Centro de Investigación en Alimentación y Desarrollo, A.C.

CARACTERIZACIÓN DE LAS VARIANTES DE SARS-CoV-2 PREDOMINANTES EN LA POBLACIÓN DE SINALOA, MÉXICO Y SUS POSIBLES EFECTOS EN LA METILACIÓN GLOBAL DEL ARN Y EN LA RESPUESTA INMUNE

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RESUMEN

El virus del Síndrome Respiratorio Severo Agudo (SARS-CoV-2), agente causal de la enfermedad del coronavirus-19 (COVID-19), presenta una alta tasa de mutación. Las mutaciones en la proteína spike pueden modificar su afinidad al receptor de la enzima convertidora de angiotensina 2 (ACE2) del hospedero, y por tanto la infectividad viral. El presente estudio tuvo como objetivo caracterizar las variantes de SARS-CoV-2 en la población de Sinaloa e identificar las diferencias variante-específicas en la metilación global del ARN y su posible relación con el sistema inmune. El estudio consistió en tres etapas, en la primera se describió el comportamiento de la pandemia de COVID-19, integrando factores como edad y sexo, en la población de Sinaloa desde el 1 de marzo del 2020 hasta el 28 de agosto del 2022. En la segunda, se evaluaron las diferencias en la metilación global del ARN relacionadas a las variantes de SARS-CoV-2 en muestras nasofaríngeas de pacientes que resultaron positivos al virus en la población de Mazatlán, Sinaloa. Finalmente, en la tercera etapa se evaluó la posible influencia de SARS-CoV-2 en el desarrollo de autoinmunidad post-COVID. Los resultados mostraron que, el riesgo relativo de infección (RR) para personas mayores de 60 años disminuyó en la tercera ola, alcanzando un máximo en el grupo de 30 a 45 años, lo que se asocia a la circulación de la variante delta en esta etapa y al programa de vacunación que inició con adultos mayores ($p < 0.0001$). Adicionalmente, los hombres mayores de 60 años tuvieron en general un RR más alto que las mujeres del mismo grupo de edad, aunque esta diferencia no fue significativa ($p = 0.137$). Por otro lado, las variantes de SARS-CoV-2 influyeron significativamente en los niveles globales de metilación del ARN ($p < 0.0001$), siendo las variantes de preocupación (delta y ómicron) las que presentaron los menores niveles de metilación. El mecanismo mediante el cual las variantes de SARS-CoV-2 pueden alterar los niveles de metilación del ARN, así como las implicaciones de estas alteraciones en la infección y/o replicación viral no es claro y requiere más investigación. Por otra parte, se propusieron los mecanismos implicados en el desarrollo de la diabetes mellitus en la era post-COVID-19, con particular interés en el mimetismo molecular y el papel de las variantes del SARS-CoV-2, así como la metilación m6A del ARN viral y del hospedero.

Palabras claves: variantes virales de SARS-CoV-2, muestras nasofaríngeas, metilación global m6A, proteína Spike, diabetes mellitus, síndrome post-COVID-19.

ABSTRACT

The Severe Acute Respiratory Syndrome virus (SARS-CoV-2), the causative agent of the coronavirus-19 disease (COVID-19), show a high mutation rate. Mutations in the spike protein can modify the affinity to the host's angiotensin-converting enzyme 2 (ACE2) receptor and therefore the viral infectivity. The objective of this study was to characterize the variants of SARS-CoV-2 in the population of Sinaloa and identify variant-specific differences in global RNA methylation and its possible relationship with the immune system.

The study consisted of three stages, the first described the behavior of the COVID-19 pandemic, integrating factors such as age and sex, in the population of Sinaloa from March 1st, 2020, to August 28th, 2022. In the second, differences in global RNA methylation related to SARS-CoV-2 variants were evaluated in nasopharyngeal samples from patients who tested positive for the virus in the population of Mazatlán, Sinaloa. Finally, in the third stage, the possible influence of SARS-CoV-2 on the development of post-COVID autoimmunity. The results indicated that, the relative risk of infection (RR) for people older than 60 years decreased in the third wave, reaching a maximum in the 30-45 age group, which was associated with the circulation of the delta variant at this stage and the vaccination program that began with older adults ($p < 0.0001$). Additionally, men older than 60 years generally had higher RR than women of the same age group, although this difference was not significant ($p = 0.137$). On the other hand, SARS-CoV-2 variants significantly influenced global m6A methylation levels ($p < 0.0001$), with variants of concern (delta and omicron) showing the lowest methylation levels. The mechanism by which SARS-CoV-2 variants can modify RNA methylation levels, as well as the implications of these alterations for viral infection and/or replication, is unclear and requires further investigation. On the other hand, mechanisms implicated in the development of diabetes mellitus in the post-COVID-19 era were proposed, with particular interest in molecular mimicry and the role of SARS-CoV-2 variants, as well as viral/host m6A RNA methylation.

Keywords: SARS-CoV-2 viral variants, nasopharyngeal swabs, m6A global methylation, RNA, epitopes, Spike protein, human leukocyte antigen (HLA), diabetes mellitus, post-COVID-19 syndrome.

1. SINOPSIS

1.1. Justificación

El coronavirus SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2) que causa la enfermedad llamada COVID-19, constituye un problema de salud pública a nivel mundial. La Organización Mundial de la Salud (OMS) declaró el estado de Emergencia Sanitaria Mundial ante la expansión del virus SARS-CoV-2 en enero de 2020 (Lai *et al.,* 2020). La gravedad de la COVID-19 se asoció al elevado número de pacientes con infecciones severas y con alto riesgo de muerte, aunado al alto porcentaje de personas asintomáticas capaces de transmitir el virus sin saberlo (de Diego-Castell *et al.,* 2023). COVID-19 afectó de forma grave a pacientes de edad avanzada y/o personas con comorbilidades como hipertensión, diabetes, obesidad o inmunosupresión (Pallarés Carratalá *et al.,* 2020). SARS-CoV-2 presenta una alta tasa de mutación, lo cual originó variantes virales con diferentes grados de transmisión y severidad (Majumdar *et al.,* 2021; Wu *et al.,* 2021). El estudio del comportamiento de la pandemia de COVID-19 es de interés mundial ya que proporciona información para sustentar las estrategias de salud pública para contrarrestar eficazmente nuevas epidemias. Por tal motivo, en el presente trabajo se describió el comportamiento de la pandemia de COVID-19 durante las primeras cuatro olas epidémicas, en una muestra de la población de Sinaloa, del 1 de marzo del 2020 al 28 de agosto del 2022.

El genoma del SARS-CoV-2 es una molécula de ARN de cadena sencilla y sentido positivo que se comporta como un ARN mensajero (ARNm) (Fernández-Pérez *et al.,* 2021); consta de aproximadamente 30,000 nucleótidos y codifica varias proteínas necesarias para su replicación y la infección de las células huésped. El proceso de replicación del SARS-CoV-2 comienza cuando el virus se une a receptores específicos en la superficie de las células huésped, particularmente las células epiteliales del tracto respiratorio. Una vez que se une, el ARN viral se libera en el interior de la célula. Luego, el ARN viral actúa como un molde para la síntesis de ARN complementario (ARNc) en sentido negativo utilizando la maquinaria de la célula huésped. Este ARNc viral se utiliza para producir proteínas virales, incluidas las proteínas estructurales (como la proteína spike (S), la proteína de la envoltura (E), la proteína de la membrana (M) y la nucleocápside (N)) y

proteínas no estructurales (como las enzimas involucradas en la replicación del ARN viral). Estas proteínas virales se ensamblan junto con el ARN viral para formar nuevas partículas virales (Mingaleeva *et al.,* 2022). Recientemente se han descrito mecanismos involucrados en las modificaciones epigenéticas en los ARNm (Bhat *et al.,* 2022). La modificación m6A en el ARN es la más prevalente en las moléculas de ARN. La m6A consiste en la adición de un grupo metilo de S-adenosil-L-metionina (AdoMet/SAM) en la posición 6 de la adenina del ARN. Algunos sitios m6A de ARNm virales propician la evasión de los receptores de reconocimiento de patrones de la respuesta inmune innata celular y promueven la eficiencia de la replicación viral (revisado por Brocard *et al.,* 2017), por ejemplo, se ha reportado que los ARNs virales metilados del SARS-CoV-2 que se encuentran en el citoplasma evaden el reconocimiento por el gen inducible por ácido retinoico (RIG-I) y no se promueve la señalización antiviral y la expresión de interferón tipo I (Li *et al.,* 2021). Antes de este estudio, no existían reportes que determinaran los niveles de metilación m6A del ARN por variante viral, bajo este contexto, en este trabajo se evaluó la influencia de las variantes virales en la metilación global m6A del ARN total en muestras nasofaríngeas de pacientes infectados con SARS-CoV-2 en una muestra de la población de Mazatlán, Sinaloa.

La afinidad del SARS-CoV-2 por el receptor ACE2, que también participa en el metabolismo de la glucosa, podría conducir potencialmente al desarrollo de diabetes de nueva aparición después de la COVID-19 (NODAC por sus siglas en inglés) (Muniyappa, 2020). La pandemia también ha provocado cambios en el estilo de vida y los hábitos alimentarios, lo que a su vez podría contribuir al desarrollo de diabetes mellitus tipo 2 (DM2) (Muniyappa, 2020). Los mecanismos moleculares subyacentes a NODAC, incluida la metilación m6A del ARN y el mimetismo molecular, son áreas de investigación en curso (Laxminarayana, 2022; Vaid *et al.,* 2023), por tanto, se realizó una revisión bibliográfica sobre los mecanismos implicados en el desarrollo de la diabetes mellitus en la era post-COVID-19, con particular interés en el papel de las variantes del SARS-CoV-2 en el mimetismo molecular, así como la metilación m6A tanto del ARN viral como del hospedero.

1.2. Antecedentes

El estudio de la naturaleza infecciosa del SARS-CoV-2, así como del impacto de factores externos

no virales ayuda a mitigar la prevalencia y la severidad de la infección mediante estrategias de salud pública. La comprensión de la distribución y la gravedad de los casos de COVID-19 es crucial para evaluar el impacto de la pandemia y diseñar estrategias efectivas de salud pública para controlar la propagación del virus (Batista-Roche *et al.,* 2022); esta distribución de casos se vio influenciada por las disparidades demográficas en relación con la edad, el sexo, y/o el estado de salud, la infraestructura de salud pública (como recursos médicos, protocolos y tratamientos), factores socioeconómicos (como el nivel de ingresos, las condiciones de vivienda y el acceso a la atención médica); y factores ambientales (como el clima, la contaminación del aire, la radiación solar, entre otros). Por ejemplo, la calidad del aire es un factor relacionado con los casos de COVID-19 ya que el aire contaminado aumenta la susceptibilidad a enfermedades respiratorias (Liu *et al.,* 2020); la radiación solar y la presencia de ozono se han asociado con la reducción del ciclo de vida del SARS-CoV-2 en algunas altitudes (Semple *et al.,* 2020); la velocidad y dirección del viento se han relacionado con la propagación del virus (Zhu *et al.,* 2020). Además, la disposición de aguas residuales y la eliminación del hábitat de la vida silvestre también se han asociado con la transmisión del SARS-CoV-2 (Ahmed *et al.,* 2020).

La progresión de la COVID-19 involucra la metilación del ARN viral. La modificación m6A es la más prevalente en las moléculas de ARN. Esta modificación consiste en la adición de un grupo metilo de S-adenosil-L-metionina (AdoMet/SAM) en la posición 6 de la adenina del ARN. La incorporación de un grupo metilo en la posición 6 de la base nitrogenada adenina es la misma tanto para la molécula de ADN como para la del ARN, pero las enzimas que participan son diferentes al igual que las implicaciones de ambas modificaciones. Esta modificación fue descubierta inicialmente en los ARNm de células tumorales (Desrosiers *et al.,* 1974) y posteriormente fue identificada en algunos virus (Tirumuru *et al.,* 2016), y bacterias (Deng *et al.,* 2015).

Las N6-metiladenosinas en los ARNm (m6A ARNm) juegan un papel importante en el control de la expresión génica (Chen y Wong, 2020). La mayoría de los ARNm modificados con m6A contienen solo un sitio m6A, pero algunos ARNm contienen 20 o más sitios m6A. Las modificaciones m6A del ARNm son reguladas dinámicamente por enzimas metiltransferasas (*"writers"* como la metiltransferasa 3 (METTL3), y la metiltransferasa 14 (METTL14) que transfieren grupos metilo al nitrógeno 6 de la adenosina del ARN) y desmetilasas (*"erasers"* como fat mass and obesity associated protein (FTO) y AlkB homolog 5 (ALKBH5) que eliminan grupos metilo). La metilación m6A se completa con la liberación del ARNm al nucleoplasma (Fig. 1). La

metilación y desmetilación en los ARNm citoplasmáticos es poco frecuente (Ke *et al.,* 2017), una vez en el nucleoplasma, los ARNm metilados son reconocidos por proteínas de unión a m6A ("readers") y exportados del núcleo luego de ser procesados por splicing alternativo. Más aún, los "readers" guían los ARNm metilados hacia los ribosomas para su traducción o hacia otros complejos para su degradación (Zaccara *et al.,* 2019). Por ejemplo, YTH domain family member 2 (YTHDF2) es un reader cuyo dominio carboxilo terminal se une al ARNm metilado mientras que su dominio amino-terminal recluta proteínas que degradan el ARNm; en general, los mecanismos de degradación de ARNm metilado son llevados a cabo por complejos proteicos que involucran a YTHDF2 (Lee *et al.,* 2020).

Las metiltransferasas del hospedero modulan los ciclos replicativos de algunos virus como el SARS-CoV-2 (Gu *et al.,* 2021). Los ARNs genómicos y sub genómicos del SARS-CoV-2 que se encuentran en el citoplasma de la célula huésped son metilados por la metiltransferasa METTL3 del hospedero. Recientemente se reportó que el ARN del SARS-CoV-2 y de la célula huésped se metilan dinámicamente in vitro (Li *et al.,* 2021), por ejemplo, se ha observado que en células de hepatocarcinoma humano (Huh7), el ARN genómico del SARS-CoV-2 se metila durante la infección y la modificación m6A ocurre con mayor frecuencia hacia la región 3' del genoma (Liu *et al.,* 2021). Las proteínas del SARS-CoV-2 también pueden interactuar con la maquinaría de metilación del huésped para modular la replicación viral. Por ejemplo, un aumento en la expresión de METTL3 48 horas después de la infección en células Vero E6 se asoció positivamente con la replicación del SARS-CoV-2 (Zhang *et al.,* 2021). Adicionalmente, otros estudios muestran que la infección por SARS-CoV-2 modifica el metiloma m6A de célula huésped in vitro, promoviendo la expresión diferencial de los genes del huésped (Li *et al.,* 2021); e in vivo, alterando los niveles de m6A en linfocitos de muestras de sangre periférica de pacientes con COVID-19 severo al aumentar la expresión de la metiltransferasa proteína 15 con motivo de unión a ARN (RBM15) (Meng *et al.,* 2021). Por otro lado, la ARN polimerasa dependiente de ARN (RdRp) de SARS-CoV-2 entra al núcleo de la célula huésped, se une a la METTL3 y posibilita la translocación de la metilasa del núcleo al citoplasma (Zhang *et al.,* 2021); posteriormente en el citoplasma, los ARNs virales metilados evaden el reconocimiento por el gen inducible por ácido retinoico I (RIG-I) y no se promueve ni la señalización antiviral ni la expresión de interferón tipo I (Li *et al.,* 2021) (Fig. 1). Los ARNs metilados en algunos virus pueden tener una función antiviral o proviral en el ciclo replicativo mediante mecanismos aún poco entendidos (Baquero-Perez *et al.,* 2021).

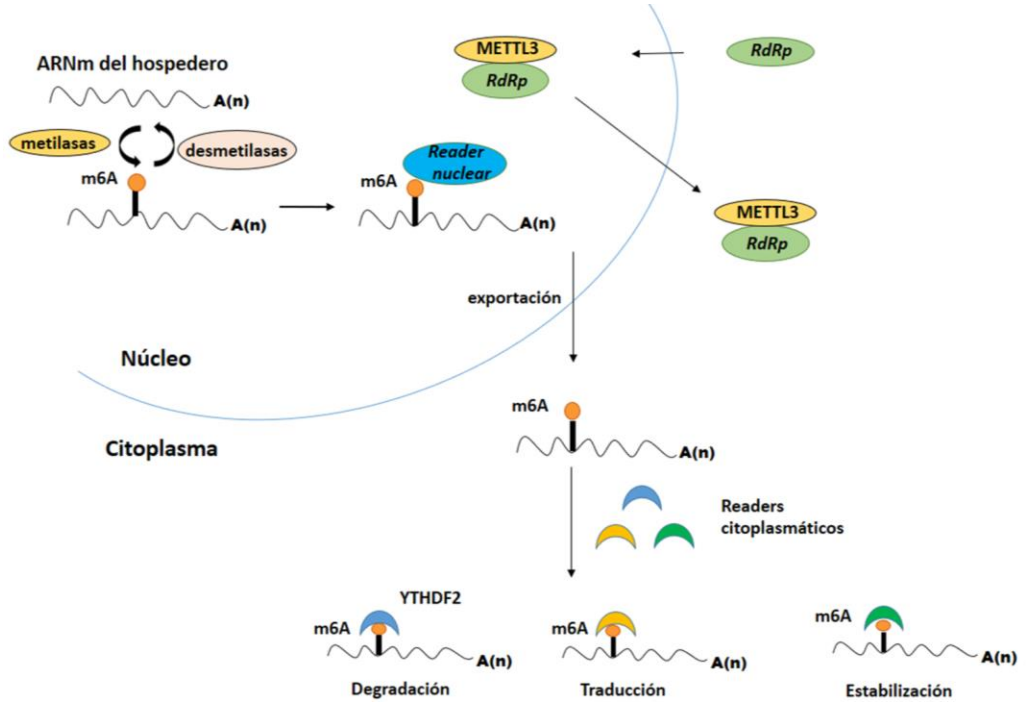

Figura 1. Funciones relacionadas con la modificación m6A del ARN y las enzimas participantes. Las principales funciones moleculares relacionadas con esta modificación epigenética son pre-ARNm *splicing*, translocación, traducción y estabilidad del ARNm, procesamiento de micro-ARN y la regulación de los ARN codificantes largos. Figura creada en BioRender

Finalmente, el mimetismo molecular se refiere al fenómeno de reactividad cruzada inmunitaria entre un patógeno como SARS-CoV-2 y proteínas humanas, donde una célula inmunitaria reconoce a ambos debido a su similitud de secuencia (Moody *et al.*, 2021). Este fenómeno se relaciona con la promoción o exacerbación de enfermedades autoinmunes como el síndrome de Guillain-Barré, fenómenos trombóticos, anemia hemolítica y otros (Liu *et al.*, 2021). Los autores de un estudio computacional sobre mimetismo molecular entre la proteína spike de SARS-CoV-2 y epítopos conocidos reportaron dos epítopos, TQLPP y ELDKY, que se asociaron con el mimetismo molecular en el SARS-CoV-2 (Nunez-Castilla *et al.*, 2022). Por su parte, Churilov y colaboradores (2022) reportaron pentapéptidos compartidos entre los autoantígenos relacionados con la diabetes y la proteína spike del SARS-CoV-2. Otros autores destacaron un posible parecido en la secuencia de aminoácidos de la insulina humana, representadas por el código 4F0N, y el ácido glutámico descarboxilasa-65 (GAD65), representado por el código 2OKK con ciertas proteínas en el SARS-CoV-2, específicamente la proteína spike (representada por el Código 6ZB5) (de Oliveira *et al.*, 2021). Estas observaciones sugieren una similitud molecular que podría tener implicaciones para

la respuesta del sistema inmunológico, potencialmente conduciendo a reactividad cruzada entre anticuerpos dirigido a la insulina humana o GAD65 y ciertas proteínas del SARS-CoV-2, particularmente la proteína spike.

1.3. Hipótesis

Las variantes de SARS-CoV-2 predominantes en la población de Sinaloa desencadenan respuestas diferenciales en la metilación global del ARN y en el sistema inmune en cuanto al reconocimiento de antígenos y autoinmunidad.

1.4. Objetivo General

Caracterizar las variantes de SARS-CoV-2 predominantes en una muestra de la población de Sinaloa y sus posibles efectos en la metilación global del ARN y en la respuesta inmune.

1.5. Objetivos Específicos

1. Describir el comportamiento de la pandemia de COVID-19 y las variantes de SARS-CoV-2 detectadas en la población de Sinaloa
2. Evaluar el efecto de las variantes de SARS-CoV-2 en la metilación global m6A de ARN de muestras nasofaríngeas de personas infectadas en la población de Mazatlán, Sinaloa
3. Proponer mecanismos que expliquen la autoinmunidad post-COVID, con énfasis en el desarrollo de diabetes mellitus

1.6. Sección Integradora del Trabajo

La presente tesis está integrada por cinco capítulos que incluyen: la sinopsis de la tesis (Capítulo 1, presente capítulo), tres artículos originales publicados (Capítulos 2, 3 y 4), el capítulo de discusión general (Capítulo 5) y finalmente las Conclusiones y Recomendaciones, la Bibliografía citada y los Anexos. Los artículos se presentan en el idioma que fueron publicados. En los siguientes párrafos se describen brevemente las publicaciones que forman parte de los capítulos 2, 3 y 4 de esta tesis.

El capítulo 2 “SARS-CoV-2 variants and associated cases during four epidemic waves in Sinaloa, Mexico”, cubrió el primer objetivo de la tesis. En este se describe el comportamiento de la pandemia de COVID-19 durante las primeras cuatro olas epidémicas, por edad y sexo, en la población de Sinaloa desde el 1 de marzo de 2020 hasta el 28 de agosto de 2022. Todos los análisis se realizaron a partir de bases de datos públicas como la iniciativa internacional para compartir datos genómicos del virus de la gripe y del SARS-CoV-2 (GISAID en inglés, https://www.gisaid.org) y la Dirección General de Epidemiología (DGE en español, https://www.gob.mx/salud/documentos/datos-abiertos-bases-historicas-direccion-general-de-epidemiologia?idiom=es). El número de olas en Sinaloa se identificó a partir de la curva epidemiológica (número de casos por día de inicio de síntomas); y se realizó la prueba de mediana de Mood y la prueba de mediana post-hoc por pares para detectar diferencias de edad entre las olas, con un tamaño de muestra total de 75 para los casos y 150 para las muertes, estimados a partir de análisis previos con un tamaño de muestra total de 10 por ola, un nivel de significancia de $\alpha = 0.050$ y una potencia de $\gamma = 0.9$. Además, se utilizaron las pruebas ANOVA multifactorial y Sidak HSD para evaluar la influencia del sexo, la edad y las olas epidémicas en el Riesgo Relativo de infección por SARS-CoV-2 (RR). El RR se define como la proporción de individuos enfermos en un determinado grupo de edad con respecto a la población general perteneciente al mismo grupo de edad. Los análisis estadísticos se realizaron en R 4.1 (RStudio Team, 2021) y RStudio 2021.09.1 (RStudio Team, 2024). El riesgo relativo de infección (RR) para personas mayores de 60 años disminuyó en la tercera ola, mientras que aumentó en el grupo de 30 a 45 años, lo que se asocia a la circulación de la variante delta y al programa de vacunación que se inició con adultos mayores ($p < 0.0001$). Los hombres mayores de 60 años tuvieron RR más altos que las mujeres del mismo grupo de edad, aunque esta diferencia no fue significativa ($p = 0.137$). Estos hallazgos resaltan la

importancia de la vigilancia epidemiológica y la adaptación de las estrategias de prevención y control de la pandemia.

El capítulo 3 “Global m6A RNA methylation in SARS-CoV-2 positive nasopharyngeal samples in a Mexican population: a first approximation study” cubrió el segundo objetivo de la tesis. En esta sección se determinó la relación que existe entre las variantes de SARS-CoV-2, y la metilación del ARN. El ARN total se obtuvo a partir de muestras nasofaríngeas de individuos ambulatorios y hospitalizados en la ciudad de Mazatlán, Sinaloa, México. Las muestras positivas a SARS-CoV-2 (analizadas por RT-qPCR) se secuenciaron para identificar las variantes virales y se determinaron los niveles de metilación global m6A del ARN total mediante un método de inmunoensayo competitivo. Como grupo control se utilizaron muestras negativas al virus. Se identificaron las variantes de preocupación B.1.1.7 (alfa), B.1.617.2 (delta), B.1.1.529 (omicron) y P.1 (gamma), así como la variante de interés B.1.429 (epsilon) y el linaje B.1.1.519 (originado en México) en la población de Mazatlán en el periodo comprendido entre febrero del 2021 y enero del 2022. Se realizó un ANOVA bifactorial con interacciones para evaluar la influencia del sexo y la variante viral en los niveles de metilación global. Los análisis estadísticos se realizaron en R 4.1 (RStudio Team, 2021). El sexo de los individuos no influyó significativamente en los niveles globales de metilación m6A ($p = 0.291$), ni tampoco hubo interacción con las variantes virales ($p=0.211$). Sin embargo, los niveles de metilación global sí se diferenciaron según las variantes virales ($p < 0.0001$). Las muestras de pacientes positivos a la variante omicron presentaron los menores niveles de metilación, seguido de pacientes positivos a la variante delta. Los niveles de metilación de las muestras con las variantes alfa, epsilon y el linaje B.1.1.519 fueron similares al control. Además, se observó que la metilación global del ARN fue menor en las variantes de preocupación que en las variantes de interés, independientemente de la vacunación.

El capítulo 4 “New-Onset diabetes mellitus after COVID-19: combined effects of SARS-CoV-2 variants, molecular mimicry, and m6A RNA methylation.” cubre el tercer objetivo de la tesis. En este capítulo se realizó una revisión bibliográfica sobre la diabetes mellitus post-COVID-19. Esta revisión identifica mecanismos implicados en el desarrollo de la diabetes mellitus en la era post-COVID-19, con particular interés en el papel de las variantes del SARS-CoV-2, en el mimetismo molecular, la metilación del ARN m6A viral y del hospedero, y posibles asociaciones entre ellos. Para ello se realizó una revisión exhaustiva de la literatura científica disponible sobre el tema, incluyendo estudios epidemiológicos, metaanálisis, revisiones sistemáticas y estudios clínicos. Se postula que las variantes del virus podrían desencadenar respuestas autoinmunes que afectan la

función de las células pancreáticas, provocando la aparición de diabetes. Además, la metilación del ARN viral podría influir en la regulación de la expresión de genes relacionados con la diabetes. Estos hallazgos sugieren la necesidad de una vigilancia continua y un enfoque multidisciplinario para comprender y abordar las implicaciones a largo plazo de la infección por SARS-CoV-2, especialmente en relación con la diabetes mellitus como síndrome post-COVID-19.

2. SARS-COV-2 VARIANTS AND ASSOCIATED CASES DURING FOUR EPIDEMIC WAVES IN SINALOA, MEXICO

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SARS-CoV-2 variants and associated cases during four epidemic waves in Sinaloa, Mexico

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Abstract

The COVID-19 pandemic is a global public health problem that has revealed deficiencies and challenges in health systems worldwide. To date, four waves (each one driven by different viral variants and showing different behaviors) have affected Mexico. Here we describe the COVID-19 pandemic behavior in the population of Sinaloa, Mexico after four epidemic waves. Epidemiological data were obtained from public federal databases from March 2020 to February 2022, and genomes of SARS-CoV-2 variants of interest (VOI) and concern (VOC) in Sinaloa were downloaded from the GISAID database from January 2021 to May 2022. The relative risk (RR) of SARS-CoV-2 infection was calculated from public data. Sinaloa presented four epidemic waves from March 2020 to February 2022, and each wave was driven by different variants with different degrees of transmissibility and severity. Interestingly, the delta variant (which dominated the third wave) was probably the most severe, producing a large number of cases per day and high mortality rates, while the omicron variant (which dominated the fourth wave) produced the largest number of cases per day but decreased mortality rates. Most of the COVID-19 cases in Sinaloa occurred among people between 30 and 45 years old, and the average age of the deceased was above 60 years old in all waves. Older people showed higher risk of infection than infants and younger people; however, the relative risk (RR) for people older than 60 years old decreased in the third and fourth waves. Men older than 60 years old showed higher RR than women of the same age group. The COVID-19 pandemic has shown changing behaviors in time, mostly derived from different emerging viral variants and the immunization of the population. Overall, these results show that SARS-CoV-2 infections appear in timely waves, each one driven by different variants (and subvariants or sublineages), with different degrees of transmissibility and severity. The population should continue with preventive measures to avoid infection.
Key words: SARS-CoV-2 variants, relative risk, COVID-19; Sinaloa, vaccine.

Variantes de SARS-CoV-2 y los casos asociados a cuatro olas epidemiológicas en Sinaloa, México

Resumen

La pandemia de COVID-19 es un problema de salud pública que ha revelado las deficiencias y los retos presentes en el funcionamiento de los sistemas hospitalarios del mundo. En México, hasta el momento de finalizar esta recopilación, se han manifestado cuatro "olas epidemiológicas", cada una dominada por variantes virales con comportamientos diferentes. En este reporte se describe el progreso de la pandemia COVID-19 en la población de Sinaloa, México, durante las cuatro olas epidemiológicas. La información se obtuvo de las bases de datos públicas federales durante el período de marzo del 2020 a febrero del 2022 y los genomas de las variantes de SARS-CoV-2 de interés y preocupación en Sinaloa se tomaron de la base de datos GISAID de enero del 2021 a mayo del 2022. El riesgo relativo (RR) de contraer SARS-CoV-2 fue calculado a partir de documentos públicos. Sinaloa presentó cuatro olas epidemiológicas, entre marzo del 2020 y febrero del 2022, cada una estuvo dominada por variantes diferentes, también en grado de transmisión y severidad. Es un hecho de interés que la variante delta (presente en la tercera ola) fue la más severa, por el alto número de enfermos por día y las altas tasas de mortalidad, a diferencia de la variante omicron (en la cuarta ola) que produjo el mayor número de pacientes por día, pero menores tasas de mortalidad. La mayoría de los contagios por COVID-19 en Sinaloa se presentaron en la población de entre 30 y 45 años de edad, con una edad promedio de los fallecidos superior a los 60 años en todas las olas; estos últimos por ser adultos mayores, fueron más vulnerables que los infantes y las personas más jóvenes, sin embargo, el riesgo relativo (RR) para personas mayores disminuyó en la tercera y cuarta olas. Los hombres mayores de 60 años presentaron un RR más alto que las mujeres de la misma edad. En el transcurso de la pandemia, los cambios de comportamiento del virus se deben a la emergencia de las nuevas variantes y a la respuesta de la población inmunizada. En general, los resultados indican que las variantes (subvariantes o sublinajes) del SARS-CoV-2 cada vez que surgen en lo que se denomina como "una ola", el grado de severidad y su transmisión es distinta, lo que conlleva a la población a una permanente prevención.
Palabras clave: variantes de SARS-CoV-2, riesgo relativo, COVID-19, Sinaloa, vacuna.

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Introduction

SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2) is the causative agent of Coronavirus Disease-19 (COVID-19) in humans. The entry of the virus into the cells is favored by the affinity of the Spike protein to the angiotensin-converting enzyme 2 (ACE2) in the host cell membrane (Gadanec *et al*., 2002), and most SARS-CoV-2 mutations detected worldwide are found within the Spike protein (Becerra-Flores & Cardozo, 2020). These mutations have produced different variants and lineages. According to the Center for Disease Control and Prevention (CDC), a lineage is a "group of closely related viruses with a common ancestor", whereas a variant refers to the "viral genome that may contain one or more mutations that differentiate it from other variants"; thus variants with similar mutations have been designated as Variants of Concern (VOC) or Variants of Interest (VOI) depending on their severity, transmissibility, immune response, or treatment efficacy (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html).

SARS-CoV-2 variants have been named after the country they were identified for the first time, the Pango lineage, and letters from the Greek alphabet convened by the World Health Organization (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). For instance, VOCs include alpha (B.1.1.7) found for the first time in the United Kingdom, gamma (P.1) first found in Japan and Brazil, delta (original lineage B.1.617.2) found in India, beta (B.1.351) and omicron (original lineage B.1.1.529) found in South Africa. Different variants show different degrees of transmissibility and severity (Davies *et al*., 2021). In Mexico, in addition to VOCs, the epsilon variants (B.1.427 and B.1.429) found for the first time in California (USA) and the B.1.1.519 lineage found in Mexico have also been reported.

The extension of the country (1.9 million km^2 of continental surface), the environmental, socioeconomic, and cultural heterogeneity, as well as a decentralized health system, caused different dynamics in the pandemic among regions and/or states (suppl. Figure S1); Sinaloa is a state located in the northwestern coast of Mexico, it sustains important economic activities such as agriculture, aquaculture, and tourism; the latter is the main source of income in the port of Mazatlán (located at the southern side of the state), which holds crowded touristic activities, creating a suitable environment for viral transmission. Thus, the aim of this work was to describe the COVID-19 pandemic behavior in Sinaloa after four epidemic waves.

Methodology

The genomes of SARS-CoV-2 variants reported for Sinaloa were obtained from the GISAID database https://www.gisaid.org (last accessed June 14th, 2022, suppl. file S2). The lineages were determined with Pangolin v4.0. COVID-19 cases and deaths reported in Sinaloa, from March 2020 to February 2022, were obtained from the General Directorate of Epidemiology (DGE in Spanish) database https://www.gob.mx/salud/documentos/datos-abiertos-bases-historicas-direccion-general-de-epidemiologia?idiom=es (last accessed March 13, 2022). Number of waves in Sinaloa was identified from the epidemiological curve (number of cases per day of onset of symptoms), and Mood's median test and the *post hoc* pairwise median test were performed to detect differences in age between the waves, with total sample sizes of 75 for cases and 150 for deaths, estimated from previous analyzes with a total sample of 10 by wave, a significance level of $\alpha = 0.05$ and power of $\gamma = 0.9$. The Relative Risk (RR) of infection with SARS-CoV-2, defined as the ratio of sick individuals in a given age group to the general population belonging to the same age group, was calculated according to Sun, Chen & Viboud (2020):

$$R_i = \frac{\frac{C_i}{\sum_i C_i}}{\frac{N_i}{\sum_i N_i}}$$

where C_i is the number of cases in age group i and N_i is the population size of age group i.

The population size of the age group (N_i) for Sinaloa was obtained from the official Mexican census of 2020 https://datamexico.org/es/profile/geo/sinaloa-si (last accessed February 28, 2022). Multifactorial ANOVA and Sidak HSD tests were used to assess the influence of sex, age, and epidemic waves in the Relative Risk (model: RR ~ sex + age + wave). All analyses were performed in R v4.0.

Results

Variants of SARS-CoV-2 detected in Sinaloa, Mexico

A total of 2,090 genomes were recovered from GISAID, of these, two were not considered as they lack a complete sampling date. Genomes were obtained from different locations in Sinaloa: Ahome (26), Culiacán (469), El Fuerte (1), Guasave (16), Los Mochis (119), Mazatlán (414), Navolato 1, and not specified (1,044); 10 samples were taken in 2020, 1,768 in 2021, and 312 in 2022. 719 were obtained from females, 801 from males, and 570 from unknown sex; 2,085 samples were human, and 5 were environmental.

According with GISAID records, predominant SARS-CoV-2 variants in Sinaloa, from February 2021 to May 2022, were alpha (B.1.1.7), gamma (P.1), delta (original lineage: B.1.617.2) and its lineages AY, lambda (C.37), Mu (B.1.621), and omicron (original lineage: B.1.1.529) and its lineages B.1 and B2, and sublineages (Figure 1).

The COVID-19 pandemic in Sinaloa from March 2020 to February 2022 showed four epidemic waves; the first wave

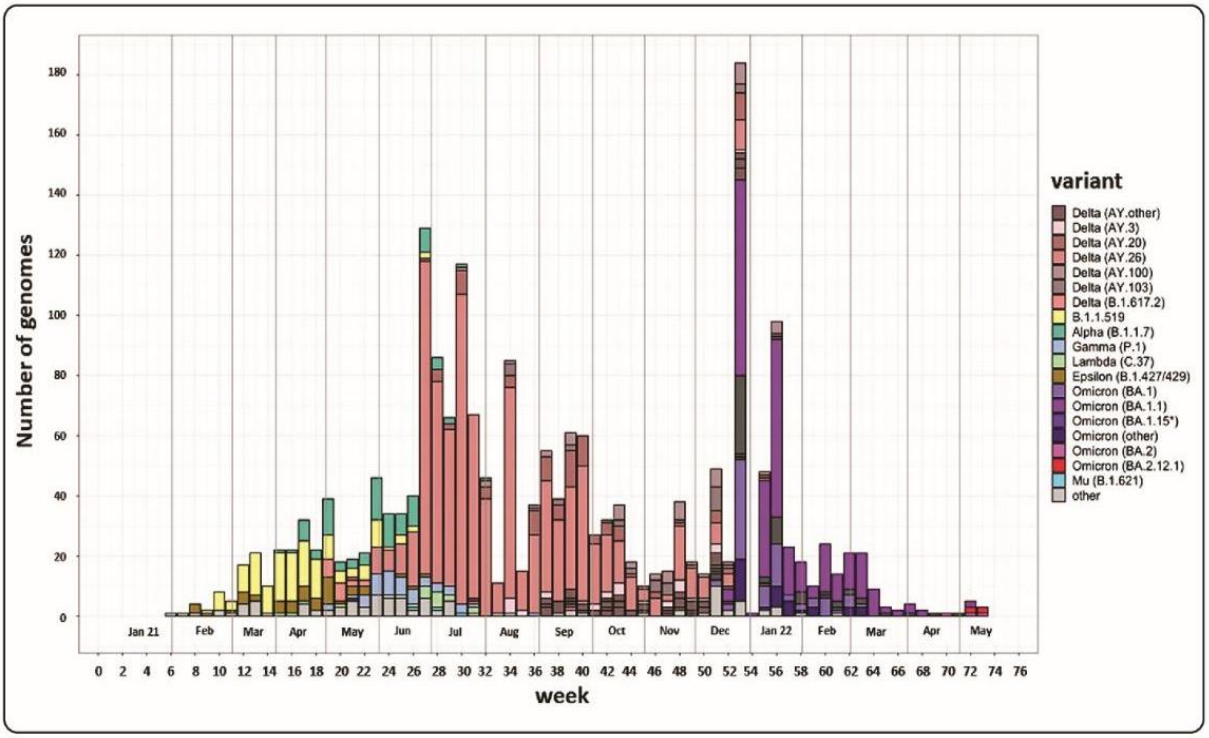

Figure 1. SARS-CoV-2 genomes (2088) sequenced from samples collected in Sinaloa, Mexico, from January 2021 to May 2022. * Lineages and sublineages.

presenting a peak between May and June 2020; the second between February and March 2021; the third between June and August 2021; and the fourth between January and February 2022. The first wave presented a plateau descent of around four months, and the highest numbers of cases per day were recorded during the third and fourth waves. Likewise, the highest numbers of deaths per day were observed during the first and third waves (Figure 2). Epsilon variants (B.1.427 and B.1.429) and the B.1.1.519 lineage dominated the second wave. During the third wave, the delta variant and its AY lineages displaced the previous ones, although alpha and gamma variants were also detected. In the fourth wave, the omicron variant and its lineages (BA.1, BA.1.1) dominated and almost displaced the delta variant. No genomic information was available in the GISAID database for Sinaloa for the first wave.

The median age of positive cases was 44 years old in the first wave, 41 years old in the second wave, 36 years old in the third wave, and 37 years old in the fourth wave (Figure 3A). Significant differences among medians of the "age" factor were also detected between the first and third waves, the second and third waves, and the third and fourth waves (Mood's median test: $p = 0.0000713$, wave1-wave3: $p = 0.01017$, wave2-wave3: $p = 0.00000272$, wave3-wave4: $p = 0.045$). The age distribution of SARS-CoV-2-associated deaths (Figure 3B) was skewed towards older age groups with a median of 67 years old for the first wave, 69 years old for the second wave, 61 years old for the third wave, and 74 years old for the fourth wave. A significant decrease in median age was detected in the third wave with respect to the first, second, and fourth waves, and between the first and second with respect to the fourth wave (Mood's median test: $p = 0.000000158$, wave1-wave3: $p = 0.000807$, wave1-wave4: $p = 0.000445$, wave2-wave3: $p = 0.000164$, wave2-wave4: $p = 0.00679$, wave3-wave4: $p = 0.00000000598$).

More COVID-19 cases were observed in the third and fourth waves compared with the first and second waves, and total mortality was lower in the fourth wave (Figure 4). Few infections among children were confirmed by the adjustment of age demographics of Sinaloa, with an RR below 0.5. The RRs were above 1 in people over 60 years old in the first and second waves; however, during the third and fourth waves the risk decreased for this age group (which was one of the first to be vaccinated). Also, men older than 60 years old showed higher RR than women of the same age group. The RRs were also above 1 in people from 30 to 59 years old mostly during the third and fourth waves, indicating that this age group was exposed during this period, presenting a high probability of infection. Also, the age group of 15-29 in the third wave

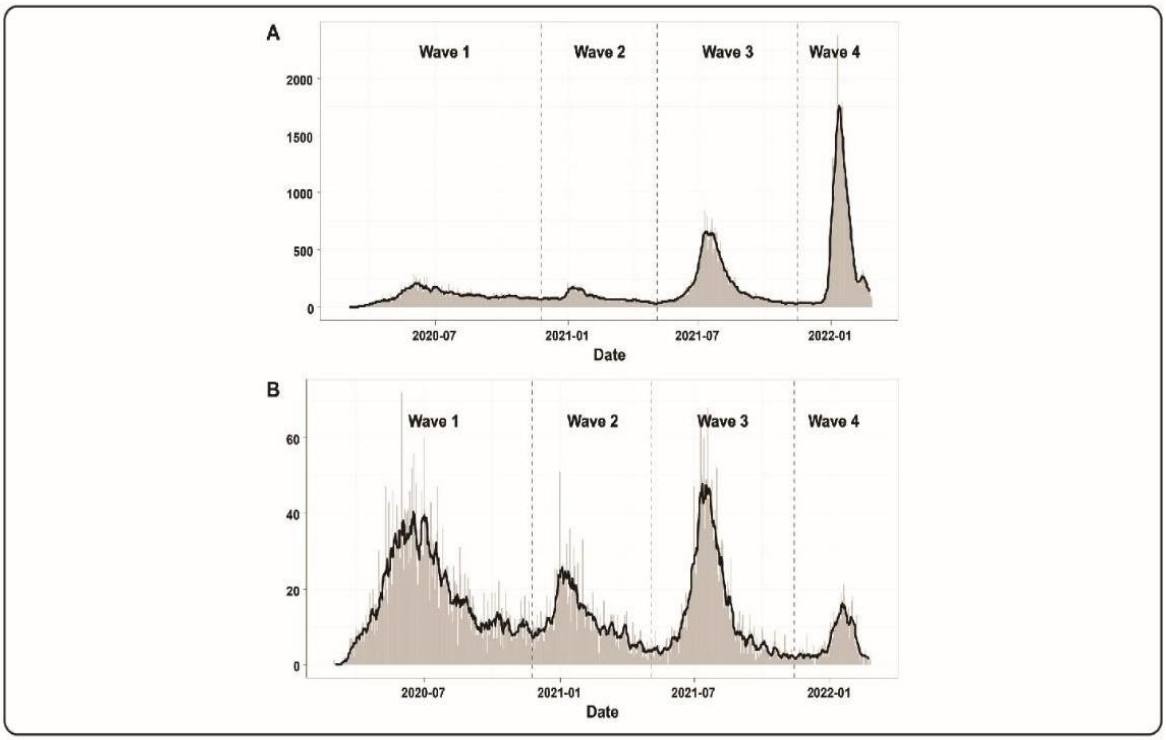

Figure 2. SARS-CoV-2 epidemic waves in Sinaloa, Mexico from March 2020 to February 2022. (A) Cases per day and (B) deaths per day.

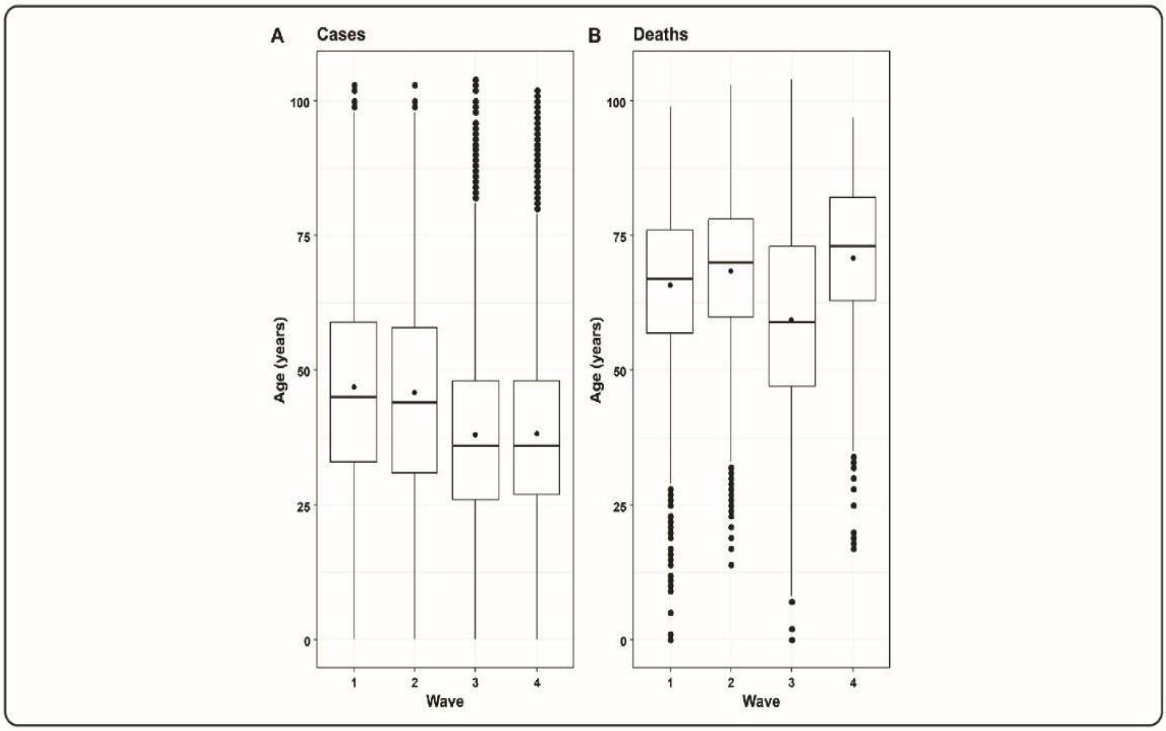

Figure 3. Age distribution of SARS-CoV-2-associated (A) cases, and (B) deaths, according to epidemic waves. Black dots indicate mean values.

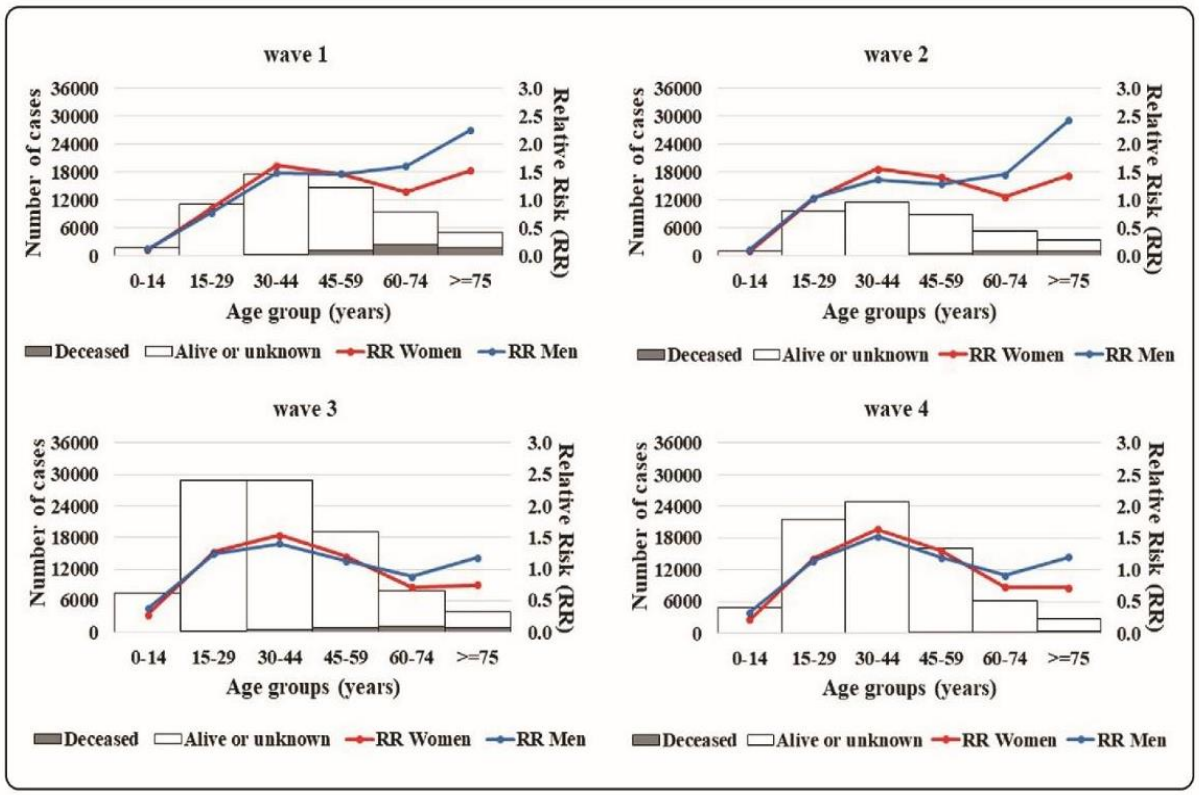

Figure 4. Distribution of SARS-CoV-2-associated cases and deaths, and the Relative Risk (RR) of infection by sex and age groups, according to epidemic waves in Sinaloa, Mexico.

showed an RR above 1, indicating that this age group was also exposed (Figure 4). ANOVA showed that the factor "age", but not the factor "wave" or "sex" influenced the RR significantly ($p = 6.33e-10$ for age; 0.154 for wave, and 0.137 for sex). In the *post-hoc* analysis, significant differences were detected for $\alpha = 0.05$ between the 0-14 age group and the other age groups ($p < 0.0001$), and for $\alpha = 0.1$ between the 30-44 age group and both 15-19 and 60-74 age groups ($p = 0.061$) (suppl. Figure S3).

Discussion

Viral variants have arisen due to different mutations, with implications for transmissibility since they can modify the affinity of the Spike protein with ACE2 receptors in humans, affecting both viral entry and replication (Zhou & Wang, 2021), plus they may also evade the immune system (García-Beltrán *et al.*, 2021). Current vaccines for SARS-CoV-2 have been selected based on their ability to generate neutralizing antibodies (Kyriakidis, López-Cortés, González, Grimaldos & Prado, 2021; Krammer, 2020). Vaccination in Sinaloa started at the beginning of the second wave, on January 12, 2021 for health professionals, and on May 16 for teachers, school workers, and people over 50 years old. During the third wave, a large percent of the adult population had received at least the first dose of the vaccine. From January 13th, 2021 to date, five vaccines have been applied in the population of Sinaloa, under the federal vaccination program: AZD1222, CoronaVac, BNT162b2, Ad5-nCOV, and mRNA-1273 from the companies AstraZeneca, Sinovac, Pfizer-BioNTech, CanSinoBio, and Moderna respectively (https://saludsinaloa.gob.mx/). Vaccines developed by AstraZeneca and CanSinoBio are based on non-replicative viral vectors (Folegatti *et al.*, 2020; Wu, 2020) such as simian or human adenovirus, which produce the Spike glycoprotein to improve humoral and cellular responses in mammalian cells. CoronaVac vaccine from Sinovac contains inactivated SARS-CoV-2 (Zhang, Zeng & Pan, 2021). Pfizer-BioNTech and Moderna successfully developed a vaccine consisting of the full-length Spike mRNA (Martínez-Flores *et al.*, 2021).

All these vaccines were initially designed to fight the original variant of SARS-CoV-2 (Wuhan strain NC_045512); however, mutation and recombination rates mostly in the receptor-binding domain (RBD) of the Spike protein have posed a challenge for acquired immunity (Duarte *et al.*, 2022). A new generation of vaccines should achieve better recognition of viral variants and boost immunity to cope with subsequent epidemic waves.

New SARS-CoV-2 variants present mutations that allow a more efficient internalization into the host cell and/or immune response evasion (McCallum *et al.*, 2022). For example, N501Y and E484K mutations are present in the RBD of B.1.1.7, B.1.351 and P.1 lineages. N501Y increases the affinity to ACE2, whereas E484K and L452R (found in the RBD of B.1.617 and B.1.427/429 linages) enable the escape from several monoclonal antibodies as well as antibodies in plasma from convalescent patients (Harvey *et al.*, 2021; Iijima *et al.*, 2022); thus, new variants with the proper combination of mutations could potentially generate a new COVID-19 wave.

Delta and omicron variants were more contagious than previous variants (Daria, Asaduzzaman, Shahriar & Islam, 2021), and dominated the third and fourth waves, respectively, in Sinaloa. The median age of the deceased was above 60 years old in all waves, and most of the cases occurred in people between 30 and 45 years old; importantly, this age group makes up a significant portion of the labor force in the state. A previous study showed that susceptibility to SARS-CoV-2 infection in individuals under 20 years old is approximately half of adults aged over 20 years old (Davies *et al.*, 2020). Older age is an especially strong and independent risk factor for hospitalization, mechanical ventilation, and death (Clarfield & Dwolatzky, 2021). Younger people are more likely to have stronger immune systems compared to older people (Turke, 2020). In addition, older adults have more incidences of pre-existing chronic diseases affecting the immune system and therefore the response against the virus (Balboa-Castillo *et al.*, 2021).

As mentioned before, there were more SARS-CoV-2-associated cases in the third and fourth waves compared with the first and second waves; however, the number of deaths per day was higher in the first and third waves. In addition, total mortality was higher in the first, second, and third waves, decreasing in the fourth wave. This behavior could be due to the combination of several factors; the presence of SARS-CoV-2 variants, with high prevalence of the contagious delta and omicron lineages during the third and fourth waves; the lack of vaccines and treatments, as well as insufficient medical facilities for patient care and hospitalization mostly in the first wave; the severity of delta linages during the third wave; the vaccination program starting in January 2021 (at the beginning of the second wave) immunizing the most exposed or vulnerable population first, achieving complete vaccination schemes (including boosting doses) during the fourth wave; and a better understanding of SARS-CoV-2 pathology as the pandemic evolved.

Recently, Torres-Ibarra *et al.* (2022) estimated infection fatality rates (IFRs) after the first epidemic wave in Mexico and found that IFRs were higher for men than for women and increased with age. They also observed that urban and metropolitan areas experienced higher IFRs than rural areas, and suggested that the large heterogeneity of IFRs across regions could be due to structural factors, such as population density, hospital saturation, or quality of care. In addition, they explained some limitations of the estimation of IFRs such as the misclassification due to lack of testing at the beginning of the pandemic, the variability in IFRs that can be introduced depending on selected dates, and the underestimation of seroprevalence because some subgroups at high risk of SARS-CoV-2 infection were not considered in ENSANUT 2020 Covid-19 database. Here, we did not estimate the IFRs for Sinaloa, but we calculated the RRs in the four epidemic waves, which considered the positive cases over the total population. We found that a higher relative risk was detected in men than in women older than 60 years old in the first and second waves, which agrees with previous reports suggesting differences in interferon responses between both sexes (Ciarambino, Para & Giordano, 2021).

This study has some limitations that should be considered because DGE bases are biased due to underreporting, and sampling of isolates for genomic sequencing may also be biased because successful sequencing depends on relatively high viral loads, and may not necessarily represent all circulating variants in Sinaloa.

Conclusions

The COVID-19 pandemic in the state of Sinaloa showed four epidemiological waves in the period from January 2020 to February 2022. Different SARS-CoV-2 variants drove each wave. The decrease in the number of deaths during the fourth wave could be related to the vaccination program, more efficient and affordable testing alternatives, and the use of preventive measures (such as masks and virtual work) during the pandemic. COVID-19 waves in Sinaloa seem to occur during summer and winter, not necessarily coinciding with massive events, and precise triggering factors are still not clear. Preventive measures might be partially relaxed only during "interwave" periods, but reinforced as the wave arrives. It is important for the population to understand the behavior of the COVID-19 pandemic, and, continue with preventive and containment measures.

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Authors' contributions

Conceptualization, Jorge Luis Batista-Roche, Marian Mirabent-Casals; methodology, Jorge Luis Batista-Roche, Marian Mirabent-Casals, César Berlanga-Robles; validation, César Berlanga-Robles, Bruno Gómez-Gil, Alejandra García-Gasca; formal analysis, Luis Batista-Roche, Marian Mirabent-Casals, César Berlanga-Robles; writing—original draft preparation, Jorge Luis Batista-Roche, Marian Mirabent-Casals, Alejandra García-Gasca; writing—review and editing, Jorge Luis Batista-Roche, Marian Mirabent-Casals, Alejandra García-Gasca, César Berlanga-Robles, Bruno Gómez-Gil; supervision, Alejandra García-Gasca. All authors read and approved the manuscript.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article (public databases).

Declaration of Competing Interest

All authors declare no conflict of interest.

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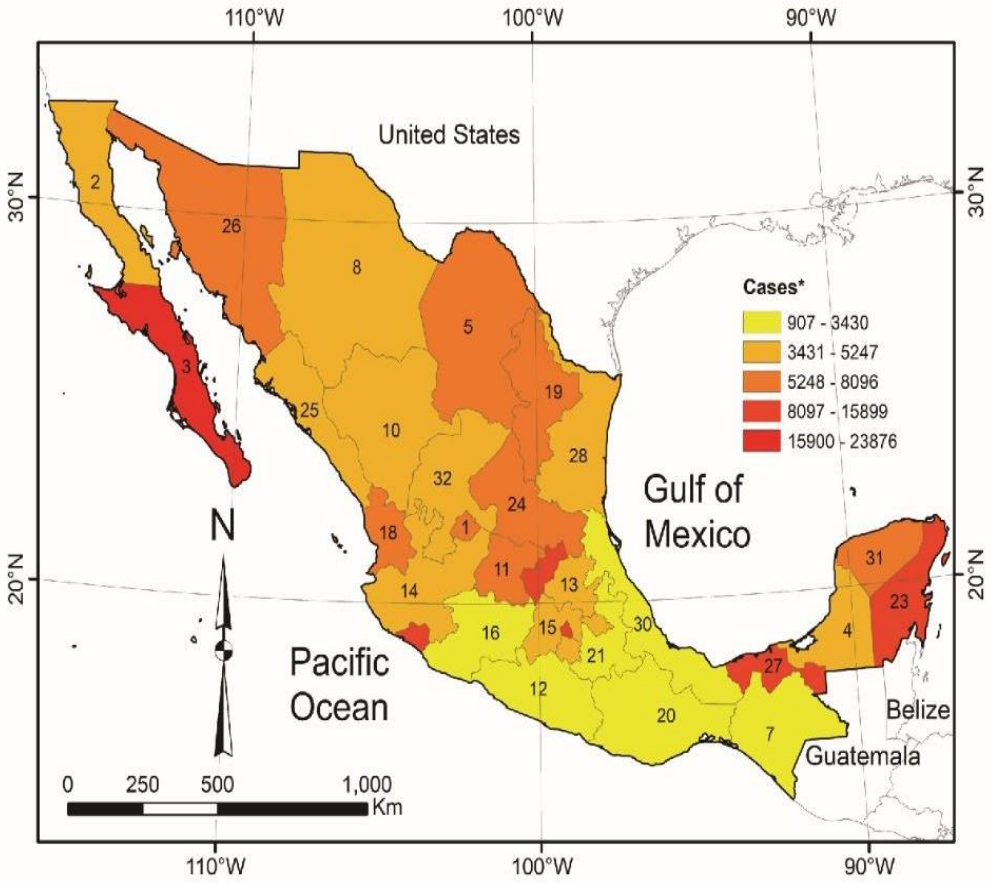
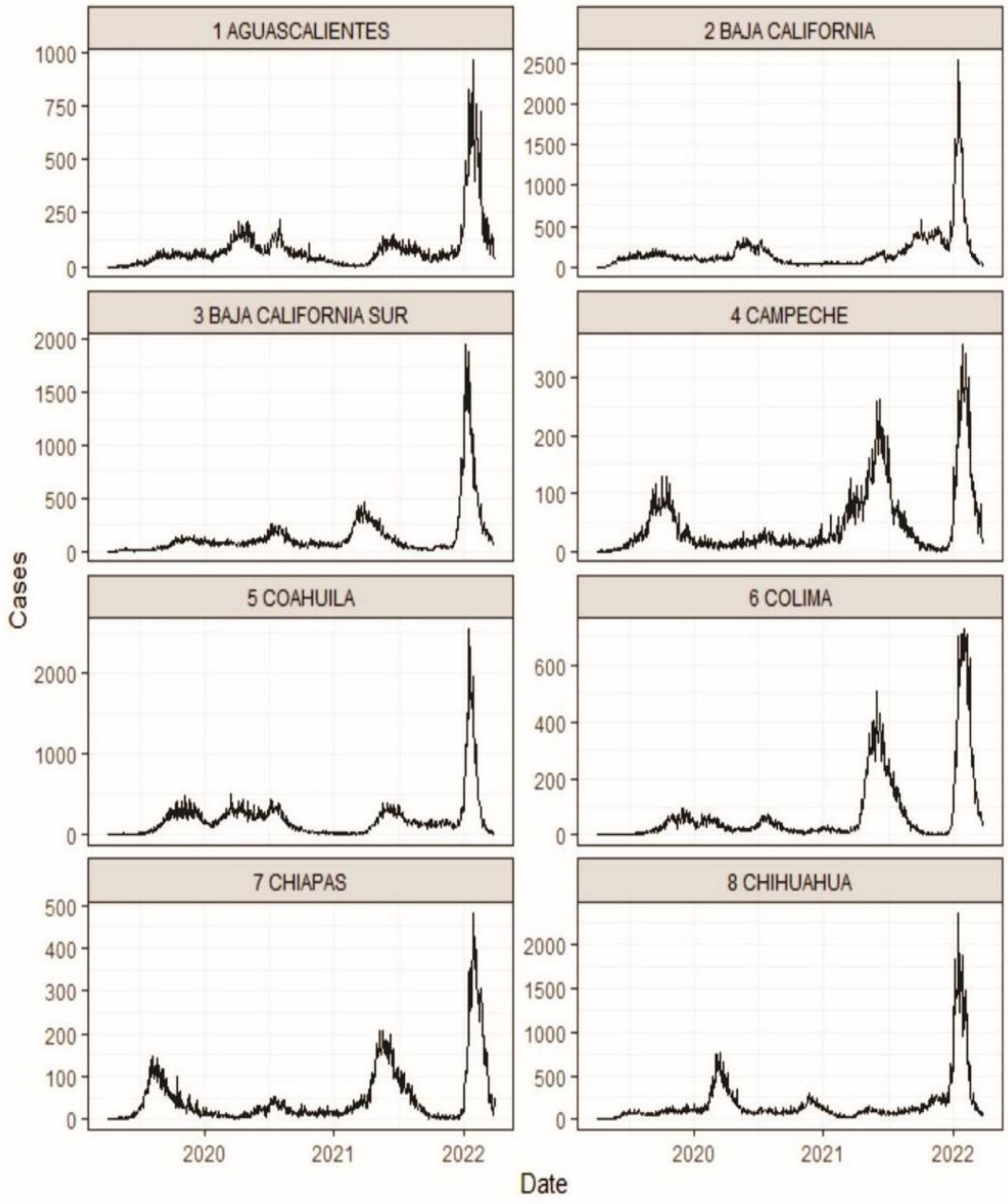
SUPPLEMENTARY FIGURE S1
COVID-19 PANDEMIC DYNAMICS IN MEXICO
(Cases by symptom onset by Federal entity)



Federal entity of Mexico. Cases*: accumulated cases from 2020-03-01 to 2022-02-26 per 100,000 people. Sources: Geostatistical Framework, June 20161; Admin or Countries 4.72. General Directorate of Epidemiology database.

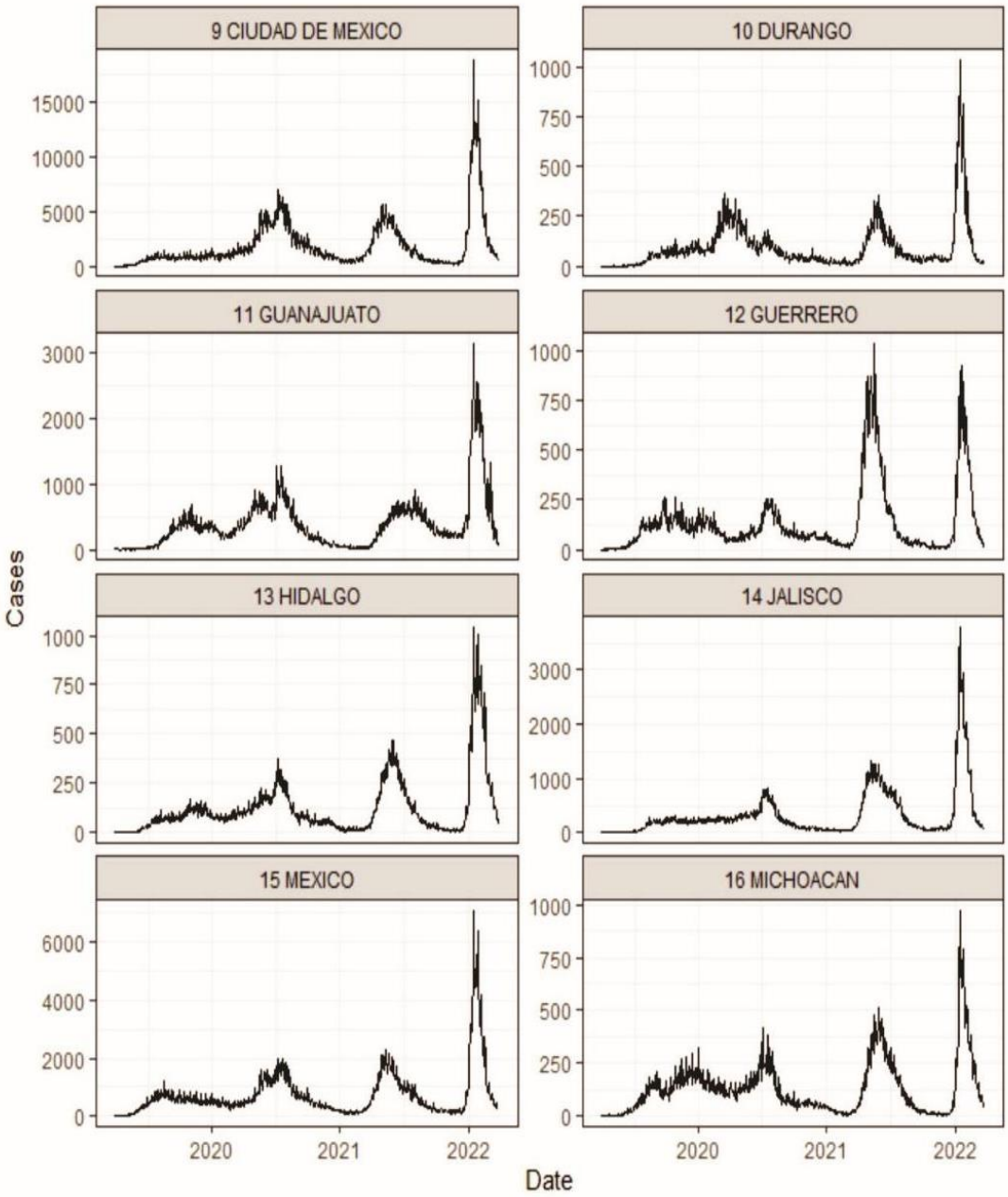
[1]INEGI, https://www.inegi.org.mx/app/biblioteca/ficha.html?upc=702825217341

[2]Natural Earth, http://www.naturalearthdata.com/downloads/10m-culturalvectors/



Source: General Direction of Epidemiology database:

https://www.gob.mx/salud/documentos/datos-abiertos-bases-historicasdireccion-general-de-epidemiologia?idiom=es



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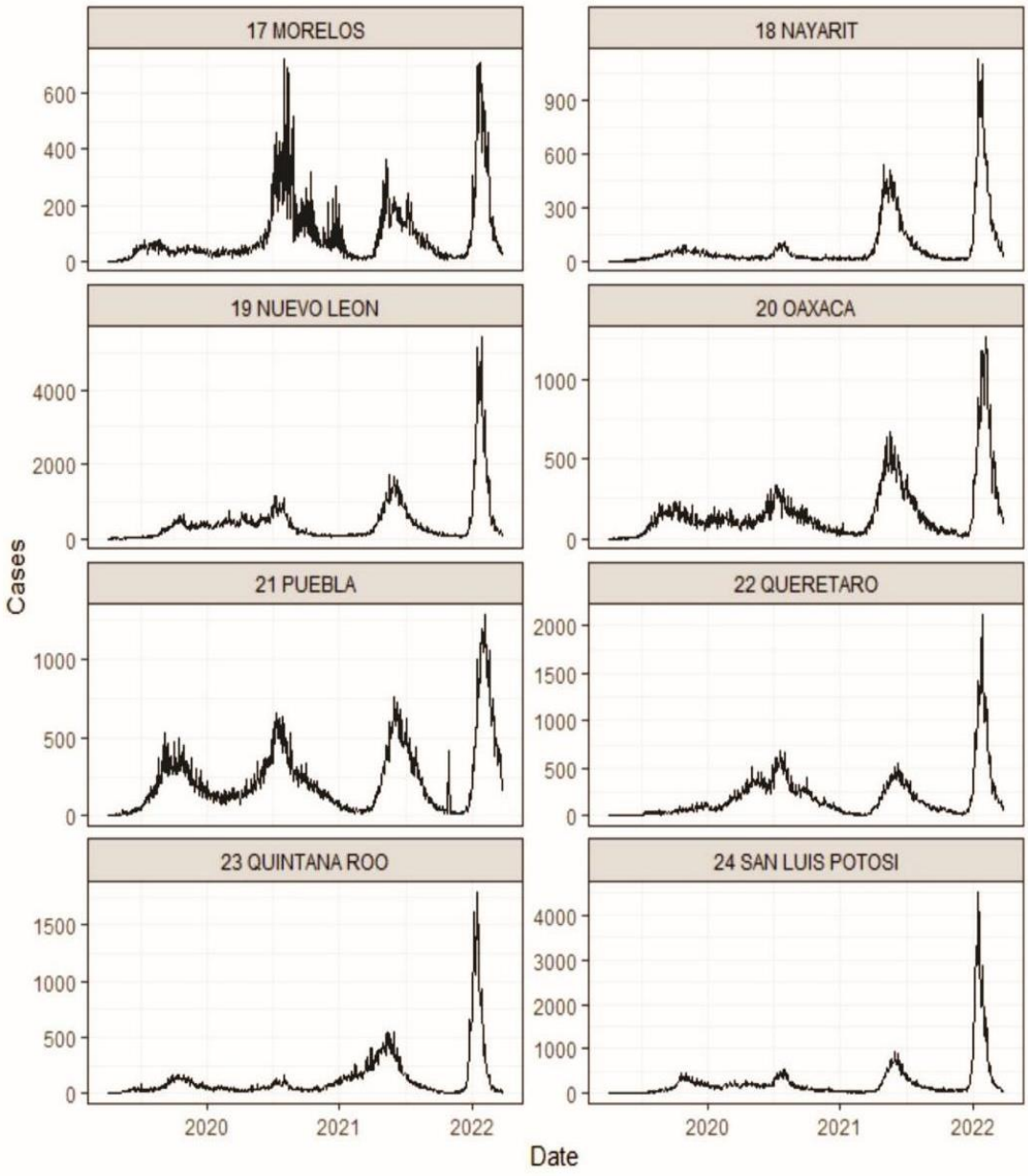

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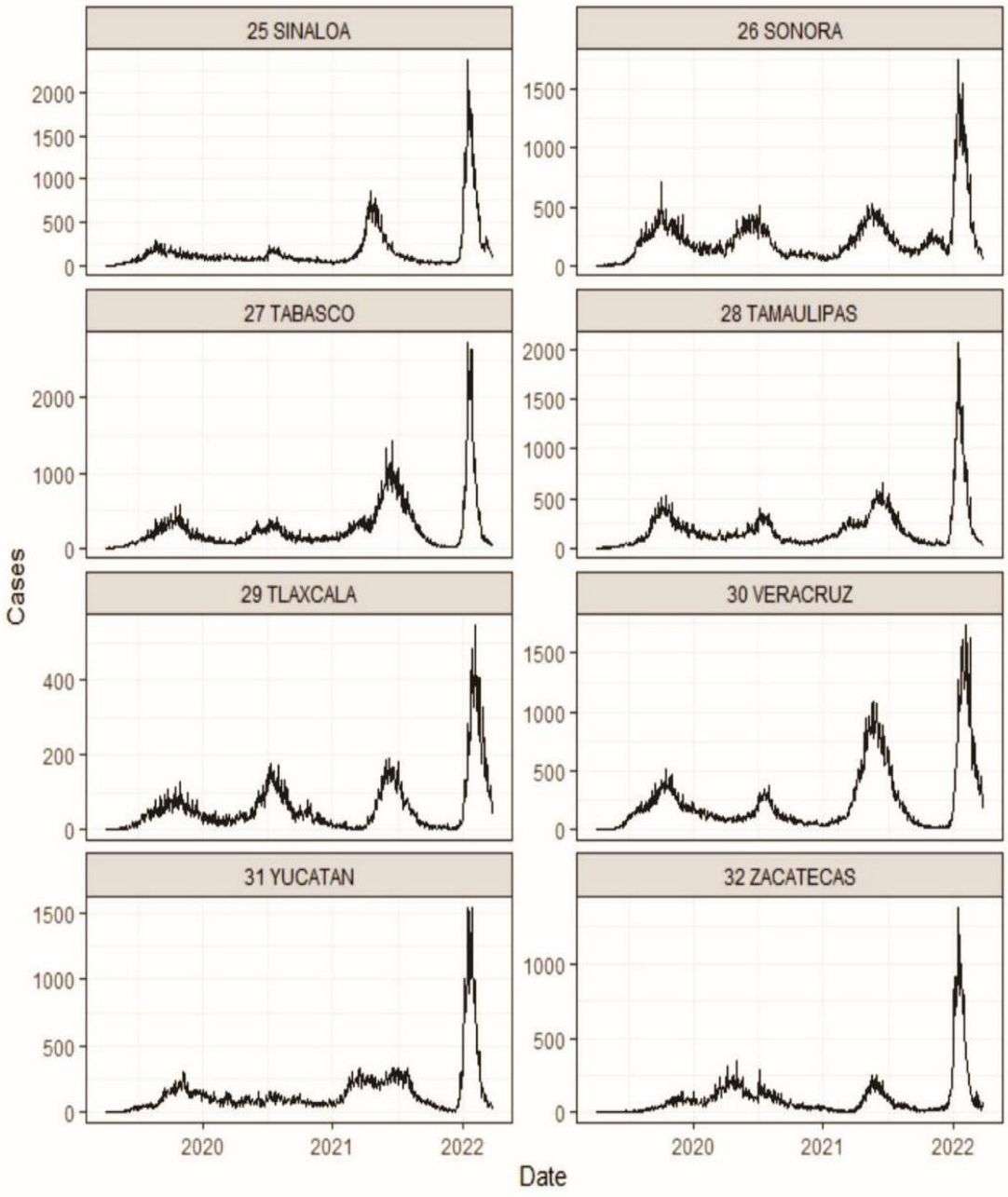

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https://www.gob.mx/salud/documentos/datos-abiertos-bases-historicasdireccion-general-de-epidemiologia?idiom=es

Supplemental table

Data Availability
GISAID Identifier: EPI_SET_20220614xp
DOI: https://doi.org/10.55876/gis8.220614xp
All genome sequences and associated metadata in this dataset are published in GISAID's EpiCoV database. To view the contributors of each individual sequence with details such as accession number, Virus name, Collection date, Originating Lab and Submitting Lab and the list of Authors, visit https://doi.org/10.55876/gis8.220614xp
Data Snapshot
• EPI_SET_20220614xp is composed of 2,090 individual genome sequences. • The collection dates range from 2020-04-02 to 2022-05-23; • Data were collected in 1 countries and territories; • All sequences in this dataset are compared relative to hCoV-19/Wuhan/WIV04/2019 (WIV04), the official reference sequence employed by GISAID (EPI_ISL_402124). Learn more at https://gisaid.org/WIV04.

Supplementary Figure S2
ANOVA for the relative risk (RR) of infection with SARS-CoV-2 for sex, age, and wave factors, in COVID-19 patients in Sinaloa, Mexico.

ANOVA table for the model RR = sex + age + wave.

Source	Sum Sq	Df	F value	p
sex	0.2022	1	2.3083	0.137
age	9.0572	5	20.6803	6.327e-10
wave	0.4869	3	1.8531	0.154
Residuals	3.3285	38		

Post-hoc comparison with Sidak method for the estimated marginal means (Least-square means) of the RR response to the age factor.

contrast	estimate	SE	df	t.ratio	p.value
0-14 - 15-19	-0.8605	0.1480	38	-5.8147	0.000015
0-14 - 30-44	-1.3115	0.1480	38	-8.8629	0.000000
0-14 - 45-59	-1.0993	0.1480	38	-7.4289	0.000000
0-14 - 60-74	-0.8558	0.1480	38	-5.7833	0.000017
0-14 - >=75	-1.2314	0.1480	38	-8.3215	0.000000
15-19 - 30-44	-0.4511	0.1480	38	-3.0481	0.060852
15-19 - 45-59	-0.2389	0.1480	38	-1.6142	0.839359
15-19 - 60-74	0.0047	0.1480	38	0.0314	1.000000
15-19 - >=75	-0.3710	0.1480	38	-2.5068	0.221815
30-44 - 45-59	0.2122	0.1480	38	1.4340	0.926535
30-44 - 60-74	0.4557	0.1480	38	3.0796	0.056082
30-44 - >=75	0.0801	0.1480	38	0.5414	0.999999
45-59 - 60-74	0.2435	0.1480	38	1.6456	0.820215
45-59 - >=75	-0.1321	0.1480	38	-0.8926	0.999187
60-74 - >=75	-0.3756	0.1480	38	-2.5382	0.207243

Results are averaged over the levels of: sex, wave. P value adjustment: Sidak method for 15 tests.

Confident interval at 95% (α = 0.05) and 99% (α = 0.1) levels for estimated marginal means (Least-square means) of the RR response to the age factor.

				α = 0.05			α = 0.1		
age	mean	SE	df	lower.CL	upper.CL	group	lower.CL	upper.CL	group
0-14	0.208	0.105	38	-0.082	0.498	a	-0.052	0.468	a
60-74	1.064	0.105	38	0.774	1.354	b	0.804	1.324	b
15-19	1.069	0.105	38	0.778	1.359	b	0.808	1.329	b
45-59	1.307	0.105	38	1.017	1.598	b	1.047	1.568	bc
>=75	1.439	0.105	38	1.149	1.730	b	1.179	1.700	bc
30-44	1.52	0.105	38	1.229	1.810	b	1.259	1.780	c

Results are averaged over the levels of: sex, wave. Confidence level used: 0.95 and 0.9. Conf-level adjustment: Sidak method for 6 estimates. P value adjustment: Sidak method for 15 tests significance level used: alpha = 0.05 and 0.01.

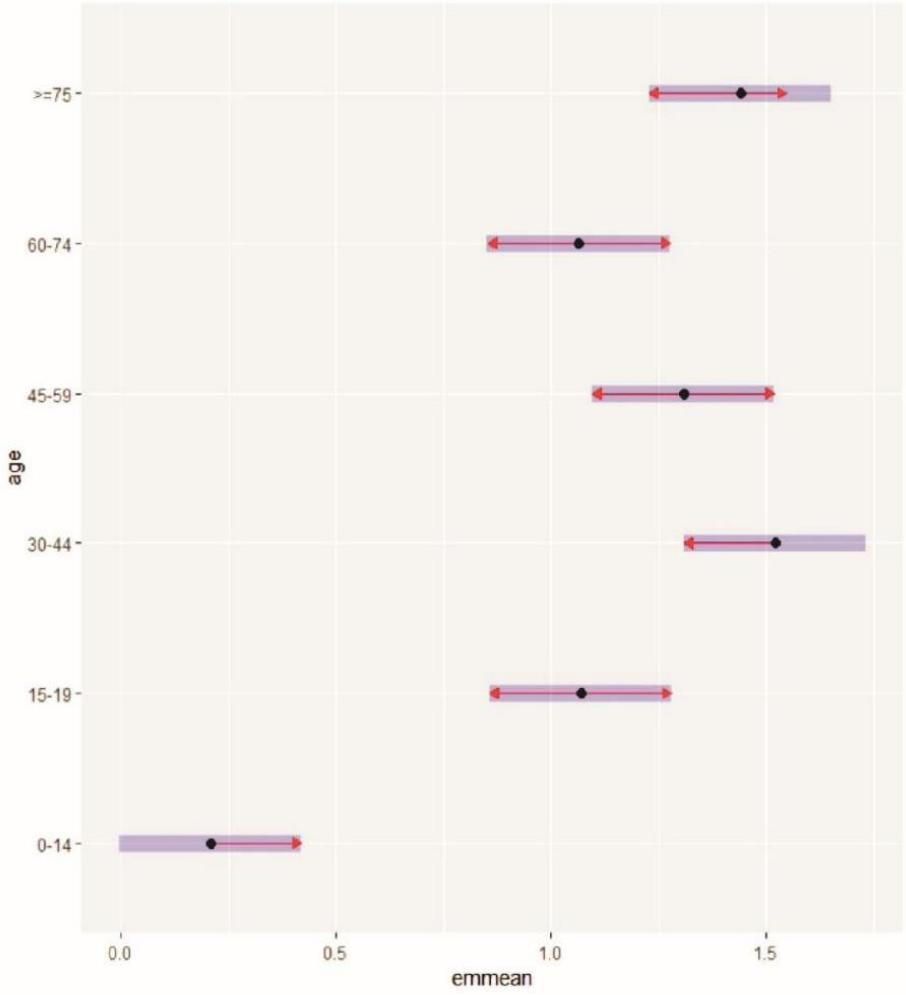

Figure S2. Estimated marginal means (Least-square means) of the RR response to the age factor in COVID-19 patients in Sinaloa, Mexico. The purple stripes indicate the 95% confidence interval, and the red arrows the contrast between age groups for alpha = 0.05.

3. GLOBAL m6A RNA METHYLATION IN SARS-COV-2 POSITIVE NASOPHARYNGEAL SAMPLES IN A MEXICAN POPULATION: A FIRST APPROXIMATION STUDY

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epigenomes

MDPI

Communication

Global m6A RNA Methylation in SARS-CoV-2 Positive Nasopharyngeal Samples in a Mexican Population: A First Approximation Study

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Abstract: The Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) is the causal agent of COVID-19 (Coronavirus Disease-19). Both mutation and/or recombination events in the SARS-CoV-2 genome have resulted in variants that differ in transmissibility and severity. Furthermore, RNA methylation of the N6 position of adenosine (m6A) is known to be altered in cells infected with SARS-CoV-2. However, it is not known whether this epitranscriptomic modification differs across individuals dependent on the presence of infection with distinct SARS-CoV-2 variants, the viral load, or the vaccination status. To address this issue, we selected RNAs (n = 60) from SARS-CoV-2 sequenced nasopharyngeal samples (n = 404) of 30- to 60-year-old outpatients or hospitalized individuals from the city of Mazatlán (Mexico) between February 2021 and March 2022. Control samples were non-infected individuals (n = 10). SARS-CoV-2 was determined with real-time PCR, viral variants were determined with sequencing, and global m6A levels were determined by using a competitive immunoassay method. We identified variants of concern (VOC; alpha, gamma, delta, omicron), the variant of interest (VOI; epsilon), and the lineage B.1.1.519. Global m6A methylation differed significantly across viral variants ($p = 3.2 \times 10^{-7}$). In particular, we found that m6A levels were significantly lower in the VOC delta- and omicron-positive individuals compared to non-infected individuals ($p = 2.541236 \times 10^{-2}$ and 1.134411×10^{-4}, respectively). However, we uncovered no significant correlation between global m6A levels and viral nucleocapsid (*N*) gene expression or age. Furthermore, individuals with complete vaccination schemes showed significantly lower m6A levels than unvaccinated individuals ($p = 2.6 \times 10^{-4}$), and differences in methylation levels across variants in unvaccinated individuals were significant ($p = 3.068 \times 10^{-3}$). These preliminary results suggest that SARS-CoV-2 variants show differences in global m6A levels.

Keywords: SARS-CoV-2; m6A methylation; nasopharyngeal samples; viral variants

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Citation: Batista-Roche, J.L.; Gómez-Gil, B.; Lund, G.; Berlanga-Robles, C.A.; García-Gasca, A. Global m6A RNA Methylation in SARS-CoV-2 Positive Nasopharyngeal Samples in a Mexican Population: A First Approximation Study. *Epigenomes* **2022**, *6*, 16. https://doi.org/10.3390/epigenomes6030016

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1. Introduction

SARS-CoV-2 is an enveloped, spheroidal-shaped virus that causes the disease known as COVID-19 in humans. It has a capsid that contains the structural proteins spike (S), membrane (M), envelope (E), and the nucleocapsid protein (N). Its genome is a single, positive stranded ribonucleic acid (RNA) that behaves as messenger RNA (mRNA) [1]. The N protein is a crucial structural component of SARS-CoV-2 that participates in the virion assembly and is often used as a diagnostic marker of viral infection [2]. In a vaccine setting,

the N protein induces SARS-CoV-2-specific T cell proliferation and cytotoxic activity and promotes long-lasting T cell immunity [3].

The main receptor and route of entry of SARS-CoV-2 into the host cells is the angiotensin-converting enzyme 2 (ACE2), which binds with the S protein of SARS-CoV-2 and mediates the fusion of viral and cell membranes. ACE2 is found in the membranes of different human cells such as pneumocytes, enterocytes, proximal tubular cells of the kidney, and endothelial cells of veins and arteries [4]. Various mutations in the viral genome have given rise to SARS-CoV-2 variants, which differ not only in nucleotide sequences but also in transmissibility and severity [5,6].

Following the discovery of RNA methylation at N6- position of adenosine (m6A), the most prevalent modification of RNA in mammalian cells [7,8], this modification has also been detected in several viral genomes [9]. Most m6A is found in the consensus sequence motif DRACH (where A* denotes the methylated adenosine, D denotes A, G or U, R denotes A and G, and H denotes A, C or U) and mRNAs may contain from one to up to 20 m6A sites or more [10,11]. Genomic and viral levels of m6A are dynamically regulated by the activity of methyltransferase enzymes (i.e., "writers" such as METTL3, METTL14 that transfer methyl groups to nitrogen 6 of the adenosine) and demethylases ("erasers" such as fat mass and obesity-associated protein (FTO) and a ketoglutarate dependent dioxygenase homolog 5 (ALKBH5)). Once methylated, m6A is recognized by "reader" proteins (e.g., YTH N6-Methyladenosine RNA Binding Protein domain-containing proteins YTHDC1 and C2, YTHDF1, F2 and F3) that influence the fate of m6A RNA, either by guiding mRNAs to ribosomes for translation or to other complexes for their degradation [11,12].

It was recently reported that SARS-CoV-2 RNA, as well as negative-stranded viral RNAs, are dynamically methylated in vitro, as is the host cell RNA [13]. In human hepatocarcinoma cells (Huh7), SARS-CoV-2 genomic RNA is gradually methylated during infection and m6A occurs more frequently towards the 3′ region of the genome [14]. In addition, SARS-CoV-2 proteins interact with the host m6A methylation machinery to modulate viral replication. For example, an increase in METTL3 expression 48 h post-infection in Vero E6 cells was positively associated with SARS-CoV-2 replication [15]. In accordance, depletion of METTL3 leads to a reduction in SARS-CoV-2 replication [13,15,16], although the opposite has also been reported [14]. Furthermore, knockdown of specific erasers (ALKBH5) and readers (YTHDF1, YTHDF2, YTHDF3) have also been shown to impact on viral replication [14,16].

Recent studies show that SARS-CoV-2 infection changes the host cell m6A methylome in vitro, promoting differential expression of host genes [13], and in vivo, altering m6A modification levels in lymphocytes from peripheral blood samples by increased expression of the m6A methyltransferase RNA-binding motif protein 15 (*RBM15*) [17] or decreased expression of *METTL3* in epithelial cells of bronchoalveolar lavage fluid of COVID-19 patients [13].

To date, only a few studies have probed for alterations in global m6A levels in a limited number of COVID-19 positive individuals (n = 2–20) [13,17,18]. These studies found increased levels of m6A in peripheral blood samples [17] or in epithelial cells of bronchoalveolar lavage fluid [13] in infected individuals relative to non-infected individuals that are associated with increased expression of *RBM15*, which encodes for a protein that facilitates m6A by guiding METTL3 to the target RNA sequence [17]. To our knowledge, no studies have compared m6A levels across individuals infected with distinct COVID-19 variants. Therefore, we measured global levels of this modification in nasopharyngeal samples from adult (30- to 60-year-old) male and female patients infected with different SARS-CoV-2 variants between February 2021 and March 2022.

2. Results

2.1. Viral Variants Detected in Mazatlán, Mexico during the COVID-19 Pandemic (February 2021–March 2022)

Analysis of nasopharyngeal samples from a total of 404 individuals (219 males and 185 females) revealed the presence of distinct SARS-CoV-2 variants during the second, third, and fourth waves of the COVID-19 pandemic in Mazatlán, Mexico from February 2021 to March 2022 (Figure 1). During the second wave, epsilon and B.1.1.519 lineage variants predominated in the population, peaking between February and March 2021. During the third wave, peaking between June and August, the delta variant dominated, although the alpha and gamma variants were also detected. Finally, the fourth wave, peaking between January and February, was dominated by the omicron variant.

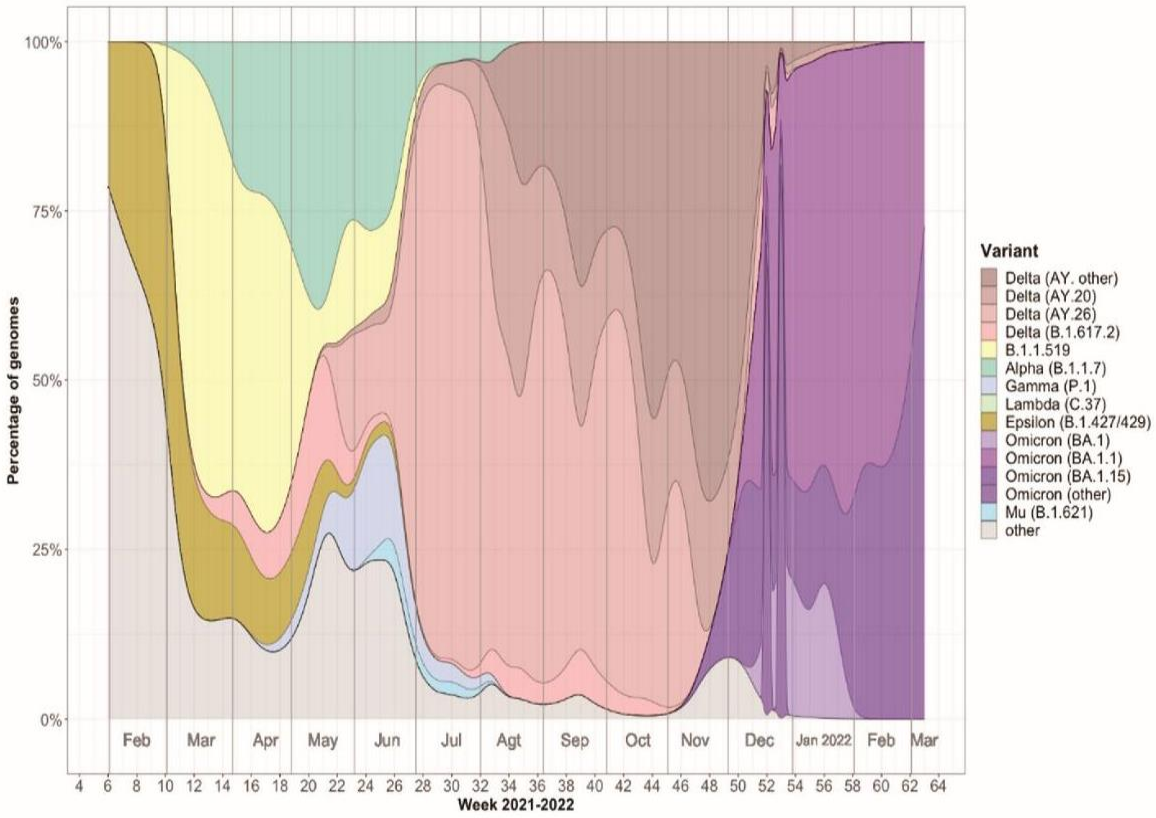

Figure 1. Percentage of genomes of SARS-CoV-2 variants sequenced in Mazatlán, Mexico, from February 2021 to March 2022 (n = 404). Delta (AY. other) = AY.100, AY.103, AY.113, AY.119, AY.122.4, AY.25, AY.3, AY.39, AY.43, AY.44. AY.53. Omicron (other) = BA.1.1.16, BA.1.1.2, BA.1.1.8, BA.1.13, BA.1.14, BA.1.17.

2.2. Global m6A Levels and Nucleocapsid (N) Gene Expression in Nasopharyngeal Samples

Global m6A levels were determined with 60 randomly selected sequenced samples of each of the following variants: omicron (BA.1), delta (B.1.617.2), gamma (P.1), alpha (B.1.1.7), and epsilon (B.1.429), as well as the B.1.1.519 lineage (Figure 2A). Table 1 shows the average age, sex distribution, and vaccination status of individuals in each variant group. Vaccination data were available for 64 of the 70 samples analyzed; 8 and 4 samples were from patients with a complete or partial vaccination schemes, respectively, while 52 samples came from unvaccinated patients.

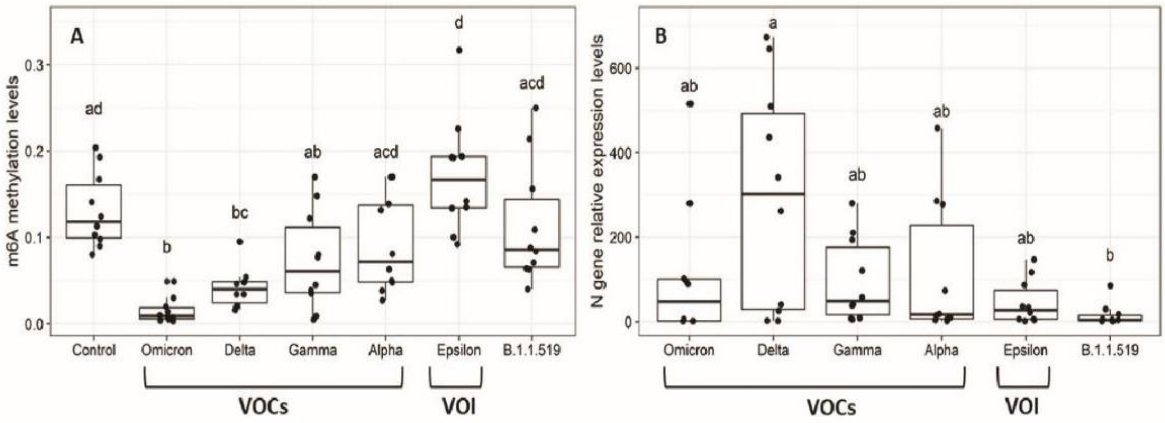

Figure 2. (**A**) Global m6A levels of RNAs extracted from human nasopharyngeal samples, tested for SARS-CoV-2 by real-time PCR based on viral variant ($n = 10$ each). Control: SARS-CoV-2 negative samples (Kruskal-Wallis $p = 3.2 \times 10^{-7}$); different letters indicate significant differences in methylation levels. (**B**) Relative expression of the *N* gene in RNAs extracted from human nasopharyngeal samples, tested for SARS-CoV-2 by real-time PCR based on viral variant ($n = 10$ each) (Kruskal-Wallis, $p = 0.041$; Dunn, $p = 0.024$); different letters indicate significant differences in gene expression levels. VOC: omicron, delta, gamma, and alpha. VOI: epsilon; B.1.1.519: lineage found in Mexico during the second wave.

Table 1. Characteristics of patients (average age, sex distribution, and vaccination status) included in each variant group. The Control group refers to SARS-CoV-2 negative patients.

Variant	Average Age ± SD	Sex Distribution ($n = 70$)		Vaccination Status ($n = 64$)
		Males	Females	
Control	46 ± 7	5	5	Not vaccinated ($n = 10$)
Omicron	42 ± 8	5	5	Complete ($n = 8$) Not vaccinated ($n = 2$)
Delta	39 ± 10	5	5	Partial ($n = 4$)
Gamma	44 ± 7	5	5	Not vaccinated ($n = 10$)
Alpha	49 ± 8	5	5	Not vaccinated ($n = 10$)
Epsilon	45 ± 10	5	5	Not vaccinated ($n = 10$)
B.1.1.519	47 ± 11	5	5	Not vaccinated ($n = 10$)

A Kruskal–Wallis test revealed significant differences in global m6A according to viral variant ($p = 3.2 \times 10^{-7}$). In particular, delta and omicron-positive patients showed significantly lower m6A levels than the control group (SARS-CoV-2 negative) ($p = 2.541236 \times 10^{-2}$ and 1.134411×10^{-4} for delta and omicron respectively).

To understand whether the differences in variant m6A levels were related to viral load, we compared expression levels of the viral nucleocapsid (*N*) gene across variants. Only delta and B.1.1.519 variants showed significant differences in *N* expression levels (Kruskal-Wallis, $p = 4.1 \times 10^{-2}$; Dunn, $p = 2.4 \times 10^{-2}$) (Figure 2B). However, we found no significant correlation, neither between m6A levels and relative expression of the *N* gene of all, or only unvaccinated individuals ($r = -0.06$, $p = 0.63$ and $r = 0.004$, $p = 0.9803$, respectively) (Figure 3), nor between m6A and age ($r = 0.15$, $p = 0.21$).

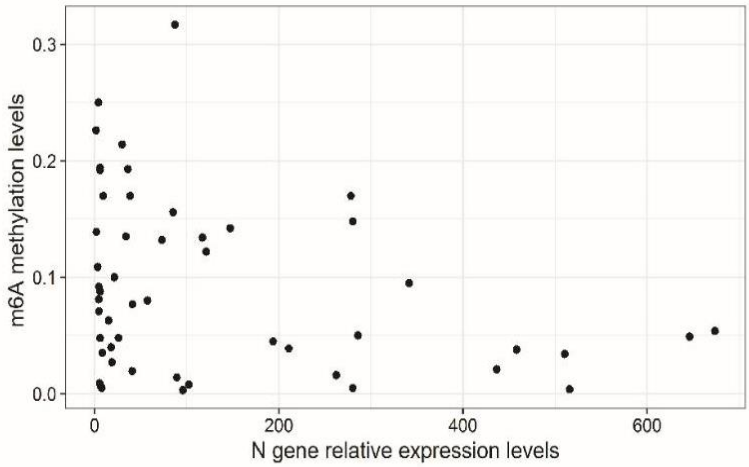

Figure 3. Scatter plot between relative expression of the *N* gene and m6A levels (Spearman's rank correlation, rho = −0.063, $p = 0.63$).

Finally, we evaluated whether patient vaccination status affected global m6A RNA methylation levels. Samples from patients with complete vaccination schemes showed significantly lower levels of RNA methylation compared to unvaccinated patients (Kruskal-Wallis, $p = 2.6 \times 10^{-4}$; Dunn test, $p = 2.5 \times 10^{-4}$) (Figure 4A). Importantly, samples from vaccinated patients were obtained during the third and fourth waves, dominated by delta and omicron variants, respectively. To exclude that vaccination status affected the observed differences between variants, we performed a Kruskal-Wallis analysis restricted to unvaccinated individuals. As previously observed, m6A levels differed significantly across variants ($p = 3.068 \times 10^{-3}$), with omicron and some gamma-infected patients showing the lowest methylation levels (Figure 4B). The delta variant was not represented in unvaccinated individuals.

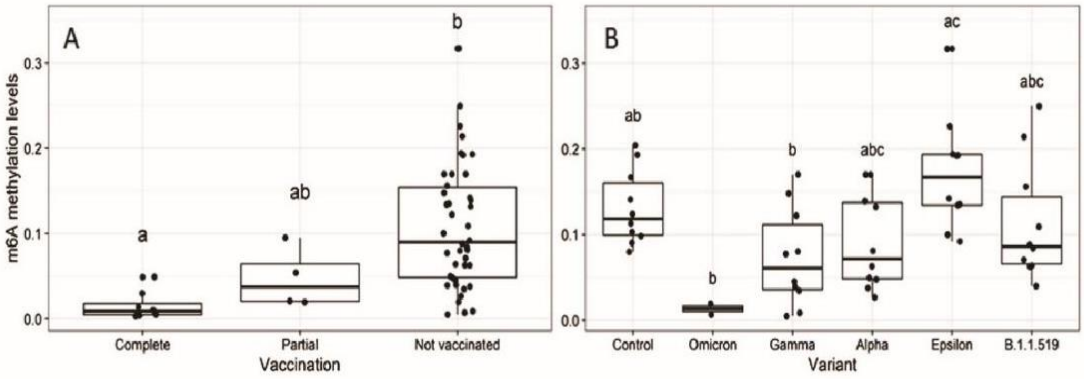

Figure 4. (**A**) Global m6A levels in of RNAs extracted from human nasopharyngeal samples, tested for SARS-CoV-2 by real-time PCR with different vaccination schemes ($n = 64$). Partial and complete vaccination refers to the application of one or two doses, respectively, of authorized vaccines in Mexico (AZD1222, CoronaVac, BNT162b2, Ad5-nCOV, and mRNA-1273 from the companies AstraZeneca, Sinovac, Pfizer-BioNTech, CanSinoBio, and Moderna, respectively) (Kruskal-Wallis, $p = 2.6 \times 10^{-4}$; Dunn test, $p = 2.5 \times 10^{-4}$); $n = 8$, 4, and 52 for complete, partial, and unvaccinated individuals, respectively. (**B**) Global m6A levels of RNAs extracted from human nasopharyngeal samples from unvaccinated patients, tested for SARS-CoV-2 by real-time PCR based on viral variant ($n = 52$). Control: SARS-CoV-2 negative samples (Kruskal–Wallis $p = 3.068 \times 10^{-3}$); different letters indicate significant differences in methylation levels.

3. Discussion

RNA modifications such as m6A play an important role in different physiological processes and human diseases. In the innate immune response, m6A-modfied mRNAs are essential for the translation of co-stimulatory molecules that allow dendritic cell maturation and subsequent T cell activation [19]. Also, the immunosuppressive functions of regulatory T cells are maintained by m6A-driven degradation of the suppressor of cytokine signaling (SOCS) transcripts [8]. In addition, RNAs from several viral families acquire m6A modifications as a common strategy to evade innate host immunity [20].

It has been reported that m6A deficiency in the vesicular stomatitis virus (VSV) genome triggers significantly higher levels of type I interferon [20]. However, some m6A sites of viral RNAs favor the evasion of the pattern recognition receptors of the cellular innate immune response and promote viral replication [21]. Conversely, certain transcripts of some RNA viruses and adenoviruses contain m6A-methylated sites that negatively influence infection [14]. Currently, there is a controversy in the literature regarding the effects of m6A methyltransferases on SARS-CoV-2 replication. Apparently, depending on the cell type, depletion of m6A methyltransferases can decrease viral load [13] or increase viral replication and the percentage of SARS-CoV-2 infected cells [14].

To our knowledge, this is the first report showing that global m6A levels of nasopharyngeal RNA samples of patients infected with SARS-CoV-2 differ among viral variants. Interestingly, the two most contagious variants (delta and omicron)—with omicron being the most contagious globally—[22] showed the lowest methylation levels. These differences are unlikely to reflect variation in disease severity across variants, as COVID-19 patients with beta and delta variants generally are at higher risk of developing severe disease compared to patients with alpha, gamma [23], and omicron variant(s), whose mutations have been suggested to contribute to the host immune escape [24]. Interestingly, DRACH motifs are highly conserved among SARS-CoV-2 variants [25], thus the contribution of DRACH motifs to differential methylation levels among variants remains unclear and require further research.

From a public health perspective, identifying a reliable marker of severe COVID-19 risk is of utmost importance. Unfortunately, we have no information regarding disease severity of the individuals analyzed in this study since most samples were from outpatients. In addition, we found no correlation between viral load and m6A levels across variants. However, previous studies have reported a higher m6A level in lung or peripheral blood from patients with moderate or severe COVID-19 disease compared to healthy individuals [13,17,18,26]. Interestingly, in peripheral blood these changes were associated with increased expression of *RBM15* [17]. Moreover, Qiu et al. [26] proposed a predictive "m6A score" to quantify and model the m6A pattern in blood leukocytes for each COVID-19 patient based on m6A levels and nine selected differentially expressed genes (m6A-DEGs) mostly related to the immune response; in this model, patients displaying higher (protective) scores showed a better prognosis related to T-cell activation compared to patients with lower scores; in addition to clinical prognosis, the model may predict the possibility of contracting COVID-19 in patients infected with SARS-CoV-2, as well as the detection of SARS-CoV-2 carriers [26].

Our study also revealed significantly lower levels of m6A in samples from fully vaccinated compared to unvaccinated individuals (n = 8 and 52, respectively). Most unvaccinated individuals were positive for alpha, gamma, epsilon, and B.1.1.519 variants, whereas delta and omicron were the dominant variants in vaccinated individuals. Importantly, we show that the variation in m6A levels across variants, in particular the low levels in delta and omicron, could not be explained by vaccination status alone. While changes in DNA methylation levels have been reported after influenza vaccination [27,28], there is no information regarding RNA methylation after vaccination. Potential vaccination-mediated m6A is intriguing and deserves further investigation.

The delta variant showed significant differences in the mean levels of *N* expression when compared with the B.1.1.519 variant, indicating that the delta variant had the highest

viral loads. Similarly, additional studies show that patients infected with the delta variant presented increased viral loads compared to other SARS-CoV-2 variants [29,30]. Conversely, a recent study that compared a larger number of individuals positive for alpha or delta to other variants (n = 36 and 41, respectively), neither found significant differences between mean viral load across variants, nor between vaccinated and unvaccinated participants (37 vaccinated and 136 unvaccinated) [31]. In our study, no significant differences in *N* gene expression were observed among VOC- and VOI-positive samples, consistent with the previous report.

The results presented in this study are preliminary and should be interpreted with caution due to the following limitations. First, although all patients enrolled in this study were Mexican and living in the city of Mazatlán, genetic background, health and immune conditions, as well as lifestyle, greatly differ across individuals. Furthermore, we received samples from different hospitals which may differ in the sampling process and/or the social/economic status of the patients. Second, global m6A data represented the sum of methylation of both human and viral RNAs; therefore, additional studies are needed to determine their relative contributions. Third, due to the small amounts of RNA obtained from each nasopharyngeal sample, we were unable to perform technical duplicates of m6A content. Fourth, since we only had access to amplification data of the viral *N* and human *Rp* genes, calculations of viral *N* expression were performed relative to *Rp*. Fifth, increasing the sample size of analyzed individuals is important in order to improve the reliability of the conclusions presented.

The port of Mazatlán is a very touristic location, receiving thousands of visitors from Mexico and other countries around the world every year. Rapid changes of the dominant variants over the sampling period could be due to the crowded environment allowing the virus to spread. In addition, high mutation and recombination rates of RNA viruses, as well as asymptomatic infections, could also contribute to the rapid diversification of SARS-CoV-2 [32] even among fully vaccinated individuals. Despite the limitations of this study (which can be addressed in future studies), our results suggest that viral variants modulate genome and/or viral m6A levels differentially (Figure 5). Further research is needed to fully unravel and understand the molecular basis of these differences and its relevance in viral infection and transmission, as well as the implications in human immunity and health.

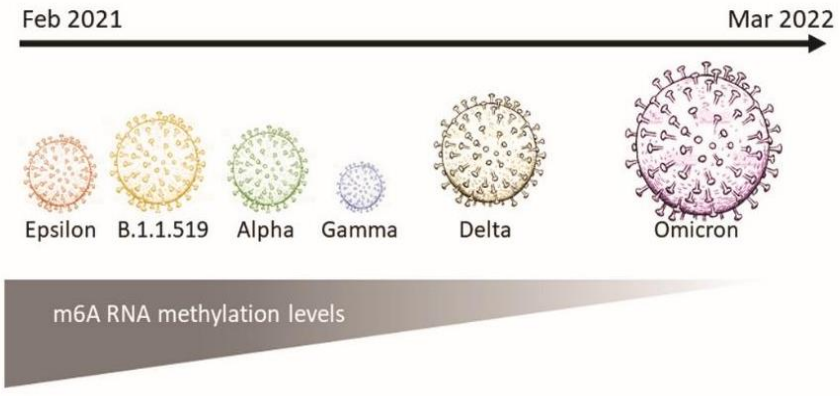

Figure 5. Schematic representation of m6A levels (grey triangle) among different SARS-CoV-2 variants from February 2021 to March 2022. Circle size represents number of cases per variant (smaller circles = less cases; larger circles = more cases) in the studied population.

4. Materials and Methods

4.1. Samples

This study used 404 SARS-CoV-2 PCR-positive sequenced samples from the National Epidemiological Surveillance Consortium (see Figure 1), from which 60 samples (plus 10 samples from SARS-CoV-2 negative patients) were randomly selected for further analysis.

Patients <30 or >60 years old, as well as SARS-CoV-2 PCR-positive samples with Cts <30 were excluded.

4.2. Identification and Sequencing of SARS-CoV-2 Positive Samples

Nasopharyngeal samples were taken by health professionals from different hospitals in Mazatlán (Mexico) and transported to the Molecular Diagnostic Laboratory at the Research Centre for Nutrition and Development (LDM-CIAD, official authorization number: DGE-DDYR-DSAT-04471-2020) for SARS-CoV-2 detection by real-time PCR (2019-nCov CDC® Integrated DNA Technologies, Coralville, IA, USA). Relative expression levels of the viral *N* gene were determined for each sample as follows [33]:

$$\Delta C_T = \frac{C_T N1 + C_T N2}{2} - C_T Rp \quad (1)$$

where *N*1 and *N*2 are regions of the viral *N* gene and *Rp* is the human *RNAse P* gene (Supplementary File S1).

RNA samples (n = 404) with higher *N* levels (C_T < 30) were selected for sequencing through the National Epidemiological Surveillance Consortium (COVIGEN). Complementary DNAs (cDNAs) were synthesized from total RNAs using the GoScript™ Reverse Transcription System (Promega, Madison, WI, USA). Libraries were prepared for sequencing using the COVID-Seq test (Illumina, San Diego, CA, USA), and the MiniSeq™ sequencing system.

4.3. Global RNA Methylation Assay

Sequenced SARS-CoV-2-positive RNA samples (n = 60; 10 per variant) were randomly selected from adult (30–60 years old) patients (men and women). In addition, 10 negative samples were used as controls. RNA concentration and purity were measured with a spectrophotometer (Nanodrop, Thermo Fisher Scientific, Waltham, MA, USA Thermo). Because of the small amounts of RNA in nasopharyngeal samples, all samples were vacuum-lyophilized (LABCONCO, Kansas City, MO, USA) at −56 °C and 0.123 mBar, for 48 h. The samples were then resuspended in nuclease-free water for a final concentration of 60 ng/µL. The methylation assay was performed with the EpiQuik™ m6A RNA Methylation Quantification Kit (Epigentek, Farmingdale, NY, USA), according to the manufacturer's instructions. Absorbance at 450 nm was measured in a microplate reader (EPOCH2, BioTek, Thermo Fisher Scientific, Waltham, MA, USA). The standard curve was obtained and the relative methylation level *m6A* (%) was determined according to the following formula:

$$m6A\ (\%) = \frac{\left(OD_{sample} - OD_{NC}\right) \div S}{\left(OD_{PC} - OD_{NC}\right) \div P} \times 100 \quad (2)$$

where OD_{sample} is the optical density of the sample, OD_{NC} is the optical density of the negative control, OD_{PC} is the optical density of the positive control, S is the amount of RNA (ng) applied to the well, and P is the amount of RNA (ng) applied to the positive control well (Supplementary File S2).

4.4. Statistical Analysis

Normality and homoscedasticity were evaluated with Shapiro-Wilk and Leneve tests, respectively. Kruskal–Wallis and *post-hoc* Dunn tests were performed with relative m6A RNA methylation and *N* gene expression data to detect differences among variants. Spearman correlations between relative m6A methylation and relative expression levels of the *N* gene or age were also done. In addition, differences in m6A methylation levels among vaccination schemes were analyzed by Kruskal–Wallis (unbalanced) and Dunn tests. The significance level was 0.05. All analyses were performed in RStudio 4.0.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/epigenomes6030016/s1, Supplementary File S1: Real-time PCR data; Supplementary File S2: m6A RNA methylation data.

Author Contributions: Conceptualization, A.G.-G. and J.L.B.-R.; methodology, A.G.-G., J.L.B.-R., B.G.-G. and G.L.; formal analysis, J.L.B.-R., C.A.B.-R. and B.G.-G.; investigation, A.G.-G. and J.L.B.-R.; resources, A.G.-G. and B.G.-G.; data curation, J.L.B.-R., B.G.-G. and A.G.-G.; writing—original draft preparation, A.G.-G. and J.L.B.-R.; writing—review and editing, A.G.-G., J.L.B.-R., B.G.-G., G.L. and C.A.B.-R.; visualization, A.G.-G., J.L.B.-R. and B.G.-G.; supervision, A.G.-G.; project administration, B.G.-G. and A.G.-G.; funding acquisition, B.G.-G. and A.G.-G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee for Research at CIAD (CONBIOETICA-26-CEI-001-20200122CEI/028-1/2021).

Informed Consent Statement: Patient consent was waived because of the health emergency and retrospective nature of the study. All health providers were required to inform the patients that their samples could have one out of three different destinations: (1) destruction; (2) storage (in case the Mexican authority needs the sample), or (3) research and sequencing. All patients used in this study were identified with a code provided by the health provider, without sharing any personal information.

Data Availability Statement: Data supporting reported results are provided as supplementary files.

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Conflicts of Interest: The authors declare no conflict of interest.

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4. NEW-ONSET DIABETES MELLITUS AFTER COVID-19: COMBINED EFFECTS OF SARS-CoV-2 VARIANTS, MOLECULAR MIMICRY, AND m6A RNA METHYLATION

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Review

New-Onset Diabetes Mellitus after COVID-19: Combined Effects of SARS-CoV-2 Variants, Molecular Mimicry, and m6A RNA Methylation

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Abstract: Post-COVID syndrome, also known as long COVID, includes a range of symptoms that persist for months or even years after initial infection such as fatigue, shortness of breath, joint pain, chest pain, muscle aches, and heart palpitations, among others. In addition, long COVID is related with new-onset diseases such as diabetes mellitus. The association between SARS-CoV-2 infections and the development of diabetes mellitus is complex and not fully understood. Therefore, the objective of this article was to summarize the state of the art in possible mechanisms involved in the development of diabetes mellitus in the post-COVID-19 era, particularly the impact of SARS-CoV-2 variants on molecular mimicry, the role of viral m6A RNA methylation, and the potential associations between these factors. A better understanding of the combinatorial effects of these mechanisms is paramount for both clinicians and researchers alike because it could help tailor more effective treatment strategies, enhance patient care, and guide future research efforts.

Keywords: diabetes mellitus; molecular mimicry; SARS-CoV-2 variants; m6A methylation

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1. Introduction

Coronavirus disease-19 (COVID-19) is caused by Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) in humans. The entry of the virus into the cells is favored by the affinity of the spike protein to the angiotensin-converting enzyme 2 (ACE2) receptors in the host [1]. Most SARS-CoV-2 mutations detected worldwide are found within the viral spike protein and to a lesser degree in other structural and non-structural proteins [2]. Multiple variants of SARS-CoV-2 have emerged worldwide and have been associated with greater transmissibility and/or severity [3–5]. According to the Centers for Disease Control and Prevention (CDC), these variants are classified as variants of concern (VOC), variants of interest (VOI), variants of high consequence (VOHC), or variants being monitored (VBM) [6].

Post-COVID syndrome is characterized by persistent symptoms following the acute phase of COVID-19 infection [7]. While COVID-19 symptoms typically resolve within weeks [8], long COVID manifestations can persist for months or years [9], and include conditions such as cardiovascular diseases, thrombotic events, cerebrovascular diseases [10], myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [11], and diabetes mellitus [10]. Diabetes mellitus requires careful management as it can produce serious health consequences if not properly controlled [12]. It is a chronic disease associated with the destruction or dysfunction of beta pancreatic insulin-secreting cells. The three main classes

of diabetes are Type 1 (T1DM), Type 2 (T2DM), and gestational diabetes mellitus (GDM). Worldwide, approximately 1 in 11 adults live with diabetes mellitus, with around 90% of them diagnosed with T2DM; nevertheless, the occurrence of T1DM has been rising globally, accounting for approximately 5% of all diagnosed diabetes cases [13].

Recent findings from a systematic review involving insights from 20 selected articles suggest a possible connection between SARS-CoV-2 infection and the development of new-onset diabetes after COVID-19 (NODAC). This association operates as a pathophysiological mechanism supported by epidemiological data and by the clinical and biological observations obtained from the affected individuals [14]. In another study, the rate of NODAC was reported to be 29 cases per 1000 person-years over an average monitoring period of 4.6 months [15]. Moreover, a systematic review and meta-analysis, encompassing nine investigations with nearly 40 million participants, demonstrated a significant correlation between COVID-19 infection and an increased risk of diabetes. The incidence of diabetes following COVID-19 was 15.53 per 1000 person-years, exhibiting an elevated relative risk of 1.62 (1.45–1.80). Type 1 diabetes showed a relative risk of 1.48 and type 2 diabetes a relative risk of 1.70 compared to non-COVID-19 patients, with statistically significant positive associations across all age groups and a higher risk observed in patients with severe COVID-19, particularly within the first three months post-infection [16].

Chourasia et al. [17] performed another systematic review focusing on NODAC patients, using the following diagnostic criteria: hemoglobin A1C $\geq$ 6.5%, fasting blood glucose $\geq$ 126 mg/dL, and two-hour blood glucose $\geq$ 200 mg/dL with the oral glucose tolerance test (OGTT). For the review, the authors included adult patients who developed diabetes mellitus at least four weeks after the initial COVID-19 infection; all studies included sample sizes >35,000, with statistical and/or clinical significance. Patients with a pre-existing diagnosis of diabetes or diabetes diagnosed earlier than four weeks after the initial COVID-19 infection were excluded. The major findings of this study included an increased risk of new-onset diabetes mellitus four weeks after acute COVID-19 infection, especially within the first six months, and this was correlated with the severity of the initial infection. Another study reported that the diagnosis of new-onset diabetes after COVID-19 requires confirmation that there is no history of diabetes, that HbA1c is normal at the time of diagnosis, and that there is persistent hyperglycemia after infection [18]. According to a new-onset diabetes mellitus post-COVID-19 study in India, individuals who developed diabetes during the pandemic exhibited elevated glycemic indices and C-peptide levels compared to those who developed diabetes before the pandemic. This underscores the impact of SARS-CoV-2 on glucose metabolism [19].

Based on the current evidence reported, individuals with documented COVID-19 exhibited a higher incidence of T2DM compared to those with acute respiratory infection (AURI), with an incidence rate ratio (IRR) of 1.28 (95% CI 1.05, 1.57) per 1000 person-years, while no significant increase in incidence rate ratio was observed for other forms of diabetes, after matching for demographic and clinical characteristics [20]. Qeadan et al. [21] reported that individuals diagnosed with COVID-19 in the United States had a 42% higher risk of developing new-onset T1DM compared to those without COVID-19, with males exhibiting a slightly higher risk than females. A study carried out by Xie et al. [22] reported that patients with COVID-19 (n = 181,280) had a 40% higher risk of new diabetes compared with the control population (n = 4,118,441) after 1 year. This is consistent with another meta-analysis, which showed a 66% higher risk of developing diabetes in people who had COVID-19 (n = 4,270,747) compared with the control group (n = 43,203,759) [23].

Several uncertainties exist due to study limitations and the dynamic nature of the pandemic [24,25]. The multifactorial etiology involves host characteristics, social determinants, and various pandemic-related factors (e.g., psychosocial stress). For example, GDM prevalence was significantly higher in 2020 than in 2019, probably due to the stress induced by the pandemic that may have led to chronic inflammation [26] and greater gestational weight gain [27]. Changes in lifestyle and dietary habits during the pandemic, such as the increased consumption of processed foods, comfort foods, and sugary beverages, may have

contributed to a higher calorie intake [28]. Excess calorie consumption, especially from high sugar processed foods, could contribute to insulin resistance, obesity, and, ultimately, the development of T2DM [29]. Furthermore, the lockdowns implemented during the pandemic may have led to increased sedentary behavior among individuals; this behavior is a known risk factor for the development of T2DM and GDM, and can lead to weight gain, insulin resistance, and other metabolic disturbances [30,31]; therefore, there is a need to consider diabetes mellitus as a post-COVID-19 syndrome for proper prevention and management [32].

2. Molecular Mechanisms Involved in NODAC Development

Different molecular mechanisms have been proposed to explain NODAC. For instance, the pathogenesis of T2DM has been associated with dysregulation of non-coding RNA expression, as well as alterations in epigenetic modifications to DNA or RNA [33]. Particularly, N6-methyladenosine (m6A) RNA methylation is one of the most relevant modifications of coding and non-coding RNAs, which consists of the addition of a methyl group in position 6 of the adenine of the RNA [34].

In a study carried out by De Jesus et al. [35], m6A RNA sequencing in human T2DM islets revealed several hypomethylated transcripts involved in insulin secretion; in addition, the depletion of m6A methylation levels in beta cells induced cell cycle arrest, decreased beta cell proliferation, and impaired insulin degranulation and secretion. We previously reported that m6A levels were significantly lower in individuals infected with SARS-CoV-2 variants delta and omicron compared to other variants and uninfected individuals [36]; however, the possible alterations to m6A RNA methylation levels by SARS-CoV-2 variants in beta cells have not been reported yet, even though RNA methylation is a potential key player in understanding and treating COVID-19 [37].

RNA methylation and the expression levels of m6A regulators may be altered in autoimmune diseases, and these changes may contribute to the initiation and progression of the disease. Wang et al. [38] analyzed the expression of m6A regulators and methylation patterns in immune cells obtained from T1DM patients (14–25 years old, n = 12, 6 with T1DM and 6 controls) and found that the increase in m6A methylation levels was accompanied by upregulated gene expression, while the decrease was accompanied by reduced gene expression.

Molecular mimicry is another suggested mechanism attempting to explain viral infection-related autoimmunity. Molecular mimicry involves the activation of T cells and the production of autoantibodies, which cross-react with host antigens. This process can be initiated by the presence of dual T-cell receptors, which can react to both foreign and self-antigens [39,40]. This mechanism is particularly relevant in the context of autoimmune diseases such as insulin-dependent diabetes [41], including those cases triggered by SARS-CoV-2 [42–44]. Thus, this review offers a more holistic understanding of the possible mechanisms involved in the development of NODAC, focusing on m6A RNA methylation, molecular mimicry, and inflammatory processes.

2.1. Alterations of m6A RNA Methylation Patterns

RNA methylation and demethylation are mediated by a series of enzymes, such as methyltransferases (or "writers" that transfer methyl groups to the nitrogen 6 of the adenosine of the RNA) and demethylases (or "erasers" that remove methyl groups), and have been associated with multiple functions within the cell such as RNA splicing, translocation, degradation, and stability, as well as the translation and regulation of non-coding RNAs [34] (Figure 1). Diabetic patients show alterations in the m6A methylation machinery in both immune and beta cells, which could be aggravated by SARS-CoV-2 infection, though the information available is scarce.

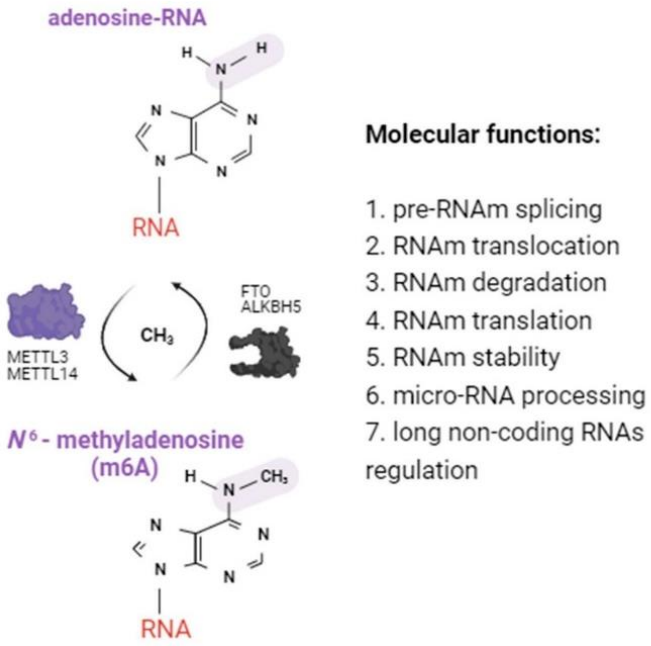

Figure 1. Functions related to m6A RNA modifications and participating enzymes. These modifications are dynamically regulated by methyltransferase enzymes ("writers" such as methyltransferase-like 3 and 14 (METTL3 and METTL14) that transfer methyl groups to the nitrogen 6 of the adenosine of the RNA) and demethylases ("erasers" such as fat mass and obesity-associated protein (FTO) and alkB homolog 5 (ALKBH5) that remove methyl groups).

2.1.1. Alterations in Immune Cells from Diabetic Patients

Information regarding alterations to RNA methylation in immune cells from diabetic patients is scarce. One study by Wang et al. [38] reported that the genes involved in the regulation of m6A modifications showed notable changes in patients with T1DM (n = 6) compared to healthy individuals (n = 6). Specifically, the writer methyltransferase-like 3 (*METTL3*) and the reader insulin-like growth factor 2 binding protein 2 (*IGF2BP2*) showed decreased expression, while the readers YTH N6-methyladenosine RNA binding protein C1 (*YTHDC1*) and the heterogeneous nuclear ribonucleoprotein A2B1 (*HNRNPA2B1*) had increased expression. In addition, a microarray analysis showed that hypermethylated transcripts were enriched in the Janus kinase/signal transducers and activators of the transcription (JAK/STAT) signaling pathway, hypomethylated transcripts were enriched in the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway, and both methylation patterns were enriched in the mitogen-activated protein kinase (MAPK) pathway. Another study carried out by Yang et al. [45] reported a reduced level of m6A methylation in white blood cells from individuals with T2DM (n = 102) compared to healthy patients (n = 107). That study also revealed an increased expression of the demethylase fat mass and obesity-associated protein (*FTO*), and variations in expression levels of other m6A methyl-esterases. Specifically, there were elevated levels of *METTL3*, *METTL14*, and Wilms' tumor 1-associating protein (*WTAP*) in T2DM patients compared to healthy individuals. Although more studies are needed, these findings suggest potential associations between m6A methylation and demethylation processes and the development of T1DM and T2DM.

2.1.2. Alterations in Beta Cells from Diabetic Patients

Research has shown that METTL3, a key component of the m6A methyltransferase complex, is crucial for maintaining beta cell function. Li and coworkers [46] demonstrated that a decrease in METTL3 under inflammatory and oxidative stress conditions leads to

beta cell failure and hyperglycemia. This is likely due to decreased m6A modifications and the reduced expression of insulin secretion-related genes. Similarly, Liu et al. [47] found that the deletion of METTL14 results in glucose intolerance, decreased beta cell mass, and impaired insulin secretion. Furthermore, Cheng and coworkers [48] showed that METTL3 ameliorates the methylglyoxal-induced impairment of insulin secretion in pancreatic beta cells by regulating MAF BZIP transcription factor A (*MAFA*) expression. This suggests that METTL3 is a potential drug target for the treatment of beta cell failure in diabetes. Other authors have shown that FTO, a gene product associated with obesity and diabetes, plays a crucial role in maintaining beta cell function. Russell and Morgan [49] found that FTO protein enhances glucose-induced insulin secretion in pancreatic beta cells. However, Fan et al. [50] discovered that FTO overexpression inhibited insulin secretion and promoted nuclear factor kappa B (NF-κB) activation, potentially contributing to beta cell dysfunction.

In the context of T2DM, the impact of m6A methylation on the regulation of the biological function(s) of human beta cells has been recognized. m6A-sequencing of human T2DM islets revealed several hypomethylated transcripts involved in cell cycle progression and insulin secretion [35]. Moreover, beta cell-specific *Mettl14* knockout mice, which displayed reduced m6A methylation levels, mimicked the islet phenotype in human T2DM with early diabetes onset and mortality due to decreased beta cell proliferation and insulin degranulation [35]. Decreased levels of *METTL3*, *METTL14*, alkB homolog 5 (*ALKBH5*), and YTH N6-methyladenosine RNA binding protein F1 (*YTHDF1*) in beta cells from individuals with T2DM (n = 7) compared to non-diabetic counterparts (n = 5) were reported before the COVID-19 pandemic [35]. Additionally, both METTL3 and METTL14 protein levels were reduced in the entire islets of T2DM patients. These findings suggest that the m6A methylation content might serve as a specific biomarker for predicting the risk of T2DM and its associated complications. However, it is crucial to note that these results warrant replication and validation in larger populations and through diverse experiments.

2.1.3. Alterations in m6A Methylation Induced by mRNA Vaccines

Some studies suggest a potential association between COVID-19 vaccination and the risk of causing elevated blood glucose or an exacerbation of pre-existing diabetes [51]. It has been reported that COVID-19 mRNA vaccination can induce T1DM in some individuals with a genetic predisposition [52], even in adults [53]. According to Alsudais et al. [52], eight studies in 12 patients diagnosed with T1DM post-vaccination were analyzed. The Pfizer-BioNTech vaccine was the most commonly administered (7/12), followed by the Moderna mRNA-1273 vaccine (2/12), CoronaVac (1/12), ChAdox1-s (1/12), and Pfizer-BioNTech/CoronaVac (1/12). The diagnostic criteria of T1DM included low C-peptide levels, and positive antibodies (e.g., anti-glutamic acid decarboxylase antibodies, anti-GAD) and/or HbA1c levels. Only five patients had reliable HbA1c data before vaccination; however, all patients showed significantly higher HbA1c levels after vaccination with an average level of 9.96%. Fifty percent of the patients (6/12) developed T1DM after the second vaccine dose; diabetic ketoacidosis occurred in 41.7% of cases, mostly within eight days post-vaccination. Genetic susceptibility was found in 41.7% of patients, with notable mutations in RNA binding motif protein 45 (RBM45)/Major Histocompatibility Complex, Class II, DR Beta 1 (DRB1) and Major Histocompatibility Complex, Class II, DQ Beta 1 (HLA-DQB1). Some limitations of that review include the lack of crucial information such as baseline body mass index (BMI) and HbA1c levels in some case reports, making it difficult to identify vaccine-induced diabetes and link vaccination to T1DM. Therefore, the authors did not rule out the possibility of diabetes onset due to COVID-19 infection.

In another study, Moon et al. [53] reported a case of a 56-year-old woman who experienced hyperglycemia following the second dose of the COVID-19 mRNA vaccine (Moderna) despite no previous diabetes history. This is an important finding of T1DM development post-vaccination with an mRNA vaccine, and notably the oldest patient under such circumstances. Nevertheless, in a much larger cohort of individuals from Hong Kong, Xiong et al. [54] found no evidence of an increased risk of diabetes following

COVID-19 vaccination (CoronaVac (Sinovac) n = 5760 and BNT162b2 (Pfizer-BioNTech) n = 4411). In the BNT162b2 group, 2109 diabetes cases occurred after SARS-CoV-2 infection, and infection was associated with a higher risk of diabetes, mainly T2DM, irrespective of variants, although the risk was lower with Omicron.

We previously reported that vaccinated individuals showed significantly lower m6A methylation levels than unvaccinated individuals (n = 8 and 52, respectively), and that differences in m6A methylation levels across variants in unvaccinated individuals were significant; however, no significant correlation was observed between m6A methylation levels and viral load (nucleocapsid gene expression) or age [36]. According to the study, a complete vaccination scheme denoted the administration of one or two doses (depending on the vaccine specifications) of authorized vaccines in Mexico, including AZD1222, CoronaVac, BNT162b2, Ad5-nCOV, and mRNA-1273, developed by AstraZeneca, Sinovac, Pfizer-BioNTech, CanSinoBio, and Moderna, respectively. Although variation in m6A levels across variants could not be explained by the vaccination status alone, this first report of potential vaccination-mediated m6A methylation is intriguing and warrants further investigation, in particular regarding the possibility that COVID-19 vaccines (though generally considered safe) may induce an autoimmune response in susceptible individuals by mediating a decrease in m6A levels.

2.2. Diabetes Mellitus: Beta Pancreatic Cell Destruction and Dysfunction

T1DM is an autoimmune disease associated with the destruction of beta pancreatic cells. During the disease, several autoantibodies are produced such as those directed against insulin, tyrosine phosphatase, glutamic acid anti-decarboxylase enzymes, and the zinc transport protein [55]. The presence of these autoantibodies in an individual's blood serum is an indication that the immune system is attacking the pancreatic beta cells that produce insulin [56,57]. The pathogenesis and progression of T1DM has been linked (among other factors) to viral infections. Viruses may lead to T1DM through a direct cytolytic activity on beta cells or by triggering autoimmune responses against beta cells.

Beta cell dysfunction and insulin resistance are key factors in the pathogenesis of T2DM [58]. The interplay between beta cell dysfunction and insulin resistance is complex, with both states influencing each other and potentially exacerbating diabetes. Insulin resistance is caused by alterations in insulin receptors in the cells and precedes T2DM [58]. Reiterer et al. [59] proposed that SARS-CoV-2 might induce dysfunction in adipose tissue, leading to insulin resistance. The authors analyzed a COVID-19 patient cohort without prior metabolic conditions (n = 4102), observing new-onset insulin resistance and hyperglycemia in 47% of the patients. In addition, SARS-CoV-2 infection was found to elevate the expression of the RE1-silencing transcription factor (*REST*), influencing the transcriptional regulation of key metabolic factors (such as myeloperoxidase, apelin, and myostatin) and contributing to the disruption of glucose metabolism [60]. Beta cell dysfunction impairs insulin secretion and is a critical determinant of the disease. This dysfunction is influenced by genetic and metabolic abnormalities, which affect glucose homeostasis [58].

Currently, there is no evidence supporting the notion that SARS-CoV-2 infection during pregnancy results in permanent diabetes for mothers or their offspring through autoimmunity or beta cell destruction. The heightened prevalence of GDM observed during the pandemic is likely attributed to lifestyle changes during lockdown. Nevertheless, severe COVID-19 cases may contribute to the development of GDM by exacerbating glucose tolerance [61]. GDM and T2DM share insulin-related challenges, affecting both obese and lean women. Obesity contributes to pre-existing insulin resistance, while lean women predominantly face impaired first-phase insulin secretion. Pregnancy-induced insulin resistance is influenced by factors such as placental hormones and pro-inflammatory cytokines. Ultimately, by the third trimester, insulin resistance during pregnancy reaches levels similar to T2DM [62].

2.3. Direct SARS-CoV-2 Invasion of Beta Cells

The expression of *ACE2* in the pancreas (mainly in islet cells) is higher than in the lungs, and *ACE2* is overexpressed in diabetic/hyperglycemic islets compared to non-diabetic/normoglycemic [63,64], so it is possible that SARS-CoV-2 could bind to this receptor and enter beta cells, producing cellular dysfunction and acute hyperglycemia [65–67]. Viral replication within beta cells can lead to their destruction, reducing insulin production. SARS-CoV-2 can cause direct damage to the pancreas, potentially inducing T1DM in previously non-diabetic subjects [68–70]. Even though this is a non-cytolytic virus, it has been reported that SARS-CoV-2 could induce cell injury mediated by different cell death mechanisms such as apoptosis, autophagy, and necrosis [71]. These mechanisms triggered by SARS-CoV-2 might facilitate viral clearance as part of the host's antiviral immunity and also contribute to virus-induced tissue injuries and disease progression. SARS-CoV-2 may negatively affect human pancreatic islet function and survival by creating inflammatory conditions, which may in turn lead to metabolic abnormalities observed in patients with COVID-19. A direct tropism of SARS-CoV-2 for beta cells was suggested with the detection of SARS-CoV-2-specific viral RNA from pancreatic sections of newly hyperglycemic deceased patients who had COVID-19 [72]. The diabetogenic effect induced by SARS-CoV-2 infection can also be mediated by a possible direct viral cytotoxic mechanism against human pancreatic islets [72].

2.4. Immune Responses (Autoimmunity and Inflammation) Induced by SARS-CoV-2

The autoimmune responses induced by viral infections are mediated by peripheral blood mononuclear cells (PBMCs) such as T, B, natural killer (NK), dendritic cells (DCs), and monocytes. These responses are attributed to the structural similarity between viral antigens and motifs found in proteins of beta cells [73]. Boddu et al. [13] proposed that antigen exposure triggers the activation of autoreactive lymphocytes, initiating an autoimmune response. Then, an autoimmune reaction progresses to the destruction of the remaining beta cell mass, leading to the onset of insulin-dependent T1DM. This theory may not fully elucidate the immediate onset of diabetes during the acute phase of COVID-19 infection. However, it could explain the later emergence of diabetes in the weeks or months following recovery from COVID-19 [74]. Figure 2 shows the possible mechanisms of beta cell destruction induced by SARS-CoV-2 infection.

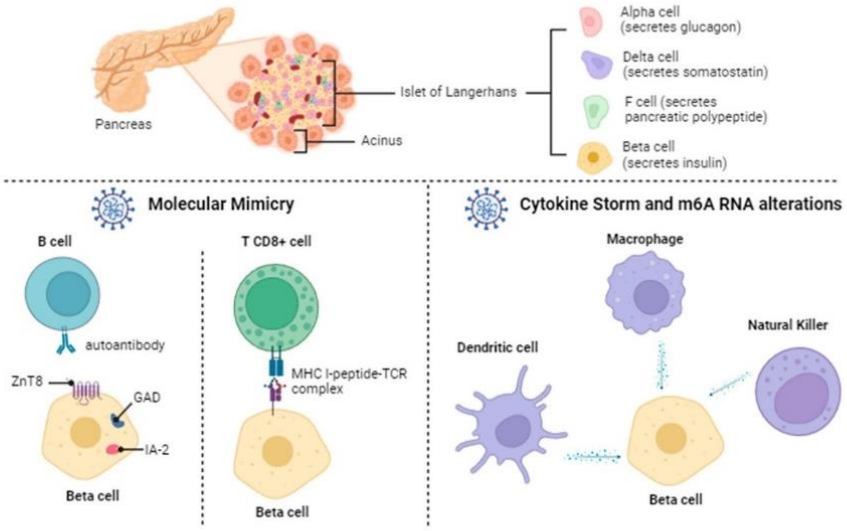

Figure 2. Mechanisms of cytotoxicity involved in pancreatic beta cell destruction after SARS-CoV-2 infection. Beta pancreatic cells can be destroyed by autoreactive T and B lymphocytes due to molecular

mimicry. The immune cross-reactivity between a SARS-CoV-2 protein and one or various human proteins occurs when an immune cell recognizes both the antigen and the auto-antigen due to their sequence similarity [75]. Human antibodies against SARS-CoV-2 could have cross-reactivity with the Zinc Transporter 8 (ZnT8), Glutamic Acid Decarboxylase (GAD), Insulinoma Antigen-2 (IA-2), or other highly expressed pancreatic proteins. T Lymphocytes (CD8+) recognize, through the T-cell receptor (TCR), the peptides derived from SARS-CoV-2 proteins that are presented by the Major Histocompatibility Complex I (MHC-I) of infected beta cells. Beta cells can also be destroyed by innate immune cells, such as dendritic cells, macrophages, and natural killer cells, through different mechanisms that involve pro-inflammatory cytokines.

2.4.1. Molecular Mimicry

The spike protein from SARS-CoV-2 contains mimotopes that resemble human antigen epitopes, potentially leading to antigenic mimicry and the activation of immune receptors [76]. Computational methods revealed that the spike protein of SARS-CoV-2 contains clusters of molecular mimics with significant autoimmune potential. Within these clusters, multiple molecular mimics share motifs that are recurrently present in the human proteome [77]. This computational study provided insights into the autoimmune potential of SARS-CoV-2 for a therapeutic intervention or vaccine design, to avoid any autoimmune interference. During SARS-CoV-2 infections, the host immune system can activate expression of the apolipoprotein B editing catalytic polypeptide 3G and F (*APOBEC3G/F*) and adenosine deaminase RNA1-specific (*ADAR1*) genes, which leads to editing the viral RNA and host transcriptome. This response can trigger the production of autoantigens, perceived as foreign by the immune system, and therefore promotes the production of autoantibodies and potentially induces transient or chronic autoimmune diseases [78]. Thus, the viral infection could activate T cells that recognize pancreatic antigens, leading to an autoimmune attack on beta cells and the subsequent development of T1DM. Autoimmunity is also related to molecular mimicry between peptides of the SARS-CoV-2 spike protein and human antigens mainly expressed by endocrine glands such as the pituitary, adrenal [79], thyroid, and pancreas [80]. It is possible that the similarity of spike peptides with the membrane receptors in pancreatic beta cells in humans could be related to the development of T1DM in previously non-diabetic patients.

Moody et al. [75] employed in silico immuno-informatic tools to predict B-cell epitopes of eight SARS-CoV-2 variants and the original Wuhan variant. Subsequently, they studied the similarity between the predicted B-cell epitopes and human proteins. The authors reported an association between human proteins with sequences shared with SARS-CoV-2 and autoimmune diseases such as T1DM, systemic lupus erythematosus (SLE), and multiple sclerosis (MS), among others. For example, histone H3 shares an identical six amino acid sequence with the SARS-CoV-2 protein Orf8 and is in a region identified as an epitope in COVID-19 patients. Furthermore, autoantibody-targeting proteins found in autoimmune diseases, such as SLE and T1DM, among others, share similar sequences with some of the new predicted epitopes of SARS-CoV-2. Additionally, the authors identified 11 new predicted B-cell epitopes in host proteins, explaining key aspects of the extrapulmonary pathology of COVID-19. In another study, de Oliveira et al. [81] reported the potential similarity between the amino acid sequences of human insulin (4F0N) and glutamic acid decarboxylase-65 (GAD65) (2OKK) with SARS-CoV-2 proteins, such as the spike protein (6ZB5), to explain the possible trigger of T1DM. The authors found a sequence identity from 5 to 45.45%. The data suggest a possible pathogenic link between T1DM and SARS-CoV-2. Collectively, these studies (Supplementary Table S1) suggest that molecular mimicry may play a significant role in the pathogenesis of autoimmune diseases such as T1DM in the context of SARS-CoV-2.

2.4.2. Cytokine Storm

A cytokine storm can lead to inflammation and immune system dysfunction, potentially contributing to the destruction of pancreatic beta cells. During severe COVID-19, there is an increased release of counter-regulatory hormones and proinflammatory cytokines, including interleukin 6 (IL-6) and tumoral necrosis factor alpha (TNF-alpha), collectively referred to as a cytokine storm. This overwhelming surge of cytokines is recognized for its ability to induce insulin resistance, leading to elevated blood sugar levels or hyperglycemia [82]. Viral infections in diabetic animal models trigger natural killer cells and T cells to release inflammatory cytokines, destroying beta cells [83]. In COVID-19 patients, a dual immune response occurs, with T helper (Th) 1 cells activated by interferon-gamma (IFN-gamma) and monocyte chemoattractant protein-1, and Th2 cells expressing IL-4 and IL-10 to suppress inflammation. Macrophage activation syndrome in COVID-19 involves elevated levels of IL-6, IL-1-beta, TNF-alpha, INF-gamma, and ferritin [84]. Additionally, Th17 cells are activated in the cytokine storm, releasing IL-17 and granulocyte-colony stimulating factor. Th17 cells are abundant in the pancreas of T1DM patients [85]. In T2DM, elevated IL-17 levels are linked to adipose tissue inflammation, regulating pro-inflammatory cytokines, and contributing to insulin resistance [86]. The cytokine storm induced by SARS-CoV-2 in diabetic patients exacerbates the systemic immune imbalance, potentially worsening their clinical condition [87,88].

2.5. Alterations in m6A Methylation by Different SARS-CoV-2 Variants

Rangu et al. [89] suggested that new SARS-CoV-2 variants might have differential effects on the development of diabetes due to mutations associated with an alternative route of entry into beta cells, or the maintenance of higher infection loads and high levels of circulating cytokines. The authors recommended determining invasion mechanisms of SARS-CoV-2 variants in beta cells, as well as the differential effects on insulin secretion. The lack of reliable markers of in vivo beta cell death limits the studies of the potential effects induced by the different viral variants. Further research is needed to comprehensively investigate the functional consequences of changes in m6A RNA methylation patterns within beta cells caused by distinct SARS-CoV-2 variants.

We previously reported that the m6A levels of nasopharyngeal samples were significantly lower in individuals infected with SARS-CoV-2 variants delta and omicron compared to other variants and uninfected individuals [36]. Vaid et al. [90] reported that SARS-CoV-2 variants could cause a global loss of m6A methylation levels in cellular RNAs of air/liquid interface cultures of human airway epithelia, while viral RNA remains m6A-modified. METTL3 showed an unusual cytoplasmic localization post-infection. The B.1.351 variant presented a less pronounced effect on METTL3 localization and loss of m6A than the B.1 and the B.1.1.7 variants. Furthermore, transcripts with m6A modifications were preferentially downregulated post-infection [90]. Altogether, this information suggests that SARS-CoV-2-related m6A alterations could (hypothetically) lead to beta cell dysfunction (Figure 3). SARS-CoV-2 variants may differently alter the m6A methylation enzymatic machinery in pancreatic beta cells, affecting the global levels of m6A methylation of insulin-related gene transcripts. Further research is needed to confirm this hypothesis. On the other hand, the genetic background and the epigenetic status of the host pancreatic cells could also contribute to the outcome following viral infection. Epigenetic marks before viral infection may differ between individuals depending on lifestyle (e.g., physical activity or diet), previous viral infections, pharmacological treatments, and exposure to pollutants, among other factors.

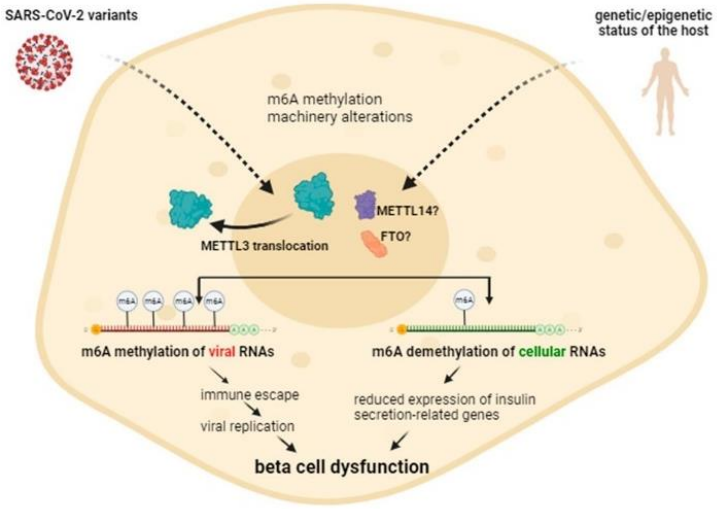

Figure 3. A simplified model illustrating hypothetic functional consequences of changes in RNA (m6A) methylation patterns within beta cells caused by SARS-CoV-2 variants. SARS-CoV-2 variants could impact the m6A methylation process in pancreatic beta cells of susceptible individuals with certain genetic and epigenetic backgrounds. These variants could reduce the global levels of m6A methylation of cellular RNAs, particularly those associated with insulin secretion-related genes, which could result in reduced expression and further beta cell dysfunction. Moreover, the m6A methylation of viral RNAs could facilitate immune escape, enhance viral replication, and ultimately contribute to beta cell dysfunction. Hence, the interplay between viral RNA methylation, cellular RNA demethylation, and genetic and epigenetic factors in pancreatic cells could collectively influence the dysfunction of beta cells following viral infection.

3. Concluding Remarks

The onset of diabetes mellitus following COVID-19 infection appears to be influenced by a complex interplay of factors, including the emergence of SARS-CoV-2 variants, inflammation, molecular mimicry, and m6A RNA methylation. The evolving landscape of SARS-CoV-2 variants introduces new challenges in understanding their potential impact on the development of diabetes. Molecular mimicry, wherein viral components resemble host tissues, may contribute to autoimmune responses triggering diabetes in susceptible individuals. Additionally, the involvement of m6A RNA methylation, a crucial epigenetic modification, suggests a potential role in dysregulating gene expression and insulin sensitivity. As research continues, it is essential to unravel the intricate mechanisms linking COVID-19 to diabetes onset. This understanding would guide public health strategies, early detection, and targeted interventions to mitigate the long-term consequences of the pandemic on metabolic health. Furthermore, an interdisciplinary collaboration between virologists, immunologists, geneticists, and epigenetic researchers is crucial for a comprehensive grasp of the multifaceted relationship between COVID-19 and diabetes mellitus. Addressing these complexities will not only enhance our understanding of pathophysiology but will also pave the way for more effective prevention and management strategies for individuals at risk. Further research related to global, viral, and host RNA methylation is required, as well as in vivo trials assessing beta cell death, auto-antibody titers, insulin production and secretion, and the expression of insulin-related genes, pro-inflammatory cytokines, and RNA methylation-related genes.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/covid4040032/s1, Table S1: Molecular mimicry of SARS-CoV-2 and the pathogenesis of autoimmune diseases.

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Table S1. Molecular mimicry of SARS-CoV-2 and the pathogenesis of autoimmune diseases

Association with T1DM onset	Key Findings	References
Yes	The researchers found shared pentapeptides between four distinct autoantigens associated with diabetes: Islet cell autoantigen 1 (Q05084), protein tyrosine-phosphatase receptor-type N (Q16849), glutamate decarboxylase (Q99259), Carboxypeptidase H (P16870) and the Spike protein of SARS-CoV-2 (UniProt, Id = P0DTC2). This discovery suggests a molecular commonality between certain components involved in diabetes and the viral Spike protein, indicating a potential link or interaction that could have implications for the immune response and diabetes-related processes during a SARS-CoV-2 infection. Further research is needed to elucidate the significance of these shared pentapeptides in the context of diabetes and COVID-19.	Churilov et al. (2022)
No	The researchers demonstrated that a specific motif, TQLPP, present in the Spike protein of the virus, shares antibody binding properties with thrombopoietin. Additionally, the ELDKY motif is found in various human proteins, including PRKG1, associated with platelet activation and calcium regulation, and tropomyosin, linked to cardiac disease. This suggests a potential cross-reactivity where antibodies produced against the Spike protein might also interact with these human proteins, raising concerns about the broader impact of the immune response and its potential involvement in platelet activation and cardiac issues.	Nunez-Castilla et al. (2022)
Yes	The authors highlighted a potential resemblance in the amino acid sequences of human insulin (represented by the code 4F0N) and glutamic acid decarboxylase-65 (GAD65, represented by the code 2OKK) with certain proteins in SARS-CoV-2, specifically the Spike protein (represented by the code 6ZB5). This observation suggests a molecular similarity that could have implications for the immune system's response, potentially leading to cross-reactivity between antibodies targeting human insulin or GAD65 and certain proteins of the SARS-CoV-2 virus, particularly the Spike protein. Further investigation is needed to understand the immunological consequences of such sequence similarities.	de Oliveira et al. (2021)
No	The authors identified 136 alignments of 6–23 amino acids in 129 human proteins that are immunologically likely to be cross-reactive with SARS-CoV-2. This suggests that certain parts of these human proteins share similarities with the virus, raising the possibility that the immune system might react to both the virus and these human proteins due to these shared amino acid sequences. This finding underscores the complexity and interconnectedness of the immune response in the context of SARS-CoV-2 infection.	Moody et al. (2021)

5. DISCUSIÓN GENERAL

5.1. Comportamiento de la Pandemia de COVID-19 en Sinaloa

El presente trabajo inició con el estudio del comportamiento de la pandemia de COVID-19 en cuatro olas epidemiológicas desde enero del 2020 a febrero del 2022 en el estado de Sinaloa, México. Aparentemente, las olas de COVID-19 en Sinaloa ocurrieron durante el invierno (menor temperatura y humedad) y el verano (mayor temperatura y humedad), lo que indica una posible estacionalidad. Estos resultados fueron consistentes con publicaciones donde reportaron que las temperaturas más bajas se relacionaron con el aumento de casos positivos en otros países (*Liu et al.,* 2020; Le *et al.,* 2021). Adicionalmente, Liu y colaboradores (2020) reportaron que la baja humedad también favoreció la transmisión del virus. Karim y colaboradores (2022) emplearon modelos lineales generalizados para examinar el efecto de la temperatura y la humedad en el número de casos confirmados de infectados por SARS-CoV-2 (cepa original y la variante alfa B.1.1.7) y en el número total de muertes por COVID-19. Los autores encontraron que las altas temperaturas y la alta humedad redujeron significativamente la transmisión del SARS-CoV-2 y el número de muertes por COVID-19. Las altas temperaturas y humedades absolutas pudieron haber disminuido la supervivencia del virus en los aerosoles (Karim at al., 2022). Adicionalmente, Liu y colaboradores (2021) utilizaron un método de análisis de series temporales para detectar las señales estacionales en la serie temporal de casos confirmados de COVID-19 en 10 países, y emplearon un modelo modificado para simular la evolución de la pandemia de COVID-19 y cuantificar el impacto de la estacionalidad en ella. Los autores reportaron que la infección por SARS-CoV-2 y la mortalidad por COVID-19 fue mayor en países con climas fríos y la estacionalidad de COVID-19 fue más pronunciada en latitudes altas. El ciclo estacional es una característica de diversas infecciones virales respiratorias como la influenza en climas templados (Martínez, 2018). El análisis de las tendencias temporales puede proporcionar información sobre la eficacia de las medidas de salud pública y sobre el potencial de futuros brotes, sin embargo, se requieren más estudios para establecer con certeza dicha estacionalidad e incluir los factores sociales como eventos masivos con aglomeración de personas.

El SARS-CoV-2 presenta alta tasa de mutación que también se relaciona con la transmisibilidad del virus y la severidad de la enfermedad COVID-19 (Davies *et al.*, 2021). En este estudio se reporta que las variantes delta y omicron fueron las más contagiosas en Sinaloa, México, lo que coincide con otras publicaciones en México (Loza *et al.*, 2023; Zárate *et al.*, 2023). La variante delta predominó durante la 3ra ola epidemiológica en Sinaloa, México donde se encontró un mayor número de fallecidos. Otros autores también reportan que la variante delta y sus sublinajes como delta plus fueron más letales que las variantes previamente reportadas (Dhawan *et al.*, 2022). Adicionalmente, la mortalidad en la 4ta ola en Sinaloa fue menor que en las olas previas, lo que podría estar relacionado con el programa de vacunación en esta población. La vacunación en Sinaloa comenzó al inicio de la segunda ola, el 12 de enero de 2021 para profesionales de la salud, y el 16 de mayo para maestros, trabajadores escolares y personas mayores de 50 años. Durante la tercera ola, gran parte de la población adulta recibió al menos la primera dosis de la vacuna. Las vacunas aplicadas inicialmente el 13 de enero de 2021 en la población de Sinaloa bajo el programa federal de vacunación fueron: AZD1222, CoronaVac, BNT162b2, Ad5-nCOV y mRNA-1273 de las compañías AstraZeneca, Sinovac, Pfizer-BioNTech, CanSinoBio y Moderna respectivamente (https://saludsinaloa.gob.mx/).

Publicaciones recientes concluyen que los enfoques de inmunización con las vacunas disponibles no pueden prevenir la infección por SARS-CoV-2, pero sí reducen el riesgo de mortalidad grave y las tasas de mortalidad (Dhawan *et al.*, 2022). Por tal motivo, se han propuesto nuevas estrategias para el desarrollo de vacunas de nueva generación tales como la inmunización de refuerzo con vacunas aprobadas, vacunas con fuentes más complejas del sitio de unión al receptor (RBD) de la proteína spike, vacunas de virus atenuados vivos que liberan antígenos del SARS-CoV-2, vacunas subunitarias con epítopos de células T y vacunas desarrolladas utilizando tecnologías de inteligencia artificial (Li *et al.*, 2022; Al-Fattah Yahaya *et al.*, 2023). Estos nuevos enfoques de vacunas pueden proporcionar una protección inmune más fuerte y brindar una mayor protección a personas de alto riesgo (Atmar *et al.*, 2022; Li *et al.*, 2022; Abdul-Karim *et al.*, 2022). Sin embargo, estas nuevas vacunas aún están en fase 1 o preclínica, por lo que se necesita más tiempo para demostrar que son seguras y eficaces. Según los CDC, las vacunas de Pfizer y Moderna se han actualizado con el tiempo para combatir las nuevas variantes del virus. Inicialmente, estas vacunas protegían contra el virus SARS-CoV-2 original, sin embargo, fueron reemplazadas en septiembre de 2022 por vacunas “bivalentes”, que se dirigen tanto al virus original como a las variantes

omicron BA.4 y BA.5. Las nuevas vacunas actualizadas en septiembre de 2023 reemplazaron las vacunas bivalentes por otras dirigidas al linaje XBB de la variante omicron. Las vacunas original y bivalente ya no se utilizan y aún no se ha decidido si se administrarán dosis de vacunas actualizadas anualmente. Las vacunas actualizadas de Pfizer y Moderna a pesar de ser eficaces contra los linajes recientes de omicron y ser efectivas contra COVID-19 severo, presentaron efectos secundarios 7 días posteriores a la segunda dosis como fiebre, escalofríos, cansancio y dolor de cabeza, además de reacciones adversas menos frecuentes como miocarditis y pericarditis principalmente entre personas de sexo masculino de entre 12 y 39 años de edad (https://espanol.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/overview-COVID-19-vaccines.html).

Actualmente, el desarrollo de vacunas de ADN resulta prometedor ya que podían promover altos títulos de anticuerpos neutralizantes capaces de neutralizar de forma cruzada diferentes variantes virales y está orientado a prevenir las infecciones por nuevas variantes de SARS-CoV-2 (Al-Fattah Yahaya *et al.,* 2023). Inovio Pharmaceuticals desarrolló la vacuna INO-4800, basada en ADN contra el SARS-CoV-2 que incorpora el gen S de longitud completa en el vector pGX0001 (Smith *et al.,* 2020). De manera similar, Prompetchara y colaboradores (2021) construyeron candidatos de vacunas de ADN que portaban S, S1 (pCMVkan-S1) o S2 (pCMVkan-S2) de longitud completa. Khalid y Poh. (2023) resumen las principales vacunas de ADN contra variantes de SARS-CoV-2 incluyendo omicron y sus linajes. Estas vacunas también han tenido sus limitaciones ya que en estudios preclínicos con animales grandes y en humanos no provocaron respuestas humorales y celulares más potentes en relación con ensayos en animales pequeños como ratones. Además, otra razón de la baja inmunogenicidad es la menor eficiencia de transfección del ADN plasmídico desnudo en estudios clínicos. A pesar de los esfuerzos por encontrar la vacuna más eficaz y con menos efectos adversos, éstas no protegen contra los efectos a largo plazo. De manera complementaria y exploratoria, realizamos un análisis adicional sobre la caracterización de los epítopos T y B predichos a partir de las proteínas spike de las variantes de SARS-CoV-2 predominantes en una muestra de la población de Sinaloa, México, lo cual sería el primer paso para el desarrollo de una vacuna multiepítopo (ANEXO 3).

5.2. Metilación M6A del ARN en Muestras Nasofaríngeas de Personas Infectadas con Distintas Variantes Virales

Las mutaciones del SARS-CoV-2 pueden ocurrir en las regiones del genoma viral con sitios m6A, y estas modificaciones m6A en el ARN viral se relacionan con la replicación viral (Liu *et al.,* 2021). Estas mutaciones originan diferentes variantes que podrían tener variaciones en su capacidad para modificar epigenéticamente el ARN viral y del huésped, lo que podría influir en las tasas de replicación viral. Adicionalmente, la maquinaria m6A de la célula huésped podría responder de manera diferente a estas variantes, lo que provocaría cambios en la respuesta inmune del huésped y en la susceptibilidad a la infección. Este trabajo presenta el primer informe que muestra que los niveles globales de m6A de muestras de ARN nasofaríngeo de pacientes infectados con SARS-CoV-2 difieren entre las variantes virales. Es notable que las dos variantes más contagiosas (delta y ómicron) mostraron los niveles de metilación más bajos, especialmente omicron, siendo la más contagiosa a nivel mundial (Duong *et al.*, 2022).

Comprender cómo las modificaciones de m6A pueden diferir en diversas variantes virales podría tener implicaciones para el desarrollo de vacunas o tratamientos antivirales. Algunas variantes podrían tener modificaciones que les confieran más o menos susceptibilidad al reconocimiento inmunológico, lo que podría afectar la eficacia de las vacunas. La maquinaria m6A podría ser una posible estrategia terapéutica para abordar las variantes emergentes. Al interferir con las modificaciones de m6A, podría ser posible interrumpir el ciclo de replicación viral. Desde el punto de vista de la salud pública, encontrar un marcador confiable para el riesgo grave de COVID-19 es crucial. Aunque no se encontró correlación entre la carga viral y los niveles de m6A entre las variantes, estudios anteriores han observado niveles más altos de m6A en sangre pulmonar o periférica de pacientes con COVID-19 moderado o grave en comparación con individuos sanos (Li *et al.,* 2021; Meng *et al.,* 2021; Qiu *et al.,* 2021; An *et al.,* 2022). Qiu y colaboradores (2021) propusieron un "puntaje m6A" predictivo para evaluar el pronóstico de cada paciente con COVID-19 basado en los niveles de m6A y nueve genes diferencialmente expresados mayormente relacionados con la respuesta inmune. Los pacientes con puntajes más altos mostraron un mejor pronóstico relacionado con la activación de células T en comparación con aquellos con puntajes más bajos. Este modelo también puede predecir la probabilidad de contraer COVID-19 y detectar

portadores de SARS-CoV-2 (Qiu *et al.,* 2021).

Adicionalmente, en esta sección de la tesis se observó que individuos vacunados presentaban niveles significativamente inferiores de metilación m6A en comparación con los no vacunados (n = 8 y 52, respectivamente). Sin embargo, no se encontró correlación entre estos niveles y la expresión del gen de la nucleocápside viral (N) (p = 0.63). Aunque la variación en los niveles de m6A entre las variantes no pudo ser explicada por el estatus de vacunación, este descubrimiento sugiere la posibilidad de que las vacunas influyan en la metilación m6A, un fenómeno que requiere de un estudio más profundo por las implicaciones que puede tener en la respuesta immune.

Mecanismos de autoinmunidad post COVID-19 con énfasis en el desarrollo de diabetes mellitus

La infección por SARS-CoV-2 puede desempeñar un papel en la promoción o exacerbación de enfermedades autoinmunes como el síndrome de Guillain-Barré, fenómenos trombóticos, anemia hemolítica (Liu *et al.,* 2021) y diabetes mellitus tipo 1 (DM1) (Zhang *et al.,* 2022; Qeadan *et al.,* 2022). Los trastornos autoinmunes más comunes en la población mundial incluyen la enfermedad de Crohn, DM1, la esclerosis múltiple, la artritis reumatoide, el lupus, la esclerodermia y la soriasis (https://nationalstemcellfoundation.org/glossary/autoimmune-disease/, consultado el 24 de abril del 2024). En un estudio que incluyó 354,527 individuos con COVID-19 y 6,134,940 controles, se identificó un aumento significativo en el riesgo de múltiples trastornos autoinmunes y autoinflamatorios posteriores al COVID-19 (Anexo 2 Cuadro 1) (Lim *et al.,* 2023). Otro estudio incluyó a 1,028,721 personas con COVID-19 y 3,168,467 personas sanas como grupo control. En comparación con los controles, los pacientes con COVID-19 presentaron un mayor riesgo de desarrollar diversas enfermedades autoinmunes (Anexo 2 Cuadro 1) (Peng *et al.,* 2023). Recientemente, Hileman y colaboradores (2024) en un estudio de 3908 592 pacientes reportaron la mayor incidencia de psoriasis (0.15%), la artritis reumatoide (0.14%) y DM1 (0.13%) en el grupo de pacientes con COVID-19 durante el período de estudio.

El desarrollo de la DM1 post-COVID1-9 como una enfermedad autoinmune es de preocupación global (Caruso *et al.,* 2020). Esto se debe a la disfunción del sistema inmunológico, que puede llevar a la activación de células T auto-reactivas y la destrucción de las células beta pancreáticas (Darmarajan *et al.,* 2022). El desequilibrio entre las células T reguladoras y las células T auxiliares, particularmente el aumento de la frecuencia de las células Th17, es un factor clave en la patogénesis de la DM1 (Ryba-Stanisławowska *et al.,* 2013). Las células inmunes innatas disfuncionales también pueden contribuir a la inflamación crónica y el daño tisular, aumentando aún más el riesgo

de condiciones autoinmunes (Layseca-Espinosa *et al.,* 2019). Durante la enfermedad de COVID-19 se pueden producir autoanticuerpos como los dirigidos contra la insulina (anti-insulina, IAA), la enzima tirosina fosfatasa (IA-2A), la antidecarboxilasa del ácido glutámico (GAD) y la proteína transportadora del zinc (ZnT8A) (Vera *et al.,* 2021). La presencia de estos autoanticuerpos en el suero sanguíneo de un individuo es indicativo de que su sistema inmunológico está atacando las células beta del páncreas que producen insulina (Hayes *et al.,* 2008; Kokuina *et al.,* 2022). Por tanto, se ha hipotetizado que la asociación entre COVID-19 y el desarrollo de enfermedades autoinmunes, incluida la DM1, implica el mecanismo de mimetismo molecular, la presencia de autoanticuerpos y la inducción de señalización inflamatoria (Castro, 2023).

Más allá de la autoinmunidad por mimetismo molecular, en este trabajo se hipotetiza una posible relación entre el desarrollo de diabetes mellitus post-COVID-19 y las diferentes variantes de SARS-CoV-2 con mecanismos moleculares asociados a la metilación m6A del ARN del virus y del hospedero. Las variantes del SARS-CoV-2 podrían impactar el proceso de metilación m6A en las células beta pancreáticas. Estas variantes podrían reducir los niveles globales de metilación m6A de ARN celulares, particularmente aquellos asociados con genes relacionados con la secreción de insulina, lo que podría resultar en una mayor disfunción de las células beta pancreáticas. Además, la metilación m6A de los ARN virales podría facilitar la evasión inmune, mejorar la replicación viral y, en última instancia, contribuir a la disfunción de las células beta.

La interacción entre la metilación del ARN viral, la desmetilación del ARN celular y los factores genéticos y epigenéticos en las células pancreáticas podría influir colectivamente en la disfunción de las células beta tras la infección viral. Rangu y colaboradores (2022) sugieren que las nuevas variantes del SARS-CoV-2 podrían tener efectos diferentes en el desarrollo de la diabetes debido a mutaciones que podrían cambiar la forma en que el virus ingresa a las células beta pancreáticas o mantener cargas de infección más altas y elevados niveles de citoquinas circulantes (Rangu *et al.,* 2022). Por otro lado, nosotros reportamos que los niveles de m6A en muestras nasofaríngeas eran significativamente más bajos en individuos infectados con las variantes delta y omicron del SARS-CoV-2 en comparación con otras variantes e individuos no infectados (Batista *et al.,* 2022). Vaid y colaboradores (2023) sugieren que las variantes del SARS-CoV-2 podrían causar una pérdida global de los niveles de metilación m6A en los ARN celulares, lo que podría afectar la función de las células beta pancreáticas. En conjunto, esta información sugiere que las alteraciones m6A relacionadas con el SARS-CoV-2 podrían hipotéticamente llevar a la disfunción de las células

beta. Se necesita más investigación para confirmar estas observaciones y comprender la manera en que el estatus genético y epigenético del individuo puede influir en la respuesta al virus.

Las vacunas contra la COVID-19 han sido vitales en la lucha contra la pandemia al evitar casos graves o fallecimientos de la enfermedad (Chakraborty *et al.,* 2023; Ayalew *et al.,* 2023). Varios autores han evaluado la eficacia, inmunogenicidad y seguridad de estas vacunas en diversos grupos poblacionales, incluidos adultos mayores y personas con enfermedades autoinmunes (Sharif *et al.,* 2021; Li *et al.,* 2022; Widhani *et al.,* 2023; Frasca *et al.,* 2023). Sin embargo, existe controversia en cuanto al riesgo de desarrollar enfermedades autoinmunes tras la vacunación contra la COVID-19 (Chen *et al.,* 2022). Algunos estudios sugieren una asociación entre la vacunación y el desarrollo de enfermedades autoinmunes como el síndrome de Guillain-Barré (Waheed *et al.,* 2021; Bouattour *et al.,* 2022), así como la exacerbación de la diabetes preexistente o la inducción de diabetes tipo 1 en personas con predisposición genética (He *et al.,* 2023; Alsudais *et al.,* 2023). Recientemente, se ha informado sobre casos de diabetes tipo 1 después de la vacunación con ARNm contra la COVID-19, aunque algunos aspectos, como el índice de masa corporal inicial y los niveles de HbA1c, no están completamente documentados en todos los informes de casos, lo que dificulta establecer una relación causal clara entre la vacunación y el desarrollo de la enfermedad (Alsudais *et al.,* 2023). En otro estudio, Moon y colaboradores (2023) reportaron un caso de hiperglucemia tras la vacunación con ARNm de Moderna, indicando un posible vínculo con la diabetes tipo 1, sin embargo, Xiong y colaboradores (2023) no encontraron evidencia de un mayor riesgo de diabetes post-vacunación con las vacunas CoronaVac (Sinovac) y BNT162b2 (Pfizer-BioNTech) en un estudio más amplio en Hong Kong. Nosotros reportamos previamente que los individuos vacunados mostraron niveles de metilación de m6A significativamente más bajos que los individuos no vacunados. Aunque estas diferencias no mostraron una correlación clara con la carga viral ni la edad, el hallazgo sugiere la posible existencia de un fenómeno de metilación de m6A influenciado por la vacunación (Batista-Roche *et al.,* 2022). Esta observación inicial enfatiza la necesidad de una exploración más profunda sobre cómo las vacunas contra COVID-19 pueden modular la respuesta inmunitaria, especialmente en individuos susceptibles mediando una disminución en los niveles de m6A.

5.3. Estado Actual de la Pandemia de COVID-19 (Reporte de la OMS el 25 de Mayo del 2023)

Actualmente, la OMS está monitoreando dos variantes de interés (VOI), XBB.1.5 y XBB.1.16, junto con siete variantes bajo monitoreo (VUM) y sus linajes descendientes: BA.2.75, CH.1.1, BQ.1, XBB, XBB.1.9.1, XBB.1.9.2 y XBB.2.3. A nivel mundial se ha informado sobre la variante XBB.1.5 en 113 países. Esta variante presenta características de escape inmunológico que pueden superar a otras subvariantes de omicron (Ao *et al.,* 2023; Yue *et al.,* 2023).

Entre el 24 de abril y el 21 de mayo de 2023, la OMS informó aproximadamente 2.3 millones de nuevos casos y cerca de 15,000 muertes por COVID-19 a nivel mundial, marcando una disminución del 21% y 17%, respectivamente, respecto a los 28 días previos (27 de marzo-23 de abril de 2023). Globalmente, hasta el 21 de mayo del 2023, se han registrado más de 766 millones de casos confirmados y más de 6.9 millones de fallecidos, aunque estos números probablemente subestiman las tasas reales de infección debido a limitaciones en las pruebas diagnósticas y retrasos en la notificación (https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---25-may-2023)

La disminución en la mortalidad por COVID-19 está relacionada con varios factores como una mejor comprensión de las características biológicas del SARS-CoV-2, así como un mayor entendimiento de los mecanismos asociados a la transmisión viral (Jackson *et al.,* 2022). Además, la aplicación de tratamientos más eficaces (Panahi *et al.,* 2023), como el uso de corticosteroides en pacientes hospitalizados (Umbrello *et al.,* 2021), ha contribuido significativamente a esta disminución. La implementación de programas de vacunación a gran escala ha desempeñado un papel crucial al reducir la gravedad de la enfermedad en pacientes con comorbilidades y al disminuir la incidencia de casos graves y fatales (Hoxha *et al.,* 2022). Simultáneamente, se ha registrado un incremento en la capacidad hospitalaria, permitiendo una mejor atención y gestión de un mayor número de casos. Adicionalmente, las medidas de salud pública, como el uso generalizado de mascarillas (Aravindakshan *et al.,* 2022), el distanciamiento social y las prácticas de higiene mejoradas, fueron en su momento y siguen siendo fundamentales en la contención de la propagación del virus y en la reducción de la carga de enfermedad en la población (Talic *et al.,* 2021). Estos esfuerzos combinados han contribuido de manera significativa a la disminución de la mortalidad asociada a COVID-19 a nivel global (Ribeiro da Silva *et al.,* 2022).

Un tema que aún está en desarrollo y necesita mayor comprensión es el papel que ha desempeñado la inmunidad innata y entrenada en la protección contra la COVID-19. La inmunidad innata es la primera línea de defensa, activándose rápidamente contra patógenos; mientras que la inmunidad entrenada se desarrolla a través de la exposición a diversos estímulos como vacunas, infecciones previas o agentes de entrenamiento inmunológicos (Dagenais *et al.,* 2023). En un estudio de cohorte en los Países Bajos con 43,257 participantes reportaron que la inmunidad híbrida en personas con dos otres y hasta cuatro eventos de inmunización, ya sea por vacunación o infecciones previas, estaban mejor protegidos contra la infección por omicron que la inmunización inducida sólo por la vacuna hasta al menos 30 semanas después del último evento de vacunación (de Gier *et al.,* 2023). Otros autores también informaron sobre el efecto positivo de la inmunidad híbrida contra la infección por omicron; sin embargo, estos trabajos presentan limitaciones como la medición del momento exacto de la infección con métodos serológicos, clasificaciones erróneas de infecciones previas, tamaños de muestra limitados y posibles errores de clasificación de las variantes virales definidas por período secular en lugar de secuenciación (Tartof *et al.,* 2023). Por otro lado, se ha informado sobre diversos agentes de entrenamiento inmunológico para proteger contra infecciones virales como el SARS-CoV-2, el bacilo de Calmette-Guérin (BCG), el β-glucano y el lipopolisacárido (LPS). (Brueggeman *et al.,* 2022). Brueggeman y colaboradores (2022) sugieren que los individuos sometidos a entrenamiento inmunológico podrían desarrollar una respuesta inmunitaria innata más robusta frente a la infección por SARS-CoV-2 en comparación con aquellos no entrenados. Además, hipotetizan que el entrenamiento de la inmunidad innata podría beneficiar a las personas vacunadas contra COVID-19 al mitigar la gravedad de la enfermedad y reducir la mortalidad mediante la mejora de las respuestas inmunitarias. Sin embargo, un estudio realizado en Dinamarca con 1703 trabajadores de la salud en el grupo BCG y 1683 en el grupo control no encontró un efecto protector de la vacunación con BCG contra COVID-19 grave. A los 6 meses, el riesgo de COVID-19 grave fue del 7.6 % en el grupo BCG y del 6.5 % en el grupo placebo (diferencia de riesgo, 1,1 puntos porcentuales; IC del 95 %, -1.2 a 3.5; p = 0.34) (Pittet *et al.,* 2023). En cuanto a este tema, Lopez-Campos y Valvano (2023), en una carta al editor, comunicaron que los resultados de más de 100 estudios publicados en los primeros ocho meses de la pandemia son cuestionables. Además de las vacunas con efecto heterólogo, Larenas-Linnemann y colaboradores (2020) reportaron otras intervenciones para estimular la inmunidad innata relacionadas con el estilo de vida como hacer ejercicio, dormir, suplementación con vitaminas D, C, zinc y estimulantes

inmunológicos no específicos como vacunas bacterianas, probióticos, extracto de leucocitos dializables o moléculas sintéticas. Estas intervenciones muestran cierto grado de evidencia para potenciar la respuesta inmunitaria innata y, por lo tanto, podrían conllevar posibles beneficios; sin embargo, se deben realizar ensayos específicos en COVID-19 para respaldar recomendaciones sólidas.

6. CONCLUSIONES GENERALES

El patrón epidemiológico de la pandemia de COVID-19 en Sinaloa, México y los hallazgos sobre la metilación m6A del ARN viral revelan una interacción compleja entre las variantes virales y la respuesta del huésped.

La pandemia en esta población desde marzo de 2020 hasta febrero de 2022 se caracterizó por cuatro olas epidemiológicas, con diferencias en la prevalencia de variantes como alfa, epsilon, gamma, delta y omicron. En paralelo, el análisis de los niveles de metilación de m6A en muestras de ARN nasofaríngeo demostró que las variantes más prevalentes en su momento (delta y omicron) exhibieron los niveles más bajos de metilación m6A, lo que sugiere una posible influencia de estas variantes en los niveles m6A genómico y/o viral. Este hallazgo destaca la necesidad de investigar más a fondo las bases moleculares de estas diferencias y su impacto en la inmunidad y la salud humana. Además, el estudio señala una posible asociación entre la vacunación contra el COVID-19 y niveles reducidos de metilación m6A, lo que plantea interrogantes sobre el papel de las vacunas en la epigenética del ARN viral o del huésped y la respuesta inmune. En última instancia, estos resultados subrayan la importancia de una investigación interdisciplinaria para comprender completamente la relación entre la infección por SARS-CoV-2, la metilación m6A y el desarrollo de enfermedades metabólicas como la diabetes mellitus en la era post-COVID-19, lo que podría informar el desarrollo de estrategias de salud pública más efectivas y dirigidas.

7. RECOMENDACIONES

Diversas áreas del conocimiento en relación con la pandemia de COVID-19 requieren mayor investigación. Se debe continuar estudiando la complejidad genómica de los coronavirus como el SARS-CoV-2 ya que se desconocen muchas funciones de las proteínas no estructurales relacionadas con la replicación viral. Asimismo, se requiere también continuar investigando sobre la eficacia a largo plazo de las vacunas desarrolladas hasta el momento, así como la necesidad de dosis de refuerzo. Además, se necesita conocer a profundidad la duración y la eficacia de la inmunidad después de la infección natural por SARS-CoV-2, la vacunación, agentes de entrenamiento inmunológico, así como la posibilidad de reinfección con otras variantes virales.

Otro tema de interés implica estudiar los efectos a largo plazo de COVID-19 no sólo en la salud metabólica, sino también en la salud física y mental de las personas. Adicionalmente, se hace necesario desarrollar estrategias certeras de detección temprana, vigilancia epidemiológica y respuesta rápida para futuras apariciones de enfermedades infecciosas. El uso de tecnologías emergentes como la inteligencia artificial y el aprendizaje automático es clave para analizar grandes cantidades de datos epidemiológicos, genómicos y clínicos. Estas estrategias junto con los métodos de secuenciación genómica de próxima generación ayudarán a identificar potenciales reservorios de animales infestados con otros virus y comprender la dinámica de transmisión a humanos. Además, se insta a realizar detecciones tempranas del virus y sus variantes en aguas residuales y fluidos biológicos. Por último, se enfatiza la importancia de una comunicación efectiva de los resultados obtenidos sobre el comportamiento de la pandemia de COVID-19 a nivel mundial, lo cual fortalecerá la capacidad de respuesta ante futuras emergencias de salud pública.

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9. ANEXOS

9.1. Datos de PCR en Tiempo Real y Metilación M6A del ARN (Correspondiente al Capítulo 3)

Supplementary File S1: Real-time PCR data

Samples	Age	Sex	Variant	Vaccination	Ct1	Ct2	Average_Cts	Control (Rp)	Average_Cts-Control	Relative_expression _N_gene
1	41	Female	Omicron	Complete	23.19	24.89	24.04	30.62	-6.58	95.67035191
2	51	Female	Omicron	Complete	21.09	22.36	21.725	28.4	-6.675	102.1821935
3	30	Female	Omicron	Complete	24.46	24.31	24.385	25.28	-0.895	1.859609885
4	50	Female	Omicron	Complete	21.46	23.28	22.37	31.38	-9.01	515.5612416
5	49	Female	Omicron	Complete	30.67	32.31	31.49	32.3	-0.81	1.753211443
6	52	Male	Omicron	Complete	22.08	23.88	22.98	31.11	-8.13	280.1391875
7	38	Male	Omicron	Not vaccinated	29.19	30.75	29.97	30.33	-0.36	1.283425898
8	33	Male	Omicron	Not vaccinated	24.58	27.03	25.805	28.47	-2.665	6.342273093
9	35	Male	Omicron	Complete	27.39	29.58	28.485	29.25	-0.765	1.699369998
10	38	Male	Omicron	Complete	23.4	25.28	24.34	30.82	-6.48	89.26359465
11	50	Male	Delta	unknown	17.17	18.44	17.805	26.8	-8.995	510.2286146
12	32	Male	Delta	unknown	26.03	26.04	26.035	27.18	-1.145	2.211461307
13	35	Male	Delta	unknown	15.8	14.23	15.015	24.35	-9.335	645.8252333
14	37	Male	Delta	unknown	25.95	24.68	25.315	26.69	-1.375	2.593679109
15	30	Male	Delta	unknown	24.01	25.13	24.57	29.26	-4.69	25.8125363
16	47	Female	Delta	Partial	20.02	21.11	20.565	28.98	-8.415	341.3244613
17	30	Female	Delta	unknown	23.56	25.33	24.445	32.48	-8.035	262.2865467
18	40	Female	Delta	Partial	16.78	15.19	15.985	25.38	-9.395	673.2506932
19	30	Female	Delta	Partial	22.86	21.23	22.045	27.39	-5.345	40.64483144
20	60	Female	Delta	Partial	20.75	21.91	21.33	30.1	-8.77	436.5490646
21	32	Male	Gamma	Not vaccinated	24.52	25.98	25.25	32.17	-6.92	121.0953788
22	54	Male	Gamma	Not vaccinated	29.44	27.88	28.66	31.17	-2.51	5.696200782
23	48	Male	Gamma	Not vaccinated	21.27	21.49	21.38	26.65	-5.27	38.58585049

24	44	Male	Gamma	Not vaccinated	24.32	26.6	25.46	33.59	-8.13	280.1391875
25	45	Male	Gamma	Not vaccinated	20.24	18.84	19.54	22.5	-2.96	7.781239579
26	36	Female	Gamma	Not vaccinated	19.14	21.02	20.08	27.68	-7.6	194.0117205
27	43	Female	Gamma	Not vaccinated	23.53	22.16	22.845	28.69	-5.845	57.48047186
28	45	Female	Gamma	Not vaccinated	15.28	15.38	15.33	23.05	-7.72	210.8393004
29	40	Female	Gamma	Not vaccinated	25.33	22.89	24.11	27.22	-3.11	8.633825892
30	55	Female	Gamma	Not vaccinated	25.42	27.55	26.485	31.84	-5.355	40.9275386
31	53	Male	Mex	Not vaccinated	27.49	25.06	26.275	26.37	-0.095	1.068065408
32	32	Male	Mex	Not vaccinated	26.94	26.38	26.66	29.27	-2.61	6.105036836
33	52	Male	Mex	Not vaccinated	23.15	23.95	23.55	25.3	-1.75	3.363585661
34	35	Male	Mex	Not vaccinated	21.96	21.51	21.735	25.89	-4.155	17.81474589
35	34	Male	Mex	Not vaccinated	24.21	25.12	24.665	26.92	-2.255	4.773342972
36	39	Female	Mex	Not vaccinated	25.5	24.98	25.24	30.15	-4.91	30.06472797
37	58	Female	Mex	Not vaccinated	29.33	31.51	30.42	31.63	-1.21	2.313376368
38	55	Female	Mex	Not vaccinated	22.1	20.21	21.155	27.57	-6.415	85.33111533
39	59	Female	Mex	Not vaccinated	29.81	30.14	29.975	32.03	-2.055	4.155436413
40	51	Female	Mex	Not vaccinated	30.16	27.88	29.02	29.52	-0.5	1.414213562
41	37	Male	Alpha	Not vaccinated	22.45	25.57	24.01	32.17	-8.16	286.0255073
42	52	Male	Alpha	Not vaccinated	24.23	25.29	24.76	25.65	-0.89	1.853176124
43	43	Male	Alpha	Not vaccinated	21.42	22.42	21.92	26.16	-4.24	18.89588258
44	37	Male	Alpha	Not vaccinated	26.49	27.56	27.025	30.96	-3.935	15.29512508

45	48	Male	Alpha	Not vaccinated	25.53	29.28	27.405	29.52	-2.115	4.331900182
46	59	Female	Alpha	Not vaccinated	19.13	18.96	19.045	25.24	-6.195	73.26234628
47	45	Female	Alpha	Not vaccinated	19.05	20.25	19.65	28.49	-8.84	458.2528363
48	60	Female	Alpha	Not vaccinated	27.41	29.24	28.325	30.92	-2.595	6.041890342
49	57	Female	Alpha	Not vaccinated	22.48	23.66	23.07	26.32	-3.25	9.51365692
50	52	Female	Alpha	Not vaccinated	17.72	20.16	18.94	27.06	-8.12	278.2041248
51	59	Male	Epsilon	Not vaccinated	22.01	24.23	23.12	27.55	-4.43	21.55573723
52	51	Male	Epsilon	Not vaccinated	19.09	18.01	18.55	25.42	-6.87	116.9704256
53	36	Male	Epsilon	Not vaccinated	25.1	25.45	25.275	27.86	-2.585	6.000155957
54	46	Male	Epsilon	Not vaccinated	26.94	26.38	26.66	29.27	-2.61	6.105036836
55	56	Male	Epsilon	Not vaccinated	27.54	30.24	28.89	31.06	-2.17	4.500233939
56	55	Female	Epsilon	Not vaccinated	23.06	23.52	23.29	30.49	-7.2	147.0333894
57	33	Female	Epsilon	Not vaccinated	27.32	25.24	26.28	27.08	-0.8	1.741101127
58	39	Female	Epsilon	Not vaccinated	21.12	21.21	21.165	27.61	-6.445	87.12410346
59	41	Female	Epsilon	Not vaccinated	25.38	28.43	26.905	31.99	-5.085	33.94200772
60	30	Female	Epsilon	Not vaccinated	19.77	20.46	20.115	25.29	-5.175	36.12686095

Supplementary File S2: m6A RNA methylation data.

Samples	Age	Sex	Variant	Vaccination	RNA applied to the well: S (ng)	DO_sample	DO_PC	RNA applied CP: P(ng)	DO_NC	Relative m6A (%)
1	41	Female	Omicron	Complete	480	0.108	0.263	0.1	0.084	0.003
2	51	Female	Omicron	Complete	480	0.156	0.263	0.1	0.084	0.008
3	30	Female	Omicron	Complete	480	0.34	0.263	0.1	0.084	0.030
4	50	Female	Omicron	Complete	480	0.12	0.263	0.1	0.084	0.004
5	49	Female	Omicron	Complete	480	0.167	0.263	0.1	0.084	0.010
6	52	Male	Omicron	Complete	480	0.126	0.263	0.1	0.084	0.005
7	38	Male	Omicron	Not vaccinated	480	0.255	0.263	0.1	0.084	0.020
8	33	Male	Omicron	Not vaccinated	480	0.147	0.263	0.1	0.084	0.007
9	35	Male	Omicron	Complete	480	0.503	0.263	0.1	0.084	0.049
10	38	Male	Omicron	Complete	480	0.201	0.263	0.1	0.084	0.014
11	50	Male	Delta	unknown	480	0.096	0.078	0.1	0.05	0.034
12	32	Male	Delta	unknown	240	0.081	0.078	0.1	0.05	0.046
13	35	Male	Delta	unknown	360	0.099	0.078	0.1	0.05	0.049
14	37	Male	Delta	unknown	315	0.08	0.078	0.1	0.05	0.034
15	30	Male	Delta	unknown	355	0.098	0.078	0.1	0.05	0.048
16	47	Female	Delta	Partial	405	0.158	0.078	0.1	0.05	0.095
17	30	Female	Delta	unknown	336	0.065	0.078	0.1	0.05	0.016
18	40	Female	Delta	Partial	585	0.139	0.078	0.1	0.05	0.054

19	30	Female	Delta	Partial	256	0.064	0.078	0.1	0.05	0.020
20	60	Female	Delta	Partial	516	0.08	0.078	0.1	0.05	0.021
21	32	Male	Gamma	Not vaccinated	484	0.215	0.078	0.1	0.05	0.122
22	54	Male	Gamma	Not vaccinated	114	0.053	0.078	0.1	0.05	0.009
23	48	Male	Gamma	Not vaccinated	164	0.128	0.078	0.1	0.05	0.170
24	44	Male	Gamma	Not vaccinated	382	0.208	0.078	0.1	0.05	0.148
25	45	Male	Gamma	Not vaccinated	527	0.057	0.078	0.1	0.05	0.005
26	36	Female	Gamma	Not vaccinated	640	0.13	0.078	0.1	0.05	0.045
27	43	Female	Gamma	Not vaccinated	142	0.082	0.078	0.1	0.05	0.080
28	45	Female	Gamma	Not vaccinated	181	0.07	0.078	0.1	0.05	0.039
29	40	Female	Gamma	Not vaccinated	245	0.074	0.078	0.1	0.05	0.035
30	55	Female	Gamma	Not vaccinated	214	0.096	0.078	0.1	0.05	0.077
31	53	Male	Mex	Not vaccinated	300	0.104	0.078	0.1	0.05	0.064
32	32	Male	Mex	Not vaccinated	420	0.153	0.078	0.1	0.05	0.088
33	52	Male	Mex	Not vaccinated	480	0.197	0.078	0.1	0.05	0.109
34	35	Male	Mex	Not vaccinated	480	0.104	0.078	0.1	0.05	0.040
35	34	Male	Mex	Not vaccinated	180	0.086	0.078	0.1	0.05	0.071
36	39	Female	Mex	Not vaccinated	300	0.23	0.078	0.1	0.05	0.214

37	58	Female	Mex	Not vaccinated	240	0.092	0.078	0.1	0.05	0.063
38	55	Female	Mex	Not vaccinated	400	0.225	0.078	0.1	0.05	0.156
39	59	Female	Mex	Not vaccinated	480	0.386	0.078	0.1	0.05	0.250
40	51	Female	Mex	Not vaccinated	140	0.083	0.078	0.1	0.05	0.084
41	37	Male	Alpha	Not vaccinated	480	0.117	0.082	0.1	0.057	0.050
42	52	Male	Alpha	Not vaccinated	522	0.238	0.082	0.1	0.057	0.139
43	43	Male	Alpha	Not vaccinated	300	0.077	0.082	0.1	0.057	0.027
44	37	Male	Alpha	Not vaccinated	310	0.106	0.082	0.1	0.057	0.063
45	48	Male	Alpha	Not vaccinated	168	0.091	0.082	0.1	0.057	0.081
46	59	Female	Alpha	Not vaccinated	300	0.156	0.082	0.1	0.057	0.132
47	45	Female	Alpha	Not vaccinated	260	0.082	0.082	0.1	0.057	0.038
48	60	Female	Alpha	Not vaccinated	430	0.109	0.082	0.1	0.057	0.048
49	57	Female	Alpha	Not vaccinated	243	0.160	0.082	0.1	0.057	0.170
50	52	Female	Alpha	Not vaccinated	132	0.113	0.082	0.1	0.057	0.170
51	59	Male	Epsilon	Not vaccinated	560	0.197	0.082	0.1	0.057	0.100
52	51	Male	Epsilon	Not vaccinated	448	0.207	0.082	0.1	0.057	0.134
53	36	Male	Epsilon	Not vaccinated	190	0.148	0.082	0.1	0.057	0.192

54	46	Male	Epsilon	Not vaccinated	186	0.147	0.082	0.1	0.057	0.194
55	56	Male	Epsilon	Not vaccinated	420	0.154	0.082	0.1	0.057	0.092
56	55	Female	Epsilon	Not vaccinated	450	0.217	0.082	0.1	0.057	0.142
57	33	Female	Epsilon	Not vaccinated	258	0.203	0.082	0.1	0.057	0.226
58	39	Female	Epsilon	Not vaccinated	260	0.263	0.082	0.1	0.057	0.317
59	41	Female	Epsilon	Not vaccinated	359	0.178	0.082	0.1	0.057	0.135
60	30	Female	Epsilon	Not vaccinated	180	0.144	0.082	0.1	0.057	0.193
61		Male	Control	Not vaccinated	300	0.21	0.082	0.1	0.057	0.204
62		Male	Control	Not vaccinated	450	0.147	0.082	0.1	0.057	0.080
63		Male	Control	Not vaccinated	500	0.17	0.082	0.1	0.057	0.090
64		Male	Control	Not vaccinated	500	0.266	0.082	0.1	0.057	0.167
65		Male	Control	Not vaccinated	450	0.274	0.082	0.1	0.057	0.193
66		Female	Control	Not vaccinated	420	0.176	0.082	0.1	0.057	0.113
67		Female	Control	Not vaccinated	500	0.233	0.082	0.1	0.057	0.141
68		Female	Control	Not vaccinated	480	0.206	0.082	0.1	0.057	0.124
69		Female	Control	Not vaccinated	480	0.181	0.082	0.1	0.057	0.103
70		Female	Control	Not vaccinated	480	0.174	0.082	0.1	0.057	0.098

9.2. Riesgo de Múltiples Trastornos Autoinmunes y Autoinflamatorios Posteriores a COVID-19 (Correspondiente al Capítulo 5)

Cuadro 1. Riesgo de múltiples trastornos autoinmunes y autoinflamatorios posteriores al COVID-19

Trastorno autoinmune	**índice de riesgo ajustado [aHR]/ intervalo de confianza [IC] del 95%**	**References**
alopecia areata	1,12/ 1,05-1,19	Lim *et al.* (2023)
alopecia total	1,74/1,39-2,17	
enfermedad de Crohn	1,68/1,31-2,15	
sarcoidosis	1,59/1,00-2,52	
anemia perniciosa	1,72/1,12–2,64	Peng *et al.,* 2023
espondiloartritis	1,32/1,03-1,69	
artritis reumatoide	1,29/1,09-1,54	
otras artritis autoinmunes	1,43/1,33–1,54	
psoriasis	1,42/ 1,13-1,78	
penfigoide	2,39/1,83-3,11	
enfermedad de Graves	1,30/1,10-1,54	
síndrome de anticuerpos antifosfolípidos	2,12/1,47-3,05	
trombocitopenia mediada por inmunidad	2,1/1,82–2,43	
esclerosis múltiple	2,66/1,17–6,05	
vasculitis	1,46/1,04-2,04	

9.3. Análisis de Epítopos Comunes a las Variantes del SARS-CoV-2 que se Unen a los Alelos HLA más Frecuentes en la Población de Sinaloa como Primer Paso para el Desarrollo de una Vacuna Multiepítopo (Análisis Adicional)

INTRODUCCIÓN

La entrada del SARS-CoV-2 en las células es facilitada por la afinidad de la proteína spike a los receptores de la enzima convertidora de angiotensina 2 (ACE2) en el huésped (Gadanec *et al.,* 2021). La mayoría de las mutaciones de SARS-CoV-2 detectadas a nivel mundial se encuentran dentro de la proteína spike viral, que es fundamental para la unión a la célula huésped (Becerra-

Flores y Cardozo, 2020). Estas variaciones pueden ser utilizadas para identificar epítopos como blancos para el desarrollo de vacunas, así como para el diseño de fármacos antivirales, con el fin de encontrar tratamientos contra el SARS-CoV-2 (Zaheer *et al.*, 2020). Por lo tanto, el objetivo de este trabajo fue caracterizar los epítopos T y B predichos a partir de las proteínas spike de las variantes de SARS-CoV-2 predominantes en la población de Sinaloa, México como un primer paso para el desarrollo de una vacuna multi-epítopo.

MATERIALES Y MÉTODOS

La búsqueda de los genomas de las variantes de la proteína spike de SARS-CoV-2, previamente reportadas como relevantes en la población de Sinaloa, México (Batista *et al.*, 2022), se realizó en la base de datos GISAID (Global Initiative on Sharing Avian Influenza). Los genomas se alinearon contra un genoma de referencia para SARS-CoV-2 (ID de acceso de Genbank: NC_045512) utilizando el servidor MAFFT (https://mafft.cbrc.jp/alignment/software/closelyrelatedviralgenomes.html). Las secuencias de nucleótidos de spike (3,822 nucleótidos) fueron extraídas y traducidas a secuencias de aminoácidos con el software BioEdit Sequence Alignment Editor v7.0. Las variantes completas de Spike (sin bases ni aminoácidos ambiguos) encontradas en Sinaloa se exportaron en formato FASTA. Se seleccionó una secuencia de cada variante de Spike (B.1.1.7, B.1.617.2, P.1, B.1.1.519, B.1.1.529, B.1.427 y B.1.429) para un análisis posterior.

Los alelos de antígenos leucocitarios humanos (HLA) prevalentes en Sinaloa (frecuencia alélica ≥ 5%) se identificaron con la base de datos de frecuencia de alelos (www.allelefrequencies.net/). Los posibles epítopos de linfocitos T colaboradores y citotóxicos que se unirían a las moléculas HLA prevalentes fueron predichos con los servidores NetMHCpan-4.1 (http://www.cbs.dtu.dk/services/NetMHCpan/) y NetMHCIIpan-4.0 (http://www.cbs.dtu.dk/services/NetMHCIIpan-4.0/) (Reynisson *et al.*, 2020), respectivamente. Las longitudes de los péptidos fueron de 8, 9 y 10 aminoácidos para MHC-I y de 15 aminoácidos para MHC-II. Se seleccionaron los epítopos de unión fuerte - strong binders (rango <0.5 para los péptidos de MHC-I y rango <1 para los péptidos de MHC-II). Se utilizó el método PopCover-2.0 (https://services.healthtech.dtu.dk/service.php?PopCover-2.0) para identificar 10 péptidos de las variantes de Spike con cobertura óptima de los alelos HLA seleccionados. Los epítopos lineales B fueron predichos con el servidor BepiPred 2.0 (http://www.cbs.dtu.dk/services/BepiPred/) (Jespersen *et al.*, 2017) (probabilidad de epítopo > 0.51 y más de 3 aminoácidos). Se buscaron los

epítopos lineales reportados con ensayos positivos para la glicoproteína Spike [P0DTC2] (SARS-CoV-2) en la Base de Datos de Epítopos Inmunológicos (IEDB) y se compararon con los epítopos predichos. Se realizó un análisis de conservación de epítopos (http://tools.iedb.org/conservancy/) para los péptidos predichos por PopCover y las variantes completas de Spike encontradas en Sinaloa.

RESULTADOS Y DISCUSIÓN

Estudio de caracterización de epítopos en Sinaloa, México

Los alelos MHC-I más frecuentes en una muestra de la población de Sinaloa fueron B40:02, B14:02 y B15:01. Los alelos prevalentes de MHC-II fueron DQB103:02, DQB103:01 y DRB103:01 (cuadro 2). Las frecuencias de los alelos HLA-A y HLA-C en la población de Sinaloa no estaban disponibles. Además, no se encontró suficiente información sobre DQA para predecir péptidos que se unan a los alelos DQB1.

Cuadro 2. Alelos MHC-I y MHC-II más frecuentes en una muestra de la población de Sinaloa

Población	Alelo	MHC	Frequencia alélica
Mexico, Sinaloa, Culiacan (n=103)	B*40:02	I	0.0971
	B*14:02	I	0.0631
	B*15:01	I	0.0534
	DQB1*03:02	II	0.2524
	DQB1*03:01	II	0.1602
Mexico, Sinaloa rural (n=183)	B*40:02	I	0.1093
	B*15:01	I	0.0656
	DQB1*03:02	II	0.2760
	DQB1*03:01	II	0.1940
	DRB1*03:01	II	0.0546
Mexico, Sinaloa Mestizo (n=56)	B*14:02	I	0.0630
	B*40:02	I	0.0540
	DQB1*03:02	II	0.2050
	DQB1*03:01	II	0.1520
Mexico, Sinaloa, Capomos Mayo Yoremes (n=60)	DQB1*03:02	II	0.6250
	DQB1*03:01	II	0.1833
	DQB1*04:02	II	0.1083
	DRB1*04:07	II	0.5083
	DRB1*14:06	II	0.1167
	DRB1*08:02	II	0.0917
	DRB1*04:04	II	0.0750
	DRB1*04:03	II	0.0583

Las variantes de spike fueron sometidas a los servidores NetMHCpan y NetMHCIIpan para predecir epítopos de linfocitos T citotóxicos (CTL) y linfocitos T colaboradores (HTL), respectivamente. Para los CTL, el conjunto de datos incluyó 3 HLA-B (40:02, 14:02 y 15:01) y para los HTL incluyó 6 HLA-DRB1 (0301, 0403, 0404, 0407, 0802 y 1406) prevalentes en Sinaloa. Se encontró que el número de epítopos difiere ligeramente entre las variantes de spike. Hubo un máximo de 8 epítopos lineales B predichos dentro del dominio de unión al receptor (RBD) (Cuadro 3).

PopCover predijo que al menos 10 péptidos (HTPINLVRDLPQGFS, TRGVYYPDKVFRSSV, GINITRFQTLLALHR, TSNFRVQPTESIVRF, SQILPDPSKPSKRSF, RAAEIRASANLAATK, TQSLLIVNNATNVVI, PDKVFRSSVLHSTQD, YEPQIITTDNTFVSG, VVFLHVTYVPAQEKN), con fuertes enlaces a los HLA prevalentes en Sinaloa, son comunes a todas las variantes de spike (Fig. 2). El péptido TRGVYYPDKVFRSSV está presente eíín todas las variantes de spike con una cobertura máxima de HLA. Está contenido en un epítopo de células B reportado en la IEDB (RTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFF). Los péptidos TRGVYYPDKVFRSSV y TSNFRVQPTESIVRF se encontraron como epítopos T en la IEDB, porque inducen la liberación de interferón gamma. Los otros péptidos no han sido evaluados hasta la fecha (no tienen resultados para ensayos positivos o negativos). Aunque ninguno de los 10 péptidos predichos por PopCover estaba contenido en los epítopos B predichos por BepiPred, 7 de ellos mostraron ensayos positivos de células B en la IEDB. Los 10 péptidos resultantes del análisis de PopCover y las 140 variantes de spike encontradas en Sinaloa fueron sometidos a la herramienta de conservación de epítopos de la IEDB. Los resultados mostraron una conservación (fracción de secuencias de proteínas que contienen el epítopo) mayor al 80% para todos los epítopos (cuadro 4).

Cuadro 3. Número de epítopos T y B predichos en las variantes de spike.

	Epítopos CTL (strong binders para HLA-B)			Epítopos HTL (strong binders para HLA-DRB1)						Epítopos B	
Variante Spike	**14:02**	**15:01**	**40:02**	**0301**	**0407**	**0404**	**0403**	**1406**	**0802**	**Sequencia Completa**	**RBD**
Referencia	21	27	15	13	15	18	9	7	0	31	8
B_1_427	22	26	15	13	15	18	9	7	0	23	5
B_1_617_2	21	27	15	13	15	21	9	7	0	28	6
B_1_429	22	26	15	13	15	18	9	7	0	23	5
B_1_1_7	21	29	15	10	15	17	9	8	1	30	7
B_1_1_519	20	26	14	13	15	18	9	7	0	29	7
P_1	22	29	15	13	14	18	9	5	3	30	6
B_1_1_529	25	26	15	13	14	9	6	6	3	34	7

CTL: Linfocitos T citotóxicos; **HTL**: Linfocitos T colaboradores; **B**: Linfocitos B; **RBD**: dominio de unión al receptor.

Las variantes virales han surgido debido a diferentes mutaciones que cambian la secuencia de aminoácidos de las proteínas, y estas mutaciones pueden tener implicaciones para la transmisibilidad ya que pueden modificar la afinidad de la proteína Spike con los receptores ACE2 en los humanos, la entrada viral y la replicación (Zhou y Wang, 2021). También pueden alterar la neutralización por anticuerpos (García-Beltrán *et al.,* 2021). En este trabajo queremos resaltar las implicaciones de estas mutaciones para la activación del sistema inmunológico a través de la presentación de antígenos por parte de las células. El cuadro 3 muestra que el número de epítopos T putativos varía según la variante viral y también el HLA presentado por el huésped.

Los HLA son genes altamente polimórficos (hay varios alelos para cada locus) en el cromosoma 6 en humanos. Codifican proteínas presentadoras de antígenos en la superficie celular, también conocidas como la versión humana del MHC encontrado en muchos animales. En humanos, los HLA correspondientes al MHC de clase I son HLA-A, HLA-B y HLA-C. Además, los HLA correspondientes al MHC de clase II son HLA-DP (DPA1 y DPB1), HLA-DM (DMA y DMB), HLA-DOA, HLA-DOB, HLA-DQ (DQA1 y DQB1) y HLA-DR (DRA, DRB1, DRB3, DRB4, DRB5) (Trachtenberg *et al.,* 2007). Los loci de HLA-B y HLA-DRB1 tienen el mayor nivel de diversidad alélica, lo que significa un alto número de variantes alélicas (Tokić *et al.,* 2020). Los

HLA varían según las regiones geográficas y, por lo tanto, las proporciones cambian en diferentes poblaciones (Bühler y Sánchez-Mazas, 2011).

Recientemente, Clayton y colaboradores (2020) analizaron HLA de clase I (HLA-A, -B) y clase II (HLA-DRB1, -DQB1) e identificaron los alelos más frecuentes en la población de Sinaloa. En el presente trabajo, utilizamos esos datos reportados en la base de datos de Frecuencia de Alelos para predecir péptidos fuertemente ligantes a HLA prevalentes. El cuadro 3 muestra que las personas con HLA-B15:01 y HLA-DRB10404 pueden presentar más péptidos derivados de variantes de Spike que aquellas con otros alelos, sugiriendo un posible papel protector para este alelo contra la infección, según lo informado por Nguyen y colaboradores (2020) para HLA-B15:03. Las personas con HLA-DRB10802, que muestran un 7 % de frecuencia alélica en Sinaloa, podrían tener un déficit de respuesta de anticuerpos a las variantes de Spike, debido a la falta de "strong binder" a MHC-II (cuadro 3). Se predijo que HLA-DRB10802 se uniría al menor número de péptidos de las variantes de Spike, sugiriendo que la respuesta inmune en portadores de este alelo puede ser más débil, lo que resulta en síntomas más graves. Kachuri y colaboradores (2020) identificaron a DRB1 y DQB1 como factores genéticos clave que controlan la susceptibilidad del huésped a las infecciones virales. Curiosamente, HLA-DRB11501 se asoció con una mayor susceptibilidad a la enfermedad y a resultados graves de COVID-19 (Novelli *et al.,* 2020). HLA-B15:01 se asoció positivamente con la incidencia de la infección por SARS-CoV-2, mientras que HLA-B14 mostró una asociación inversa (Migliorini *et al.,* 2021). Por otro lado, la pérdida de epítopos en la región del RBD, que interactúa con ACE2, es un factor a tener en cuenta en el desarrollo de vacunas efectivas contra todas las variantes (cuadro 3).

Las vacunas actuales se seleccionan en función de su capacidad para generar anticuerpos neutralizantes (Kyriakidis *et al.,* 2021; Krammer, 2020). Desde el 13 de enero de 2021 hasta la fecha, se han aplicado cuatro vacunas en la población de Sinaloa: AZD1222, CoronaVac, BNT162b2 y Ad5-nCOV de las empresas AstraZeneca, Sinovac, Pfizer-BioNTech y CanSinoBio, respectivamente (https://saludsinaloa.gob.mx/). Las vacunas desarrolladas por AstraZeneca y CanSinoBio se basan en vectores virales no replicativos (Folegatti *et al.,* 2020; Wu *et al.,* 2020) como adenovirus simios o humanos, que producen la glicoproteína S para mejorar las respuestas humorales y celulares en las células mamíferas. La vacuna CoronaVac de Sinovac contiene SARS-CoV-2 inactivado (Zhang *et al.,* 2021). Pfizer-BioNTech desarrolló con éxito la BNT162b2, una vacuna que consiste en el ARNm completo de S con sitios de mutación K986P y V987P (Martínez-Flores *et al.,* 2021).

CONCLUSIONES

Los epítopos conservados en todas las variantes de SARS-CoV-2 encontradas en Sinaloa podrían ser útiles para el desarrollo de vacunas de múltiples epítopos y podrían influir en la efectividad de las vacunas basadas en la proteína Spike. Sin embargo, se requieren más estudios para evaluar el impacto de las nuevas variantes virales en los resultados inmunológicos de las personas vacunadas.

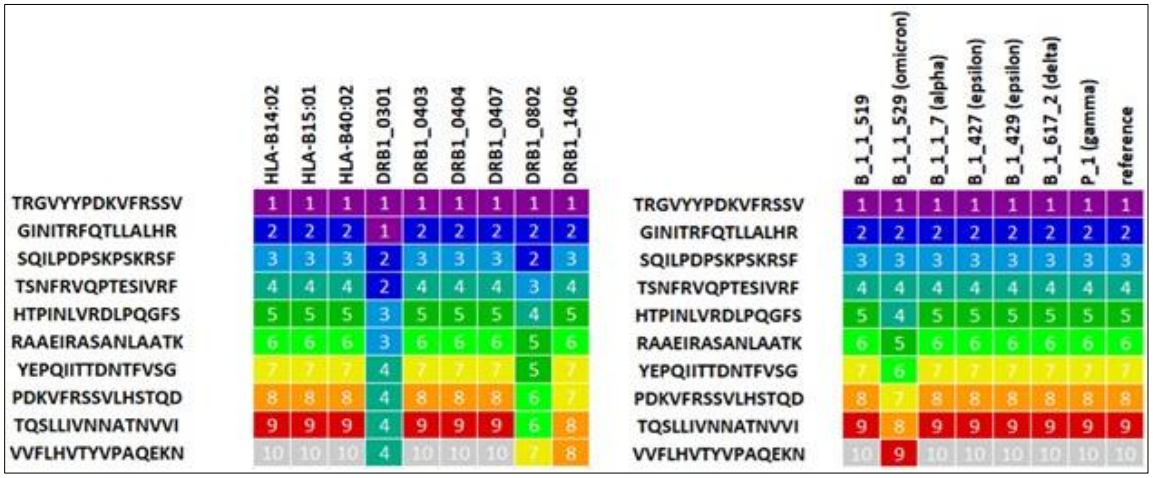

Figura 2. Resultados del PopCover

Cuadro 4. Análisis de conservancia de epítopos

Sequencia del epítopo	**Porcentaje de coincidencias de secuencias de proteínas con identidad ≤ 100%**	**Identidad mínima (%)**
HTPINLVRDLPQGFS	100.00% (140/140)	100.00
TRGVYYPDKVFRSSV	100.00% (140/140)	100.00
GINITRFQTLLALHR	96.43% (135/140)	93.33
TSNFRVQPTESIVRF	99.29% (139/140)	93.33
SQILPDPSKPSKRSF	100.00% (140/140)	100.00
RAAEIRASANLAATK	85.00% (119/140)	93.33
TQSLLIVNNATNVVI	99.29% (139/140)	93.33
PDKVFRSSVLHSTQD	96.43% (135/140)	93.33
YEPQIITTDNTFVSG	82.86% (116/140)	93.33
VVFLHVTYVPAQEKN	82.86% (116/140)	86.67