AgeAccelHannum, HorvathAge–AgeAccelHorvath, PhenoAge–AgeAccelPhenoAge, SkinHorvathAge–AgeAccelSkin, intrinsic epigenetic age acceleration (IEAA) and extrinsic epigenetic age acceleration (EEAA)). Symbols indicate significant differences according to (#) significance vs AgeAccelGrim, ($) significance vs AgeAccelHannum; (%) significance vs AgeAccelHorvath; (&) significance vs AgeAccelPhenoAge, (+) significance vs AgeAccelSkin ($P < 0.05$. One-way ANOVA with Tukey post hoc)

explore the effects of age and sex as confounding factors on our sample; results from the linear models (LM) demonstrate that none of them have a significant correlation with both variables (Supplementary Table 1S). The LM performed between the telomere length estimation and the chronological age, where the correlation is negative, suggesting that short telomere lengths correspond to older chronological ages for older adults with mild COVID-19 (Supplementary Table 1S).

Moreover, we analyzed if there were clusters between the epigenetic clocks and the corresponding accelerations. As seen in Fig. 2, the results showed that there is a marked heterogeneity among individuals in terms of their epigenetic measures and that there were two marked clusters (Fig. 2A).

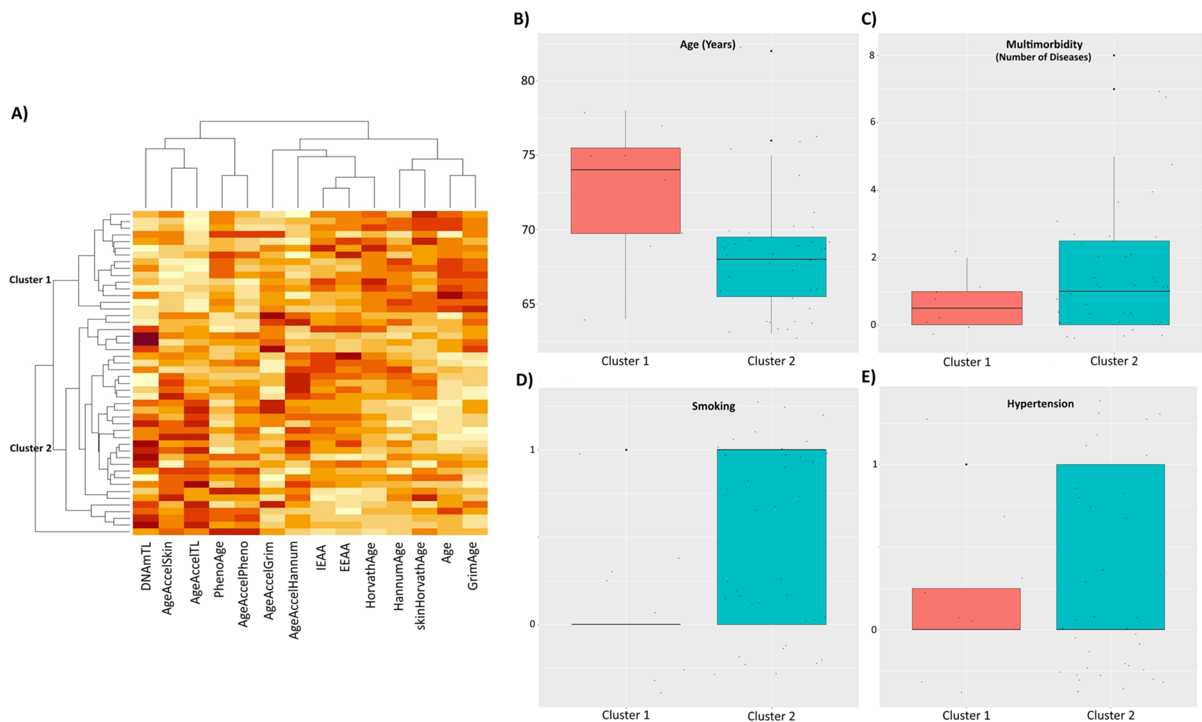

Fig. 2 Clustering analysis of epigenetic ages on patients. **A** The cladogram (left panel) indicates that there are two groups of individuals that have different sets of epigenetic data. **B** Barplot for chronological age difference between clusters (Cluster 1, red, and Cluster 2, blue). **C** Barplot for multimorbidity (number of diseases) difference between clusters (Cluster 1, red, and Cluster 2, blue). **D** Barplot for smoking (yes/no) difference between clusters (Cluster 1, red, and Cluster 2, blue). **E** Barplot for hypertension (yes/no) difference between clusters (Cluster 1, red, and Cluster 2, blue)

Interestingly, the main differences between each cluster of individuals are chronological age, multimorbidity, smoking, and hypertension (Fig. 2B–E).

Epigenetic age associations with clinical, immunological estimators and DNA damage

Once we obtained the results from the epigenetic analysis, we aimed to understand which clinical and sociodemographic variables significantly influenced the epigenetic ages. Our results from the association analyses demonstrate that HorvathAge is significantly associated with T2D and SkinHorvathAge is also significantly associated with multimorbidity; such associations indicate that both variables directly influence these epigenetic ages. Moreover, we also performed a Pearson correlation analysis between the epigenetic clocks and health disparities. As seen in Table 2, T2D is associated with HorvathAge and HannumAge. On the other hand, smoking is significantly associated with DNAmTL, HannumAge, and SkinHorvathAge. Additionally, multimorbidity is associated with HannumAge and SkinHorvathAge. Once we correlated the immunological estimates with the clocks (Table 3), we found a significant correlation with NK, DNAmTL, and CD8pCD28nCD45RAn cells (senescent CD8 + cells) estimator with HorvathAge and PhenoAge. Finally, these results are quite similar for PhenoAge and SkinHorvath when associated with tail DNA, which represents DNA damage in our patients (Table 4).

Discussion

In the present study, we aimed to understand the association between mild COVID-19, epigenetic ages, and clinical variables from a Mexican cohort of older adults. We found that GrimAge is the only epigenetic clock that is higher than the chronological age, and its acceleration tends to increase in our

Table 2 Univariate analysis for epigenetic ages and clinical variables (n=47, Pearson's product-moment correlation)

	DNAmTL		HorvathAge		HannumAge		PhenoAge		SkinHorvathAge		GrimAge	
	p-value	cor	*p*-value	cor	*p*-value	cor	*p*-value	cor	*p*-value	cor	*p*-value	cor
Type 2 diabetes	0.2497	−0.0404	0.0285*	0.3197	0.054*	−0.2829	0.4397	0.11544	0.2944	0.15620	0.7499	−0.09032
Hypertension	0.2217	−0.0699	0.5344	0.0929	0.0826	−0.2558	0.8185	0.03439	0.0786	0.25911	0.6601	−0.06585
Alcoholism	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Smoking	0.0075*	0.3852	0.1539	−0.2113	0.0018*	−0.4438	0.1826	−0.19780	0.0294*	−0.31791	0.1431	−0.21689
Multimorbidity	0.4368	−0.1162	0.6391	0.0702	0.0216*	−0.3344	0.4347	0.11669	0.0059*	0.39557	0.9918	−0.00154
Frailty phenotype	0.1374	−0.2199	0.8185	−0.0344	0.2367	−0.1760	0.10675	0.1027	0.2841	0.15954	0.9918	0.1430
Insomnia	0.6145	−0.0753	0.7731	−0.1168	0.5832	0.8215	0.0338*	−0.0059	0.6713	−0.06354	0.4929	0.1025

population; moreover, such measure is not influenced by age or sex on our dataset. GrimAge is considered an accurate predictor of functional health and performance with implications for longevity [52]; people with accelerated GrimAge have an increased risk of developing chronic diseases like heart disease [19] and cancer [53]. In accordance, an accelerated GrimAge has been associated with severe COVID-19 [27]. Moreover, it has been found that people with COVID-19 may have implications for their long-term health, probably because the virus itself may damage cells and tissues [54], leading to premature aging as indicated by an increased GrimAge.

We also found accelerated intrinsic (IEAA) and extrinsic (EEAA) epigenetic ages in mild COVID-19 older adults. IEAA captures cellular age acceleration independent of blood cell composition, which is known to change with age, giving us a more accurate measurement of the disease independent of age [55]. Interestingly, it has been reported that higher IEAA is associated with more damage to the kidney, heart, and peripheral arteries in a hypertensive cohort of African Americans [55], a phenomenon also reported for long COVID-19, where individuals show damage in organs and tissues derived from the infection [56]. Our results support the idea of epigenetic changes, specifically IEAA, as a subclinical biomarker for infection outcomes. As expected, telomere length estimation is negatively correlated. A second analysis showed that epigenetic clocks and accelerations were heterogeneous in our population (cluster analysis). However, two main clusters were identified, and according to our study, the effects may be associated with multimorbidity, hypertension, and smoking, which are different as compared between both clusters. Noteworthy is that these same variables have been associated with COVID-19 severity [57]; however, we only evaluated mild infection and cannot reach further conclusions.

We then analyzed the association of epigenetic ages with clinical variables and found an association between HannumAge and type 2 diabetes (T2D), smoking, and multimorbidity. These findings are in accordance with other studies that have shown that HannumAge is associated with an increased risk of developing metabolic diseases, such as T2D [58], higher BMI [59], and stroke [60]. Notably, people with metabolic syndrome are known to be at higher risk of developing severe COVID-19 [61], suggesting that these could be indirectly related to COVID-19 infection.

Table 3 Correlation between epigenetic ages and immunological variables ($n = 47$, multiple linear regression model)

	DNAmTL (*p*-value)	HorvathAge (*p*-value)	HannumAge (*p*-value)	PhenoAge (*p*-value)	SkinHorvathAge (*p*-value)	GrimAge (*p*-value)
CD8T	0.2961	0.4830	0.4380	0.1689	0.5276	0.3320
CD4T	0.3893	0.4970	0.5200	0.4589	0.6869	0.3780
NK	0.0057*	0.5361	0.8330	0.2408	0.0608	0.7550
Bcell	0.3777	0.4830	0.2320	0.6964	0.8217	0.3360
Mono	0.5374	0.7203	0.6130	0.6111	0.9582	0.7810
Gran	0.2520	0.3985	0.6700	0.8465	0.5091	0.7230
PlasmaBlast	0.5931	0.3521	0.3690	0.7901	0.9180	0.9320
CD8pCD28nCD45RAn	0.7220	0.0485*	0.3290	0.0034*	0.8618	0.3440
CD8.naive	0.0981	0.5308	0.5740	0.1032	0.1063	0.8220
CD4.naive	0.2748	0.4320	0.9330	0.8431	0.4090	0.5460
PropNeuron	0.9543	0.3555	0.8430	0.2399	0.4225	0.6350

Regarding immunological estimators, D8pCD-28nCD45RAn cells, also known as senescent CD8 + T-cells, were associated with PhenoAge and HorvathAge. These cells have been related to HIV-associated inflammation and immunosenescence implicated as a cause of the premature onset of other end-organ diseases present in young adults with HIV + that reflects immune system aging also associated with EEAA [62]. Senescent CD8 + T-cells can produce pro-inflammatory cytokines, which can contribute to chronic inflammation. In aging, CD8 + T-cells have been associated with age-related diseases such as cancer, cardiovascular disease, and Alzheimer's disease; finally, it has also been reported that epigenetic clocks are accelerated in CD8 + T-cells [63, 64].

On the other hand, CD8 + T-cells play a fundamental role in acute viral infections, providing subsequent protection against reinfection [65]. Furthermore, recent research suggests that active CD8 + T-cells may play a protective role in the early phase of COVID-19 but a pathogenic role in the late stages of the disease due to reduced cytotoxic function [66]. Moreover, a recent study using data from the UK Biobank England found that PhenoAge was associated with an increased risk of test positivity and COVID-19 mortality [67]. Another interesting association is that of NK and DNAmTL (DNA methylation-based estimator of telomere length); this association may be explained by the fact that telomere length can be a valuable parameter for predicting disease severity in patients, as individuals with short telomeres may have a higher risk of developing severe COVID-19 [68]. Moreover, there is an intricate relationship between DNAmTL and age, studies showed that such measures correlate negatively with age, in this sense, DNAmTL seems to be an interesting biomarker useful for age-related pathologies the relationship between DNAmTL and age is promising for new developments in aging.

Since viruses are particularly efficient at inducing DNA damage [69], we studied this phenomenon through comet assay. The percentage of tail DNA is a parameter to evaluate the severity of infectious diseases; in this sense, our results suggest a significant association of DNA damage with PhenoAge and SkinBloodHorvathAge. Interestingly, these results are in accordance with the

Table 4 Correlation between epigenetic age and DNA damage ($n = 33$, Pearson's product-moment correlation)

Epigenetic age	% tail DNA	
	p-value	Correlation
DNAmTL	0.5764	0.1009
HorvathAge	0.8007	−0.0457
HannumAge	0.0925	0.2976
PhenoAge	0.0101*	−0.4418
SkinHorvathAge	0.0103*	−0.4404
GrimAge	0.9817	−0.0041

literature since DNA damage impairs the function of immune cells, making them less able to fight off infection and concomitantly lead to the production of inflammatory molecules, which contribute to the cytokine storm seen in severe COVID-19 cases [70, 71]. As previously mentioned, PhenoAge is associated with COVID-19 severity [67], probably due to the underlying age-related changes that contribute to PhenoAge, such as inflammation, cellular senescence, and impaired immune function [72], which may also make people more susceptible to severe COVID-19.

Further research is needed to understand the relationship between DNA damage, SkinBloodHortvathAge, and COVID-19 severity. In this context, there is a growing body of evidence that suggests there is a close relationship between epigenetic clocks and the immune system. For example, recent studies have shown that epigenetic age is accelerated in people with certain immune disorders, such as HIV/AIDS [73] and rheumatoid arthritis [74]. Additionally, epigenetic changes regulate the development and function of immune cells.

Finally, an important limitation of our study is the fact that our sample is small in comparison to other studies; all the participants are Mexican beneficiaries of public health, and our results are mainly exploratory. Further analysis with more patients is needed to compare different populations. Nevertheless, to our knowledge, this is the first study where epigenetic clocks are analyzed in a Mexican population of older adults with mild COVID-19.

Conclusions

Epigenetic changes occur due to mild COVID-19 in older adults, as shown by the association with the epigenetic clocks of GrimAge, EEAA, and IEAA. Further analysis showed that inflammatory mediators (D8pCD28nCD45RAn) are associated with PhenoAge and HorvathAge, while DNA damage was associated with PhenoAge and SkinBloodHorvathAge. More research, including longitudinal studies, is needed to understand the long-term consequences of elevated GrimAge in people with mild COVID-19. However, to date, findings suggest that it is important for people who have had COVID-19 to take steps to reduce their risk of developing chronic diseases.

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Declarations

Competing interests The authors declare no competing interests.

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