

Bionatura

Latin American journal of Biotechnology and Life Sciences



Bloqueo auriculo-ventricular avanzado y bradicardia extrema como síntomas iniciales de Covid-19
Hipercoagulabilidad, trombosis intravascular y trombocitosis asociada al COVID-19.



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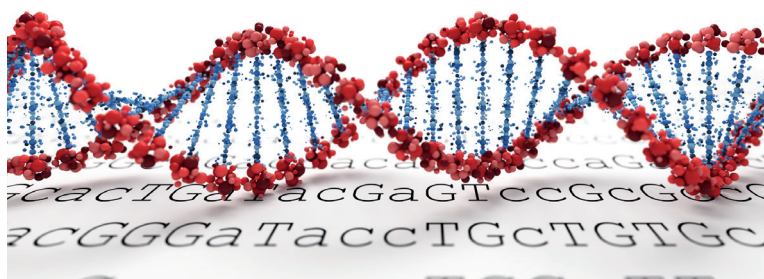
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EDITORIAL

COVID-19: An effective vaccine is needed

Lisset Hermida and Ricardo Silva

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1097

A novel coronavirus named SARS-CoV-2 is responsible for the present pneumonia outbreak called COVID-19 that started in early December 2019 in Wuhan City, Hubei province, China¹. Until March 12, SARS-CoV-2 had caused 80980 infections and 3173 deaths in China, but even worst, it is currently spreading fast in other countries, reaching 45293 infections and 1460 deaths outside China, being the most affected countries Italy, Iran and South Korea².

New cases identified in Wuhan are thought to have acquired the infection from a zoonotic source, as many reported visiting or working in the Huanan Wholesale Seafood Market. The coronaviruses are naturally hosted and evolutionarily shaped by bats^{3,4}. The whole-genome sequence identity of the SARS-CoV-2 has 96.2% similarity to a bat SARS-related coronavirus (SARSr-CoV; RaTG13) collected in Yunnan province, China^{5,6}. Nevertheless, this percentage indicates that an intermediate species should be responsible for the transmission to humans.

The incredibly rapid and effective response from the Chinese government in managing the virus widespread transmission resulted in the creation of a comprehensive epidemiological strategy that has led to the nature of newer diagnostic tools as well as a more advanced clinical management. On top of that, all scientific research has been primarily focused on the virus and its outbreak, allowing China, in a record time of just three months, to gather groundbreaking findings to share with the scientific and health community worldwide. So far, we can identify the critical issues associated with the current outbreak. First, the high virus spreads based on direct and indirect forms of transmission. SARS-CoV-2 is transmitted through drops and fomites during close and unprotected contact between an infected person and an uninfected one. Airborne spread of the virus has not been reported; however, it may be envisaged whether specific aerosol generation procedures are carried out in health care facilities⁷. Researchers are trying to relate the particular nature of the Spike protein and its interaction with the host cell to explain the high level of transmission⁸. Second, in a recent study involving 1099 patients in Wuhan province, China, fever was present in 43.8% of patients at the time of admission but developed in 88.7% during hospitalization⁹. This means that people infected with afebrile are abundant during the initial period of the disease and therefore favor the spread of the virus while escaping temperature control. Third, about 20% of confirmed patients develop severe clinical manifestations¹⁰. This situation is a challenge for any health system. Fourth, WHO has recently estimated a global fatality rate of 3.4%, but this figure must be confirmed once the epidemic is over¹¹. As for China, until March 12, the estimated fatality rate of the outbreak was 3.9%¹⁰.

The combination of the factors mentioned above indicates that we are facing an unprecedented respiratory virus infection. This is in line with the high level of required prevention and control measures implemented by China to reach an effective reduction in the number of daily new infected cases. Given the previous factors, as well as the current spreading of the virus across the globe (particularly in Italy, which is currently lockdown), the preventive vaccination appears to be the

most effective solution to contain the virus. Why is a vaccine against SARS-CoV-2 possible? Because the results obtained so far, either in preclinical or clinical trials with other two lethal Beta coronaviruses, can guide the development of a vaccine against SARS-CoV-2.^{12,13} It has been demonstrated the protective capacity of neutralizing antibodies and cell-mediated immunity in animal models^{14,15,16,17}. On the other hand, the current success of plasma therapy from the cured COVID-19 patients indicates the functionality of antibodies against the SARS-CoV-2 replication¹⁸. Finally, the rapid DNA sequencing of several isolates of the SARS-CoV-2 by Chinese scientists, up to date over 100, has provided suitable information to design the vaccine candidate¹⁹.

Accordingly, up to now, there are at least 35 vaccine candidates under research and development from different laboratories worldwide²⁰. Different approaches are being used, such as whole-inactivated, subunit vaccines, vectored vaccines, DNA, and RNAm vaccines, among others. Particularly in China, Dr. Zheng Zhongwei, Director of the Development Center for Medical Science and Technology from the National Health Commission, said that China has nine ongoing research projects on vaccine development, and some will hopefully enter clinical trials in April this year. The main types of vaccines that China is working on include inactivated, nucleic acid-based, and vector vaccines. The United States biotech company Inovio Pharmaceuticals and its partner Beijing Advaccine Biotechnology are developing a DNA vaccine called INO-4800²¹. The vaccine candidate is based on DNA technology, which has been used for its GLS-5300 MERS coronavirus vaccine candidate. This vaccine candidate GLS-5300 showed the first positive results of immunogenicity in humans against MERS-CoV²². In January, another US biotech company GeoVax partnered with Chinese vaccine developer BravoVax to develop a vector vaccine, which uses the MVA platform²¹. In addition, the first reported inactivated vaccine candidate against SARS-CoV-2 is being developed by China's state-owned pharmaceutical enterprise Sinopharm Group, as reported by Chinese officials at a press conference on February 17.

Nevertheless, despite the urgent need, the National Medical Product Administration of China claims to pass the required tests for the vaccines to be administered in healthy humans. It has been recognized immunopathogenic events associated with the most severe cases of COVID-19²³. Also, there is a possibility of antibody-dependent enhancement (ADE) since it has been described for the other two related lethal coronaviruses²⁴. Regarding the two previous elements, the WHO already published the Synopsis for designing and conducting the vaccine clinical trials to prevent COVID-19²⁵.

It is worth noting that COVID-19 is the third zoonosis related to human lethal coronaviruses. In 2002, a human pathogenic CoV, severe acute respiratory syndrome (SARS-CoV), caused 8,096 human infections with a 9.6% mortality rate²⁶. In 2012, the coronavirus Middle East respiratory syndrome MERS-CoV was another emerging pathogen that provoked outbreaks in the Arabian Peninsula and travelers from this region. As of April 24, 2016, a total of 1,728 cases with 624 dea-



Figure 1. Biosafety Level 3 containment is required for culturing and characterizing the SARS-CoV-2.

ths (36.1% mortality) were reported in 27 countries²⁷. As the human population continues to increase, demand for agricultural land grows, exposing livestock and humans to infections in the wild. Climate change is also modifying the ecosystem and the concentration of animal vectors, and rapid expansion in air traffic, the movement of people across different borders, political instability and conflicts mean these new pathogens can quickly spread across the world. Given this unfavorable scenario, the development of vaccines against SARS-CoV-2 should be accomplished with a strategic viewpoint. An effective vaccine should be able to protect not only against the three already known lethal coronaviruses, but also be able to prevent against another potentially fatal human Beta CoVs, which could cause similar outbreaks in the future. In fact, one Research Proposal based on this approach is being currently evaluated by the China Cuba Biotechnology Joint Innovation Center (CCBJIC), in the Yongzhou Municipality of Hunan Province, China²⁸. The world should be ready to combat these proven events occurring in nature.

Conclusions

The entire globe is now facing a big challenge: the effective containment of the SARS-CoV-2 transmission. Based on China's experience so far, the implementation of stringent and rigorous control measures is currently the most successful way to reduce infections. However, given the critical issues related to the current outbreak and the economic differences of the world's countries, an effective vaccine will be the ultimate goal to stop the ongoing coronavirus transmission and next virus zoonosis.

Bibliographic references

- Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506 (2020).
- OCHA Iraq. Situation Reports. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/> (2009).
- Cui, J., Li, F. & Shi, Z. L. Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology* vol. 17 181–192 (2019).
- Li, X., Song, Y., Wong, G. & Cui, J. Bat origin of a new human coronavirus: there and back again. *Sci. China Life Sci.* 63, 461–462 (2020).
- Zhou, P. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273 (2020).
- Paraskevis, D. et al. Full-genome evolutionary analysis of the nov-

- el corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infect. Genet. Evol.* 79, 104212 (2020).
- WHO. WHO Director-General's opening remarks at the mission briefing on COVID-19 - 26 February 2020. 1–6 <https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---3-march-2020> (2020).
- Mallapaty, S. Why does the coronavirus spread so easily between people? *Nature* 579, 183–183 (2020).
- Guan, W. et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* NEJMoa2002032 (2020) doi:10.1056/NEJMoa2002032.
- <https://www.worldometers.info/coronavirus/>.
- WHO. WHO Director-General's opening remarks at the mission briefing on COVID-19 - 26 February 2020. 1–6 <https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020> (2020).
- Yong, C. Y., Ong, H. K., Yeap, S. K., Ho, K. L. & Tan, W. S. Recent Advances in the Vaccine Development Against Middle East Respiratory Syndrome-Coronavirus. *Front. Microbiol.* 10, 1781 (2019).
- Jiang, S., He, Y. & Liu, S. SARS Vaccine Development - Volume 11, Number 7—July 2005 - *Emerging Infectious Diseases journal* - CDC. doi:10.3201/EID1107.050219.
- Liu, W. J. et al. T-cell immunity of SARS-CoV: Implications for vaccine development against MERS-CoV. *Antiviral Research* vol. 137 82–92 (2017).
- Zhao, J. et al. Airway Memory CD4+ T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. *Immunity* 44, 1379–1391 (2016).
- Chen, Z. et al. Recombinant Modified Vaccinia Virus Ankara Expressing the Spike Glycoprotein of Severe Acute Respiratory Syndrome Coronavirus Induces Protective Neutralizing Antibodies Primarily Targeting the Receptor Binding Region. *J. Virol.* 79, 2678–2688 (2005).
- Muthumani, K. et al. A synthetic consensus anti-spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates. *Sci. Transl. Med.* 7, (2015).
- Potential treatments for novel coronavirus - *Chinadaily.com.cn*. <https://covid-19.chinadaily.com.cn/a/202003/05/WS5e62126da31012821727d02f.html>.
- BIG Search - National Genomics Data Center - National Genomics Data Center. <https://bigd.big.ac.cn/search?dbld=2019n-CoVR&q=ncov&page=1>.
- Cadila, Z., Vector, V., Vector, V. & Vector, V. DRAFT landscape of COVID-19 candidate vaccines - 4 March. 4–6 (2020).
- Teamwork underway to develop vaccine. <https://enapp.chinadaily.com.cn/a/202003/12/AP5e69863ea3103a24b110a827.html>.
- Yoon, I. K. & Kim, J. H. First clinical trial of a MERS coronavirus DNA vaccine. *The Lancet Infectious Diseases* vol. 19 924–925 (2019).
- Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506 (2020).
- Wan, Y. et al. Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry. *J. Virol.* 94, (2019).
- WHO | Coronavirus disease (COVID-2019) R&D. WHO (2020).
- Peiris, J. S. M., Guan, Y. & Yuen, K. Y. Severe acute respiratory syndrome. *Nature Medicine* vol. 10 S88–S97 (2004).
- Middle East respiratory syndrome coronavirus (MERS-CoV) - Saudi Arabia. *Saudi Med. J.* 35, 1293 (2014)
- <https://oncubanews.com/en/cuba/cuba-and-china-to-open-bio-technological-innovative-center/>

LETTER TO EDITOR / CARTA AL EDITOR

El SARS-CoV-2 y COVID-19 en Ecuador, desafíos, oportunidad y propuestas para fortalecer la investigación en salud en el país

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El virus del síndrome agudo respiratorio severo 2 (SARS-CoV-2) es el agente etiológico de la pandemia de COVID-19 que ha paralizado a la humanidad por su elevada tasa de transmisibilidad y de mortalidad. En Ecuador su diagnóstico y vigilancia a nivel nacional está a cargo principalmente del Instituto Nacional de Investigación en Salud Pública (INSPI) Dr. Leopoldo Izquieta Pérez. Una entidad gubernamental adscrita al Ministerio de Salud Pública (MSP), con autonomía financiera y recursos provenientes del Ministerio de Economía y Finanzas. De acuerdo al decreto 1290 de creación del INSPI tiene como atribuciones y responsabilidades "Ejecutar los Proyectos de Investigación, Desarrollo Tecnológico e innovación que la Autoridad Sanitaria Nacional y la Secretaría Nacional de Educación Superior, Ciencia y Tecnología hubieren aprobado; y, Proveer servicios de laboratorio especializado en salud, en función de las prioridades establecidas por la Autoridad Sanitaria Nacional". Como en epidemias anteriores, por ej. las de Zika y Chikungunya, el INSPI cumple a cabalidad su rol de provisión de servicio especializado para el diagnóstico y vigilancia de COVID-19.

En relación a la ejecución de proyectos de investigación científica, y en concordancia con sus responsabilidades, el INSPI también lidera varias iniciativas de investigación en salud en el país. Una tarea que constantemente confronta a la institución con los desafíos intrínsecos de ejercer estas actividades en países con escasos recursos y que entre los más relevantes incluye: 1) limitados recursos económicos, 2) falta de talento humano e infraestructura, 3) intrincadas normativas para la obtención de permisos de investigación con muestras humanas, y adquisiciones, y 4) costos elevados de equipos e insumos^{1,2}. Si bien estos desafíos limitan la agilidad para hacer investigación

se cumple con este encargo con mucha disposición. La experiencia adquirida por quienes hacemos investigación en salud en el país y la sensibilización de actores claves en la importancia de esta actividad, evidenciada por la necesidad de respuestas al COVID-19, constituyen una valiosa oportunidad para promover estrategias conjuntas para facilitarla y fortalecerla.

Respecto al primer desafío, Ecuador invierte en investigación apenas un 0.36% de su PIB, un valor tres veces menor que Brasil, el país que más invierte en la región (1,26%), y entre 10 y 13 veces menos que los países que más invierten a nivel global, Corea, Israel, Alemania y EE. UU (3-5%). De acuerdo a datos de la Red Interamericana e Iberoamericana de Indicadores de Ciencia y Tecnología (RICYT), actualizados al 2019, Ecuador se ubica en el puesto 9 de 16 países de América Latina y el Caribe, en cuanto a inversión en ciencia y tecnología³. Es una comparación correspondiente a un promedio entre los años 2008 y 2014. Del 2015 al 2020 no hay datos del país en la RICYT; sin embargo, y en concordancia con la crisis económica local se evidencia una reducción considerable de inversión, reflejada por la falta de financiamiento y/o convocatorias de la SENECYT para proyectos de investigación en estos años. Una potencial estrategia para solventar este desafío es que el Estado encuentre mecanismos que garanticen su inversión continua, por ejemplo, a través de una fracción de los impuestos con los que contribuimos permanentemente. Alternativamente, los actores de la investigación en salud podríamos promover más colaboraciones interinstitucionales que permitan cofinanciar temas de interés mutuo o la presentación de propuestas fuertes a convocatorias de fondos externos.

En lo referente a talento humano los recortes en el sector público son una constante lo que dificulta la continuidad

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Figura 1. Pandemia de coronavirus en Ecuador.

de los proyectos de investigación, el posicionamiento de los investigadores como tal, su contribución y desarrollo sostenido de sus líneas investigativas. Adicionalmente algunos de los investigadores no son remunerados acorde su formación profesional¹. La solución a estos inconvenientes es que los organismos competentes den la importancia adecuada a los investigadores, garantizando su estabilidad laboral, la homologación de salarios de acuerdo a la categorización de investigadores de la SENESCYT o un salario justo de acuerdo a la formación profesional. En relación a la infraestructura, ciertas investigaciones, como la evaluación de fármacos en cultivos celulares, requieren el aislamiento y cultivo in vitro de SARS-CoV-2 y por consiguiente laboratorios con bioseguridad BSL3, Ecuador no cuenta con estos laboratorios por lo que la solución más prudente es dotar al país con este tipo de infraestructuras. Mientras tanto se deberán ejecutar investigaciones de este tipo con investigadores de países que tengan acceso a este tipo de laboratorios.

En lo que tiene que ver con las normativas de aprobación de investigación con muestras humanas la obligación más importante de acuerdo a documentos que fundamentan los principios éticos como la Declaración de Helsinki, es que los protocolos sean aprobados por un Comité de Ética de Investigación en Seres Humanos (CEISH)⁴. En Ecuador los protocolos deben aprobarse por dos instancias, primero por un CEISH y luego por el MSP, quien a su vez puede invalidar la decisión del CEISH, solicitar correcciones y reiniciar desde cero. Dada estas exigencias la aprobación de una investigación puede tardar un mínimo de seis meses en el país. Para la investigación de COVID-19 el MSP ha facilitado la revisión expedita de protocolos únicamente a través de un CEISH, lo que demuestra que es posible simplificar este procedimiento. La solución adecuada a este desafío es adoptar las recomendaciones de la Declaración de Helsinki y evitar la duplicación de esfuerzos en esta tarea. En cuanto a la compra de equipos las solicitudes de autorización deben ser hechas al Ministerio de Economía y Finanzas cuya obtención toma un tiempo prudencial. Y lo que tiene que ver con compras de insumos y reactivos requiere de múltiples gestiones internas para cumplir todas las exigencias de control. La solución a estos dos problemas es simplificar los procedimientos de control a lo mínimo posible.

Un desafío adicional es el elevado costo de equipos insumos y reactivos que puede representar cinco veces más que su costo en el país de origen¹. Una solución en concordancia con las medidas adoptadas para el COVID-19 sería eliminar impuestos para equipos, insumos y reactivos destinados a investigación en salud. Una idea alternativa es promover la producción de insumos como puntas de pipeta y tubos de colección de muestras por parte de las industrias locales, así como también la producción de reactivos y herramientas de diagnóstico local por parte de los institutos de investigación públicos o Instituciones de Educación Superior.

La tragedia y las transformaciones sociales que acarrea la pandemia de COVID-19 promoverán cambios en lo político, económico y social de nuestro país. Es el momento oportuno para que se revalorice la investigación en salud y que se convierta en una prioridad en la agenda de los tomadores de decisiones, y en los planes de gobierno de quienes aspiren a gobernar nuestra nación. Quienes estamos involucrados en investigación en salud debemos proponer y promover diferentes soluciones y estrategias que la faciliten y la fortalezcan. Luego de esta pandemia es muy probable que los actores políticos hayan entendido la importancia de la investigación en salud para responder mejor a la amenaza de un agente infeccioso, pero probablemente muchos de ellos desconocen las múltiples barreras que esta actividad enfrenta cotidianamente.

Referencias bibliográficas

1. Ciocca DR, Delgado G. The reality of scientific research in Latin America; an insider's perspective. *Cell Stress Chaperones*. 2017;
2. Moloney A. Latin America faces hurdles in health research. *Lancet*. 2009.
3. Red Interamericana e Iberoamericana de Indicadores de Ciencia y Tecnología. El estado de la ciencia. Principales Indicadores de Ciencia y Tecnología Iberoamericanos Interamericanos 2019 [Internet]. 2019. Available from: www.ricyt.org
4. Asociación Médica Mundial. Declaración de Helsinki de la AMM – Principios éticos para las investigaciones médicas en seres humanos – WMA – The World Medical Association. 5 de mayo del 2015. 2019.

RESEARCH / INVESTIGACIÓN

Development of an ecological strategy for the control of downy mildew (*Pseudoperonospora cubensis*) in cucumber cultivation (*Cucumis sativus* L.)Julio Gabriel-Ortega^{1*}, Edwin Pereira-Murillo², Fernando Ayón-Villao³, Carlos Castro-Piguave⁴, Isaías Delvalle-García⁵, José A. Castillo^{6*}

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Abstract: Downy mildew is a severe disease of cucumber worldwide. The oomycete *Pseudoperonospora cubensis* cause it and once it is established in a region, the infection spreads rapidly, causing significant loss of yield and fruit quality. The objective of the research was to develop an ecological strategy for the control of downy mildew in cucumber. The treatments were arranged in a completely randomized experimental design with an alternation of chemical and biological fungicides. The treatments were: T1: systemic fungicide (Ridomil Gold, 2.5 g/l) alternating with a contact fungicide (Bravo 2.5 ml/l), T2: CustomBio 5 (Bacillus-based fungicide, 3ml/l), T3: control (water), T4: *Trichoderma sp.* (3 ml/l), T5: systemic fungicide (Ridomil Gold 2.5g/l) alternating with CustomBio 5 (3 ml/l), and T6: systemic fungicide (Ridomil Gold 2.5g/l) alternating with *Trichoderma sp.* (3 ml/l). The following variables were evaluated: stem thickness, plant height, number and weight of fruits, yield, the area under the relative progress curve (AUDPCr), and economic analysis of each treatment. The results showed that the best treatments were T1 and T6, with an AUDPCr of 11.89% and 12.10%, respectively. Treatments T6 and T1 showed the best yield, as well. The profitability analysis showed that all the alternatives were profitable with a Benefit/Cost>1 ratio. However, the treatments T6 and T1 were the most useful. We recommend this control strategy to reduce the use of chemical fungicides and, at the same time, obtain an efficient control of the disease, which guarantees a significant yield of high-quality fruit.

Key words: downy mildew; ecological control; cucumber; AUDPCr, biological fungicide.

Introduction

Cucumber (*Cucumis sativus* L.) is the fourth most important vegetable in the world¹. It was originated in India, where two botanical cultivars arose in the Himalayas, the domesticated (*Cucumis sativus* var. *sativus*), and wild cucumber (*Cucumis sativus* var. *hardwickii* Royle, Alef)². Cucumber is a short-cycle crop (3-4 months) that could be grown in the open field, or under greenhouse conditions³. Although the cucumber can be reproduced by vegetative propagation, it has substantial genetic diversity because it also spreads sexually⁴. Current cultivars show a remarkable variety of agricultural features, including the color, shape, rib, diameter, spines, and the brightness of the fruit and the overall resistance to stress. Some cucumber cultivars are well adapted to specific environments because of genetic selection⁵. In Ecuador, this vegetable is cultivated in the warm valleys of the Andean mountains and the dry tropics of the coastline corresponding to 87.20% and 12.80% of total land devoted to this vegetable, respectively⁶. The Ecuadorian production reaches an annual average of 3,045 tons, from an area of 364 hectares with an average yearly growth of 1.03%⁶.

Various diseases affect cucumber. Among the most critical conditions is downy mildew caused by the oomycete *Pseudoperonospora cubensis*, which is one of the most severe threats to cucumber production worldwide and in Ecuador. It is spread in temperate and semiarid regions, and the pathogen infects plants of all ages, although the disease is more severe at early stages. Usually, the disease infects foliage, causing a reduction in early photosynthetic activity, which affects plant development, delaying growth, and reducing yield. The inflicted chlorotic lesions on the leaf surface could progress until they become necrotic, engaging the entire leave, which may die within days after the infection⁷. The disease cause premature defoliation and then the

sunburn of the fruit due to overexposure to direct sunlight. In the United States, host resistance in past decades successfully controlled the downy mildew; however, the pathogen has overcome this resistance and now has become a very severe threat to cucumber growers, mainly because it is also evolving fungicide resistance⁸. In Ecuador, the disease severely affects cucumber production damaging more than 60% of the output^{7,9}.

The application of chemical pesticides is the most commonly used control method, either through using contact and systemic fungicides or the combination of both¹⁰. However, the new trends are directed towards the integrated pest management (IPM)¹¹, which includes the application of alternative products such as resistance inducers (i.e., *Trichoderma atroviride*)^{12,13} or beneficial microorganisms such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Derris gummosa* and *Trichoderma harzianum*^{12,14}. These microorganisms show the potential to interact with local microflora, and usually, their products are biodegradable in situ to non-toxic compounds by environmental organisms. The search for new and varied products of natural origin for disease management programs represents an essential alternative in sustainable agriculture¹⁵. Therefore, the objective of this work is to develop an ecological strategy for the control of downy mildew (*Ps. cubensis*) in cucumber farming.

There is a need to develop strategies for the control of *Ps. cubensis* in an ecological and environmentally friendly way, thereby safeguarding people's health, and contributing to reducing production costs due to the lower application of pesticides. Here, we present preliminary data on the use of a combination of chemical fungicides and microorganisms suitable for control of *Ps. cubensis* in cucumber production under greenhouse conditions¹⁶.

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Materials and methods

Study site

This investigation was carried out from June to November 2017 in the 'Puerto La Boca' community in Manabí province, Ecuador (1°18'20"S, 80°45'42" W, 53 m altitude). Its climate has of 24.8°C and 298 mm of average temperature and annual rainfall, respectively, corresponding to dry tropical forest ecosystems.

Treatments, growth conditions and research management

Sexual seeds of the hybrid Humocaro were pre-germinated in blotting paper moistened with distilled water. The seeds were placed in a closed environment with white light, RH > 80% and a temperature of 20°C±2. For the transplanting of the seedlings, plastic trays with 128 alveoli (15 x 20 10 cm/alveolus) were used. The seedbed was prepared with topsoil (60%), Biocompost (30%), and Earthworm Humus (10%). The pre-germinated seeds were transplanted and then sprayed to field capacity. Nine days after the seedlings were put on plastic trays, they were transplanted to soil beds (36 x 1.20 x 0.60 m), containing topsoil supplemented with Biocompost in a 3:1 ratio. The transplant was performed to holes 0.15 m deep, at a distance of 1.2 m and 0.20 m between rows and plants, respectively, totaling 12 rows. Each experimental unit had an area of 26 m² with 55 plants/units. The total area of the experiment was hosted in a 500 m² greenhouse with a photoperiod of 12h light:12h dark, an average temperature of 22°C, and RH>60%. The chemical fungicides and biological organisms that we tested in various treatments are described in Table 1. The organic fungicide CustomBio 5 contains five species of the genus *Bacillus* (*B. subtilis*, *B. laterosporus*, *B. licheniformis*, *B. megaterium* y *B. pumilus* at 8x10⁸ CFU/ml) and is produced by Ecuadorian company Naturalite S.A., Guayaquil, Ecuador.

The treatments followed a completely randomized design (DCA)¹⁷, and the experimental units of each treatment consisted of 330 plants, totaling about 1980 plants. Twenty-five random plants were chosen from each experimental group in each treatment for evaluation.

Response Variables

The area under the relative disease progress curve (AUDPCr) was calculated using five affected leaves of the upper middle third of the plant and a -100% damage scale¹⁸.

Stem diameter (SD, in mm) was measured with a Vernier caliper at 30 cm from the ground when the crop had 50% flowering. Fruit weight (FW, in g) was measured by weighting fruits in each harvest. The number of fruits (NF) was measured by counting all fruits of each harvest. The number of not-set fruits (NNSF) was assessed, by counting the number of dead fruits in each treatment.

Plant height (PH, in cm) was measured when the plant had 50% of the flowering.

Profit/cost analysis was estimated using the methodology suggested by CIMMYT¹⁹ that analyzes all the benefits and costs of the different treatments.

Statistical analysis

We performed an analysis of variance to test hypotheses about the fixed effects of treatments and the comparisons of means to determine the response of the best treatment using SAS University software, Cary, NC. For this, we contrasted ways using one degree of freedom and $\alpha=0.01$. Likewise, the analysis of variance allowed estimating the variance components for random effects. The comparison of means was performed using the multiple Tukey tests at $\alpha = 0.05$, and the association between the variables studied were analyzed using Pearson's correlation test¹⁷.

Results

Before the analysis of variance, analysis of normality and homogeneity of variances of the variables and the treatments were made. This analysis showed that the variables AUDPCr, NF, FW, and NNSF were not normally distributed, so the data was transformed to square root to normalize it. For AUDPCr, severity data were arcsine transformed¹⁷.

The analysis of variance of the following variables, AUDPCr, SD, NF, and FW, showed that the coefficients of variation (CV) are within the ranges allowed for this type of research (CV of

Code	Treatment
T1:	Systemic fungicide (Metalaxyl-Macozeb) 2.5 g/l + contact fungicide (Clorotalonil) 2.5 ml/l (alternate application)
T2	CustomBio B5 3 ml/l.
T3	Control (water)
T4	<i>Trichoderma</i> sp. 3 ml/l.
T5	Systemic fungicide (Metalaxyl-Macozeb) 2.5 g/l + CustomBio B5 3 ml/l (alternate application)
T6	Systemic fungicide (Metalaxyl-Macozeb) 2.5 g/l + <i>Trichoderma</i> sp. 3 ml/l (alternate application)

Table 1. Treatments analyzed in this work.

Plants were fertilized with Biocompost Organic Fertilizer (Organicgreen S.A., Ecuador) (50 g/hole), and EcoFungi (Eco-Microbials, Florida, USA) a mix of mycorrhizae (20 g/20 l of water) and Starlite bioregulator (Organicgreen S.A., Ecuador) (50 ml/20 l of water) applied to the ground at the first day of transplanting and along the crop cycle totaling six fertilizations.

Nine harvests were made at intervals of four to five days for each one. This began when the cucumber fruits presented the right color and size. The second, third, fourth, fifth, sixth, and seventh applications of the treatments were performed at 27, 34, 41, 48, 55, 62, and 68 days after the transplant, respectively. In total, seven crops were made throughout the crop cycle.

7.61 to 35.43%) (Table 2). Highly significant differences were observed at the probability of $p < 0.01$ for all the variables evaluated. This result indicates that at least one of the treatments was different from the others (Table 2). The analysis of variance of the variables NNSF and PH showed that the CV, are within the ranges allowed for this type of investigation (CV from 8.08 to 29.44 %) (Table 2). Highly significant differences were observed at the probability of $p < 0.01$ only for PH. This indicated that at least one of the treatments is different in PH. In contrast, there were no significant differences in NNSF (Table 2).

The analysis of means performed by the Tukey test at $p < 0.05$ probability for the variables AUDPCr, SD, NF, FW, NNSF, and PH,

Source of variation	DF	Medium squares					
		AUDPCr	SD	NF	FW	NNSF	PH
Total	119						
Treatment	5	60.63**	60.63**	30.11**	64.18**	1.26ns	21.67**
Error	114	6.70	6.70	4.91	9.90	0.77	2.71
CV (%)		35.43	7.61	19.02	19.19	29.44	8.05

** : Highly significant differences at $p < 0.01$ probability

DF: Degrees of freedom

CV: Coefficient of variation

DF: Degrees of freedom

AUDPCr: Area under the disease progress curve relative

SD: Stem diameter (mm)

NF: Number of fruits

FW: Fruit weight (g)

NNSF: Number of not set fruits

PH: Plant height (cm)

Table 2. Analysis of variance for AUDPCr, SD, NF, FW, NNSF and PH in cucumber plants, Puerto La Boca, Ecuador, 2017.

showed significant differences in all cases except for NNSF, where all treatments are statistically equal (Table 3).

The best disease reduction was achieved with treatments T1 and T6 relative to T3 (control), observing AUDPCr of 11.89%, 12.10%, and 29.84 %, respectively. It is worthy to note that other secondary diseases caused by different fungi were also controlled. Regarding the SD, results indicate that the T4 treatment showed a higher diameter (36.80 mm) compared to the T1 treatment (32.01 mm), being significantly different at a $p < 0.05$ probability. The T3 and T6 treatments were statistically the same instead; treatments T4, T2, T5, and T6 were not statistically different from each other. About NF, the T4 treatment was significantly different from the T3, T6, and T1 treatments at $p < 0.05$ probability, but not substantially

different from the T2 and T5 treatments. The T3 treatment was not statistically different from the T2, T5, T6, and T1 treatments for this variable. Regarding the FW, the T4 treatment was significantly different from the T3, T6, and T1 treatments at $p < 0.05$ probability. The treatments T2, T5, and T4 were not significantly different from each other, nor treatments T3, T2, T5, T6, and T1. Concerning PH, it was found (Table 4) that treatments T3, T2, T5, T4, and T1 were not significantly different from each other, but were significantly different from the T6 treatment. There were no significant differences for NNSF for any of the treatments. The Pearson's correlation analysis (Table 4) showed a high and significant negative correlation for AUDPCr concerning to NF (-0.94) and FW (-0.94).

The profitability analysis (Table 5) showed that, in general, all

Treatment	AUDPCr	SD	NF	FW	NNSF	PH
T3	29.84 a	34.30 bc	139.45 b	378.90 b	9.90 a	417.80 a
T2	21.34 ab	32.85 bc	144.85 ab	289.70 ab	7.50 a	417.80 a
T5	21.02 ab	33.00 bc	147.25 ab	294.50 ab	12.00 a	427.30 a
T4	20.27 ab	36.80 a	190.30 a	380.60 a	8.80 a	466.00 a
T6	12.10 b	35.05 ab	119.95 b	229.40 b	7.70 a	318.20 b
T1	11.89 b	32.05 c	103.15 b	206.30 b	8.00 a	478.50 a
HSD	10.19	2.37	48.78	97.3	6.56	84.6

Means with the same letter are not significantly different at $p < 0.05$ probability.

HSD: Honest Significant Difference

For treatments, description see Table 1.

Table 3. Analysis of means for AUDPCr, SD, NF, FW, NNSF and PH, Puerto La Boca, Ecuador, 2017.

	AUDPCr	SD	NF	FW	NNSF	PH
AUDPCr	1.00	0.10 ns	-0.94**	-0.94**	0.06ns	0.045ns
SD		1.00	0.19*	0.19*	-0.166ns	0.00ns
NF			1.00	0.00**	0.000ns	-0.0ns
FW				1.00	0.00ns	0.00ns
NNSF					1.00	0.21ns
PH						1.00

ns: Not significant

*: Significant differences at $p < 0.05$ probability

** : Highly significant differences at $p < 0.01$ probability

SD: Stem diameter (mm)

NF: Number of fruits

FW: Fruit weight (g)

NNSF: Number of not-set fruits

PH: Plant height (cm)

Table 4. Correlation analysis.

Treatments	NP (1000 m ²)	Weight/harvest (Kg)	Price/Kg (USD)	Gross Profit (1000 m ²)	Cost (1000 m ²)	Net profit (USD)	Ratio B/C
T6	1980	955.43	2.1	2006.40	400.00	1606.40	4.02
T1	1980	952.64	2.1	2000.55	400.00	1600.55	4.00
T2	1980	950.40	2.1	1995.84	400.00	1595.84	3.99
T4	1980	942.56	2.1	1979.38	400.00	1579.38	3.95
T3	1980	941.31	2.1	1976.75	400.00	1576.75	3.94
T5	1980	924.68	2.1	1941.83	400.00	1541.83	3.85

B/C>1: Profitable. For treatments, description see Table 1.

Table 5. The profitability of treatments for the control of downy mildew in cucumber, Puerto La Boca, Ecuador, 2017.

the alternatives were profitable with a benefit to cost ratio ($B/C > 1$). However, the treatments T6 (Systemic fungicide + *Trichoderma* sp) and T1 (Systemic fungicide + contact fungicide) were distinguished, as the most profitable with USD 1606 and USD 1600 respectively.

Discussion

The downy mildew of cucumber is traditionally controlled with chemical fungicides; however, chemical control is not always feasible because of the high costs associated with the application especially for poor farmers of South American countries and the high environmental liabilities that the use of chemical fungicides means. In this work, we developed an ecological control strategy for *Ps. cubensis* based on the alternation of systemic and contact fungicides and the replacement of the contact fungicide by a microorganisms-based one. This strategy allowed satisfactorily controlling the pathogen and reaching a high production of fruits. Likewise, it helped to reduce production costs due to the lower application of pesticides. Although we present preliminary results, we undoubtedly think this is a promising strategy to control fungal pathogens in cucumber that can be adopted in IPM programs because of their affinity with the environment and lost cost^{20,21}. We also determined that all control alternatives evaluated here were profitable with a benefit/cost ratio greater than 1 ($B/C > 1$). Still, particularly the treatments alternating systemic fungicide with *Trichoderma*, as well as systemic with contact fungicide, were the most profitable. This suggests that applying an ecological strategy with microorganisms is useful and beneficial.

The contact fungicides that we used (Chlorothalonil), acted in the first hours after the application. Still, once oomycete penetrates the plant, systemic fungicides (Metalaxyl) should be used to control fungal infection because these are mobilized in the internal tissues and organs of the plant after their application^{22,23}. In our trials, the alternate form of *Bacillus*-based fungicide (CustomBio 5) with chemical counterparts allowed reducing the use of pesticides at least 50% of the applications. The mode of action may rely on the production of antibiotic molecules because it is well known that the *Bacillus* species contained in CustomBio 5 are efficient producers of antibiotic molecules^{24,25} and antifungal volatiles²⁶. Likewise, *Trichoderma* sp. worked properly when it was applied alone or in alternate applications with the systemic and contact fun-

gicide²⁷. Adnan *et al.*²⁷ mention that the species of the genus *Trichoderma* are the most commonly used antagonists for the control of plant diseases caused by fungi, due to their rapid growth in a large number of substrates, ample abiotic stress tolerance, quick ability to colonize, easy establishment after inoculation and sufficient capacity to compete for space and nutrients with pathogens. Besides, *Trichoderma* can stimulate plant growth due to its ability to produce plant growth regulators, vitamins, and recycle nutrients such as P (phytate) and Zn in the soil²⁸. In our experiments, the systemic fungicide did not harm *Trichoderma* since the applications were alternate, letting at least one week pass between applications. Additionally, some *Trichoderma* strains show resistance or tolerance to fungicides^{29,30}, although this particular property was not tested in the *Trichoderma* strains used in this work.

Conclusions

Ps. cubensis is a significant fungal disease for cucumber farming in Ecuador and worldwide, causing the disease called downy mildew. Traditionally, the application of fungicides has helped to hinder the spreading of the disease; however, chemical control is not always feasible because of the high costs associated with the implementation of fungicides and the implied environmental damage. In this work, we developed an ecological control strategy for *Ps. cubensis* based on the alternation of systemic and contact fungicides and the replacement of the contact fungicide by a microorganisms-based one. This strategy allowed satisfactorily controlling the pathogen and reaching a high production of fruits. Likewise, it helped to reduce production costs due to the lower application of pesticides. Undoubtedly, this strategy constitutes an excellent alternative to control pathogens in cucumber, and it can be adopted in IPM programs because of their affinity with the environment and lost cost.

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Conflict of interest

All authors declare that they have no conflict of interest.

Bibliographic references

1. Lv, J., Qi, J., Shi, Q., Shen, D., Zhang, S., Shao, G., Li, H., Sun, Z., Weng, Y., Shang, Y., Gu, X., Li, X., Zhu, X., Zhang, J., van Treuren, R., van Doijeweert, W., Zhang, Z. and Huang, S. Genetic diversity and population structure of cucumber (*Cucumis sativus* L.). *PLoS ONE*. 2012. 7:e46919. <https://doi.org/10.1371/journal.pone.0046919>
2. Sebastian, P., Schaefer, H., Telford, I.R.H. and Renner, S.S. Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proc Natl Acad Sci USA*. 2010. 107:14269–73. <https://doi.org/10.1073/pnas.1005338107>
3. Gruda, N., Sallaku, G. and Balliu, A. Cucumber. In: Baudoin, W., Nersisyan, A., Shamilov, A., Hodder, A., Gutierrez, D., de Pascale, S., Nicola, S., Gruda, N., Urban, L., Tanny Good, J. (eds) *Agricultural Practices for greenhouse vegetable production in the South East European countries - Principles for sustainable intensification of smallholder farms*. FAO Plant Production and Protection Paper 230. 2017. pp 287–300. ISBN 978-92-5-109622-2
4. Innark, P., Khanobdee, C., Samipak, S. and Jantasuriyarat, C. Evaluation of genetic diversity in cucumber (*Cucumis sativus* L.) germplasm using agro-economic traits and microsatellite markers. *Sci Hortic*. 2013. 162:278–84. <https://doi.org/10.1016/j.scienta.2013.08.029>
5. Naegele, R.P. and Wehner, T.C. Genetic Resources of Cucumber. In: Grumet R, Katzir N, Garcia-Mas J, (eds). *Genetics and genomics of Cucurbitaceae*. Springer International Publishing. 2016. pp. 61–86
6. Brussil, C.S. Proyecto de factibilidad para la exportación de pepino a los Países Bajos. Thesis. Universidad Tecnológica Equinoccial, Ecuador. 2012.
7. Savory, E.A., Granke, L.L., Quesada-Ocampo, L.M., Varbanova, M., Hausbeck, M.K. and Day, B. The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*: Cucurbit downy mildew. *Mol Plant Pathol*. 2011. 12:217–26. <https://doi.org/10.1111/j.1364-3703.2010.00670.x>
8. Burkhardt, A. and Day, B. A genomics perspective on cucurbit-oomycete interactions. *Plant Biotech*. 2013. 30:265–71. <https://doi.org/10.5511/plantbiotechnology.13.0315a>
9. Lebeda, A., Pavelková, J., Urban, J. and Sedláková, B. Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. *J Phytopathol*. 2011. 159:589–596. doi: 10.1111/j.1439-0434.2011.01811.x
10. Khare, C.P., Srivastava, J.N., Tiwari, P.K., Kotesthane, A. and Thrimurthi, V.S. Downy Mildew of Cucurbits and Their Management. In: Awasthi LP. (ed) *Recent Advances in the Diagnosis and Management of Plant Diseases*. Springer, New Delhi. 2015. pp 47–54. https://doi.org/10.1007/978-81-322-2571-3_5
11. Haveri, N., Thulasiram, K. and Shashidhar, K.R. Integrated approach for management of downy mildew of cucumber caused by *Pseudoperonospora cubensis*. *J Pharmacogn Phytochem*. 2019. 8:510–513.
12. Scherf, A., Schuster, C., Marx, P., Gärber, U., Konstantinidou-Doltsinis, S. and Schmitt, A. Control of downy mildew (*Pseudoperonospora cubensis*) of greenhouse grown cucumbers with alternative biological agents. *Commun Agric Appl Biol Sci*. 2010. 75:541–54.
13. Szczech, M., Nawrocka, J., Felczyński, K., Małolepsza, U., Sobolewski, U., Kowalska, B., Maciorowski, B., Jas, K. and Kancelista, A. *Trichoderma atroviride* TRS25 isolate reduces downy mildew and induces systemic defence responses in cucumber in field conditions. *Sci Hortic*. 2017. 224:17–26. <https://doi.org/10.1016/j.scienta.2017.05.035>
14. Elsharkawy, M.M., Kamel, S.M., Nagwa, M., El-Khateeb, M. Biological control of powdery and downy mildews of cucumber under greenhouse conditions. *Egypt J Biol Pest Co*. 2014. 24:407–414.
15. Brzozowski, L. and Mazourek, M.A. Sustainable agricultural future relies on the transition to organic agroecological pest management. *Sustainability*. 2018. 10:2023. <https://doi.org/10.3390/su10062023>
16. Cohen, Y. and Coffey, M.D. Systemic fungicides and the control of oomycetes. *Ann. Rev. Phytopathol*. 1986. 24:311–338. <https://doi.org/10.1146/annurev.py.24.090186.001523>
17. Gabriel, J., Castro, C., Valverde, A. and Indacochea, B. Diseños experimentales: Teoría y práctica para experimentos agropecuarios. Grupo COMPAS, Universidad Estatal del Sur de Manabí (UNESUM), Jipijapa, Ecuador. 2017. 116 p. ISBN 978-9942-750-50-1. <http://142.93.18.15:8080/jspui/handle/123456789/116>
18. Gabriel, J., Ortuño, N., Vera, M., Castro, C., Narváez, W. and Manobanda, M. Manual para evaluación de daños de enfermedades en cultivos agrícolas. Grupo COMPAS, Universidad Estatal del Sur de Manabí, Jipijapa, Ecuador. 2017b. 53 p. ISBN 978-9942-760-04-3. https://www.researchgate.net/publication/317356316_Manual_para_evaluacion_de_danos_de_enfermedades_en_cultivos_agricolas
19. CIMMYT. La formulación de recomendaciones a partir de datos agronómicos: Un manual metodológico de evaluación económica. México D.F., México: CIMMYT. 1988. ISBN 968-6127-24-0
20. Colucci, S.J. and Holmes, G.J. Downy mildew of cucurbits. *The Plant Health Instructor*. 2010. <https://doi.org/10.1094/PHI-I-2010-0825-01>
21. Sharma, A., Katoch, V. and Rana, C. Important Diseases of Cucurbitaceous Crops and Their Management. Important diseases of cucurbitaceous crops and their management. In: Pessarakli M. (ed). *Handbook of cucurbits growth, cultural practices, and physiology*. CRC press. 2016. pp. 301–324.
22. Fernández-Northcote, E.N., Navia, O. and Gandarillas, A. Bases de las estrategias de control químico del tizón tardío de la papa desarrolladas por PROINPA en Bolivia. *Rev Latinoam Papa*. 1999. 11:1–25.
23. Ruiz-Sánchez, E., Tún-Suárez, J.M., Pinzón-López, L.L., Valerio-Hernández, G. and Zavala-León, M.J. Evaluación de fungicidas sistémicos para el control del mildiú vellosa (*Pseudoperonospora cubensis* Berk. & Curt.) Rost. en el cultivo del melón (*Cucumis melo* L.). *Rev Chapingo*. 2008. 14(1): 79–84.
24. Raaijmakers, J.M., Vlami, M. and de Souza, J.T. Antibiotic production by bacterial biocontrol agents. *Anton Leeuw*. 2002. 81:537–547. <https://doi.org/10.1023/A:1020501420831>
25. Stein, T. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mol Microbiol*. 2005. 56:845–57. <https://doi.org/10.1111/j.1365-2958.2005.04587.x>
26. Kai, M., Hausteine, M., Molina, F., Petri, A., Scholz, B. and Piechulla, B. Bacterial volatiles and their action potential. *Appl Microbiol Biot*. 2009. 81:1001–12. <https://doi.org/10.1007/s00253-008-1760-3>
27. Adnan, M., Islam, W., Shabbir, A., Khan, K.A., Ghramh, H.A., Huang, Z., Chen, H.Y.H. and Lu, G.D. Plant defense against fungal pathogens by antagonistic fungi with *Trichoderma* in focus. *Microb Pathogenesis*. 2019. 129:7–18. <https://doi.org/10.1016/j.micpath.2019.01.042>
28. Li, R.X., Cai, F., Pang, G., Shen, Q.R., Li, R. and Chen, W. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *PLoS ONE*. 2015. 10:e0130081. <https://doi.org/10.1371/journal.pone.0130081>
29. Chaparro, A.P., Carvajal, L.H. and Orduz, S. Fungicide tolerance of *Trichoderma asperelloides* and *T. harzianum* strains. *Agr Sci*. 2011. 2:301–307. <https://doi.org/10.4236/as.2011.23040>
30. Silva, M.A.F. da, Moura, K.E. de, Moura, K.E. de, Salomão, D. and Patricio, F.R.A. Compatibility of *Trichoderma* isolates with pesticides used in lettuce crop. *Summa Phytopathol*. 2018. 44:137–42. <https://doi.org/10.1590/0100-5405/176873>

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RESEARCH / INVESTIGACIÓN

Determinación microbiológica y molecular mediante PCR en tiempo real de dos bacterias del género *Bacillus* de interés agro biotecnológico

Microbiological and molecular determination by real-time PCR of two bacteria of the genus *Bacillus* of agro biotechnological interest

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Resumen: Se efectuó la comparación de curvas de crecimiento a diferentes rangos de pH, salinidad y temperatura, además se determinó el tamaño, forma y posición de endospora de dos diferentes cepas del género *Bacillus*. Para la identificación molecular se realizó la extracción de ácidos nucleicos, utilizando el kit High Pure PCR Template Preparation Kit de Roche® y posteriormente se analizaron las curvas de amplificación producto de la PCR en Tiempo Real.

Se realizaron cinéticas de crecimiento a cada uno de los cultivos de bacterias durante 72 horas y se analizaron estadísticamente las curvas mediante los métodos estadísticos ANOVA y Tukey para determinar significancia ($p < 0.001$). El análisis factorial para la variable "temperatura" no mostró una diferencia significativa entre cepas y tampoco con los valores fijados (28, 32, 37 °C), la variable "pH" sí presentó una interacción con valores ($p < 0.0001$) entre cepas; la variable "salinidad" también presentó diferencia significativa entre cepas y con sus respectivas interacciones. En el análisis del área bajo la curva, la variable "temperatura" no mostró diferencia significativa, mientras que las variables "pH y salinidad" presentaron valores correspondientes a ($p < 0.0001$). Para la determinación molecular, se analizaron las curvas de amplificación, confirmando la presunción bioquímica de la cepa BM como *B. megaterium* mediante el análisis del gen *phaC*, así como también se pudo corroborar que la cepa BL corresponde a *B. licheniformis* al analizar el gen *lchAA*. La cinética bacteriana permitió establecer las horas clave para el análisis del crecimiento, confirmando que las características analizadas corresponden a especies pertenecientes a la familia *Bacillaceae*, con aplicación como controladores biológicos en la agricultura.

Palabras clave: *B. licheniformis*, *B. megaterium*, gen *lchAA*, gen *phaC*, PCR en Tiempo Real.

Abstract: The comparison of growth curves was carried out at different ranges of pH, salinity, and temperature, and the size, shape, and position of the endospore of two different strains of the genus *Bacillus* were also determined. For molecular identification, nucleic acid extraction was performed using the Roche® High Pure PCR Template Preparation Kit and the amplification curves resulting from Real-Time PCR were subsequently analyzed.

Growth kinetics were performed on each of the bacteria cultures for 72 hours and the curves were statistically analyzed using the statistical methods ANOVA and Tukey to determine significance ($p < 0.001$). The factorial analysis for the variable "temperature" did not show a significant difference between strains and neither with the fixed values (28, 32, 37 °C), the variable "pH" did show an interaction with values ($p < 0.0001$) between strains; the variable "salinity" also presented a significant difference between strains and with their respective interactions. In the analysis of the area under the curve, the variable "temperature" did not show a significant difference, while the variables "pH and salinity" presented values corresponding to ($p < 0.0001$). For molecular determination, the amplification curves were analyzed, confirming the biochemical presumption of the BM strain as *B. megaterium* through the analysis of the *phaC* gene, as well as it was possible to corroborate that the BL strain corresponds to *B. licheniformis* when analyzing the gene. *lchAA*. Bacterial kinetics allowed establishing the key hours for growth analysis, confirming that the characteristics analyzed correspond to species belonging to the *Bacillaceae* family, with the application as biological controllers in agriculture.

Key words: *B. licheniformis*, *B. megaterium*, *lchAA* gene, *phaC* gene, Real-time PCR.

Introducción

El género *Bacillus* forma parte de la familia *Bacillaceae*, está compuesto por bacilos gram positivos grandes, caracterizados por su capacidad de producir endosporas¹. El género incluye microorganismos aerobios estrictos y anaerobios facultativos; algunas especies son parásitas o patógenas para animales, principalmente insectos, ya que crecen bien en los medios comunes, fermentan carbohidratos y descomponen proteínas. Las técnicas microbiológicas brindan información sobre la morfología de las colonias, la cual es fundamental en la identificación preliminar para la diferenciación de los microorganismos, la observación del fenotipo permite ubicarlo dentro del género *Bacillus*, pero muchas veces no resulta

suficiente para una determinación precisa. Por esto, se acuden a técnicas más sofisticadas, como los ensayos moleculares basados en el análisis del genotipo de los microorganismos, que proporcionan información más específica al momento de hacer una amplificación². La presente investigación se llevó a cabo con el objetivo principal de identificar bacterias de interés agro biotecnológico, que presentan presunción bioquímica dentro del género *Bacillus*. Para efectuar la presente investigación se reactivaron dos cepas crió conservadas, se determinó la concentración inicial de las muestras mediante la técnica McFarland junto con la comparación de curvas de crecimiento a diferentes rangos de pH, salinidad y temperatura, además

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se realizó la determinación de tamaño, forma y posición de endospora de las cepas. Se desarrolló la extracción de ácidos nucleicos, utilizando el kit "High Pure Template Preparation Kit" de Roche® con la finalidad de analizar las curvas de amplificación mediante regiones específicas producto de la PCR en Tiempo Real.

Materiales y métodos

La investigación se llevó a cabo en los Laboratorios de Ciencias de la Vida de la Universidad Politécnica Salesiana Sede Quito, Campus el Girón con cepas bacterianas crio conservadas obtenidas del cepario del grupo de investigación Biotecnología Aplicada a los Recursos Naturales "BIOARN".

Reactivación de cepas bacterianas

El material biológico se obtuvo del Criobank codificado como BM (*Bacillus megaterium*) y BL (*Bacillus licheniformis*), cada perla se sembró en cajas Petri de 25 mL con medio Agar nutriente (AN) a 30 ± 2 °C por 24 horas³.

Resiembra de cepas

De las placas Petri con crecimiento bacteriano se realizó la siembra con un asa de metal, las muestras se suspendieron con 7 mL de medio Tryptic Soy Broth (TSB) siendo sus condiciones óptimas de crecimiento de 30 ± 2 °C y pH de $7 \pm 0,2$ en tubos de vidrio de 35 mL y con un tiempo de 24 horas³.

Caracterización microbiológica de cepas

Se seleccionaron colonias blanquecinas, de bordes regulares que se sembraron en forma individual en Agar nutriente para determinar tamaño, forma bacilar y además se realizó tinción de Gram positiva y la tinción de endosporas⁴.

Determinación de curvas de crecimiento

Se realizaron curvas de crecimiento a cada uno de los cultivos de bacterias (BM y BL) durante 72 horas; esto con el objetivo de evaluar la capacidad de desarrollo de los microorganismos después de haber estado en contacto con los analitos (NaCl, pH, temperatura). Se realizó 3 repeticiones con un total de 54 muestras. Para cada proceso se colocó 95 mL de caldo nutritivo (TSB) con 5 mL de inóculo (BM y BL) con un volumen final de 100 mL. Se determinaron las temperaturas de 28 °C, 32 °C y 37 °C. En el caso de las curvas para pH se establecieron tres diferentes rangos: uno ácido de 5, uno neutro de 7 y un básico de 8. La salinidad se midió en tres porcentajes correspondientes a 2 %, 4 % y 6 % de NaCl. Todos los ensayos se midieron mediante espectrofotometría mediante un lector de Elisa a una absorbancia de 495 nm cada dos horas, hasta llegar a un tiempo de 72 horas.

Extracción de ácidos nucleicos

Se colocó 200 µL de muestra (BM y BL) en un tubo eppendorf libre de nucleasas de 1.5 mL agregando 5 µL de lisozima e incubó 15 minutos a 37 °C. A continuación, se añadió 200 µL de Buffer de Unión (Binding Buffer) y 40 µL de Proteinasa K, se mezcló inmediatamente e incubó por 10 minutos a 70 °C.

Posterior a esto, se añadió 100 µL de etanol absoluto al 99 % y se mezcló bien; después, se pipeteó el contenido de la muestra en un Tubo con filtro (High Pure Spin Filter Tube) junto con un tubo colector, centrifugando por 1 minuto a 8000 xg. El residuo final en el tubo con filtro se procedió a lavar.

Para los lavados se armó un nuevo tubo colector, al cual se le añadió 500 µL de Buffer removedor de inhibidores (Inhi-

bitor Removal Buffer), centrifugando por 1 minuto a 8000 xg. Posterior a esto, se removió el tubo colector y se armó uno nuevo, se añadió 500 µL de Buffer de lavado (Wash Buffer) centrifugando por 1 minuto a 8000 xg, este paso se lo realiza por duplicado.

Finalmente, para la elución del ADN se agregó 200 µL de Buffer de elución (Elution Buffer). El ADN se conservó a -20 °C para su análisis posterior.

Amplificación mediante PCR en Tiempo Real

El protocolo que se estableció fue de 5 µL para cada muestra (BM y BL) y sus controles. Se añadió 10,2 µL de Agua grado Biología Molecular, 0,4 µL del Primer FW para BL *ACTGAA-GCGATTGCAAGTT* y para BM *CGTGCAAGAGTGGGAAAAAT*, 0,4 µL del Primer RW para BL *TCGCTTCATATTGTGCGTTC* y BM *TCGCAATATGATCACGGCTA* obtenidas de regiones específicas (*lchAA*) en el caso de *B. licheniformis* y (*phaC*) para *B. megaterium*. Para la elaboración de la Master Mix se utilizó el Kit LightCycle® Fast Start DNA MasterPLUS SYBR Green I.

Análisis Estadístico

Una estructura factorial permitió estudiar los efectos producidos por dos o más factores y sus interacciones. Los resultados previamente obtenidos sobre curvas de crecimiento a tres diferentes variables permitieron un análisis factorial de tratamientos, en este caso se seleccionó un diseño completamente al azar de 2x3 (correspondiente a 2 cepas y 3 niveles de un factor condicional, que corresponde a 6 tratamientos). La comparación de los resultados obtenidos se realizó mediante el test estadístico de ANOVA. Se calculó el área bajo la curva de crecimiento de la población bacteriana (ABCCP) acumulada en el tiempo⁵. Se compararon más de dos grupos entre sí, las diferencias entre éstos fueron determinadas con el test de Tukey y en todos los casos el límite de significancia fue fijado en 5 % ($p=0,05$)⁶. Para el análisis de los datos se utilizó el programa estadístico Infostat 2018⁷.

Resultados y discusión

Caracterización microbiológica de *B. licheniformis* y *B. megaterium*

Las colonias correspondientes a BM fueron identificadas de forma macroscópica como colonias circulares, con borde entero y de elevación convexa. Según Pérez & Mota (2008)⁸, la tinción de gram corresponde a bacilos grampositivos. Se definió el tamaño en colonias grandes con una determinación microscópica de 560 µm. Para la tinción de endospora según Nacato & Valencia (2016)⁹, se estableció endosporas muy visibles en posición terminal debido al gran tamaño de BM. La cepa BL, presentó características macroscópicas como: colonias irregulares, borde ondulado y con elevación plana. Mediante observación microscópica se constató un tamaño de 116 µm. Se implementó la misma metodología señalada en BM para la tinción de endosporas obteniendo un bacilo grampositivo, con endosporas centrales.

Curvas de crecimiento

Se efectuó la curva de crecimiento microbiano de BM y BL por medio de ensayos espectrofotométricos y dos análisis estadísticos siguiendo la metodología antes mencionada con tres variables: temperatura, pH y salinidad. La cepa BL para la variable "temperatura" presentó una fase de adaptación entre las 0 y 10 horas, mientras que a partir de las 24 hasta las 48

horas se da una fase de latencia, a las 72 horas a la temperatura de 32 °C fue la primera en decaer. La cepa BM en cuanto a la temperatura tuvo una etapa de adaptación muy parecida a la cepa BL, pero en la etapa exponencial la temperatura de 28 °C dio una diferencia ya que se encontró entre los valores más bajos, lo que indicó que no fue la temperatura óptima para su crecimiento, pero sin embargo si logra crecer a dichas condiciones. Según Tortoló-Cabañas & Bell-García (2015)¹⁰, *B. megaterium* presenta buenos resultados de crecimiento entre las 45 y 48 horas. El pH en la cepa BL presentó una etapa de adaptación entre la hora 0 y 5; la fase exponencial se dio a partir de las 20 a las 48 horas notándose que a un pH de 5 se obtuvo el valor más alto. La hora de muerte se dio a las 72 horas siendo la curva de pH 5 la primera en decaer. Mientras que la cepa BM no presentó diferencia en cuanto a un rango de pH que favoreciera su crecimiento ya que en los tres casos se desarrolló de forma similar, notándose una fase exponencial entre las 24 a 48 horas y la fase de muerte a las 72 horas. En congruencia con otros estudios¹¹, se concluyó que el crecimiento celular de la cepa BL ocurría básicamente durante las primeras 24 horas. La salinidad se evidenció en la cepa BL con una fase de adaptación entre la hora 0 y 5, la fase exponencial se dio a partir de la hora 20 hasta la hora 60; la concentración de salinidad no influyó directamente en el crecimiento de BL ya que en los tres porcentajes de salinidad presentó un crecimiento sin diferencia significativa. La cepa BM tuvo una fase de adaptación entre la hora 0 hasta la hora 10 siendo la curva más alta de 4 % de salinidad, en la fase exponencial se logró evidenciar que a una concentración baja se dio el mayor pico de crecimiento que corresponde al 2 % de salinidad.

Análisis estadístico factorial

Se realizó un análisis factorial con tratamientos (tem-

peratura, pH y salinidad) mediante la prueba de Tukey con ($p < 0.05$)⁶, este valor se tomó como referencia para determinar si existe o no diferencia significativa con horas clave (hora 0, 5, 24, 48, y 72). La variable "temperatura" a la hora 0 presentó una diferencia significativa en cuanto al crecimiento entre cepas, pero no se dio una interacción entre las tres temperaturas y tampoco se presentó una diferencia significativa para la interacción cepa*temperatura. No se presentó una interacción entre cepa*temperatura en ninguna de las horas establecidas lo que indicó que la temperatura no influye directamente en el crecimiento de estas cepas. Montor-Antonio y otros (2013)¹², expresan que la cepa BL presenta una extensa aplicación en diversos procesos industriales debido a sus amplios rangos de operación de temperatura (25-90 °C). La variable "pH" obtuvo una interacción cepa*pH que denotó claramente que la cepa BM forma un solo grupo, sin diferencia en el crecimiento de los valores de pH y en el caso de la cepa BL se notó la diferencia que existe entre los valores de pH 7 y 8. Según, Pérez y otros (1998)¹³ el crecimiento de *Bacillus* está muy influenciado por el pH.

Análisis estadístico de área bajo la curva

El área bajo la curva arrojó resultados que se pueden observar en la Tabla 1. Para la variable "temperatura" existió una diferencia significativa entre cepas y en los tres valores establecidos no se encontró diferencia alguna ya que los tres se asignaron en un mismo grupo, es decir la temperatura no influyó directamente en el crecimiento de estas cepas. En cuanto a la variable "pH" si existió una diferencia significativa entre cepas siendo la cepa BL la que tuvo mayor crecimiento y en cuanto a los valores específicos (5, 7, 8 pH) es notable que a un pH de 8 ésta tuvo mejores resultados, además se dio una diferencia significativa para la interacción cepa*pH en la cual

	BM	BL	p-valor
CEPA	9107.05 ± 25.85 b	8848.98 ± 25.85 a	<0.0001
TEMPERATURA	28	8915.83 ± 31.65 a	0.08483
	32	8996.55 ± 31.65 a	
	37	8915.83 ± 31.65 a	
CEPA	6802.81 ± 23.79 a	6962.28 ± 23.79 b	0.0005
pH	5	6982.16 ± 29.13 b	0.0001
	7	6947.37 ± 29.13 b	
	8	6718.10 ± 29.13 a	
CEPA*pH	BM	BL	
5	7005.48 ± 41.20 b	6958.84 ± 41.20 b	<0.0001
7	6996.01 ± 41.20 b	6898.73 ± 41.20 b	
8	6406.95 ± 41.20 a	7029.25 ± 41.20 b	
CEPA	5499.35 ± 10.19 a	5549.12 ± 10.19 b	0.0048
SALINIDAD	2	5594.52 ± 12.47 c	<0.0001
	4	5523.86 ± 12.47 b	
	6	5454.31 ± 12.47 a	
CEPA*SALINIDAD	BM	BL	
2	5620.08 ± 17.64 d	5568.95 ± 17.64 cd	0.0012
4	5477.64 ± 17.64 ab	5570.09 ± 17.64 cd	
6	5400.31 ± 17.64 a	5508.31 ± 17.64 bc	

Tabla 1. Valores de medias, error experimental y (p) del análisis de área bajo la curva para las variables "Temperatura, pH y Salinidad" de las cepas BM y BL.

se formaron claramente dos grupos entre las dos cepas de los cuales solo BM a un pH de 8 tuvo diferencia alguna. Para la variable "salinidad" se dio una diferencia significativa entre cepas notándose que la cepa BL es la que presenta mayor crecimiento y para los valores específicos (2, 4, 6 %) existió diferencia significativa entre las tres concentraciones de salinidad en el caso de las dos cepas. También existió diferencia para la interacción cepa*salinidad siendo la cepa BM la que presentó una diferencia entre las dos a una concentración del 6 % de NaCl.

Identificación molecular de *B. licheniformis* y *B. megaterium* mediante PCR en Tiempo Real

La cepa BM y BL fueron sometidas a estudio mediante la técnica PCR en Tiempo Real; en la Figura 1 se muestran los productos de amplificación de la cepa BL mientras transcurren los ciclos de la PCR. La cepa BL presentó una hibridación con los primers codificados utilizados en otros estudios¹⁴, que permitieron la amplificación del gen *lchAA* región que ha demostrado ser capaz de identificar de forma taxonómica a esta cepa ya que el operón de Lichenysin (*lchA*) es responsable de la síntesis de lichenysin en *B. licheniformis* y se confirmó la cepa BL correspondiente a *Bacillus licheniformis*. Además, se observan los resultados obtenidos en la amplificación de la cepa BM en la cual se da la hibridación del ADN molde mediante los cebadores específicos utilizados en estudios previos¹⁵, que permitieron la amplificación del gen *phaC* cuyas secuencias sirven para reconocer regiones únicas presentes, que fueron utilizadas para diseñar los cebadores indicando la singularidad de las secuencias de *phaC* en la identificación de *B. megaterium*. Con estos resultados se pudo confirmar la cepa BM como *B. megaterium*.

Existen estudios como el de Konzl (1997)¹⁶, que presenta a la PCR en Tiempo Real como la técnica adecuada para la caracterización molecular de genes de la bacitracina producido por *B. licheniformis*. Dado que los miembros del género bacteriano gram positivo *Bacillus* son bien conocidos por su potencial para secretar un número de enzimas de interés, no fue sorprendente que las cepas de estudio correspondientes a BL y BM aisladas mediante cebadores específicos, resultaron

ser miembros de este taxón. *B. licheniformis* es una bacteria que se puede aislar de varias fuentes: como el suelo, el agua y en los desperdicios¹⁷. En el caso de la cepa BL, este estudio se basó en la amplificación del gen *lchAA* para su identificación molecular, existe un estudio que ha demostrado que la cepa *B. licheniformis* ATCC 14580 y algunas otras cepas de *B. licheniformis* albergan el gen *lchAA*¹⁷.

Existen varios estudios implicados en la identificación de la cepa BM, este estudio se centró específicamente en el gen *phaC*. Algunos autores como Pramoda & Ajeet (2013)¹⁵ proponen utilizar el gen *polihidroxialcanoato sintasa C* de *B. megaterium* para el diseño de cebadores que amplifican la región *phaC* para su identificación. Por otra parte Chen (1991)¹⁸ informa que muchas especies de *Bacillus* producen *pha* y desde entonces el gen para la síntesis de *pha* ha sido clonado a partir de *B. megaterium*.

Conclusiones

Las concentraciones bacterianas obtenidas mediante la técnica McFarland permitieron generar las curvas de crecimiento para establecer las horas clave en el análisis, confirmando que las características analizadas: forma, tamaño, tinción de gram y posición de endospora corresponden a especies pertenecientes a la familia *Bacillaceae*. Los modelos estadísticos ANOVA y Tukey permitieron el análisis particionado de los componentes para aprobar la hipótesis y con el análisis del área bajo la curva se concluye que los datos obtenidos frente a las variables planteadas presentan un alto grado de precisión. Finalmente la técnica molecular de PCR en Tiempo Real mediante las curvas de amplificación¹⁹, permitió confirmar la presunción bioquímica de la cepa BM que corresponde a *Bacillus megaterium* mediante el análisis de la región *phaC*; además se pudo corroborar que la cepa BL corresponde a *Bacillus licheniformis* al analizar la región *lchAA*.

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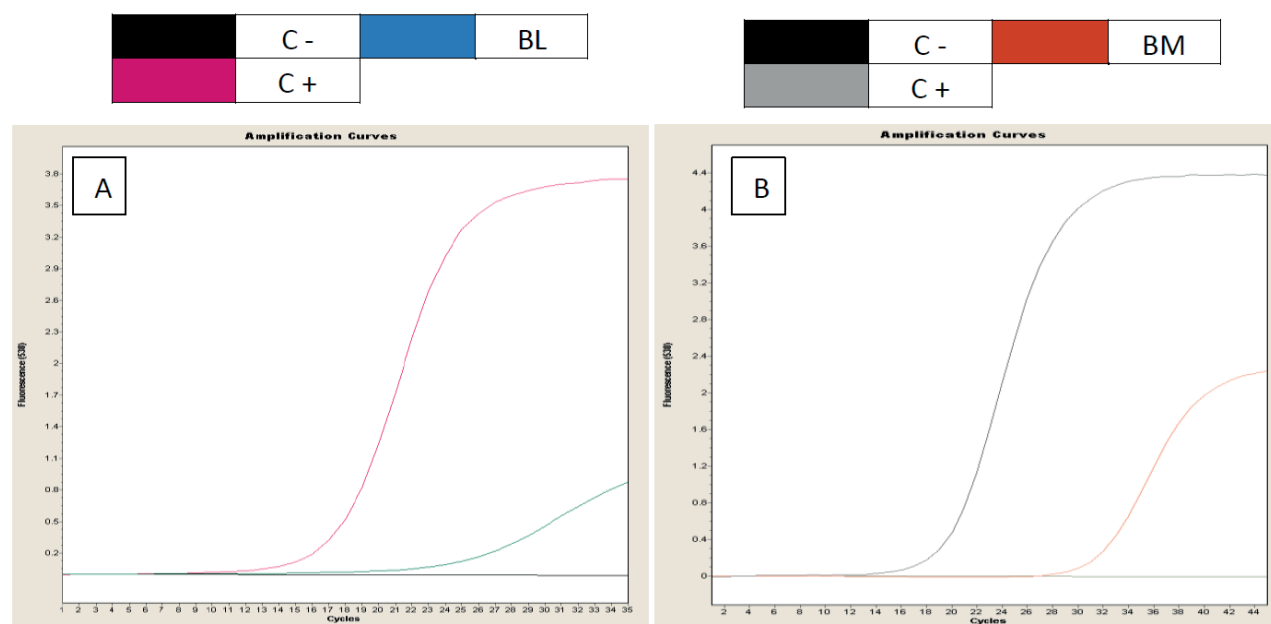


Figura 1. A) Curva de amplificación PCR en Tiempo Real con SYBR Green de la cepa BL: fluorescencia vs ciclos. C-: control negativo; C+: *Bacillus licheniformis* ATCC®14580. B) Curva de amplificación PCR en Tiempo Real con SYBR Green de la cepa BM: fluorescencia vs ciclos. C-: control negativo; C+: *Bacillus megaterium* ATCC®14581. En equipo PCR LightCycler® 2.0.

Referencias bibliográficas

- Macedo M. Temas de Bacteriología y Virología Médica [Internet]. Montevideo: Universidad de la República; 2008. 646 p. Available from: http://www.enfermeriaaps.com/portal/?wpfb_dl=4348%0Ahttp://www.higiene.edu.uy/cefa/2008/enterovirus.pdf
- Sosa-López A, Álvarez-Rivera V, Torres-Campos D, Casasús-Romero L. Identificación y caracterización de seis aislados pertenecientes al género *Bacillus* promisorios para el control de *Rhizoctonia solani* Kühn y *Sclerotium rolfsii* Sacc. *Fitosanidad*. 2012;15(1):39–43.
- Egas C, Tinajero M. Aislamiento de microorganismo capaces de producir antibióticos, a partir de suelos de Regiones Naturales del Ecuador [Internet]. Tesis. Universidad Politécnica Salesiana; 2016. Available from: <http://dspace.ups.edu.ec/bitstream/123456789/5081/1/UPS-CYT00109.pdf>
- Reyes A, Ruiz E, Yam C, Dzúl M. Selección De *Bacillus* Spp . Con Actividad Antagón Goid . En Diferentes Medios De Cultivo. *Fitosanidad* [Internet]. 2011;15(2):112–21. Available from: <https://www.redalyc.org/articulo.oa?id=2091/209122297007>
- Cecilia B, Gustavo G, Guillermo G, Alejandra B. Componentes de resistencia a *Xanthomonas vesicatoria* raza T2 en genotipos de tomate en condiciones de invernadero y cámara de crecimiento. *Agrociencia Uruguay*. 2014;18(1):86–96.
- Giacomino N, Notaro U, Cerra M, Gumiy D, Stiefel S, Baroni E, et al. Actividad antibacteriana in vitro de Cefalexina sobre *Escherichia coli* en suero y orina de canino. *FAVE Sección Ciencias Vet*. 2014;11(1/2):15–30.
- Balzarini M, Gonzalez L, Tablada M, Casanoves F, Di Rienzo J, Robledo C. *Infostat: manual del usuario*. Córdoba: Editorial Brujas. Brujas; 2008.
- Pérez M, Mota M. Morfología y Estructura Bacteriana. In: TEMAS DE BACTERIOLOGÍA Y VIROLOGÍA MÉDICA. 2008. p. 48–60.
- Ñacato C, Valencia M. Aislamiento, identificación y pruebas in vitro de cepas autóctonas de *Bacillus subtilis* como agente de biocontrol de *Alternaria* spp en *Brassica oleracea* var. *Itálica*. [Internet]. Tesis. Universidad Politécnica Salesiana; 2016. Available from: <http://dspace.ups.edu.ec/bitstream/123456789/5081/1/UPS-CYT00109.pdf>
- Tortoló-cabañas K, Bell-garcía A. Producción de proteínas recombinantes en *Bacillus megaterium*: estado del arte. *ICIDCA Sobre los Deriv la Caña Azúcar* [Internet]. 2015;49(1):22–6. Available from: <https://www.redalyc.org/articulo.oa?id=2231/223136961004>
- Cascante J. Fermentación de bacterias del género *Bacillus* en altas concentraciones de oxígeno. *J Chem Inf Model*. 2013;53(9):1689–99.
- Montor-Antonio J, Olvera-Carranza C, Reyes-Duarte D, Sachman-Ruiz B, Ramírez-Coutiño L, Del Moral S. Biochemical characterization of AmiJ33 an amylase from *Bacillus amyloliquefaciens* isolated of sugarcane soils at the Papaloapan region *Revista Electrónica Nova Scientia Caracterización bioquímica de AmiJ33 , una amilasa de Bacillus amyloliquefaciens*. *Nov Sci*. 2013;6(12):39–59.
- Gonzalez RA, Pérez M, Florido G, Piad R, González G, Sol G. Isolation and characterization of strains of *Bacillus* spp. Production of proteolytic enzymes for the production of protein hydrolysates. *Rev Av Científica*. 1998 Jan 1;1:2–9.
- Madslén EH, Rønning HT, Lindbäck T, Hassel B, Andersson MA, Granum PE. Lichenysin is produced by most *Bacillus licheniformis* strains. *J Appl Microbiol*. 2013 Oct;115(4):1068–80.
- Nayak PK, Mohanty AK, Gaonkar T, Kumar A, Bhosle SN, Garg S. Rapid Identification of Polyhydroxyalkanoate Accumulating Members of Bacillales Using Internal Primers for phaC Gene of *Bacillus megaterium*. *ISRN Bacteriol*. 2013;2013:1–12.
- Konzl D, Klenzl A, Schbrgendorfe K, Marahiell M. The bacitracin biosynthesis operon of *Bacillus licheniformis* ATCC 10716: molecular characterization of three multi-modular peptide synthetases. *Chem Biol* [Internet]. 1997 [cited 2019 Dec 2];4(12):27–37. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9427658>
- Waldeck J, Daum G, Bisping B, Meinhardt F. Isolation and molecular characterization of chitinase-deficient *Bacillus licheniformis* strains capable of deproteinization of shrimp shell waste to obtain highly viscous chitin. *Appl Environ Microbiol*. 2006;72(12):7879–85.
- Chen G. Occurrence of poly-D(-)-3-hydroxyalkanoates in the genus *Bacillus*. *FEMS Microbiol Lett*. 1991 Nov 15;84(2):173–6.
- Chiluisa-Utreras V, Echeverría A. Identificación y cuantificación de *Salmonella* sp. y ADNr 16S bacteriano mediante PCR en tiempo real en muestras de alimentos. *BIONATURA*. 2017; 1-6.

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RESEARCH / INVESTIGACIÓN

Clinical and Epidemiological characterization of an outbreak by carbapenemase-producing *Klebsiella pneumoniae*. January-March, 2017, Portoviejo, Ecuador

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Abstract: Infection Control reported three cases of Kpn-KPC with a probable relation among them in the adult intensive care unit UCI-A in the hospital "Dr. Verdi Cevallos Balda" from Portoviejo, Manabí, Ecuador. This observational and descriptive study aims to characterize this outbreak, according time, space and person. Laboratory tests are analyzed, and the correlation between cases and positive result of the study is shown. Clinical epidemiological aspects of patients with Kpn-KPC carbapenemases producing *Klebsiella pneumoniae* infection were recorded (January to March 2017). The ICU-A Adult Intensive Care Unit and family environment are a probable factor of dissemination of the disease. The isolation of KPC is an alternative to clinical suspicion. The implementation of infection control measures is essential to reduce nosocomial transmission of Kpn-KPC.

Key words: Kpn-KPC, epidemic outbreak, infected case, colonized case.

Introduction

Klebsiella pneumoniae producing carbapenemase type KPC (Kpn-KPC) is part of the emerging pathogens, with high capacity for in-hospital dissemination. The resistance of this species to third-generation cephalosporins through the production of extended-spectrum beta-lactamases (ESBL) is frequent, placing carbapenemes as the treatment of choice in infections caused by these isolates¹. However, in 1996, the first clinical isolation of carbapenemase-resistant *K. pneumoniae* was performed, through the production of carbapenemase type KPC in North Carolina, United States. From this first isolation, outbreaks are recognized in various regions of the world, identifying a worldwide dominant clone, the ST258².

Ecuador begins in 1985, from the publication of a Manual of Standards and Procedures by the Ministry of Health, and three years later there is the construction of a committee in the National Directorate of Epidemiology, in 2006 the Ministry of Public Health established the Norms of Control and Prevention of nosocomial infections.

In Eugenio Espejo Specialties Hospital in the City of Quito, an observational, retrospective and descriptive study was carried out of a series of 57 cases that register cultures with KPC type carbapenemase-producing *Klebsiella pneumoniae* isolates. The diagnosis was made between July and December of 2014. We worked with the universe of 59 patients with isolation of *Klebsiella pneumoniae* producing KPC type carbapenemases according to the daily census issued by the Statistics Unit of the said hospital.

Due to the exclusion criteria used, work was done with a total of 57 patients who died from this cause; the median age corresponded to 58, highlighting the group between 19 and 65 years of age ($n = 30$). The hospital stay time reached ($p_{10} = 30$) and ($p_{90} = 79.6$). The proportion of patients who received surgical procedures was 0.877.

In 40 cases of the deceased, the germ isolates came from orotracheal secretions, bronchoalveolar aspirate, or pleural fluid (70.2%); of these, 37 patients presented mechanical ventilation (64.9%). In 22 of the deceased cases, the isolates were performed in the secretion of surgical wounds or secretion of

bedsores (38.6%). In 11, their isolates were presented in blood cultures, and in 4, they were isolated in urinary tract cultures³.

There is no history of this type of outbreak at the Hospital "Dr. Verdi Cevallos Balda" of Portoviejo, and neither any kind of study carried out in it. Hence, the objective of the present study is to epidemiologically characterize an IH Intrahospital outbreak by Kpn-KPC in hospitalized patients the ICU-A of the Hospital "Dr. Verdi Cevallos Balda". For the present study, the exceptional and unexpected increase in the number of Kpn-KPC cases was considered as an outbreak since two cases of Kpn-KPC infection and one case colonized by Kpn-KPC and the final decision of the Control Committee for Infections prevailed at the time of declaring an outpatient outbreak. A propagation pattern that favors the hypothesis of transmission from person to person was considered.

Materials and methods

The Hospital "Dr. Verdi Cevallos Balda" is a provincial hospital located in the city of Portoviejo, province of Manabí, Ecuador, and has 140 hospitalization beds distributed in surgical, clinical, and intensive care areas.

Study period

From the detection of the index case in January 2017 to the outbreak closure in March 2017.

Study design

Observational and descriptive study. The relevant epidemiological and clinical aspects of patients with Kpn-KPC infection (cases) were recorded. All patients hospitalized in ICU-A with Kpn-KPC isolation from a clinical sample plus clinical and laboratory manifestations of the disease were defined as a case. It was described as colonization to the separation of this agent in surveillance rectal swab samples in patients without clinical or laboratory manifestations of the disease. Evolution was defined as: favorable (resolution of symptoms and signs

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of infection), attributable unfavorable nonattributable (death in the context of active infection plus positive culture outcome), and unfortunate (mortality in the context of active infection plus the result of negative culture).

A multidisciplinary infection prevention and control strategy were immediately implemented when the outbreak was detected and continued until the outbreak was declared over.

Statistical analysis

A descriptive statistical analysis of the variables was performed.

Results

During the study period, 2 cases of infection and 1 of colonization by Kpn-KPC were documented. The clinical characteristics and the evolution of the cases are described in Table 1.

Characteristics	Number (Percentage %)
Gender	
Masculine	3 (100%)
Age (range)	
Median	65 (Range 63-70)
Type of Hospitalization room	
UCI-A	3 (100%)
Presence of comorbidities	
Diabetes Mellitus type 2	1 Index case (33.3%)
Days of hospitalization before isolation (range)	
Median	5 (Range 4-7)
Site of infection	
Urinary tract	1 (33.3%)
Respiratory tract	1 (33.3%)
Digestive tract	1 (33.3%)

Table 1. Clinical and epidemiological characteristics of 3 cases of Kpn-KPC-producing *Klebsiella pneumoniae* infection.

The first case was detected on January 25, 2017, in the intensive care unit (ICU-A), in a patient admitted to the hospital with a diagnosis of septic Shock of nondetermined focus. The second case is detected in the intensive care unit (ICU-A) on January 30, 2017, in a patient admitted with a diagnosis of chest trauma. The third colonized case is detected in the intensive care unit (ICU-A) on January 30, 2017, with a diagnosis of Chronic Obstructive Pulmonary Disease COPD.

The hospitalization room for patients at the time of diagnosis of Kpn-KPC infection was the adult intensive care unit (ICU-A). The overall mortality was 66.6%, while the mortality attributable to Kpn-KPC infection was 33.3% of the cases. (Table 2)

	Number (Percentage %)
Presence of invasive	3 (100%)
Global mortality	2 (66.6%)
Attributable mortality	1 (33.3%)

Table 2. Mortality.

Within the strategies implemented for the control of the outbreak, surveillance swabs were performed in the three patients, of which 1 (33%) was colonized.

Among the strategies used to control the outbreak are the following: the education of the patient, family members, and health personnel, intensification of the control of compliance with isolation measures (handwashing, use of gloves and disposable gowns). It was possible to have staff that only worked in the UCI-A, besides

the cleaning staff that was exclusive and unique for this room. It can be considered that these measures were effective in controlling the outbreak since, once implemented, there were no more cases of infection with Kpn-KPC in hospitalized patients or ICU-A staff. The outbreak closes with the death of the second infected case on 03/04/2017.

Discussion

Our epidemiologic study describes the evolution of carbapenem-resistant *K. pneumoniae* over time in a hospital in Portoviejo, Ecuador. The hospital's Infection Control Committee declares an outbreak of Kpn-KPC with a high dissemination capacity from patient to patient in the ICU-A Intensive Care Unit due to its relationship between time, space, and person. One patient in the 3 cases of our study presented comorbidities such as type 2 diabetes mellitus and risk factors before hospitalization and administration of antimicrobials. These findings coincide with previous studies that evaluated risk factors for the acquisition of Kpn-KPC infections.

In one of them, the presence of severe disease and the previous use of antimicrobials, mainly cephalosporins and quinolones, were identified as risk factors⁴.

The sensitivity of the Kpn-KPC isolates of patients during the outbreak decreased therapeutic options, the empirical treatment being inadequate in most cases. The optimal treatment for infections caused by Kpn-KPC is not established. Moreover, the clinical data are minimal and are mainly based on case reports or small case series². Colistin was the most frequently used antimicrobial in our patients.

Although all patients received a targeted and adequate antibiotic treatment, the overall mortality was high 2 of 3 patients for 66.6%.

The strategies used to control Kpn-KPC infections, such as increased environmental cleanliness, surveillance cultures, contact precautions, and prudent use of antimicrobials, were necessary to control the outbreak. The implementation of surveillance swabs allowed to identify patients with colonization with Kpn-KPC, which are an essential source of transmission during nosocomial outbreaks⁵.

The ICU-A adult Intensive Care Unit room and the family environment are a likely factor of dissemination of the disease. The isolation of KPC is an alternative to clinical suspicion. The implementation of infection control measures is essential to reduce nosocomial transmission of Kpn-KPC.

To conclude, it is essential to note that the Infection Control team and health personnel must be alerted to identify the risk factors for Kpn-KPC infection, and thus improve the detection of probable cases and establish an adequate empirical antibiotic treatment. In this way, preventive measures could be taken earlier and so avoid the patient-to-patient spread of this menacing bacterium and prevent future outbreaks in the hospital.

Conclusions

The implementation of infection control measures is essential to reduce nosocomial transmission of Kpn-KPC. Infection Control team and health personnel must be alerted to identify the risk factors for Kpn-KPC infection, and thus improve the detection of probable cases and establish an adequate empirical antibiotic treatment. In this way, preventive measures could be taken earlier and so avoid the patient-to-patient spread of this

menacing bacterium and prevent future outbreaks in the hospital. Intensive Care Unit room and the family environment are a probable factor of dissemination of the disease. The isolation of KPC is an alternative to clinical suspicion.

Limitations

Our study has some limitations. The main one is the sample size. The delusion of these infections is relatively low. Exposure to previous antibiotics was not collected. It was not possible to obtain molecular and phenotypic results of the samples of these patients.

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Conflict of Interest

The author declares no conflict of interest.

Bibliographic references

1. Asensio A, Oliver A, González-Diego P, Baquero F, Pérez-Díaz JC, Ros P, et al. Outbreak of a multiresistant *Klebsiella pneumoniae* Strain in an Intensive Care Unit: antibiotic use as risk factor for colonization and Infection. *Clin Infect Dis*. 2000;30:55–60.
2. Hirsch E, Tam V. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multi-drug-resistant infection. *J Antimicrob Chemother*. 2010;65:1119–25.
3. Buitrón G., Quezada I., Coronel A., Rosero C., Armijos L., Dávila E., Comportamiento de casos de *Klebsiella Pneumoniae* productora de Carbapenemas en pacientes de un Hospital de tercer nivel de la ciudad de Quito. *Rev Med Reflexiones* 2015; 16: 9-13.
4. Gasink LB, Edelstein PH, Lautenbach E, Synnestvedt M, Fishman NO. Risk factors and clinical impact of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*. *Infect Control Hosp Epidemiol*. 2009;30:1180–5.
5. Lledo W, Hernández M, López E, Molinari OL, Soto RQ, Hernández E, et al., Centers for Disease Control and Prevention (CDC). Guidance for control of infections with carbapenem-resistant or carbapenemase-producing enterobacteriaceae in acute care facilities. *MMWR Morb Mortal Wkly Rep*. 2009;58: 256–60.

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RESEARCH / INVESTIGACIÓN

Factores que predisponen a la elevación de la tensión arterial en pacientes normotensos operados en cirugía electiva

Factors predisposing to elevated blood pressure in normotensive patients operated on in elective surgery

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Resumen: La estabilidad circulatoria es uno de los propósitos principales durante el acto anestésico-quirúrgico. Por eso, el objetivo propuesto fue determinar la relación de los factores de riesgo identificados, con la elevación de la tensión arterial en pacientes normotensos operados en cirugía electiva, bajo anestesia general orotraqueal. Se realizó un estudio de caso-control con n=88 normotensos que fueron operados en el transcurso del año 2018, distribuidos normalmente ($p=0,000$) en compensados ($n=44$) y descompensados ($n=44$). Se analizaron variables: anestésico-quirúrgicas, factores de riesgo (independientes) y variable dependiente: descompensación hemodinámica (dicotómica). Se realizaron pruebas de hipótesis: Kolmogorov-Smirnov (normalidad), ONEWAY (diferencias tensionales entre grupos) y de asociación: Ji-cuadrado, Fisher y Regresión logística ($p<0,05$) y Odds Ratio ($OR>1$). Los resultados fueron representados en tablas y gráficos estadísticos y a través del método de análisis y síntesis fueron expresados textualmente. Se concluye que los antecedentes patológicos personales y familiares, el tabaquismo, cafeísmo, sedentarismo y el hipercolesterolemia constituyen factores de riesgo de hipertensión arterial, que en la medida que se conjugan se produce una potenciación de sus efectos y se elevan considerablemente los riesgos de los pacientes operados en cirugía electiva, aun cuando estos sean considerados como normotensos en la evaluación preoperatoria y al entrar al salón.

Palabras clave: Hipertensión arterial, factores de riesgo, cirugía electiva, anestesia.

Abstract: Circulatory stability is one of the main factors during the anesthetic-surgical act. Therefore, the proposed objective was to determine the relationship of the identified risk factors with the derivation of blood pressure in normotensive patients operated on in elective surgery under general orotracheal anesthesia. A case-control study was performed with $n = 88$ normotensive patients who underwent surgery in 2018, normally distributed ($p = 0.000$) is compensated ($n = 44$) and decompensated ($n = 44$). Variables were analyzed: anesthetic-surgical, risk factors (independent), and dependent variable: hemodynamic decompensation (dichotomous). Hypothesis tests were performed: Kolmogorov - Smirnov (normality), ONEWAY (blood pressure differences between groups), and association: Chi-square, Fisher, and Logistic regression ($p < 0.05$) and Odds Ratio ($OR > 1$). The results were represented in statistical tables and graphs, and through the analysis and synthesis method, they were expressed verbatim. It is concluded that personal and family pathological history, smoking, caffeine, sedentary lifestyle and hypercholesterolemia are risk factors for high blood pressure. These combinations produce a potency of their effects and increase the risks of the patients, operated in elective surgery, even when they are treated as normotensive in the preoperative evaluation and upon entering the ward.

Key words: Arterial hypertension, factors of risk, elective surgery, anesthetizes.

Introducción

La hipertensión arterial (HTA) es una enfermedad de etiología múltiple caracterizada por elevación persistente de la presión arterial sistólica, diastólica o de ambas, representa por sí misma una enfermedad, como también un factor de riesgo importante para otras enfermedades, fundamentalmente para la cardiopatía isquémica, insuficiencia cardíaca, enfermedad cerebro-vascular, insuficiencia renal y para la retinopatía^{1,2}. Para el año 2014 se estimó que más del 20% de los adultos mayores de 18 años, en el mundo, eran hipertensos y casi 10 millones de muertes fueron causadas por HTA³. A nivel mundial, afecta a más de mil millones de personas y aunque puede diagnosticarse y tratarse de forma satisfactoria, sólo una de cada siete personas hipertensas tiene la presión arterial controlada⁴.

La HTA es el factor de riesgo que está presente en la mayoría de las muertes causadas por enfermedades circulatorias, enfermedades coronarias y vascular encefálicas. En la

mayor parte de las regiones existe una prevalencia del 15 al 30 % de la enfermedad. En Cuba, en el año 2015, la tasa de prevalencia de HTA fue de 217,5 por 1 000 habitantes, siendo el sexo femenino el más afectado con una tasa de 236,5 por 1 000 habitantes, así como el grupo etario de 60 a 64 años⁵.

La administración de anestesia puede suponer un riesgo para el paciente superior al de la cirugía y por eso la valoración preanestésica es un paso crucial que puede afectar a la seguridad clínica e integridad de todo el proceso quirúrgico. La aparición de HTA está generalmente condicionada a la existencia de uno o más factores predisponentes que son los mismos que contribuyen a una mayor morbilidad y mortalidad cardiovascular. Se resumen en: tabaquismo, sobrepeso, vida sedentaria, falta de ejercicios, ingesta excesiva de alcohol e hipercolesterolemia. Se añade la exagerada ingesta de sal⁶⁻¹⁰.

La estabilidad circulatoria es uno de los elementos principales en el perioperatorio de pacientes de alto riesgo. Por eso,

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el objetivo propuesto en esta investigación fue determinar la relación de los factores de riesgo identificados, con la elevación de la tensión arterial en pacientes normotensos operados en cirugía electiva, bajo anestesia general orotraqueal.

Materiales y métodos

Diseño de la investigación

Se realizó un estudio descriptivo observacional de caso-control, donde el universo estuvo conformado por 239 pacientes con sus correspondientes historias clínicas. Los mismos se caracterizaban por no ser hipertensos y fueron intervenidos con cirugía abdominal electiva, bajo anestesia general orotraqueal, a lo largo de un año en el Instituto Superior de Medicina Militar "Dr. Luis Díaz Soto" de La Habana.

A través del programa Epidat versión 3.1 se efectuó el diseño muestral y se obtuvo $n=88$ que se distribuyó equitativamente a razón de 1:1, correspondiente a: Grupo 1 pacientes normotensos descompensados, $n=44$ y grupo 2 pacientes normotensos compensados, $n=44$, por intervención del muestreo simple aleatorio.

Criterios de inclusión

Pacientes no hipertensos que expusieron su consentimiento para la obtención de las variables clínicas y de laboratorio especificadas en el trabajo.

Criterios de Exclusión

Pacientes con hipertensión arterial conocida o de debut y los que no manifestaron interés en formar parte del estudio.

Se clasificaron como normotensos a los pacientes con cifras tensionales iguales o menores a 140/90 mmHg^{3,7,10,11} y mayores a 90/60 mmHg e hipotensión cuando era inferior a 90/60 mmHg^{12,13}. Estos pacientes no tenían antecedentes conocidos de la enfermedad y durante las consultas realizadas no se detectaron cifras de HTA. La cifra de TA se monitoreó en la consulta interna, al arribo al salón, a la entrada al quirófano y durante el acto anestésico-quirúrgico cada intervalo de 10 minutos.

Variables anestésico-quirúrgicas

Se contemplaron la tensión arterial y los factores de riesgo que condicionan su elevación, clasificados en modificables: Índice de Masa Corporal (IMC)^{14,15}, hábito de fumar, el consumo de café, de bebidas alcohólicas, de sal, la hipercolesterolemia, el sedentarismo, el estrés y la ansiedad. No modificables: Edad, apariencia racial, sexo, antecedentes patológicos personales (APP) relacionados con eventos mórbidos crónicos anteriores al ingreso, antecedentes familiares (APF) de HTA y si estuvo compensado o descompensado durante el tiempo quirúrgico.

Procedimiento anestésico empleado en el estudio

Con todos los pacientes se siguió la misma metodología según lo normado en el Centro hospitalario para los que son intervenidos quirúrgicamente con anestesia general endotraqueal: Consulta interna el día antes de la intervención quirúrgica y el día de la operación, fueron trasladados en camillas al departamento de preanestesia. La premedicación fue con benzodiacepinas (midazolán 0.15 mg/kg), se realizó un registro de tensión arterial y de frecuencia cardiaca, antes y después de la premedicación. En el salón se les monitoreó el electrocardiograma, la tensión arterial (TA) sistólica, diastólica y media, la frecuencia cardiaca (FC) y la oximetría de pulso (SO_2). La in-

ducción anestésica se inició con la administración endovenosa (EV) de Tiopental con una dosis de 5 mg/kg, fentanil 5mcg/kg, Vecuronio 0,1 mg/kg y lidocaína 2% a 1mg/ kg. Se conectó el flujo de gases anestésicos, óxido nitroso, con una FiO_2 de 0,4 y el halotano en correspondencia con las necesidades individuales de cada paciente. En el transoperatorio se repusieron los egresos con cristaloides y coloides según lo calculado individualmente.

Análisis Estadístico

Una vez finalizado el acto quirúrgico se recopilaron los datos correspondientes al acto anestésico quirúrgico en las HC para su procesamiento estadístico. Con la información recolectada se creó una base de datos en el programa SPSS 11.5.1 y se analizó mediante técnicas de estadística, descriptiva e inferencial paramétrica. Previamente se determinó el tipo de distribución que siguió la muestra por medio de la prueba Kolmogorov-Smirnov que resultó normal, $p < a$.

Resultados y discusión

En la Tabla 1, se observa que en el período preanestésico los normotensos descompensados hemodinámicamente durante el transquirúrgico (TQ) presentaron mayores valores de presión sistólica ($p=0,034$) y diastólica ($p=0,001$) que los normotensos que se mantuvieron compensados. Se observa que, aun siendo los valores de TA correspondiente a cada grupo normales, este hecho no constituye una garantía absoluta para que el transoperatorio transcurra sin riesgos.

Otros investigadores han referido que el acto anestésico quirúrgico representa un factor considerablemente estresante, por lo que muchas veces resulta impredecible la posible respuesta del paciente^{16,17,18}. Se han investigado pacientes con antecedentes de haber elevado sus cifras de presión arterial ante situaciones como acudir al consultorio médico y la obtención de cifras tensionales normales en casa, es lo que se denomina "hipertensión de bata blanca"¹⁰. Una prueba de estimulación por frío en 30 pacientes dio como resultado que 24 experimentaron hipertensión arterial. A estos pacientes les fue indicado tratamiento previo y a los 6 negativos no se les prescribió tratamiento por no expresar hiperreactividad. Se obtuvo como resultado que no se produjera hipertensión perioperatoria, ni suspensiones del acto quirúrgico; concluyéndose que esta prueba es un valioso instrumento para la predicción tensional transoperatoria y auxiliar en la decisión de prescribir hipotensores, como medicación previa^{19,20}.

En la Tabla 2, se observa que, aunque en el grupo de pacientes descompensados el valor promedio de la edad es superior, no se encontró diferencia estadísticamente significativa, por lo que a pesar de la tendencia referida no se prueba en este estudio que exista asociación de la edad ($ETA=0,765$) con la descompensación de la TA en el acto anestésico quirúrgico. Varios son los autores que han referido la edad avanzada como uno de los factores que deben tenerse en cuenta antes, durante y posterior al acto anestésico quirúrgico, resaltando que esto no constituye una contraindicación, pero sí una alerta para incrementar las precauciones^{5,10,21}.

Como se ilustra en la tabla 3, de las 56 mujeres investigadas sólo se descompensaron 22, que representa el 39,3% de las mismas, entretanto, de los 32 hombres considerados se descompensaron la misma cifra, pero esta constituye el 68,7 % de la muestra analizada en este grupo, de modo que se demuestra la vinculación del sexo a la descompensación de la TA durante el TQ ($p=0,015$), con mayor prevalencia el sexo masculino.

Se ha señalado que la prevalencia es altamente variable y se ve incrementada en pacientes jóvenes, del sexo masculino que son fumadores, con excesivo consumo de alcohol y que presentan estrés laboral, entre otros factores¹³. Sin embargo en el año 2015 la tasa de prevalencia de HTA en Cuba fue mayor en el sexo femenino, así como el grupo etario de 60 a 64 años⁵.

Tanto el sexo como la raza han sido reportado en la literatura como factores de riesgo para la HTA, no modificables^{5,7,13,14,22}, lo cual se hace más evidente, en este estudio, pues los recientes descubrimientos del mapa genético tienden a demostrar la participación de múltiples genes dentro del contexto de una predisposición genética multifactorial, entre los que destacan la expresión de los genes de la enzima convertidora de angiotensina (ECA), del gen del angiotensinógeno y la no expresión de la enzima sintetizadora de óxido nítrico endotelial^{23,24}.

En otras investigaciones se ha encontrado también, una prevalencia mayor en el sexo masculino sobre el femenino, lo que se plantea es válido hasta los 50 años de edad. Después estas cifras tienden a invertirse, al parecer por la privación de estrógeno que acompaña la menopausia^{5,22,25,26}. Lo señalado, no constituye una regla que se cumpla bajo cualquier condición, ya que como se observa en los resultados de la presente investigación son múltiples los factores que influyen en la aparición de la HTA.

Al analizar la variable apariencia racial no se encontraron diferencias estadísticamente significativas. Se plantea que el color de la piel no es determinante en la aparición de la hipertensión arterial, aunque existen evidencias de que los individuos de color de piel negra sufren de complicaciones más graves, comienzan en edades tempranas de la vida y tienen mayor susceptibilidad a la lesión en órganos diana^{7,15}.

Los resultados expuestos en la tabla 4, reflejan que los antecedentes patológicos personales juegan un papel importante en la descompensación de la TA durante el TQ (84,1% vs. 15,9 %) destacándose particularmente las enfermedades renales, la epilepsia, las enfermedades cerebro vasculares (ECV), la diabetes mellitus y la cardiopatía isquémica, con 60% o más; así como en los antecedentes personales familiares (APF) de hipertensión arterial.

Los resultados pueden deberse a que el individuo es portador del gen que determina la predisposición para una enfermedad determinada, según ha sido demostrado en distintos estudios de heredo patología, aunque se plantee una mayor probabilidad del padecimiento^{7,27,28,29} y además a que en el grupo sin antecedentes influyen otros factores de mayor importancia también aquí considerados.

En cuanto a la práctica de hábitos tóxicos (Tabla 5), las frecuencias relativas en los descompensados fueron superiores para todos los factores de riesgo, donde el tabaquismo (n=32) y el cafeísmo (n=40) constituyeron el 72,7 y 90,9%, respectivamente. Por esta razón, tanto el primer factor de riesgo como al segundo han sido abordado por varios autores^{1,30-33}.

El consumo de bebidas alcohólicas se caracterizó en 79,5% (n=35) como bebedores sociales y la ingesta excesiva de sal en 36,4% (Tablas 5). Un mayor índice de masa corporal, una mayor circunferencia de la cintura, el tabaquismo y el alto consumo de alcohol se asocian de manera independiente con mayor PA sistólica^{6,35-37}. Por el contrario, el consumo leve a moderado de alcohol se ha asociado a un menor riesgo de muerte súbita cardíaca (MSC) en los varones³⁴.

En la tabla 6, se ilustra que la hipercolesterolemia se presenta en 14 pacientes (31,8%) descompensados, cifra mayor

que en los compensados ($p<0,05$). En la esfera emocional, se encontró ansiedad alta en 11 operados (25%) que se descompensaron en el TQ; en tanto en el grupo de los compensados no hubo ninguno. Se identificó en 63,6% vulnerabilidad severa al estrés (n=28) y un paciente con extrema vulnerabilidad en aquellos descompensados; mientras que en los no descompensados la frecuencia fue menor (6,8%) y nula respectivamente.

Se destaca que factores de riesgo modificables como el tabaco, el consumo nocivo de alcohol, la dieta poco saludable, la insuficiente actividad física, la obesidad, así como la hipertensión, la hiperglucemia y el hipercolesterolemia, constituyen factores de riesgo principales que contribuyen a la patología subyacente de las enfermedades cardiovasculares^{1,32,38-44}.

El término infarto agudo de miocardio, hace referencia a un riego sanguíneo insuficiente con lesión miocárdica, producido por una obstrucción en una o varias arterias coronarias. Acorde a los reportes de la Organización Mundial de la Salud (OMS), la exposición a estrés crónico hace más propensas a las personas a enfermar y morir de enfermedades cardiovasculares, además de doblar el riesgo de aparición de un infarto agudo miocárdico (IAM). La OMS pronostica que en el 2020 la enfermedad isquémica del corazón (EIC) será responsable de 11 millones de muertes, por lo que su prevención constituye un reto para las autoridades sanitarias de todos los países del mundo⁴⁵⁻⁴⁷. La evaluación preoperatoria y las alternativas durante el proceso de la anestesia son claves en la reducción de estos riesgos⁴⁸⁻⁵².

Nutricionalmente en ambos grupos, los mayores porcentajes se ubicaron en la categoría normal (Tabla 7); sin embargo, con bajo peso, sobrepeso y obesos son más en el grupo de los descompensados con n=8 (18,2%) y n=7 (15,9%), correspondientemente.

Algunos investigadores destacan haber encontrado dentro de las causales de la HTA el sedentarismo^{8,14,27,29}, igualmente en este estudio, se encontró asociación entre este factor de riesgo y el mayor porcentaje de descompensación, 60,0 %, en los sedentarios y 11,1% en los no sedentarios, durante el TQ. No se puede considerar que por sí solo, el sedentarismo constituya un elemento capaz de producir HTA. Se deben conjugar varios factores de riesgos como los antecedentes patológicos personales, la ansiedad, la vulnerabilidad al estrés y el tabaquismo que pueden influir en la descompensación hemodinámica de los pacientes. Según la literatura, existen muchos factores más que se conjugar para desencadenar la HTA, la que desencadena otros procesos que representan un gran peligro para la vida^{3,5,7,44,53}.

Al analizar el riesgo, se obtuvo que influyen de forma determinante en el desequilibrio hemodinámico, el sexo masculino, los APP, el tabaquismo, cafeísmo y la hipercolesterolemia, dado valores de OR>1 y al considerar el Intervalo de Confianza al 95% se tuvo que el 1 no cae en dichos intervalos; lo que representa que la probabilidad de descompensación de la TA durante el TQ aumenta (según el valor obtenido de OR) en presencia de los factores evaluados, por lo que constituyen riesgo.

Se ha demostrado otros efectos negativos sobre la morfología y hemodinámica cardiovascular inducidos por la nicotina y el humo de los cigarrillos, como la de inhibir la síntesis vascular y miocárdica de prostaciclina (PGI2) que unida al daño endotelial son factores de riesgo que predisponen a la hipertensión arterial, aterosclerosis, trombosis y alteraciones isquémicas^{5,15,54,55}.

TENSIÓN ARTERIAL		Tensión arterial preanestésica (mmHg) en pacientes que resultaron:	
		Descompensados	Compensados.
Promedio de TA sistólica		120	112
Desviación estándar TA sistólica		11	11
Promedio de TA diastólica		77	70
Desviación estándar TA diastólica		8	9
ANOVA	TA SISTÓLICA	p=0,034	
	TA DIASTÓLICA	p=0,001	

Fuente: Planilla Recopiladora de Datos

TA: Tensión Arterial TQ: Tiempo Quirúrgico

Tabla 1. Cifras de tensión arterial preanestésica de pacientes descompensados y compensados en el TQ.

EDAD		Elevación de la TA durante el TQ	
		SI (n=44)	NO (n=44)
Años	Media	54	49
Años	Desviación estándar	12	16
	Edad mínima	28	19
	Edad máxima	78	81
Asociación		Coeficiente ETA=0,765 p>0.05	

Fuente: Planilla Recopiladora de Datos

TA: Tensión Arterial TQ: Tiempo Quirúrgico

Tabla 2. Edad, su relación con las cifras de TA.

VARIABLES		Elevación de la TA durante el TQ				% descompensado en relación al Grupo.	
		SI (n=44)		NO (n=44)		n _T	D (%)
		n	%	n	%		
Sexo	Masculino	22	50,0	10	22,7	32	68,7
	Femenino	22	50,0	34	77,3	56	39,3
Asociación		p=0,015					
Apariencia racial	Negra	10	22,7	8	18,2	18	55,5
	Mestiza	16	36,4	12	27,3	28	57,1
	Blanca	18	40,9	24	54,5	42	42,8
Asociación		p=0,483					

Fuente: Planilla Recopiladora de Datos

TA: Tensión Arterial TQ: Tiempo Quirúrgico

Tabla 3. Comportamiento de la TA según el sexo y la apariencia racial.

VARIABLES		Elevación de la TA durante el TQ				% descompensado en relación al Grupo.	
		SI (n=44)		NO (n=44)		n _T	D (%)
		n	%	n	%		
APP	Sin antecedentes	7	15,9	18	40,9	25	28,0
	Con antecedentes	37	84,1	26	59,1	63	58,7
	Diabetes Mellitus	10	22,7	6	13,6	16	62,5
	Asma Bronquial	7	15,9	8	18,2	15	46,7
	Cardiopatía Isquémica	6	13,6	4	9,1	10	60,0
	ECV	2	4,5	0	0,0	2	100
	Enfermedad Renal	2	4,5	0	0,0	2	100
	Epilepsia	1	2,3	0	0,0	1	100
	Hipertiroidismo	0	0,0	2	4,5	2	0
	Otras	9	20,4	6	13,6	15	60,0
Asociación		p=0,018					
APF de HTA	Sin antecedentes	12	27,3	20	45,5	32	36,4
	Con antecedentes	32	72,7	24	54,5	56	63,6
Asociación		p=0,017					

Table 4. Patologías asociadas y antecedentes familiares de HTA, repercusión en las cifras de TA.

Fuente: Planilla Recopiladora de Datos

TA: Tensión Arterial, TQ: Tiempo Quirúrgico, APP: Antecedentes Patológicos Personales, ECV:

Enfermedad Cerebro Vascular, APF: Antecedentes Patológicos Familiares, HTA: Hipertensión Arterial

VARIABLES		Elevación durante el TQ				% descompensado en relación al Grupo.	
		SI (n=44)		NO (n=44)		n _T	D (%)
		n	%	n	%		
Tabaquismo	Si	32	72,7	5	11,4	37	86,5
	No	12	27,3	39	88,6	51	23,5
Asociación		p=0,000					
Cafeísmo	Si	40	90,9	22	50,0	62	64,5
	No	4	9,1	22	50,0	26	15,4
Asociación		p=0,000					
Consumo de bebidas alcohólicas	Bebedor social	35	79,5	43	97,7	78	44,9
	Consumo de riesgo	6	13,6	1	2,3	7	85,7
	Consumo perjudicial	3	6,8	0	0,0	3	100
Asociación		p=0,000					
Consumo de sal	Normal	2	63,6	42	95,5	70	40,0
		8					
	Exceso	1	36,4	2	4,5	18	88,9
Asociación		p=0,000					

Tabla 5. Hábitos tóxicos y su relación con el comportamiento de la TA de los pacientes en estudio.

Fuente: Planilla Recopiladora de Datos

TA: Tensión Arterial TQ: Tiempo Quirúrgico

VARIABLES		Elevación de la TA durante el TQ				% descompensado en relación al Grupo.	
		SI (n=44)		NO (n=44)		n _T	D(%)
		n	%	n	%		
Hipercolesterolemia	Si	14	31,8	4	9,1	18	77,8
	No	30	68,2	40	90,9	70	42,9
Asociación		p=0,017					
Ansiedad	Muy baja	0	0,0	8	18,2	8	0
	Baja	17	38,6	30	68,2	47	36,2
	Normal	16	36,4	6	13,6	22	72,7
	Alta	11	25,0	0	0,0	11	100
Asociación		p=0,000					
Estrés	No vulnerable	2	4,5	21	47,7	23	8,7
	Vulnerable	13	29,5	20	45,5	33	39,4
	Severamente vulnerable	28	63,6	3	6,8	31	90,3
	Extremadamente vulnerable	1	2,3	0	0	1	100
Asociación		p=0,000					

Fuente: Planilla Recopiladora de Datos

TA: Tensión Arterial TQ: Tiempo Quirúrgico

Tabla 6. Influencia del hipercolesterolemia, la ansiedad y el estrés en el estado hemodinámico del paciente quirúrgico.

VARIABLES		Elevación de la TA durante el TQ				% descompensado en relación al Grupo.	
		SI (n=44)		NO (n=44)		n _T	D (%)
		n	%	n	%		
Valoración nutricional	Normal	29	65,9	38	86,4	67	43,3
	Bajo peso	8	18,2	4	9,1	12	66,7
	Sobrepeso y obesos	7	15,9	2	4,5	9	77,8
Asociación		p=0,017					
Sedentarismo	Si	42	95,5	28	63,6	70	60,0
	No	2	4,5	16	36,4	18	11,1
Asociación		p=0,000					

Fuente: Planilla Recopiladora de Datos

TA: Tensión Arterial TQ: Tiempo Quirúrgico

Tabla 7. Cifras de TA durante el TQ en relación a la valoración nutricional y sedentarismo.

Conclusiones

Se pudo comprobar que los antecedentes patológicos personales y familiares, el tabaquismo, cafeísmo, sedentarismo y la hipercolesterolemia constituyen factores de riesgo de hipertensión arterial, que en la medida que se conjugan se produce una potenciación de sus efectos y se elevan considerablemente los riesgos de los pacientes operados en cirugía electiva, aun cuando estos sean considerados como normotensos en la evaluación preoperatoria, pues los que resultaron descompensados fueron los que entraron al salón con las cifras de tensión arterial normales, pero superiores.

Referencias bibliográficas

1. Williams B, Mancia G, Spiering W, Rosei EA, Azizi M, Clement DL. Guía ESC/ESH 2018 sobre el diagnóstico y tratamiento de la hipertensión arterial. *Rev Esp Cardiol*. 2019;72(2):160.e1-e78.
2. Fernández GL, Seuc JAH, Rodríguez GCA. Método de mortalidad ponderado según múltiples causas de muerte. *Revista Finlay*. 2019;9(3):197-209.
3. Ortiz R, Bermúdez V, Guzmán LJA, Silva PJS, Torres OMG, Carvajal GJS, Tirado REL, Cruz CTM, Espinoza DCI, Morocho ALA, Gratzia OM. Hipertensión arterial y su comportamiento epidemiológico en la población rural de Cumbe, Ecuador. *Revista Latinoamericana de Hipertensión*. 2019; 12(5):09-118.
4. Patel P, Ordunez P, DiPette D, Escobar MC, Trevor H, Wyss F, Henis A, Asma S, Angell S. Mejor control de la presión arterial para reducir la morbilidad y mortalidad por enfermedades cardiovasculares: Proyecto de Prevención y Tratamiento Estandarizado de la Hipertensión Arterial. *Rev Panam Salud Publica*. 2017; 41:1-12.
5. González RR, Martínez CM, Castillo SD, Rodríguez MOL, Hernández VJ. Caracterización clínico-epidemiológica de la hipertensión arterial en adultos mayores. *Revista Finlay*. 2019; 7(2):74-80.
6. Sandoya E, Torres F, Rivedieu F, Machado F, Núñez E. Situación de la hipertensión arterial a nivel público y privado. *Rev Urug Cardiol* 2016; 31:219-227.
7. Rodríguez SA, Piña AAC, Díaz PA, García RR, Balcendes AS. Brechas en el manejo del paciente hipertenso en un área metropolitana de La Habana. *Revista Finlay*. 2019;9(4): 257-272.
8. Zuni ChKX, More SBE, Fernández VCD, García FBB, Ruiz OJM, Pérez RVK. Prevalencia de factores de riesgo cardiovascular en pacientes hospitalizados en un hospital de Lima. *Rev. Fac. Med. Hum*. 2019;19(4):68-73.
9. Soto EG, Moreno AL, Pádua DD. Panorama epidemiológico de México, principales causas de morbilidad y mortalidad. *Revista de la Facultad de Medicina de la UNAM*. 2016;59(6):8-22.
10. Cuellar RS. Hipertensión Arterial. [en línea] junio 2016. Disponible en: <https://www.google.com/search? Y botplusweb.portalafarma.com>. [Consultada 12 de febrero de 2020].
11. Rosas PM, Palomo PS, Borrayo SG, Madrid MA, Almeida GE, Galván OH, Magaña SJA, Saturno ChG. Consenso de Hipertensión Arterial Sistémica en México. *Rev Med Inst Mex Seguro Soc*. 2016;54 Supl 1:6-51.
12. León RC, Arroniz SR, Rodríguez-Cabello RS. Crisis hipertensivas. Atención primaria en la red. *Guías clínicas*; 2005;5(18).
13. López HMG, Meléndez FHJ, Álvarez RS, Alvarado AJL. Factores de riesgo para hipotensión en anestesia regional subaracnoidea para cesárea. *Papel del Índice Cintura Cadera e Índice de Masa Corporal*. *Rev Colomb Anestesiología*. 2018;46(1):45-51.
14. Sabio R, Valdez P. Recomendaciones latinoamericanas para el manejo de la hipertensión arterial en adultos (RELAHTA 2). *Rev. virtual Soc. Parag. Med. Int*. 2019; 6 (1):86-123.
15. Esteban FA, Fernández CA, Gómez de Diego JJ, Vicent AML, Vidal PR. *Cardiología hoy 2018. Resumen anual de los avances en investigación y cambios en la práctica clínica*. © 2018 Sociedad Española de Cardiología. Publicación oficial. Pág. 1105.
16. Bustamante R ¿Qué Hacemos los Anestesiólogos? Desde la vigilancia anestésica monitorizada hasta la anestesia general. *REV. MED. CLIN. CONDES* - 2017; 28(5) 671-681.
17. Puerto LJA, Torres AFL, Cabanes RE. Herida por cuerpo extraño en el tórax. A propósito de un caso. *Universidad y Sociedad*. 2019;12(1):381-385.
18. Ronquillo GK, Rivera OA, Marín GAL. Análisis y aplicación de una lista de verificación en anestesia en trauma y cirugía de urgencia. *Revista Mexicana de Anestesiología*. 2019;42(1):56-61.
19. Del Pozo JH, Leiva SL. Hipertensión arterial y riesgo preoperatorio en el paciente anciano. *Acta Médica*; 2002;(10):1-2.
20. Del Pozo JH, Leiva SL. Atención prequirúrgica del hipertenso eventual. *Rev Cubana Med*. 1998; 37(3):141-6.
21. Mille LJE, Ortiz MJJ, Rocha MJF. Manejo expectante de la hipertensión arterial transoperatoria: ¿es la anestesia la solución? *Revista Mexicana de Anestesiología*. 2017;40(Supl. 1):S90-S93.
22. Pomares AAJ, Vázquez NMA, Ruiz DES. Adherencia terapéutica en pacientes con hipertensión arterial. *Revista Finlay*. 2017;7(2):8-88.
23. Juárez IM, de la Fuente LMA, Fontecha AJ. Los nutrientes de la leche en la salud cardiovascular. *Nutr Hosp*. 2015;31(Supl. 2):26-32.
24. García RP, Gómez CR, Sánchez VN. Prevalencia de la Hipertensión Arterial y factores de riesgo presentes en la población de un área de Salud. *Rev Méd Electrónica*; 2006;28(1).
25. Thierier J ¿Otro escenario más para el canto del cisne de la aspirina? Estudio TWILIGHT. *Rev Argent Cardiol* 2019;87:517-520.
26. Rodríguez SPL, Alfonso HC, Valladares GA, Pomares AJ, López AL. Estrés y emociones negativas en adultos medios con hipertensión arterial esencial. *Revista Finlay*. 2018;8(1):36-45.
27. González RR. Factores de riesgo de hipertensión arterial en adolescentes. *Revista Finlay*. 2016; 6(4):262-264.
28. Costabel JP, Mandó F, Avegliano G. Miocardiopatía dilatada: ¿cuándo y cómo proceder a la investigación etiológica? *Rev Urug Cardiol* 2018;33:343-349.
29. Vázquez ME, Calderón RZG, Arias RJ, Ruvalcaba LJC, Rivera RLA, Ramírez ME. Sedentarismo, alimentación, obesidad, consumo de alcohol y tabaco como factores de riesgo para el desarrollo de diabetes tipo 2. *Journal of negative and positive results*. 2019;4(10):1011-1021.
30. Duin BA, Sosa CB, Hernández HR, Camacho C, Camacho JC. Factores de riesgo cardiovascular en adolescentes. *Revista Venezolana de Salud Pública*. 2018; 6(2):17-25.
31. Cabrera GL, Herrera CG, Díaz TO. Actividades físico-recreativas y su influencia en la disminución de los niveles de tensión arterial en mujeres hipertensas de 60 a 70 años. *Revista Conrado*. 2017;14(62):124-133.
32. Sánchez AAG, Bobadilla SME, Dimas AB, Gómez OM, González GG. Enfermedad cardiovascular primera causa de morbilidad en un hospital de tercer nivel. *Revista Mexicana de Cardiología*. *Rev Mex Cardiol* 2016;27(s3):s98-s102.
33. Borges ML, Álvarez IM, Barroso BR, Turro PA, de la Torre NE. Algunos de los nuevos factores predictores de riesgo cardiovascular. *Panorama Cuba y Salud*. 2016;11(3):51-55.
34. Martínez PRM, Carrero VAM, Cisneros SLG, Chipi RY, Olivera MA, Izquierdo RE. Estudio retrospectivo de muerte súbita cardiovascular intrahospitalaria en el Hospital Enrique Cabrera Cossío. *CorSalud*. 2018;10(1):32-39.
35. Diéguez MM, Miguel SPE, Rodríguez HR, López BJ, Ponce de León D, Reyna CJL. Prevalencia de hipertrigliceridemia y factores de riesgo cardiovascular en estudiantes de la Universidad de Ciencias Médicas. *Holguín*, 2014-2015. *Medisur*. 2018;16(1):35-45.
36. Guamialamá MJ, Salazar DD. Evaluación antropométrica según el Índice de masa corporal en universitarios de Quito. *Rev. Salud Pública*. 2018;20(3):314-318.
37. Fernández PV. Respuesta presora al estrés como marcador pronóstico de hipertensión futura. *Facultad de Medicina. Departamento de Medicina*. Universidad Autónoma de Madrid, 2013.
38. Chaves G, Britez N, Maciel V, Klinkhof A, Mereles D. Prevalencia de factores de riesgo cardiovascular en una población adulta ambulatoria urbana: estudio AsuRiesgo, Paraguay. *Rev Panam Salud Publica*. 2015;38(2):136-43.

39. León RML, García ÁY, Álvarez HR, Morales PC, Regal CVM, González LHD. Influencia del estrés psicológico y la actividad física moderada en la reactividad cardiovascular. *Revista Finlay*. 2018;8(3): 224-232.
40. Román VJA, Vázquez MVH, Loera MJ, Cantú SON, Cervantes VDA, Bernabé ACC. Panorama epidemiológico del paciente con hipertensión arterial sistémica no controlada en una unidad de medicina familiar de Reynosa, Tamaulipas. *Aten Fam*. 2016;23(1):14-18.
41. Espinosa BAD. Hipertensión arterial: cifras para definirla al comenzar 2018. *Revista Finlay*. 2018;8(1): 66-74.
42. Vélez AC, Vidarte CJA. Efecto de un programa de entrenamiento físico sobre condición física saludable en hipertensos. *Rev. Bras. Geriatr. Gerontol*. 2016;19(2):277-288.
43. Herrera AP, Pacheco MJ, Valenzuela RG, Málaga G. Autoconocimiento, adherencia al tratamiento y control de la hipertensión arterial en el Perú: Una revisión narrativa. *Rev Peru Med Exp Salud Pública*. 2017;34(3):497-504.
44. Espinoza AM, Bollini C. Cirugía de hombro en posición silla de playa: estrategias y recomendaciones para la prevención de complicaciones isquémicas. *Rev Chil Anest* 2020; 49: 114-124.
45. Moreno MG, Mejía MCS, Fernández NMI, Sánchez NR. Incidencia y manejo del infarto agudo miocárdico con elevación del segmento ST, Hospital José María Velasco Ibarra, 2014. *Enfermería Investiga, Investigación, Vinculación, Docencia y Gestión*- 2016;1(4):151-157.
46. Short H. Isquemia miocárdica perioperatoria en cirugía no cardíaca. *Anaesthesia Tutorial of the Week*. 2018; www.wfsahq.org/resources/anaesthesia-tutorial-of-the-week.pp 1-8.
47. Kunze S. Evaluación preoperatoria en el siglo XXI. *REV. MED. CLIN. CONDES*. 2017;28(5):661-670.
48. Ronquillo GDK, Quezada MEL, Saltos MSE, González MSM. Alternativas a la intubación orotraqueal ante una vía aérea difícil. *RECIMUNDO*. 2020;4(1):26-38.
49. Salazar DLC, Neira EFE. Terapia endovascular múltiple para el manejo del vasoespasmo cerebral refractario. *Rev CES Med* 2020;34(1):74-82.
50. Domínguez LB, Ortega CG, Díaz PA, Broullón DA. Incidencias y causas de la cancelación de cirugía en un hospital universitario, Barranquilla, Colombia. *Revista Electrónica Trimestral de Enfermería. Enfermería Global*. 2020(57):507-515.
51. Recart A. cirugía mayor ambulatoria. una nueva forma de entender la medicina quirúrgica. *REV. MED. CLIN. CONDES*. 2017; 28(5) 682-690.
52. Coloma R. Manejo de vía aérea no difícil. Desde la ventilación con bolsa hasta intubación oro traqueal. *REV. MED. CLIN. CONDES*. 2017; 28(5) 691-700.
53. Salas P, González C, Carrillo D, Bolte L, Aglony M, Peredo S, Ibarra X, Rojo A, Delucchi A, Pinto V, Saieh C, Ceballos ML. Hipertensión arterial en la infancia. Recomendaciones para su diagnóstico y tratamiento. Parte 2. Rama de Nefrología Infantil, Sociedad Chilena de Pediatría. *Rev Chil Pediatr*. 2019;90(3):336-342.
54. López Farre A, Casado S. Heart failure, redox alterations and endothelial dysfunction. *Hypertension*. 2001; 38(6):1400-5.
55. Cote MC. Vascular nurse as a smoking cessation specialist. *J Vasc Nurs*. 2000; 18(2):47-53

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RESEARCH / INVESTIGACIÓN

Morphology and histology of the male reproductive system of *Collaria oleosa* (Distant, 1883) (Heteroptera: Miridae)

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Abstract: *Collaria oleosa* (Distant, 1883) is a phytophagous bug often observed causing injuries in wheat, barley, and oat crops, besides grass pastures. The male reproductive system of *C. oleosa* has been studied anatomically and histologically. It consists of a pair of testes, each one with two follicles, one pair of seminal vesicles, two pairs of external accessory glands, and an ejaculatory bulb. The testicular follicles exhibit cysts with spermatogonia, spermatocytes, spermatids, and spermatozoa bundles. The testes are connected to the seminal vesicles by the vas deferens. The seminal vesicles are connected to the ejaculatory bulb and are bound by the accessory glands. In mirids belonging to the Stenodemini tribe, the general morphology of the male reproductive system is similar. However, two follicles per testis may be characteristic of the genus *Collaria*. The anatomy and histology of the male reproductive system in *C. oleosa* provides new information and also contributes to the understanding of the systematics of Stenodemini as well as the Miridae family in general.

Key words: *Collaria oleosa*, internal morphology, grass-feeding, plant bugs, Stenodemini.

Introduction

Miridae is the largest family of Heteroptera (Hemiptera) with 11,139 species described, distributed in eight subfamilies: Bryocorinae, Cylapinae, Deraeocorinae, Isometopinae, Mirinae, Orthotylinae, Phyllinae, Psallopinae, and 39 tribes^{6,27}. The mirids exhibit a cosmopolitan distribution, occurring in all biogeographic regions of the planet^{6,30}. The genus *Collaria* (Provancher, 1872), belongs to the Stenodemini (Mirinae) tribe with 15 recognized species¹⁷. Among these species, *Collaria oleosa* (Distant, 1883) (Fig. 1) has a significant impact, because it is considered as a potential pest in wheat, barley, oats crops, grass pastures and other plants belonging to Poaceae family^{8,16}.

The plant bug *C. oleosa* presents economic importance due to the damages that produce when sucks the sap of plants. The youngest leaves attacked by *C. oleosa* can partially or dry out, delaying the growth, reducing yield and nutritional value of the crops and grasses palatability^{1,5,16}. Even though the world distribution, host plants, and injuries of this pest is well known, the morphology and histology of the reproductive system have not been studied before.

Studies of Morales *et al.*¹⁷ suggest the importance of the reproductive system to identify the species of *Collaria* genus. The external male genitalia of *C. oleosa* exhibits an outstretched pygophore with a discreet spherical apex, U-shaped left paramere, an almost aligned dorsal margin of the basal sensory lobe, a convex ventral margin, and an aligned right paramere. The endosome has two sclerites, with an expanded lobe that further reaches the secondary gonophores, surrounded with micro-hair fixed on the integument and a distal sclerite, C-shaped with spines. Although this study provides data of the genitalia of *C. oleosa*, detailed information on the male reproductive system of *C. oleosa* is still needed.

Therefore, the purpose of this study is to describe the morphological and histological structures of the male reproductive system of *C. oleosa*, besides, detailed photography of histological sections was included. This contribution will be essential to understand the evolutionary history and morphology of the Miridae family in the Neotropical region and reveal characters that can also allow the understanding and the study of

Phylogeny, Taxonomy, physiological and behavioral aspects, as well as methods of controlling this pest.

Materials and methods

Fifteen sexually mature males of *C. oleosa* were collected in the campus of the Federal University of Viçosa, in Viçosa (20° 45' 14" S, 42° 52' 55" W 648 m), state of Minas Gerais, Brazil, in the pastures of Bermuda grass (*Cynodon sp.* (Poaceae)).

Reproductive system morphology

Adult males were cryoanesthetized and the reproductive system was dissected in 0.1 M sodium phosphate buffer at pH 7.2. After dissection, it was photographed with a stereoscopic microscope (Discovery V-20 Zeiss) coupled with an AxioCam MRc Zeiss camera (Zeiss, Goettingen, Germany). Length measurements of the structures were made with the software Image Pro-Plus version 4.5 (Media Cybernetics Inc., MD, USA).

Histological analysis

To obtain the histological sections, reproductive structures were fixed for twenty-four hours in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer. The material was washed for two hours in the same phosphate buffer, post-fixed in 1% osmium tetroxide for two hours, and dehydrated in a series of ethanol solutions (30%, 50%, 70%, 90%, 100%). Reproductive structures were embedded in a mixture of historesin (Leica Historesin) and alcohol in a proportion of 1:1 and then immersed in pure historesin. Semithin sections (0.5 µm thick) were obtained using a microtome Leica RM 2155 with a glass knife, after that, they were transferred to histological slides, stained with Harris hematoxylin, eosin, toluidine blue and washed in running water for one minute. The observations and photographs were made using an Olympus BX-60 microscope. All the measurements were obtained with the software Image Pro-Plus, version 4.5.

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Figure 1. *Collaria oleosa* male (dorsal view). Scale 1 mm

Results

Male reproductive system

The male reproductive system of the *C. oleosa* consists of a pair of testes, each one with two follicles. Each testis opens into a long vas deferens, which has the posterior extremity dilated, forming the seminal vesicle where the sperms are stored until mating. The seminal vesicles connect to the ejaculatory bulb, which is balloon-shaped (0.2 mm in diameter) and bounded to the accessory glands (Fig. 2A–D).

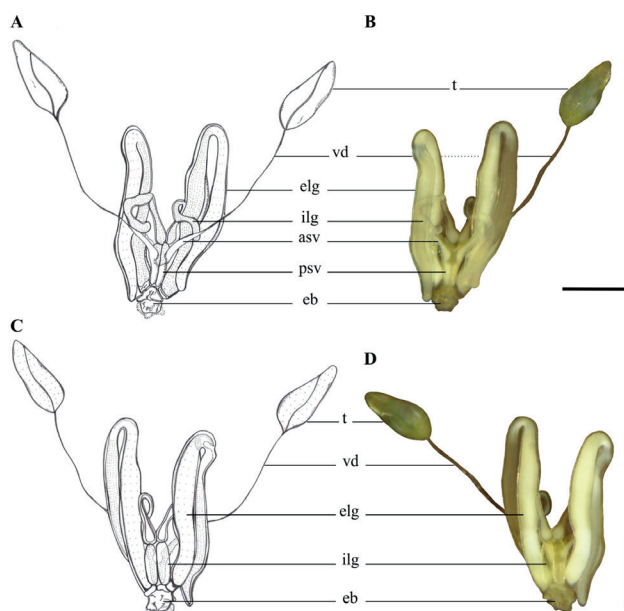


Figure 2. Overview of male reproductive system of *Collaria oleosa*. A–B, Diagram and light microscopy ($\times 40$) (dorsal view); C–D, Diagram and light microscopy ($\times 40$) (ventral view); testes (t); vas deferens (vd); anterior seminal vesicle (asv); posterior seminal vesicle (psv); external lateral gland (elg); internal lateral gland (ilg); ejaculatory bulb (eb). Scale, 0,5 mm

Morphology and Histology

The testes are elongated (~ 0.68 mm in length) $\times 0.26$ mm in width). Each testis consists of two follicles and is surrounded by a testicular capsule with green pigmentation (Fig. 2B and D). The images obtained from histological sections show that the testes of *C. oleosa* are surrounded by a peritoneal membrane, and cysts have been found inside the follicles, from the apex to the base, with spermatogonia, spermatocytes, spermatids, and spermatozoa (Fig. 3A). The spermatogonia are located at the apical region of the follicle, and the cysts with spermatocytes and spermatids are distributed along the follicle (Fig. 3B and C). Subsequently, cysts with spermatozoa were found in the region posterior to the periphery of the follicle (Fig. 3D).

The vasa deferentia are elongated (~ 0.96 mm in length) and form the seminal vesicles. These are divided by an asymmetric constriction in the anterior and posterior regions. The last one opens into the ejaculatory bulb. The vasa deferentia, especially in the vesicular region, exhibit a pearly white color due to the presence of spermatozoa (Fig. 2B and D). Histological analyses show that the seminal vesicles are surrounded by a single layer of epithelial cells. External to the epithelium is observed a well-developed muscle layer. The light of vesicular seminal is entirely filled by free spermatozoa, therefore, not disposed of in bundles (Fig. 4D).

The pair of external accessory glands are elongated with distinguishable ascending and descending arms, of a milky color. The internal accessory glands are shorter and are distinctly divided into two regions, the anterior and posterior regions. The anterior part is elongated and has the shape of a pear with a slight constriction that separates the posterior region. In essence, this last one is translucent, with a cylindrical shape (Fig. 2B and D). The walls of both gland types are lined by a single layer of columnar epithelium with at least two different cell types and externally enveloped by a thin muscle layer (Fig. 4A–C). The lumen of the external accessory gland has its secretion arranged inside of the spherical structures, whereas, in the internal accessory gland, the lumen has granular and irregular secretion (Fig. 4A–C).

Discussion

Some features of the male reproductive system of *C. oleosa*, such as, the elongated vasa deferentia, position of the seminal vesicles, presence of an ejaculatory bulb and accessory glands, resemble the other species of Stenodemini^{18,19}, however, among them there are variations, and they differ from other species usually in the number of testicular follicles.

In the Stenodemini tribe the modal number of testicular follicles is seven, however, it is possible to find species with three, (*Myrmecoris gracilis*, *Notostira elongata*, *N. erratica*, *Stenodema* (*Brachystira*) *calcarata*), six (*Dolichomiris linearis*, *Stenodema* (*Stenodema*) *laevigata*, *S. (S.) holsata*), or eight (*Leptopterna dolabrata*; *L. ferrugata*) testicular follicles^{21,19}. Our study showed that *C. oleosa* exhibited only two follicles, as found by Akingbohunge² in *Collaria meilleurii*, the presence of two follicles may be characteristic of the genus, however, studies on more species of *Collaria* in different regions where they are usually found (e.g. Nearctic, Neotropical and Afrotropical regions) are still necessary.

On the other hand, the number of follicles is an important characteristic that can be used to evaluate the age of the individual genus or species, such us, taxa with a more significant number of follicles are believed to be older than others with a smaller quantity. According to Akingbohunge and Wheeler^{2,30},

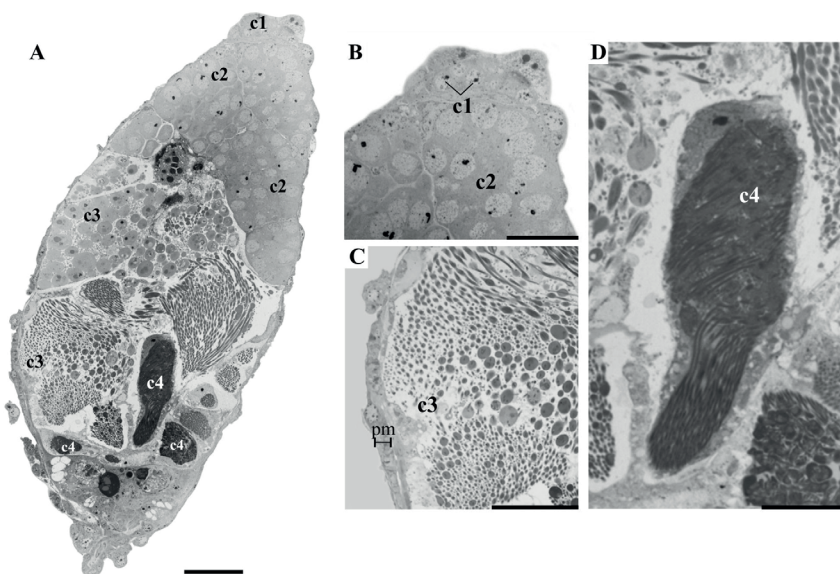
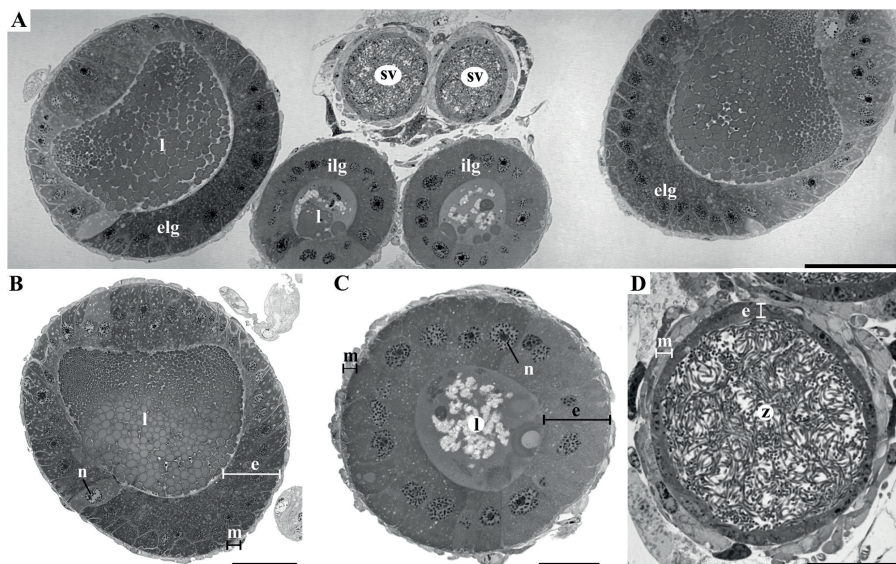


Figure 3. Longitudinal section of testis of *Collaria oleosa* showing cysts containing cells at different stages of spermatogenesis; A, Overview of testicular follicle; B, apical region with spermatogonia cysts (c1) and spermatocytes cysts (c2); C, middle region with spermatids cysts (c3); D, basal region with sperm cyst (c4). Peritoneal membrane (pm). Scales, A 30 μ m B–C 20 μ m; D 10 μ m

Figure 4. Male reproductive structures of *Collaria oleosa*, details of the transversal histological slices; A, Overview of seminal vesicle and accessory glands; B, external lateral gland; C, internal lateral gland; D, seminal vesicles (sv); external lateral gland (elg); internal lateral gland (ilg); lumen (l); muscle (m); nucleus (n); epithelium (e). Scales, A 70 μ m; B 30 μ m; C–D 20 μ m



lower amounts may be a plesiomorphic characteristic in the family. This number can be reduced in the process of oligomerization or can also increase in the process of polymerization¹⁹. In this study, the smaller number of follicles found in *C. oleosa* is probably a result of oligomerization, and hence, can be considered a young genus among the Stenodemini tribe. However, more analyses are necessary, because the cause of the variable follicle number in the Miridae family is not clear^{2,30}.

The testicular follicles have three developed regions: (1) the growth zone, where the spermatogonia become separated from the germarium and increase, allowing the occurrence of mitoses and differentiation into spermatocytes; (2) the maturation zone, where two meiotic divisions occur forming the spermatids; and (3) the differentiation zone, where the spermatids develop into spermatozoa⁷. In *C. oleosa* it is possible to find this structural organization, which is similar to that found in other mirids such as, *Adparapropa gabrieli* (Orthotylinae) and *Platyscytus decempunctatus* (Phylinae)^{26,29}.

In *C. oleosa* the vasa deferentia expand twice to form the anterior and posterior seminal vesicles. These structures are located in the posterior region of the vas deferens. This feature is also observed in the other species of Stenodemini^{18,19,20}; nevertheless, in the species of the Mirini tribe, the anterior seminal vesicle is frequently located in the middle of the vas deferens and is some distance away from the posterior seminal

vesicle^{19,28}. The sperms of *C. oleosa* in the seminal vesicle are not organized in bundles but are full and free within the lumen. In Miridae, no information exists in this respect; however, it has been reported that the sperms are arranged in bundles, as described for some species of Pentatomidae and Cimicidae^{9,22,23,24}.

The male accessory glands comprise two pairs of lateral ectodermal accessory glands (a pair of external lateral glands and a pair of internal lateral glands), besides two unpaired mesodermal medial glands (a dorsal medial gland and a ventral medial gland)^{15,19}.

In males of *C. oleosa*, it was only possible to observe a pair of lateral ectodermal accessory glands. The mesodermal medial glands were not observed in the histological preparations. Some functions of the accessory gland secretions are, facilitation of sperm transfer, to further insemination, sometimes playing a role in spermatophore production, producing some active peptides that stimulate ovary activation after mating, and disruption of female behavior^{7,10,12,13,25,26}.

The larger size of the ectodermal accessory glands observed in *C. oleosa* as well as the presence of basophilic and acidophilic secretion inside the glands suggests that the production of different substances may occur along the entire length of the glands and may be associated to the output of spermatophores. The presence of spermatophores in Miridae has been reported in *Lygus hesperus*, *L. lineolaris*, *Lygocoris pabulinus*,

Stenodema holsata, and *Stenotus rubrovittatus*^{3,4,11,9,21}; although, in *C. oleosa*, it never has been reported. Further investigations are necessary to confirm this statement.

The analysis of the male reproductive system in *C. oleosa* shows that there are similar characteristics among other species that belong to the Stenodemini tribe, and the main difference is in the number of testicular follicles. Nevertheless, additional studies on the species of this tribe and genus are necessary. Furthermore, the obtained results provide new information and also contribute to the knowledge of the male reproductive biology of Miridae. Some of the characteristics revealed may be helpful for future studies, in their taxonomy, phylogeny, reproductive physiology, and behavior, which have not yet been fully explored.

Conclusions

The *C. oleosa* male reproductive system consists of a pair of testes; each teste showed two follicles, one pair of seminal vesicles, two pairs of external accessory glands, and an ejaculatory bulb. The histological analysis showed that the testicular follicles have cysts with spermatogonia, spermatocytes, spermatids, and spermatozoa bundles. The presence of two follicles per testis on *C. oleosa* may be characteristic of the genus, however studies on more species of *Collaria* are still necessary. Morphological and histological analysis provides basic information about the male reproductive system of *C. oleosa*. These findings reveal the presence of characters that may contribute to future studies on the taxonomy and phylogeny of the Stenodemini tribe as well as the Miridae family, also could provide information to understand the biology of *C. oleosa* to search methods of controlling this pest.

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Bibliographic references

- Auad, A., Pimienta, D., Silva, D., Monteiro, P., Resende, T. 2011. *Collaria oleosa* (Hemiptera: Miridae) on *Brachiaria ruziziensis* and *Penisetum purpureum* (Poaceae): Characterization of injury and biological aspects. *Rev. Colomb. Entomol.* 37, 244–248.
- Akingbohunbe, A.E., 1983. Variation in testis follicle number in the Miridae (Hemiptera, Heteroptera) and its relationship to the higher classification of the family. *Ann. Entomol. Soc. Am.* 76, 37–43.
- Brent, C.S., 2010. Reproductive refractoriness in the western tarnished plant bug, *Lygus hesperus*. *Ann. Entomol. Soc. Am.* 102, 300–306.
- Carayon, J., 1984. Faits remarquables accompagnant l'insemination chez certains Hétéroptères Miridae. *Bull. Soc. Entomol. Fr.* 89, 982–998.
- Carlesii, L., Corseuil, E., Salvador, J. 1999. Aspectos Biológicos e Morfométricos de *Collaria scenica* (Stål) (Hemiptera: Miridae) em Trigo. *An. Soc. Entomol. Brasil.* 28, 65–73.
- Cassis, G., Schuh, R., 2012. Systematics, Biodiversity, Biogeography, and Host Associations of the Miridae (Insecta: Hemiptera: Heteroptera: Cimicomorpha). *Annu. Rev. Entomol.* 57, 377–404.
- Chapman, R.F., 1998. *The Insects: Structure and Function*. 4th edition. Cambridge Univ. Press, Cambridge.
- Da Silva, D., Alves, R., Ferreira, P., Camargo, A., 1994. *Collaria oleosa* (Distant, 1883) (Heteroptera: Miridae) uma praga potencial na cultura do trigo na região dos Cerrados. *Pesq. Agropec. Bra.* 29, 2007–2012.
- Davis N.T., 1956. The morphology and functional anatomy of the male and female reproductive systems of *Cimex lectularius* L. (Heteroptera, Cimicidae). *Ann. Entomol. Soc. Am.* 49, 466–493.
- Freitas, S.P.C., Gonçalves, T.C.M., Serrão, J.E., Santos-Mallet, J.R., 2007. Fine structure of the male accessory glands of *Triatoma rubrofasciata* (Hemiptera: Triatominae). *Microsc. Res. Tech.* 70, 355–360.
- Groot, A.T., Van Der Wal, E., Schuurman, A., Visser, J.H., Blommers, L.H.M., Van Beek, T.A., 1998. Copulation behavior of *Lygocoris pabulinus* under laboratory conditions. *Entomol. Exp. Appl.* 88, 219–228.
- Kubli, E., 2003. Sex-peptides: Seminal peptides of the *Drosophila* male. *Cell. Mol. Life Sci.* 60, 1689–1704.
- Leopold, R.A., 1976. The role of male accessory glands in insect reproduction. *Annu. Rev. Entomol.* 21, 199–221.
- Leston, D., 1961. Testis follicle number and the higher systematics of Miridae (Hemiptera: Heteroptera). *Proc. Zool. Soc. London.* 137, 89–106.
- Masner, P., 1965. The structure, function and the imaginal development of the male inner reproductive organs of *Adelphocoris lineolatus* (Goeze) (Heteroptera: Miridae). *Acta Entomol. Bohemoslov.* 62, 254–276.
- Menezes, M., 1990. *Collaria oleosa* (Distant, 1883) (Hemiptera: Miridae), nova praga de gramíneas forrageiras no sudeste de Bahia, Brasil. *Agrotrópica* 2, 113–118.
- Morales I., Ferreira P.S.F., Forero D., 2016. Taxonomic revision of *Collaria Provancher, 1872* (Hemiptera: Miridae) with the description of a new species from the Afrotropical region. *Zootaxa* 4138, 201–246.
- Mróz, E., 2007. Anatomical and molecular studies of *Stenodema* LAPORTE genus (Heteroptera: Miridae). *Genus, Supplement* 14, 77–81.
- Mróz, E., Wojciechowski, W., 2011. The systematic position the tribe Stenodemini (Heteroptera: Cimicomorpha: Miridae: Mirinae) in the light of the male internal reproductive system. *J. Nat. Hist.* 45, 1563–1588.
- Mróz, E., 2012. The structure of the male reproductive system of the genus *Psallus* FIEBER (Hemiptera: Heteroptera: Miridae). *Pol. Pismo Entomol.* 81, 3–10.
- Oku K., Kitsunozuka, K., 2011. Effects of male mating interval on spermatophore formation, transfer, and subsequent female receptivity and fecundity in the sorghum plant bug, *Stenotus rubrovittatus*. *Entomol. Exp. App.* 140, 134–138.
- Özyurt N., Candan S., Suludere Z., 2013a. The morphology and histology of the male reproductive system in *Dolycoris baccarum* Linnaeus 1758 (Heteroptera: Pentatomidae)—Light and scanning electron microscope studies. *Micron* 44, 101–106.
- Özyurt, N., Candan, S., Suludere, Z., Amutkan, D., 2013b. Morphology and histology of the reproductive system in *Graphosoma lineatum* (Heteroptera: Pentatomidae) based on optical and scanning electron microscopy. *J. Entomol. Zool. Stud.* 1, 40–46.
- Özyurt, N., Candan, S., Suludere, Z., 2014. The morphology and histology of the male reproductive system in *Apodiphus amygdali* (Germar, 1817) (Hemiptera: Heteroptera: Pentatomidae). *Life Excit. Biol.* 2, 31–41.
- Pendergrast, J.G., 1957. Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification. *Trans. R. Entomol. Soc. Lond.* 109, 1–63.
- Pires, E.M., Ferreira P.S.F., Guedes, R.N.C., Serrão, J.E., 2007. Morphology of the Phytophagous Bug *Platyscytus decempunctatus* (Carvalho) (Heteroptera: Miridae). *Neotrop. Entomol.* 36, 510–513.
- Schuh, R.T., 2002–2013. On-line Systematic Catalog of Plant Bugs (Insecta: Heteroptera: Miridae). <http://research.amnh.org/pbi/catalog/>
- Spurgeon, D.W., Brent, C.S., 2010. Morphological Characters of Diapause in *Lygus hesperus* Knight (Hemiptera: Miridae). *J. Entomol. Sci.* 45, 303–316.
- Uceli, L.F., Pirovani, V.D., Vicente, N.M.F., Pikart, T.G., Ferreira, P.S.F., Serrão, J.E., 2011. Morphology of the reproductive and digestive tracts of *Adparapropa gabrieli* (Heteroptera: Miridae). *Int. J. Trop. Insect Sci.* 31, 219–224.
- Wheeler A.G. Jr., 2001. *Biology of the Plant Bugs* (Hemiptera: Miridae). Pests, Predators, Opportunists. Comstock Publishing Associates. Cornell Univ. Press, Ithaca, New York.

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RESEARCH / INVESTIGACIÓN

Hipertensión arterial, diabetes mellitus tipo 2 y su asociación con el estado nutricional en adultos ecuatorianos

High blood pressure, type 2 diabetes mellitus and its association with nutritional status in Ecuadorian adults

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Resumen: La hipertensión arterial (HTA) y diabetes mellitus tipo 2 (DM2) representan las mayores causas de mortalidad mundial. El estado nutricional está fuertemente asociado con alteraciones en la composición corporal que influyen en el desarrollo de estas patologías, por ello el objetivo del estudio fue determinar la asociación de la hipertensión arterial y diabetes mellitus tipo 2 con el estado nutricional de pacientes que asisten al Servicio de Nutrición del Hospital San Vicente de Paúl, Ibarra-Ecuador. Presentamos un estudio descriptivo, transversal en pacientes adultos con diagnóstico médico de HTA o DM2. Se comparó el índice de masa corporal (IMC), porcentaje grasa corporal (%GC), circunferencia de cintura (Cc) y edad con la presencia o no de HTA o DM2. Se identificaron 198 adultos con edad de 47.28 ± 10.57 años; 87.10% fueron mujeres. El 30.80% presentaban HTA y el 27.27% DM2. El IMC ($p=0.002$), %GC y Cc ($p<0.001$) presentaron diferencias estadísticamente significativas entre pacientes con y sin HTA, no así con la presencia o ausencia de DM2. La edad fue significativamente distinta entre sujetos con y sin presencia de HTA o DM2, mientras que, el sexo no mostró asociación significativa con ninguna de las patologías. Tres de cada diez pacientes que acuden al Servicio de Nutrición padecen de HTA o DM2, existen diferencias significativas en el IMC, %GC y Cc entre hipertensos y no hipertensos. No se encontraron diferencias significativas en el estado nutricional de pacientes con o sin diabetes.

Palabras clave: diabetes mellitus tipo 2, índice de masa corporal, estado nutricional, hipertensión.

Abstract: Hypertension and type 2 diabetes mellitus (T2DM) represents the significant causes of world mortality. Nutritional status is strongly associated with alterations in body composition that influence the development of these pathologies, so the objective of the study was to determine the association of arterial hypertension and type 2 diabetes mellitus with the nutritional status of patients attending the Nutrition Service of the San Vicente de Paúl Hospital, Ibarra-Ecuador. A descriptive, cross-sectional study in adult patients with a medical diagnosis of hypertension or T2DM. The body mass index (BMI), body fat percentage (BFP), waist circumference (WC), and age were compared with the presence or absence of hypertension or DM2. One hundred ninety-eight adults with age 47.28 ± 10.57 years were identified; 87.10% were women. 30.80% presented HTA and 27.27% T2DM. The BMI ($p=0.002$), BFP, and WC ($p<0.001$) showed statistically significant differences between patients with and without hypertension, but not with the presence or absence of T2DM. Age was significantly different between subjects with and without the presence of hypertension or T2DM, while sex showed no significant association with any of the pathologies. Three out of ten patients who go to the Nutrition Service suffer from hypertension or type 2 diabetes mellitus; there are significant differences in BMI, BFP, and WC between hypertensive and non-hypertensive patients. Significant differences were not found in the nutritional status of patients with or without diabetes.

Key words: type 2 diabetes mellitus, body mass index, nutritional status, hypertension.

Introducción

La hipertensión arterial (HTA) es una de las principales causas de las muertes por enfermedades cardiovasculares (ECV) a nivel mundial con aproximadamente 17 millones de muertes por año, de las cuales 9.4 millones se le atribuyen específicamente a la HTA¹. A nivel latinoamericano 1.6 millones de defunciones anuales se le asignan a la HTA y 500 mil de estas son en menores de 70 años², afectando al rededor del 20 a 40% de adultos³. Se ha estimado que el 25,8% de adultos ecuatorianos de zonas urbanas padecen de HTA, siendo esta ligeramente mayor en hombres⁴. Sin embargo, poco se conoce de estudios actualizados sobre este problema de salud en nuestro medio.

Por otro lado, la diabetes mellitus tipo 2 (DM2) presenta una prevalencia mundial de 108 millones en mayores de 18 años, con mayor crecimiento en países con ingresos bajos y medios⁵, afectando así al índice de desarrollo humano y debido a sus causas multifactoriales su manejo es complejo⁶.

De la misma manera en que la HTA y la DM2 han ido en aumento, las cifras de sobrepeso y obesidad también han sufrido un incremento relevante, ya que más de 1 900 millones de adultos en el mundo presentan estas condiciones⁷, y el 63,6% de ecuatorianos de la Sierra urbana entre 19 y 59 años presentan sobrepeso u obesidad⁸. El excesivo incremento de peso en forma de grasa corporal y el aumento de la circunfe-

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rencia de la cintura (Cc) se han relacionado con el desarrollo de la HTA y de la DM2⁹.

Otro de los aspectos que contribuye al desarrollo de HTA y DM2 es el sedentarismo¹⁰, ya que la inactividad física sumada a la causa esencial de un consumo dietético excesivo conduce a un aumento de peso no saludable incrementando de este modo la probabilidad de padecer enfermedades crónicas no transmisibles.

Bajo esta perspectiva, este estudio tuvo como objetivo determinar la asociación de la HTA y DM2 con el estado nutricional de los pacientes que asisten a consulta externa del Servicio de Nutrición del Hospital San Vicente de Paúl (HSVP) en Ibarra-Ecuador.

Materiales y métodos

Estudio descriptivo, transversal a través de muestreo por conveniencia en un periodo de 6 meses comprendidos entre el último trimestre del 2018 y el primer trimestre del 2019. Se recolectaron datos de pacientes referidos al Servicio de consulta externa de Nutrición mediante interconsultas desde los distintos servicios de especialidades médicas del HSVP de Ibarra-Ecuador y casi en su totalidad debido a problemas de sobrepeso u obesidad, así como enfermedades crónicas no transmisibles.

Se incluyeron pacientes entre 18 y 64 años con diagnóstico médico definitivo de HTA o DM2 a través de CIE10 (I10 y E11 respectivamente)¹¹ que después de ser informados accedieran a firmar el consentimiento. De un total de 209 pacientes se descartaron 11 debido a datos incompletos y se obtuvo un número final de 198 pacientes. No se incluyeron embarazadas ni aquellos con patologías catastróficas o psiquiátricas.

La talla se obtuvo con un tallímetro incorporado en la báscula de columna digital Seca® 703; la Cc se midió localizando el punto medio entre la cresta iliaca y la última costilla mediante una cinta métrica metálica Lufkin® W606PM; el peso, %GC e IMC se obtuvieron con una balanza de bioimpedancia eléctrica Tanita® BF679F con capacidad máxima de 150 kg. Para estas medidas se siguió estrictamente las normativas de la Organización Mundial de la Salud (OMS).

Los puntos de corte para el IMC se establecieron en base a las recomendaciones de la OMS como normal (18.5 – 24.9 Kg/m²), sobrepeso (25 – 29.9 Kg/m²), obesidad I (30 – 34.5 Kg/m²), obesidad II (35 – 39.9 Kg/m²) y obesidad III (≥ 40 Kg/m²). Además, se recolectaron datos sociodemográficos y de actividad física diaria la cual se clasificó como No, < 30 minutos, 30 - 60 minutos y > 60 minutos.

Para el análisis estadístico se utilizó el programa SPSS versión 23 para Windows en español. Se efectuaron comparaciones de medias a través de t-Student para las variables con distribución normal y la U de Mann Whitney para aquellas que no cumplieran este supuesto. Se ejecutó la prueba de Chi-cuadrado para variables cualitativas dicotómicas y no dicotómicas; valores p < 0.05 se consideraron significativos.

Resultados

Se recolectaron datos de un total de 198 pacientes de los cuales el 87.10% fueron de género femenino (n=172), la edad media de la muestra de estudio fue de 47.28 ± 10.57 años y más de la mitad era de estado civil casado (62.40%). El 62.38% de los pacientes refirieron no realizar algún tipo de actividad física, mientras que, solo el 9.41% y 9.90% la practican de 30

a 60 minutos y por más de 60 minutos correspondientemente Tabla 1. EL 30,80% y 27,27% de los estudiados presentaban HTA o DM2 respectivamente. Adicionalmente, el 16.16% de los estudiados tuvieron sobrepeso, en tanto que, el 83.84% presentaba obesidad en los distintos grados. Las puntuaciones medias de las variables antropométricas tomadas fueron: IMC 36.08 ± 6.21 kg/m², Cc 107, 87 ± 12.96 cm y %GC 47.34 ± 9.47 % Tabla 2.

Variable	% (n=198)	Media ± DE
Sexo femenino	87.10	
Edad		47.28±10.57
EC: Casado	62.40	
EC: Soltero	27.70	
EC: Otros ^a	9.90	
AF: No	62.38	
AF: < 30 min	18.32	
AF: 30 - 60 min	9.41	
AF: > 60 min	9.90	

DE, desviación estándar; EC, estado civil; AF, actividad física.

^a Unión libre, divorciado, viudo.

Tabla 1. Características de la muestra estudiada.

Variable	% (n=198)	Media ± DE
HTA	30.80	
DM2	27.27	
Sobrepeso	16.16	
Obesidad I	31.82	
Obesidad II	28.79	
Obesidad III	23.23	
IMC (kg/m²)		36.08±6.21
Cc (cm)		107.87±12.96
%GC		47.34±9.47

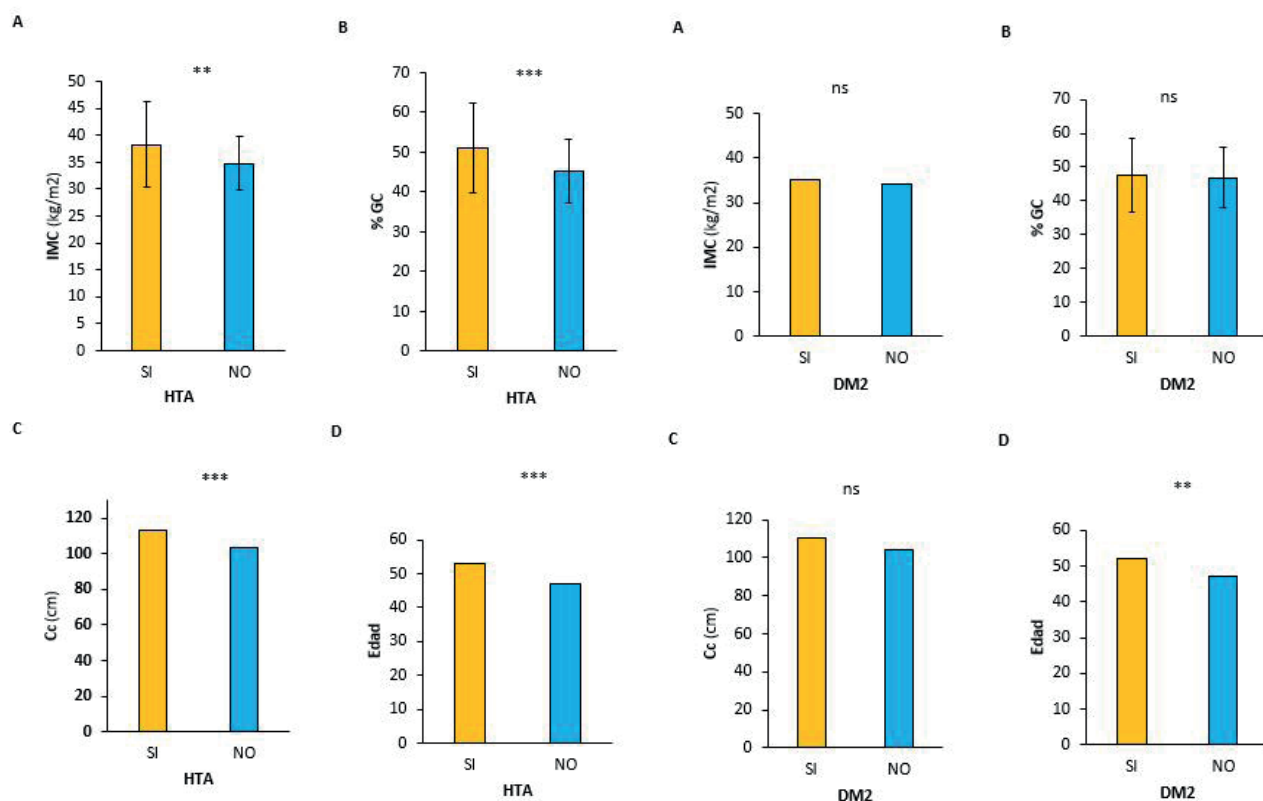
DE, desviación estándar; HTA, hipertensión arterial; DM2, diabetes mellitus tipo 2; IMC, índice de masa corporal; Cc, circunferencia de la cintura; %GC, porcentaje de grasa corporal.

Tabla 2. Prevalencia de HTA, DM2 e indicadores antropométricos.

El IMC continuo fue significativamente diferente entre los pacientes con y sin diagnóstico de HTA (38.28 ± 7.91 kg/m² vs 34.80 ± 7.08 kg/m², p=0.002) Figura 1A. El %GC fue más alto en los pacientes con HTA (51.10 ± 11.30%) frente a los que no presentaban HTA (45.28 ± 8.03 %) siendo significativamente diferentes (p < 0.001) Figura 1B. Análogamente, las medianas de la Cc y edad también fueron significativamente diferentes (p < 0.001) entre los diagnosticados con HTA en comparación a aquellos sin esta patología Figuras 1C y 1D.

En relación con el sexo de los pacientes con y sin presencia de HTA o DM2 este no presentó una relación significativa en ninguno de los casos. Al realizar el análisis del IMC categórico frente a la HTA y DM2 se observó que este se asociaba significativamente con la HTA (p=0.002), siendo mayor la proporción de pacientes con HTA en el grado de obesidad III (39.34% vs 16.06%), sin embargo, esto no sucedió con respecto a la DM2 Tabla 3.

El IMC continuo, el %GC y la Cc no fueron significativamente diferentes entre los pacientes con y sin DM2 Figuras 2A – C, mientras que, la edad fue notablemente distinta entre



IMC, índice de masa corporal; HTA, hipertensión arterial; %GC, porcentaje de grasa corporal; Cc, circunferencia de la cintura.

** $p < 0.01$; *** $p < 0.001$ (Figuras 1A y 1B, t-Estudent; Figuras 1C y 1D, U de Mann Whitney)

Figura 1. Indicadores antropométricos y edad en pacientes con y sin hipertensión arterial.

IMC, índice de masa corporal; HTA, hipertensión arterial; %GC, porcentaje de grasa corporal; Cc, circunferencia de la cintura.

ns = no significativo (Figuras 1A y 1C, U de Mann Whitney; 1B, t-Estudent); ** $p < 0.01$ (Figura 1D, U de Mann Whitney)

Figura 2. Indicadores antropométricos y edad en pacientes con y sin diabetes mellitus tipo 2.

Parámetro	HTA		Valor p	DM2		Valor p
	SI (%) n=61	NO (%) n=137		SI (%) n=54	NO (%) n=144	
Sexo						
Masculino	14.28	12.24	0.422	19.64	10.27	0.064
Femenino	85.72	87.76		80.36	89.73	
IMC						
Sobrepeso	11.48	18.25	0.002**	20.37	14.58	0.395
Obesidad I	19.67	37.23		24.07	34.72	
Obesidad II	29.51	28.47		31.48	27.78	
Obesidad III	39.34	16.06		24.07	22.92	

IMC, índice de masa corporal; HTA, hipertensión arterial; DM2, diabetes mellitus tipo 2.

** $p < 0.01$ (significativo) a través de χ^2 de Pearson.

Tabla 3. Relación entre el sexo y el índice de masa corporal frente a la hipertensión arterial y diabetes mellitus tipo 2.

este tipo de pacientes ($p = 0.002$), siendo más alta la mediana de la edad en los pacientes que presentaban DM2 (52 vs 47 años) Figura 2D.

sentaron HTA, siendo 5% mayor a los hallazgos realizados en el 2016 en una zona urbana de la ciudad de Cuenca, ubicada en la misma región ecuatoriana, donde se identificó al 25.8% de adultos con HTA⁴. Esta diferencia podría explicarse debido al ámbito de proveniencia de los datos y el grado de similitud de los participantes en nuestro estudio en cuanto a su estado nutricional (sobrepeso y obesidad), condiciones en las que son

Discusión

En esta investigación el 30.80% de los estudiados pre-

referidos al Servicio de Nutrición.

Otro estudio realizado en una zona andina de Perú en el 2018, identificó al 21% de adultos entre 20 y 59 años con HTA de los cuales el 35.8% y 43.3% tenían sobrepeso u obesidad respectivamente¹². A pesar de ello, las cifras de HTA del actual estudio siguen siendo superiores en comparación a los mencionados.

En otros países cercanos como Argentina se encontró que el 32,34% de adultos tenían HTA y el 43.10% de estos tenían sobrepeso¹³, mientras que en Brasil el 29.9% de adultos padecen de HTA y al igual que en el actual estudio su presencia se relacionó con una edad $\geq$ a 40 años y poseer un IMC de sobrepeso u obesidad¹⁴. Análogamente, otro estudio en el mismo país encontró que una edad $\geq$ a 30 años, poseer sobrepeso y tener diabetes aumenta la probabilidad de padecer HTA¹⁵.

En México la Encuesta Nacional de Salud y Nutrición (ENSANUT) en el 2016 identificó al 25.5% de adultos con HTA, de los cuales el 40% no sabía que la tenía¹⁶. A nivel de Iberoamérica y el Caribe una revisión de 33 publicaciones sobre la HTA encontró que el 32.6% de los adultos tenían esta condición¹⁷. A diferencia de esto en Haití la prevalencia de HTA parece ser la más baja con el 20.3% de adultos hipertensos¹⁸.

Estos hallazgos confirman que la presencia de HTA encontrada en este estudio concuerda con las asociaciones significativas evidenciadas entre la edad, estado nutricional a través del IMC, el %GC y la Cc, cuyos valores fueron superiores en pacientes con diagnóstico de HTA frente a los no hipertensos. Además, se conoce que a mayor edad y %GC existe una mayor asociación con la hipertensión en adultos¹⁹.

Respecto al género y su relación con la HTA, no se observó una asociación estadísticamente significativa, esto podría ser atribuido a la no igualdad entre la cantidad de hombres y mujeres que integran este estudio. Sin embargo, se sabe que a nivel mundial varios estudios epidemiológicos describen que el género femenino es menos prevalente ante esta patología²⁰.

En lo referente a la DM2 el 27.70% de los estudiados presentaron esta condición, según las estadísticas nacionales esta enfermedad ocupa el puesto número dos de mortalidad general y representa la primera causa de mortalidad para el sexo femenino y tercera causa para el sexo masculino²¹.

A diferencia de este hallazgo un estudio reciente realizado en parroquias urbanas de la ciudad de Cuenca en la provincia de Azuay en 318 adultos solo identificó al 5.7% de estas personas con DM2²². Esta enorme diferencia podría deberse al igual que en el caso de la HTA al ámbito de procedencia de los datos, así como a la distribución geográfica. Por otro lado se conoce que las áreas urbanas presentan mayor prevalencia de DM2 en comparación a las rurales²³.

Del mismo modo que en la presente investigación se encontró diferencias significativas entre la edad de los pacientes con y sin DM2, siendo los adultos de 40 a 59 años las personas con mayor riesgo de presentar esta patología. Hay que recalcar que, en este estudio la edad fue la única variable con diferencias significativas respecto a los individuos con y sin diagnóstico de DM2.

Se conoce que un IMC alto se relaciona con la presencia de DM2 debido al excesivo aumento de peso corporal, a pesar de esto algunas investigaciones han demostrado que el %GC evidencia mayor asociación con el riesgo de esta enfermedad²⁴, puesto que a diferencia del IMC este evalúa específicamente el compartimento graso del cuerpo y discrimina entre el peso muscular y graso, situación que no sucede con el IMC.

Otra ventaja de evaluar el %GC es su sensibilidad para identificar a individuos clasificados como normales según el IMC, pero que poseen un alto %GC, logrando de este modo

tomar acciones correctoras a tiempo. Ante esto un estudio identificó que personas con un IMC normal, pero con alto %GC ya poseían alteraciones de glucosa en sangre y se estima que alrededor del 64% de personas catalogadas con un estado nutricional normal según el IMC poseen un %GC alto²⁵.

Según otro estudio sobre el %GC y su asociación con la DM2 se determinó que un porcentaje $\geq$ al 22.8% en hombres y $\geq$ al 32.9% en mujeres aumentaba significativamente el riesgo de DM2. Razón por la cual, hacer uso del %GC junto con el IMC sería una buena estrategia para la identificación de riesgos en sujetos que aparentemente se encuentran en un rango de normalidad²⁶.

Curiosamente, en este estudio no se encontró diferencias significativas entre el IMC, %GC o Cc entre pacientes con y sin DM2, en parte este comportamiento podría explicarse debido al tipo de pacientes que acuden al Servicio de Nutrición y como anteriormente se ha descrito casi la totalidad de ellos presentan obesidad o sobrepeso.

Sin duda alguna, otro aspecto que en esta investigación llama la atención es el alto porcentaje de pacientes que no realizan actividad física (62.38%). Tales datos concuerdan con los encontrados en un hospital docente donde el 54.3% de los usuarios refirieron inactividad física, razón por la cual, los porcentajes de sobrepeso y obesidad superaron el 54% y 40.4% respectivamente, además la prevalencia de HTA fue de 34.3%, dato similar con nuestro estudio²⁷.

Las altas cifras de sedentarismo en la actual sociedad han aumentado debido en parte a los avances tecnológicos principalmente en lo relacionado con el desplazamiento y entretenimiento. De forma paralela al aumento del sedentarismo la prevalencia de obesidad, DM2, HTA y demás ECV han incrementado significativamente²⁸ y recientemente un estudio en adultos brasileños mostró que un tiempo de sedentarismo (mirar televisión) mayor a 4 horas diarias se asocia con mayor riesgo de DM2, HTA y enfermedad cardíaca²⁹.

Frente a este panorama la concientización a la población sobre los beneficios de la práctica regular de actividad física podría ser de utilidad para prevenir o reducir las muertes causadas por la ECV, DM2 y algunos tipos de cáncer. Adicionalmente, efectos beneficiosos en la composición corporal, parámetros bioquímicos y sobre el estrés se le han otorgado a la práctica de actividad física³⁰.

La información presentada en este estudio sobre la asociación de la HTA y DM2 con el estado nutricional puede ser de importancia al momento de dirigir estrategias de salud pública en el ámbito de la prevención. Adicionalmente, podría ayudar a identificar la prevalencia de algunos de los 5 componentes del síndrome metabólico establecidos en el consenso entre la Federación Internacional de Diabetes (IDF), la Asociación Americana del Corazón (AHA) y el Instituto Nacional del Corazón, los Pulmones y la Sangre (NHLBI), los cuales son obesidad abdominal a través de Cc, tener HTA o consumir fármacos hipotensores, colesterol HDL disminuido, triglicéridos elevados y cifras de glucosa en ayunas elevadas o tener diabetes³¹. Nuestro estudio muestra datos sobre 3 de los 5 componentes mencionados.

Entre las limitaciones de esta investigación vale mencionar que las cifras de HTA y DM2 podrían estar sobreestimadas debido al origen de los datos ya que provienen del ámbito hospitalario; otra desventaja es no disponer de datos actualizados sobre estas problemáticas de salud a nivel nacional con los cuales realizar una comparación más realista.

Los datos disímiles en el número de hombres y mujeres no permitieron comparar la asociación de la HTA y DM2 según el sexo de los estudiados, sin embargo, este comportamiento

es en cierta manera característico de las casas de salud ecuatorianas donde las mujeres son las que acuden en mayor proporción a los controles subsecuentes de salud.

Conclusiones

Tres de cada diez pacientes que acuden al Servicio de Nutrición del Hospital San Vicente de Paúl presentan hipertensión arterial o diabetes mellitus tipo 2 y en su totalidad presentan sobrepeso u obesidad. El estado nutricional a través del IMC, porcentaje de grasa corporal y circunferencia de cintura son significativamente diferentes entre pacientes con y sin diagnóstico de hipertensión arterial, siendo estos más altos en los primeros, mientras que, estos no fueron distintos en aquellos con o sin diabetes mellitus tipo 2. Estos hallazgos muestran la necesidad de realizar más estudios de este tipo en el ámbito hospitalario y el trabajo conjunto que debe existir entre médicos, nutricionistas y demás personal sanitario de las casas de salud.

Referencias bibliográficas

1. Organización Mundial de la salud. Información general sobre la HIPERTENSIÓN en el mundo. Una enfermedad que mata en silencio, una crisis de salud pública mundial. 2013.
2. World Health Organization. GLOBAL STATUS REPORT on non-communicable diseases 2014. 2014.
3. Schargrofsky H, Hernández-Hernández R, Champagne BM, Silva H, Vinuesa R, Silva Aygüer LC, et al. CARMELA: Assessment of Cardiovascular Risk in Seven Latin American Cities. *Am J Med*. 2008 Jan;121(1):58-65.
4. Ortiz-Benavides RE, Torres-Valdez M, Sigüencia-Cruz W, Añez-Ramos R, Salazar-Vílchez J, Rojas-Quintero J, et al. Factores de riesgo para hipertensión arterial en población adulta de una región urbana de Ecuador. *Rev Peru Med Exp Salud Pública*. 2016;33(2):248-55.
5. World Health Organization. Informe Mundial de la diabetes. 2016.
6. Mendoza Romo MÁ, Padrón Salas A, Cossío Torres PE, Soria Orozco M. Prevalencia mundial de la diabetes mellitus tipo 2 y su relación con el índice de desarrollo humano. *Rev Panam Salud Pública*. 2018;47(103):1-6.
7. World Health Organization. Obesity and overweight. 2017.
8. Freire W, Ramírez-Luzuriaga M, Belmont P, Mendieta M, Silva-Jaramillo M, Romero N, et al. Tomo I: Encuesta Nacional de Salud y Nutrición de la población ecuatoriana de cero a 59 años. ENSANUT-ECU 2012. Ministerio de Salud Pública/Instituto Nacional de Estadísticas y Censos. Vol. 1. Quito-Ecuador; 2014. 251-53 p.
9. Rubio-Guerra A, Narváez-Rivera J. Hipertensión arterial en el paciente obeso. *Arch en Med Fam*. 2017;19(3):69-80.
10. Paredes DR, Orraca CO, Marimón TE, Casanova MM, González VL. Influencia del sedentarismo y la dieta inadecuada en la salud de la población pinareña. *Rev Ciencias Médicas*. 2014;18(2):221-30.
11. Organización Panamericana de la Salud, Organización Mundial de la Salud. CIE-10 Clasificación Estadística Internacional de Enfermedades y Problemas Relacionados con la Salud. 2003;(554):75-6.
12. Adams KJ, Chirinos JL. PREVALENCIA DE FACTORES DE RIESGO PARA SÍNDROME METABÓLICO Y SUS COMPONENTES EN USUARIOS DE COMEDORES POPULARES EN UN DISTRITO DE LIMA, PERÚ. *Rev Peru Med Exp Salud Pública*. 2018;35(1):39-45.
13. Díaz A, Ferrante D. Trends in prevalence of hypertension in Argentina in the last 25 years: a systematic review of observational studies. *Rev Panam Salud Pública*. 2015;38(6):496-503.
14. Lessa Í, Magalhães L, Araújo MJ, De N, Filho A, Aquino E, et al. Arterial Hypertension in the Adult Population of Salvador (BA) -Brazil *. *Arq Bras Cardiol*. 2006;87(6):747-56.
15. Barbosa JB, Augusto A, Miranda A, Monteiro C, Barbosa MM, Barbosa MM, et al. Original Article Prevalence of Arterial Hypertension and Associated Factors in Adults in São Luís, State of Maranhão. *Arq Bras Cardiol*. 2008;91(4):236-42.
16. Campos-Nonato I, Hernández-Barrera L, Pedroza-Tobías A, Medina C, Barquera S. Hipertensión arterial en adultos mexicanos: prevalencia, diagnóstico y tipo de tratamiento. *Ensanut MC 2016. Salud Pública Mex*. 2018;60(3):233-43.
17. Díaz AA, Tringler MF. Prevalence of hypertension in rural populations from Ibero-America and the Caribbean. *Rural Remote Health*. 2014;14(1):1-9.
18. Tymeczyk O, McNairy ML, Petion JS, Rivera VR, Dorélien A, Peck M, et al. Hypertension prevalence and risk factors among residents of four slum communities: population- representative findings from Port-au-Prince, Haiti. *J Hypertens*. 2018;37(4):685-95.
19. Han TS, Al-Gindan YY, Govan L, Hankey CR, Lean ME. Associations of body fat and skeletal muscle with hypertension. *J Clin Hypertens*. 2019;21(2):230-8.
20. Sandberg K, Ji H. Sex differences in primary hypertension. *Biol Sex Differ*. 2012;3(1):7.
21. Instituto Nacional de Estadística y Censos. Anuario de estadísticas vitales nacimientos y defunciones 2014. Quito-Ecuador; 2014. 586 p.
22. Cordero G, Álvarez R, Añez R, Rojas J, Bermúdez V. Prevalencia de la diabetes mellitus tipo 2 y sus factores de riesgo en individuos adultos de la ciudad de Cuenca- Ecuador. *Avan Biomed*. 2017;6(1):10-21.
23. Aryal KK, Mehata S, Neupane S, Vaidya A, Dhimal M, Dhakal P, et al. The burden and determinants of non communicable diseases risk factors in Nepal: Findings from a nationwide STEPS survey. *PLoS One*. 2015;10(8):1-18.
24. Han TS, Al-Gindan YY, Govan L, Hankey CR, Lean ME. Associations of BMI, waist circumference, body fat, and skeletal muscle with type 2 diabetes in adults. *Acta Diabetol*. 2019;56(8):947-954.
25. Jo A, Mainous AG. Informational value of percent body fat with body mass index for the risk of abnormal blood glucose: A nationally representative cross-sectional study. *BMJ Open*. 2018;8(4):1-6.
26. Park SK, Ryoo J-H, Oh C-M, Choi J-M, Jung JY. Longitudinally evaluated the relationship between body fat percentage and the risk for type 2 diabetes mellitus: Korean Genome and Epidemiology Study (KoGES). *Eur J Endocrinol*. 2018;178(5):513-21.
27. Nelson F, Nyarko K, Binka F. Prevalence of Risk Factors for Non-Communicable Diseases for New Patients Reporting to Korle-Bu Teaching Hospital. *Ghana Med J*. 2015;49(1):12-8.
28. Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Impact of Physical Inactivity on the World's Major Non- Communicable Diseases. *Lancet*. 2012;380(9838):219-29. Werneck AO, Cyrino ES, Collings PJ, Ronque ER, Szwarcwald CL, Sardinha LB, et al. TV Viewing in 60,202 Adults From the National Brazilian Health Survey: Prevalence, Correlates, and Associations With Chronic Diseases. *J Phys Act Heal*. 2018;15(7):510-5.
29. Werneck AO, Cyrino ES, Collings PJ, Ronque ER, Szwarcwald CL, Sardinha LB, et al. TV Viewing in 60,202 Adults From the National Brazilian Health Survey: Prevalence, Correlates, and Associations With Chronic Diseases. *J Phys Act Heal*. 2018;15(7):510-5.
30. Milton K, Macniven R, Bauman A. Review of the epidemiological evidence for physical activity and health from low- and middle-income countries. *Glob Public Health*. 2014;9(4):369-81.
31. Eugenia V, García B, Del M, Rivera Barragán R, Carmen M Del, Soto Olivares V, et al. Clinical components of Metabolic Syndrome. *Rev Médica la Univ Veracruzana*. 2015;2(15):67-89.

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CASE REPORTS / REPORTE DE CASO

Bloqueo auriculo-ventricular avanzado y bradicardia extrema como síntomas iniciales de Covid-19: Reporte de un caso

Advanced atrioventricular block and extreme bradycardia as initial symptoms of Covid-19: A case report

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Resumen: Estamos viviendo la pandemia generado por el beta coronavirus SARS-CoV-2, sus manifestaciones clínicas son fundamentalmente respiratorias. Sin embargo, existen pacientes que podrían debutar con clínica distinta a la respiratoria, por ejemplo, gastrointestinal (diarrea); alteraciones sensitivas en el gusto y el olfato (anosmia, disgeusia) y cardiovasculares (arritmias, muerte súbita cardíaca, trastornos de la conducción), saliendo de esta manera de la presentación típica. Los síntomas cardiovasculares y específicamente las alteraciones cardíacas, podrían ser causados por una mayor cantidad de receptores para la enzima convertidora de angiotensina 2 (ACE2), que es el sitio de unión del virus a las células humanas. Se presenta un caso con diagnóstico confirmado de COVID -19, de una paciente adulta mayor, con comorbilidad cardiovascular, que debuta con arritmias y bloqueo cardíaco.

Palabras clave: COVID-19, SARS-CoV-2, arritmias cardíacas.

Abstract: We are experiencing the pandemic generated by the beta coronavirus SARS-CoV-2; its clinical manifestations are mainly respiratory. However, some patients could debut with clinical symptoms other than respiratory, for example, gastrointestinal (diarrhea); Sensory changes in taste and smell (anosmia, dysgeusia), and cardiovascular (arrhythmias, sudden cardiac death, conduction disorders), thus leaving the typical presentation.

Cardiovascular symptoms, and specifically cardiac disturbances, could be caused by an increased number of angiotensin-converting enzyme 2 (ACE2) receptors, which is the site of binding of the virus to human cells.

We present a case with a confirmed diagnosis of COVID -19, of an older adult patient with cardiovascular comorbidity, debuting with arrhythmias and heart block.

Key words: COVID-19, SARS-CoV-2, cardiac arrhythmias.

Introducción

Las manifestaciones clínicas del SARS-CoV-2, están dominadas por síntomas respiratorios, sin embargo, existe un número no despreciable de pacientes que podrían debutar con clínica primaria de daño cardiovascular, saliendo de esta manera de la presentación típica que es la sintomatología respiratoria¹.

El SARS-CoV-2, así como el SARS-CoV, infectan a las células huésped por la unión de su proteína espiga a los receptores ACE2 (enzima convertidora de angiotensina 2), receptores estos que se encuentran predominantemente en pulmón y corazón. Estos receptores ACE2 tienen una importante función en el sistema cardiovascular e inmune, y se los ha asociado al desarrollo de hipertensión arterial y diabetes mellitus. El SARS-CoV-2 invade principalmente las células epiteliales alveolares, lo que conduce obviamente a compromiso respiratorio con la sintomatología respiratoria clásica. Estos síntomas son más graves en pacientes adultos mayores con enfermedad cardiovascular y comorbilidades preexistentes tales como: hipertensión arterial, enfermedad coronaria, insuficiencia cardíaca, diabetes². Por tal razón no es de extrañar que en este grupo de pacientes exista una mayor proporción de muertes³.

El mecanismo de lesión aguda del miocardio podría responder a una mayor secreción de ACE2. De igual forma se ha planteado la posibilidad de una mayor expresión de receptores ACE2

en los pacientes que usan inhibidores del sistema renina-angiotensina-aldosterona, por lo que se podría considerar población de mayor riesgo, aseveración que aún no ha sido definida².

Otros mecanismos propuestos para la lesión miocárdica incluyen una tormenta de citoquinas desencadenada por una respuesta desbalanceada de células *T helper* tipo 1 y tipo 2^{1,4} asociado además a disfunción respiratoria e hipoxemia, que provocan daño de las células miocárdicas.

Por lo tanto, en pacientes con SARS-CoV-2, es evidente que la incidencia de síntomas cardiovasculares no es despreciable y más bien diríamos frecuente, con desenlaces más severos y alta mortalidad por lo que deberemos ser más cuidadosos al momento del diagnóstico pues esto tendrá repercusiones no solo en la evolución, sino también en el incremento de contagios. Dato importante a tener en cuenta además es el daño cardíaco relacionado con los medicamentos durante el tratamiento, en particular el uso de medicamentos antivirales, cloroquina, hidroxiclороquina, los cuales pueden causar insuficiencia cardíaca, arritmia u otros trastornos cardiovasculares que deben ser monitoreados estrechamente^{5,6}.

Se presenta un caso con diagnóstico confirmado de COVID -19, de una paciente adulta mayor, con comorbilidad cardiovascular, que debuta con arritmias y bloqueo cardíaco que precede a la clínica respiratoria.

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Caso clínico

Mujer de 81 años de edad, procedente de Quito, mestiza, con antecedente de hipertensión arterial en tratamiento con losartán 50 miligramos y carvedilol 12.5 miligramos. Presentó un cuadro clínico de síncope con una duración de un minuto, motivo por el cual es llevada a una unidad de salud donde se le realizan exámenes, evidenciándose en el electrocardiograma bloqueo aurículo-ventricular de tercer grado por lo que se indica colocación de marcapaso, sin embargo, los familiares solicitan alta voluntaria.

Presenta un nuevo episodio de síncope en el domicilio, es trasladada a emergencia, donde presentó parada cardiorrespiratoria de 2 minutos con retorno a circulación espontánea. Valorada por Terapia Intensiva (UTI), se confirmó bloqueo A-V de tercer grado así como signos de bajo gasto decidiendo su ingreso.

En UTI se presentó consciente, bradicárdica con frecuencia cardíaca entre 30 a 40 latidos por minuto, que no genera hipotensión TAM: 90 - 100 mmHg, Respiratoriamente, 14 respiraciones por minuto, con cánula nasal 2 litros por minuto tiene SatO₂: 89%. Al examen físico, consciente, sin signos neurológicos, en corazón ruidos cardíacos arrítmicos, sin soplos; en pulmones, murmullo vesicular disminuido en bases.

En analítica como hallazgos relevantes se encuentra una troponina I elevada (0.33 VN: 0.00 a 0.01). Gasometría sin hipoxemia, ni hipercapnea. Dímero D elevado (5788 VN: 45-500), electrocardiograma mostró bloqueo aurículo-ventricular de tercer grado. (Figura 1)

Durante su hospitalización, la paciente presenta seis eventos más de parada cardiorrespiratoria sin buena respuesta a marcapaso transcutáneo, atropina y aminos vasoactivas; 36 horas después de su ingreso a UTI presentó tos seca y cefalea, incrementó su trabajo respiratorio con taquipnea e hipoxemia

gasométricamente; se solicitó radiografía de tórax que mostró infiltrado alveolointersticial bilateral difuso más cardiomegalia grado III en control radiográfico (Figura 2).

Teniendo en el deterioro clínico respiratorio, los hallazgos en la placa de tórax, el valor del dímero D muy elevado, la edad y comorbilidad, asociado al estado de epidemia por SARS-Cov2, se solicitó PCR el cuál es positivo.

La paciente es llevada a un sitio de aislamiento respiratorio, permanece hospitalizada, a los 8 días revierte el bloqueo de forma espontánea (sin apoyo de marcapaso). No requirió ventilación mecánica. Realiza hipoxemia súbita que lleva para- da cardiorrespiratoria, la paciente fallece.

Discusión

El monitor del impacto cardiovascular de las infecciones virales ha sido la influenza. La miocarditis, el infarto agudo de miocardio y la exacerbación de la insuficiencia cardíaca, han sido reportadas durante epidemias históricas previas y la consecuencia común ha sido el aumento de la mortalidad⁷. Situaciones similares han sido descritas en brotes previos de coronavirus como el SARS y el MERS.

La edad avanzada, las enfermedades cardiovasculares preexistentes, la gravedad del impacto en la oxigenación, determinan resultados adversos⁸. La hipotensión, la taquicardia, la bradicardia, las arritmias e incluso la muerte cardíaca súbita son comunes en pacientes con SARS. Los cambios electrocardiográficos compatibles con lo descrito y la elevación de la troponina sugieren miocarditis viral, en la ecocardiografía suele hallarse insuficiencia diastólica ventricular izquierda subclínica, los que desarrollan insuficiencia sistólica y fracción de eyección reducida, tienen alta probabilidad de ingresar a ventilación mecánica^{9,10}.

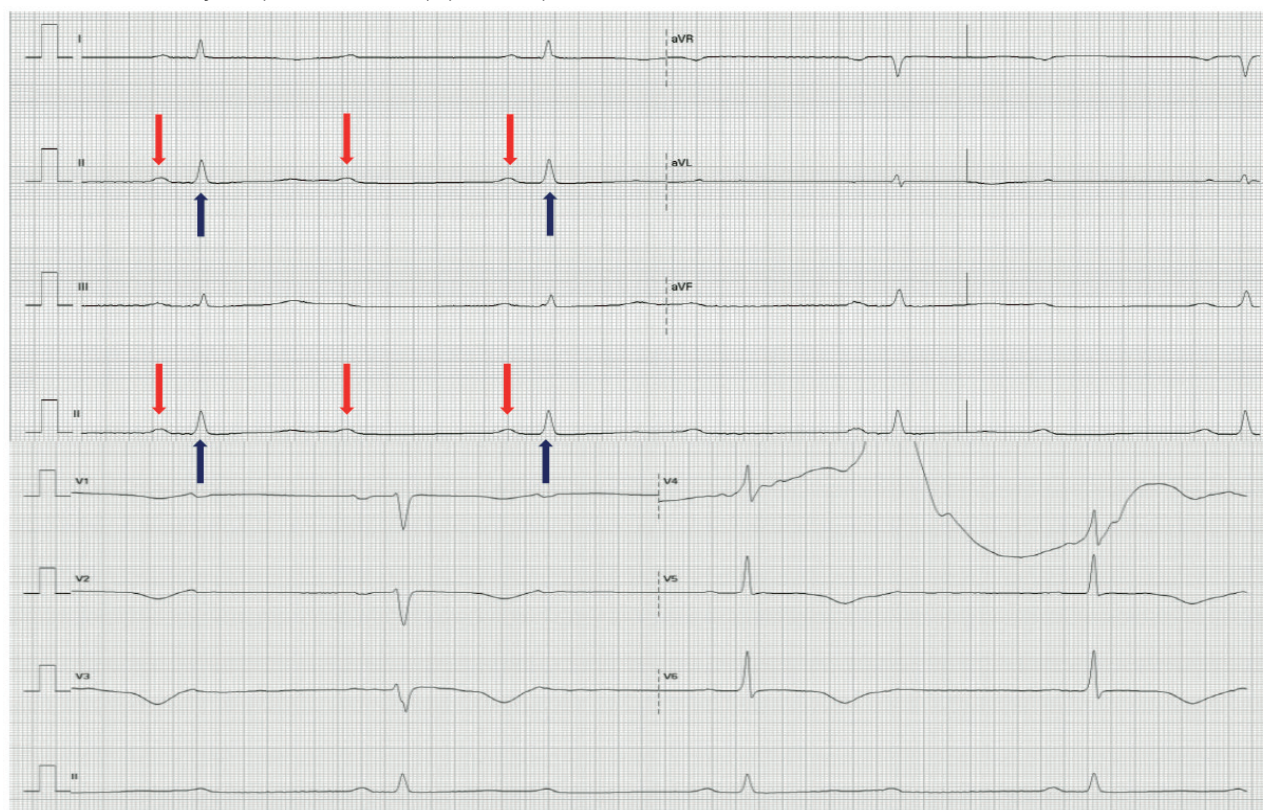


Figura 1. Electrocardiograma que demuestra bloqueo aurículo-ventricular de tercer grado. Flechas rojas: ritmo auricular, flechas azules: ritmo ventricular. Frecuencia cardíaca: 20 latidos por minuto.

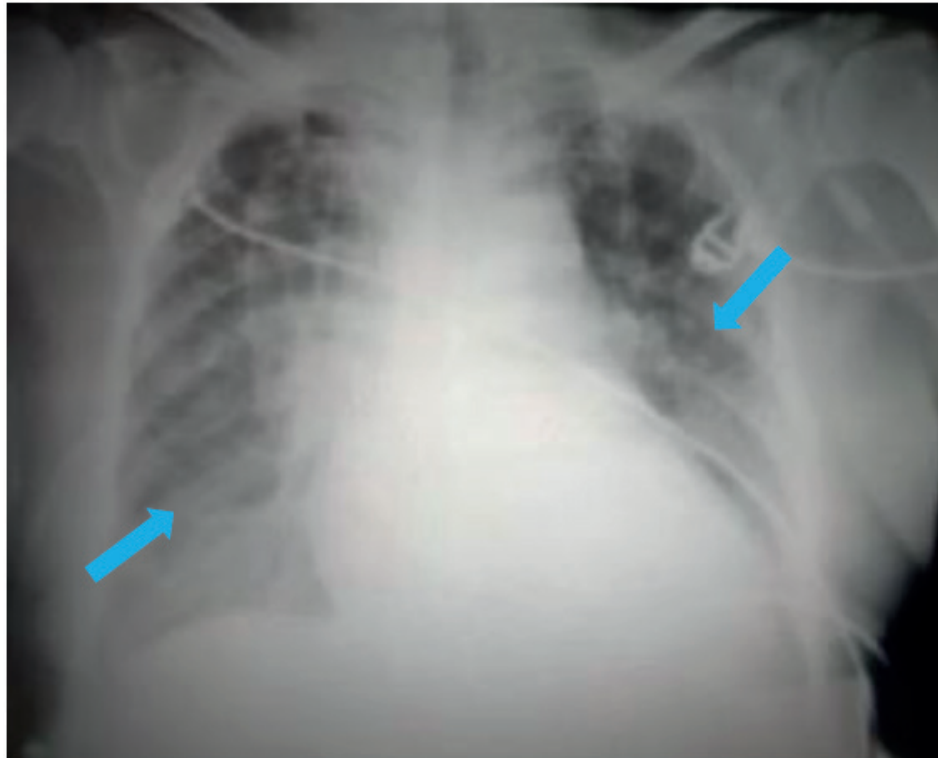


Figura 2. Radiografía antero-posterior de tórax. Flechas celestes indican infiltrado en parches bilateral difuso.

Las enfermedades virales, como toda infección sistémica generan aumento en la demanda metabólica y esto en pacientes con reserva cardíaca reducida por enfermedad cardiovascular crónica descompensa agudamente a éstos enfermos. La coexistencia de enfermedad coronaria e insuficiencia cardíaca expone a un riesgo particular de ruptura de la placa aterosclerótica secundaria a la inflamación sistémica, es por ello que el infarto agudo de miocardio es una complicación reportada.

El SARS y el SARS-CoV-2 son beta-coronavirus que se unen a las células que expresan receptores para la enzima convertidora de angiotensina 2 (ACE2)¹¹. La ACE 2 también se expresa en el corazón, permitiendo así que entren los coronavirus al sistema cardiovascular.

Los modelos murinos y las muestras de autopsias humanas demuestran que el SARS-CoV puede regular a la baja las vías de ACE2 miocárdicas y pulmonares, por lo tanto, mediando la inflamación del miocardio, el edema pulmonar y la insuficiencia respiratoria aguda.

En el caso reportado, se presenta un bloqueo aurículo-ventricular de tercer grado de instauración aguda, en una paciente de edad avanzada e hipertensa, que lleva a síncope cardíaco y eventos sucesivos de parada cardiorrespiratoria. Seguramente la etiología del bloqueo, sin síntomas respiratorios iniciales, se la debe atribuir en ésta paciente a causas isquémicas o farmacológicas, ya que la paciente tiene factores de riesgo cardiovascular, es tomadora de betabloqueantes y hubo elevación enzimática cardíaca. Sin embargo, lo agudo de la instauración del cuadro clínico en una paciente previamente asintomática, la refractariedad a marcapasos transcutáneo, atropina y aminas vasoactivas, y sobre todo que los síntomas cardíacos preceden en 36 horas a la clínica respiratoria y a una imagen pulmonar en radiografía de tórax con infiltración difusa bilateral, con PCR para SARS Cov -2 positivo; nos posiciona en un COVID -19 atípico; más aún cuando el bloqueo revierte espontáneamente, sin la necesidad de marcapaso.

Sobre este tema, hay amplio material reportado en expe-

riencias epidémicas previas por otros coronavirus. En SARS, Yu *et al.*¹² en 121 pacientes reportaron síntomas cardiovasculares como hipotensión, taquicardia, bradicardia, cardiomegalia y arritmias; Pan *et al.*¹³ en 15 pacientes describen muerte súbita cardíaca y Li *et al.*⁹ en 46 pacientes con ecocardiografía detectan insuficiencia diastólica subclínica sin fallo sistólico y que revirtió al superar la enfermedad. En MERS, Alhogbani¹⁴ en un paciente reportó miocarditis aguda que debutó con falla cardíaca y fue reversible. En relación al COVID-19; Huang *et al.*¹⁵ en 41 pacientes, observó daño miocárdico, expresado con incremento de troponina I ultrasensible con requerimiento de cuidado en algunos pacientes críticos y Wang *et al.*¹⁶ en una serie de 138 pacientes encuentran daño cardíaco agudo (7.2%), choque (8.7%), y arritmias (16.7%). Con la evidencia escrita y la elevación enzimática cardíaca, el cuadro clínico de nuestra paciente se corresponde a una probable miocarditis viral con trastornos severos de la conducción, que explicaría la refractariedad a marcapasos transcutáneo, atropina y aminas vasoactivas.

Conclusiones

El presente caso clínico muestra una probable miocarditis viral asociada a SARS CoV-2, que genera síncope, bloqueo cardíaco avanzado, bradicardia extrema y paradas cardiorrespiratorias sucesivas, que preceden a sintomatología respiratoria. La importancia de éste reporte radica en informar a la comunidad médica presentaciones clínicas atípicas del COVID -19 que alerte la necesidad de ampliar la bioseguridad en la atención en etapa de pandemia.

Referencias bibliográficas

1. Wang, D. et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA <https://doi.org/10.1001/jama.2020.1585> (2020).

2. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506 (2020).
3. Turner, A. J., Hiscox, J. A. & Hooper, N. M. ACE2: from vasoepitax to SARS virus receptor. *Trends Pharmacol. Sci.* 25, 291–294 (2004).
4. Alhogbani, T. Acute myocarditis associated with novel Middle East respiratory syndrome coronavirus. *Ann. Saudi Med.* 36, 78–80 (2016).
5. Wong, C. K. et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin. Exp. Immunol.* 136, 95–103 (2004).
6. Sakabe, M., Yoshioka, R. & Fujiki, A. Sick sinus syndrome induced by interferon and ribavirin therapy in a patient with chronic hepatitis C. *J. Cardiol. Cases* 8, 173–175 (2013).
7. Nguyen JL, Yang W, Ito K, Matte TD, Shaman J, Kinney PL. Seasonal influenza infections and cardiovascular disease mortality. *JAMA Cardiol* 2016; 1: 274.
8. Badawi A, Ryoo SG. Prevalence of comorbidities in the Middle East respiratory syndrome coronavirus (MERS-CoV): a systematic review and meta-analysis. *Int J Infect Dis* 2016; 49: 129 – 133.
9. Li SS, Cheng C, Fu C, Chan Y, Lee M, Chan JW, Yiu S. Left ventricular performance in patients with severe acute respiratory syndrome: a 30-day echocardiographic follow-up study. *Circulation* 2003; 108: 1798 – 1803.
10. Harris JE, Shah PJ, Korimilli V, Win H. Frequency of troponin elevations in patients with influenza infection during the 2017–2018 influenza season. *Int J Cardiol Heart Vasc* 2019; 22: 145 – 147.
11. Wit E D, Doremalen N. V, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 2016; 14: 523 – 534.
12. Yu C-M. Cardiovascular complications of severe acute respiratory syndrome. *Postgrad Med J* 2006; 82: 140 – 144.
13. Pan S, Zhang H, Li C, Wang C. [Cardiac arrest in severe acute respiratory syndrome: analysis of 15 cases]. *Zhonghua Jie He He Hu Xi Za Zhi* 2003; 26: 602 – 605.
14. Alhogbani T. Acute myocarditis associated with novel Middle East respiratory syndrome coronavirus. *Ann Saudi Med* 2016; 36: 78 – 80.
15. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497 – 506.
16. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020; doi:10.1001/jama.2020.1585.

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CASE REPORTS / REPORTE DE CASO

Alopecia Triangular congénita temporal en edad pediátrica. A propósito de un caso

Temporary congenital triangular alopecia in pediatric age. About a case

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Resumen: Se presenta el caso de una niña de 9 años de edad, valorada en el servicio de Dermatología provincial por ausencia de cabello presente desde el nacimiento. Se evalúa en conjunto y se concluye clínicamente como una Alopecia Triangular Congénita Temporal, sin necesidad de biopsia cutánea. Esta entidad es un trastorno de prevalencia desconocida, aunque quizá más frecuente de lo que podamos pensar, pero que en ocasiones puede constituir motivo de consulta al pediatra y/o al dermatólogo. Es una forma de Alopecia no cicatricial. Es necesario conocerla para evitar tratamientos y pruebas innecesarias para su diagnóstico por la gran similitud con otras dermatosis.

Palabras clave: Alopecia, congénita, dermatología.

Abstract: The case of a 9-year-old girl is presented, valued in the service of provincial Dermatology, by the absence of present hair from the birth. They were carried out several probable diagnoses, It is evaluated on the whole, and you conclude clinically like a Triangular Alopecia, without the necessity of cutaneous biopsy. This entity is a dysfunction of the unknown. However, the maybe more frequent prevalence of what we can think, but that on occasions it can constitute consultation reason to the pediatrician and the dermatologist. It is a form of non-cicatricial Alopecia. It is necessary to know it to avoid treatments and unnecessary tests for their diagnosis for the similarity with another skin disease.

Key words: Alopecia, congenital, dermatology.

Introducción

La Alopecia Triangular congénita, también llamada nevus de Brauer, es una forma de alopecia no cicatricial y circunscrita que fue descrita por primera vez por Sabouraud, dermatólogo francés, en el año 1905¹.

Comenzó a tomar importancia cerca de 5 años después cuando se describen las causas de alopecia en el recién nacido, sobre todo la asociada a la sífilis congénita. Se trata de una condición benigna, no progresiva y muy poco conocida, probablemente más por infradiagnóstico y la escasez de publicaciones que por su frecuencia, se sitúa en torno al 0,11% de las consultas de Dermatología en el mundo, más frecuente en la raza blanca, sin predilección de sexo, y se ha sugerido una herencia paradominante, que explica que sea de aparición esporádica; su patogenia es desconocida^{2,3}.

Clínicamente consiste en una placa de alopecia circunscrita, permanente y asintomática, no se conoce el estímulo que produce la regresión folicular irreversible. La histopatología muestra unos folículos pilosebáceos hipoplásicos sin alteraciones inflamatorias ni cicatriciales añadidas, siendo el número de folículos pilosos normal pero todos de carácter velloso, con presencia de glándulas sebáceas normales en número y tamaño^{2,3,4}.

El diagnóstico es fundamentalmente clínico, solo precisa de estudio histológico en casos de duda diagnóstica. Es importante referir el caso siempre al servicio de Dermatología Pediátrica para evitar exámenes y estudios innecesarios, en ocasiones invasivos para el niño^{3,5}.

Se han descrito casos asociados a otros trastornos como síndrome de Daw, leuconiquia, facomatosis pigmento vascular, nevus de cabello lanoso, hiperpigmentación sectorial del

iris, retraso mental, epilepsia, malformación de Dandy-Walker, síndrome de Leopard, aplasia cutis congénita^{2,3}, síndrome GLH (cráneo sinostosis, displasia cerebelosa, displasia trigeminal y retraso mental) y otros síndromes polimalformativos (cardiopatía congénita y anomalías renales y genitales), que posiblemente sean asociaciones causales⁶, aunque otros autores consideran que las alteraciones neurológicas del desarrollo y la facomatosis pigmento vascular no son simples asociaciones causales³.

El diagnóstico diferencial se realiza con otras formas de alopecia, que pueden observarse en la edad pediátrica⁵.

Esta dermatosis representa un reto diagnóstico para los dermatólogos, y casi siempre el paciente acude a la consulta con modificación de la piel alopecica o cicatrices por procedimientos invasivos^{4,6}.

La tasa de incidencia en E.U no se precisa bien, aunque estudios reportan que representa el 1,4% de las consultas de dermatología en edad pediátrica, siendo más frecuente en el sexo femenino sobre el masculino, en proporción de 2:1. En Europa se han registrado formas clínicas de otra localización, como la frontal y occipital, asociadas a trastornos sistémicos del aparato genitourinario y malformaciones cardiovasculares^{6,7}.

En Cuba, solo se ha reportado un caso de alopecia triangular congénita temporal en la Habana, se considera que se debe al subregistro en las consultas de otras especialidades o la rareza de la entidad en la Atención Primaria de Salud.

En Villa Clara no se recogen registros de esta dermatosis⁸.

En la actualidad no se dispone de un tratamiento efectivo. Por este motivo deberemos asegurarnos de explicarles bien en qué consiste esta entidad y en la ausencia de implicaciones

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clínicas, para evitar actuaciones infructuosas o contraproducentes para la salud del paciente⁶.

Caso clínico

Se presenta la paciente YPR, femenina, de 9 años de edad, valorada en el servicio de Dermatología del Hospital Pediátrico Provincial José Luis Miranda, en Santa Clara, en septiembre del año 2019 por ausencia de cabello en un área del cuero cabelludo, presente desde el nacimiento. Había sido valorada anteriormente en otro centro de Salud con diagnóstico de alopecia areata y le indicaron varios tratamientos médicos con esteroides tópicos de mediana potencia en forma de masajes compresivos, sin mejoría clínica alguna; motivo por el cual acude a nuestro centro. Se procedió a examinarla e interrogarla en compañía de sus padres. Se revisa la literatura y se plantea como diagnóstico clínico una alopecia triangular congénita temporal.

Examen Físico:

Se observa área alopécica de aproximadamente 15 cm de diámetro, con bordes difusos y pequeños pelos alrededor, localizada en región frontotemporal derecha, de forma más o menos triangular, congénita, de superficie lisa, no presencia de escamas, ni atrofia, ni cambios de coloración, ni otro tipo de lesión en el momento del examen. (Figura 1 y 2)

APP: Negativos

APF: Madre con HTA con tratamiento con enalapril, 20 mg diarios.

No se precisa de biopsia cutánea teniendo en cuenta el diagnóstico clínico de esta entidad.

Se indican exámenes complementarios de rutina:

Hemograma completo, eritro sedimentación, coagulograma completo, glicemia, perfil hepático y renal, LDH, VDRL, Antígeno de superficie para hepatitis B y VIH dentro de parámetros normales.

Rx de tórax AP: normal

Ya le habían realizado Rx de cráneo que fue normal.

Se lleva a discusión el caso en el servicio de Dermatología y se interconsulta con el servicio de Pediatría para descartar

otros trastornos asociados, y se concluye como:

Comentarios

La Alopecia Triangular Congénita Temporal representa sin dudas una dermatosis en ocasiones de complejo diagnóstico por la similitud con otras entidades^{7,8}.

El diagnóstico se basa en su naturaleza estable, su clínica característica, su localización y el aspecto normal de la piel afectada, no siendo necesario su estudio histológico.

Las manifestaciones clínicas de este cuadro son características y consisten en el hallazgo de una placa de alopecia circunscrita, no inflamatoria ni cicatricial, más habitualmente de forma unilateral que bilateral, como es el caso de nuestro paciente²⁻⁸.

Su localización preferente es frontoparietal, aunque existen casos descritos en otras localizaciones^{3,8}.

Se caracteriza por ser asintomática y permanente, de crecer lo haría en la misma proporción que el resto del cuero cabelludo⁹.

Puede estar presente desde el nacimiento o desarrollarse en los primeros meses de vida, haciéndose evidente habitualmente en los nueve primeros años de vida o, menos frecuentemente, en la adolescencia o en edad adulta³⁻⁹, por ello, en la actualidad se tiende a considerar adquirida más que congénita, teoría con la cual no están de acuerdo otros autores^{9,10}.

La forma de la lesión es triangular con ángulos romos pero también ovalados o redonda²⁻⁵.

En el interior de la lesión se aprecia una piel normal con ausencia de pelos terminales y la existencia de pelos finos vellosos⁵⁻¹⁰, que se pueden apreciar mejor con lupa⁹.

En los bordes de la lesión puede haber pelos terminales raeados que se mezclan con el pelo del cuero cabelludo normal⁵.

El diagnóstico diferencial debe hacerse con otras causas de alopecia circunscrita: la alopecia areata (pilotracción+, presencia de pelos en signo de exclamación y zonas de piel con ausencia total de pelo); la aplasia cutis (ausencia total de pelo, piel deprimida y blanda al tacto y localización de elección en vértex); la alopecia cicatricial (abarca una amplia variedad de procesos como el lupus eritematoso cutáneo crónico, el liquen plano pilar o la alopecia mecánica por tracción, donde el resultado final es un cuero cabelludo brillante y duro al tacto, sin pelo o con cabellos en penacho); la presencia de un nevo sebáceo (tumoración amarillomarrón); la tricotilomanía (presencia de cabellos de distintas longitudes y grosores variables, en ocasiones expresión de un conflicto emocional, alopecia de forma no triangular); la alopecia sutural; la alopecia vertical y la displasia focal bitemporal¹⁰.

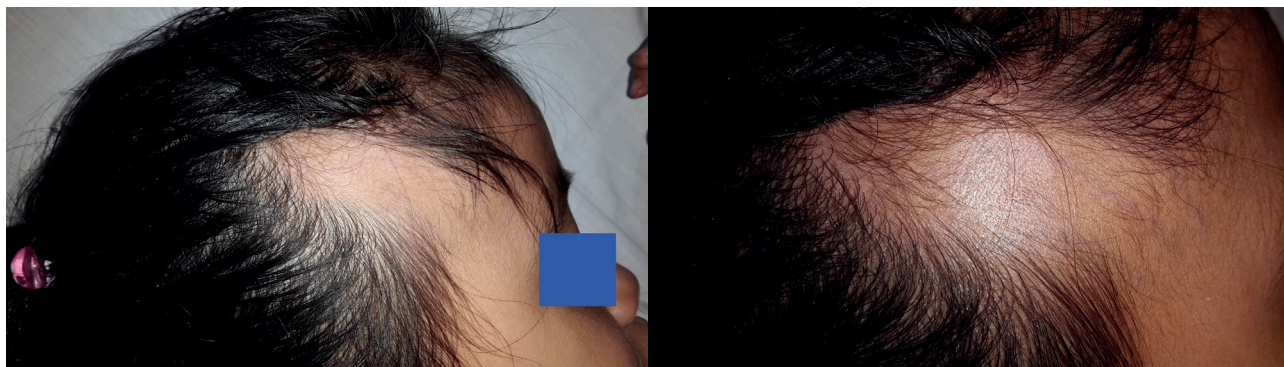


Figura 1 y 2. En ambas imágenes se observa el típico patrón de alopecia triangular temporal. En la figura 2 nótese que no existen cambios de textura ni color de la piel.

La alopecia se mantiene sin modificación hasta el momento.

La Alopecia Triangular Congénita Temporal carece de tratamiento efectivo, que tampoco suele ser necesario. Se debe explicar la naturaleza de la entidad a los padres para que eviten tratamientos infructuosos o perjudiciales (sobre todo el uso de esteroides tópicos). El cuadro permanece estable de por vida, y tanto en varones como mujeres puede realizarse una extirpación quirúrgica del área afectada¹¹.

Nuestro paciente muestra el patrón clínico y distribución característicos de esta dermatosis¹².

Conclusiones

La Alopecia Triangular Temporal representa una entidad muy infrecuente pero cuyo reconocimiento es interesante para evitar tratamientos inútiles y con posibles efectos secundarios.

Recomendaciones

Esta dermatosis pertenece a las alopecias no cicatriciales de la infancia, trastorno subdiagnosticado en el mundo y en nuestro medio, motivo por el cual se recomienda que todo recién nacido con alopecia sea valorado por el servicio de Dermatología Pediátrica más cercano.

Bibliographic references

1. Saboraud R. Manuel. Elementaire de dermatology topographique regionale. Paris: Masson and Cie; 2014. ,p.197.
2. García -Hernández MJ, Rodri  ez Pichardo A, Camacho F. Cong  nita triangular alopecia (Brauer nevus) Pediatric Dermatol. 2014.
3. Le  n-Mui     E, Monteagudo B, Labandeira J, Cabanillas M. Alopecia triangular cong  nita bilateral asociada a cardiopat  a cong  nita y anomal  as renales y genitales. Actas Dermosifilogr.2015; 99:573-82.
4. Lannicelli JC.Alopecia temporal triangular. Diagn  stico visual. Pediatr  a pr  ctica (en l  nea) (actualizado el 27/12/2015; consultado el 19/03/2019).
5. Monteagudo B,Ginrte M,Le  n-Mui     E, Monteagudo JL, Toribio J. Alopecia triangular cong  nita. An pediatric (Barc). 2016;63(2): 175-84.
6. Sarifakioglu E, Esra Yilmaz A, Gorpelioglu C, Orun E. Prevalence of scalp disorders and hairs loss in children. Cutis. 2016, 90: 225-9.
7. Jim  nez-Acosta F, Ponce I. Incidencia y prevalencia de las alopecias. Actas Dermosifilogr.2016; 100: 907:22.
8. Registro Estad  stico de Salud Nacional. Cuba 2017.
9. Jim  nez-Acosta F, Ponce I. Transplante de pelo en la alopecia temporal triangular. Actas Dermosifilogr. 2018; 28: 202-13.
- 10.Kudligi C, Vittal Bhagwat P, Shendre Eshwarrao M, Tandon N. Giant congenital triangular alopecia mimicking alopecia areata. Int J Trichology. 2018; 103(5): 367:87.
11. Elmer KB, George RM. Congenital triangular alopecia: A case report and review. Cutis, 69(2019), pp.255-6.
12. Tosti A.Congenital triangular alopecia. Report of fourteen cases. J Am Acad Dermatol, 16 (2019), pp.991-3.

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CASE REPORTS / REPORTE DE CASO

Hipercoagulabilidad, trombosis intravascular y trombocitosis asociada al COVID-19. Reporte de un caso

Hypercoagulability, intravascular thrombosis, and thrombocytosis associated with COVID-19. Case report

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Resumen: Una nueva pandemia en el siglo XXI, generada por un agente viral del tipo coronavirus denominado SARS CoV-2, que produce la enfermedad COVID-19, la misma que debutó a finales del 2019 en China, con una mortalidad importante, que afecta el tracto respiratorio inferior y se manifiesta como neumonía en humanos.

A medida de su expansión mundial, el virus ha presentado mutaciones y por ende variantes fenotípicas que han generado cuadros clínicos que distan mucho de los síntomas descritos inicialmente; más bien, las alteraciones de la coagulación y la intensa inflamación (tormenta de citoquinas) han generado síntomas atípicos y enfoques terapéuticos diferentes; situando a la ferritina y el dímero D como biomarcadores cardinales para decidir anticoagulación y/o terapia anti inflamatoria como corticoterapia, el monoclonal anti IL6 Tocilizumab o el metotrexate, entre otros.

A continuación, presentamos el caso de un paciente diagnosticado de COVID-19 con RT-PCR, que presenta a más de sus manifestaciones respiratorias, un estado protrombótico que desencadenó trombosis intravasculares y posiblemente embolismo pulmonar, con valores elevados de ferritina y dímero D, que luego de anticoagulación y administración de corticoides superó su estado de gravedad extrema y egreso de Terapia Intensiva.

Palabras clave: Trombosis, dímero D, ferritina, SARS CoV-2, COVID-19.

Abstract: A new pandemic in the 21st century, generated by a coronavirus-like viral agent called SARS CoV-2, which produces the disease COVID-19, the same that debuted in late 2019 in China, with significant mortality, affecting the lower respiratory tract and It manifests as pneumonia in humans.

As its worldwide expansion, the virus has presented mutations and therefore phenotypic variants that have generated clinical pictures that are far from the symptoms described initially; rather, coagulation disturbances and intense inflammation (cytokine storm) have created atypical symptoms and different therapeutic approaches; placing ferritin and D-dimer as cardinal biomarkers to decide anticoagulation and anti-inflammatory therapy such as corticotherapy, monoclonal anti IL6 Tocilizumab or methotrexate, among others.

Below we present the case of a patient diagnosed with COVID-19 with RT-PCR, who shows more than his respiratory manifestations. This prothrombotic state triggered intravascular thrombosis and possibly pulmonary embolism, with elevated values of ferritin and D-dimer, which after anticoagulation and administration of corticosteroids exceeded his state of extreme gravity and discharged from Intensive Care.

Key words: Thrombosis, D-dimer, ferritin, SARS CoV-2, COVID-19.

Introducción

A mediados de Diciembre del 2019, se presentaron en Wuhan-China, una serie de casos atribuidos a una infección viral, que cumplían con criterios clínicos de neumonía con diferentes estadios de gravedad; llegando incluso hasta la insuficiencia respiratoria e hipoxemia severa¹.

Esta infección, se atribuyó a una especie nueva de coronavirus, un beta coronavirus diferente al causante del Síndrome Respiratorio Agudo Severo (SARS)² y Síndrome Respiratorio del Medio Oriente (MERS)³. Este nuevo microorganismo fue nombrado por la OMS el 11 de febrero como SARS COV2, y es causante de la enfermedad COVID-19⁴.

Aunque la descripción inicial, fue un cuadro respiratorio agudo que en algunos casos progresa a neumonía, existe un número significativo de pacientes que presentan diferentes manifestaciones clínicas que van desde ser asintomáticos,

hasta daños vasculares y eventos trombóticos asociados a una respuesta inflamatoria al parecer autoinmunitaria exagerada^{5,6}.

Enfocándonos en las alteraciones de la coagulación y trastornos vasculares, se ha reportado que un porcentaje de pacientes pueden presentar múltiples episodios trombóticos que van desde isquemia periférica hasta tromboembolismo pulmonar y coagulación intravascular diseminada (CID), todo esto asociado a una respuesta macrofágica descontrolada, un estado de hipercoagulabilidad y activación patológica de la trombina⁷. La elevación del dímero D, un producto de la degradación de la fibrina, es la ventana que nos permite observar y definir la gravedad de este fallo y tomar decisiones terapéuticas, como la anticoagulación⁷.

Con estas consideraciones presentamos un caso con diagnóstico confirmado de COVID-19, en un paciente adulto sin co-

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morbilidades, que desarrolla fenómenos trombóticos.

Caso clínico

Varón de 51 años de edad, de la ciudad de Quito, con antecedentes de hipoacusia súbita hace 2 años, vértigo en tratamiento con dimenhidrinato, y colon irritable en tratamiento no farmacológico.

Luego de viaje al litoral, presentó con 15 días de evolución disnea, tos seca y odinofagia, que se agudizó luego de 7 días, requiriendo ingreso hospitalario; su evolución no fue favorable, 24 horas posterior a su ingreso, presentó hipoxemia severa, con necesidad de ingreso a ventilación mecánica invasiva. Estuvo hospitalizado en el cuarto crítico de emergencia con empeoramiento progresivo; tuvo fiebre, taquicardia, leucocitosis, requerimiento de alta fracción inspirada de oxígeno (FiO_2) y presentó fallos multiorgánicos, se lo admite a las 48 horas de su ingreso a la Unidad de Terapia Intensiva (UTI).

Ingresó a terapia intensiva, sedado profundamente y con uso de relajantes musculares, acoplado a ventilación mecánica invasiva, modo controlado por presión con parámetros ventilatorios altos (presión inspiratoria de 30 cm H_2O , presión positiva al final de la espiración (PEEP) de 14 cm H_2O , fracción inspiratoria de oxígeno de 80%, frecuencia respiratoria 28). Su hemodinamia estable, no requirió vasoactivos. Fallo renal Akin III, sin criterios dialíticos emergentes. En exámenes de laboratorio se evidenció leucocitosis (15550 VN: 4290-4920), linfopenia (940 VN: 1000-4000), trombocitosis (510000), elevación de ferritina (1038.90 VN: 22-322) y dímero D (1042 VN: 45-500).

Debido a los valores alterados de estos marcadores y el riesgo elevado de eventos trombóticos se consideró como terapia anticoagulante y corticoterapia. Continuó recibiendo terapia antibiótica a base de amino penicilinas y macrólido.

A las 36 horas de evolución se logró disminuir parámetros ventilatorios. Se decidió retirar anticoagulación por riesgo de sangrado secundario a la falla renal, y se inició despertar del paciente para liberación de la ventilación mecánica invasiva. De forma súbita presentó hipoxemia con caída brusca de la saturación de oxígeno a 70% pese a concentraciones altas de O_2 . La distensibilidad pulmonar estática se mantuvo normal

(mayor de 40 ml/cm² H_2O), se incrementó la presión positiva al final de espiración. PEEP, sin mejoría y aumento del CO_2 y acidosis respiratoria severa.

La ecocardiografía (FOCUS) mostró la vena cava inferior dilatada, ventrículo derecho cualitativamente aumentado de tamaño y con presión incrementada. Por lo que se consideró un posible tromboembolismo pulmonar, más sobreinfección bacteriana por gérmenes multidrogaresistentes. Se tomaron cultivos e inició terapia antibiótica a base de carbapenémico más glicopéptido, reiniciamos anti coagulación y se añadió corticoterapia con metilprednisolona a dosis de 1mg/kg.

Tras dos días de evolución, el examen físico diario reveló incremento del tamaño de miembro inferior derecho a nivel de región inguinal, con zona eritematosa y caliente; se solicitó valoración a cirugía vascular quienes realizaron rastreo ecográfico en el cual se observa trombo a nivel de vena femoral común derecha confirmándose trombosis venosa profunda (Figura1A-1B). Por lo que se mantuvo anticoagulación y corticoterapia.

Se observó mejoría clínica progresiva, se suspende la antibióticoterapia por cultivos negativos. El paciente presentó despertar adecuado, y lográndose extubación con retiro de la ventilación mecánica invasiva a los 12 días de hospitalización. Además se realizó terapia de reemplazo renal, con mejoría de los niveles de azoados.

El paciente egresa al día 13, de la unidad de terapia intensiva a piso de Medicina Interna, en buenas condiciones generales.

Discusión

El tema del origen de la hipoxemia, el estado de hipercoagulabilidad y la tormenta de citoquinas en COVID-19 en los estadios finales del cuadro séptico, que actualmente se sospechan con base a valores altos de ferritina (>500 ng/ml) y dímero D (mayor de 1000 ng/ml)²²; es un tema interesante, el cual no está comprendido en su totalidad y su entendimiento está convirtiéndose en un pilar fundamental para tratar al paciente infectado con SARS -CoV-2.

La enfermedad grave por COVID-19 ha sido descrita con hipoxemia severa asociada a distensibilidad pulmonar estática

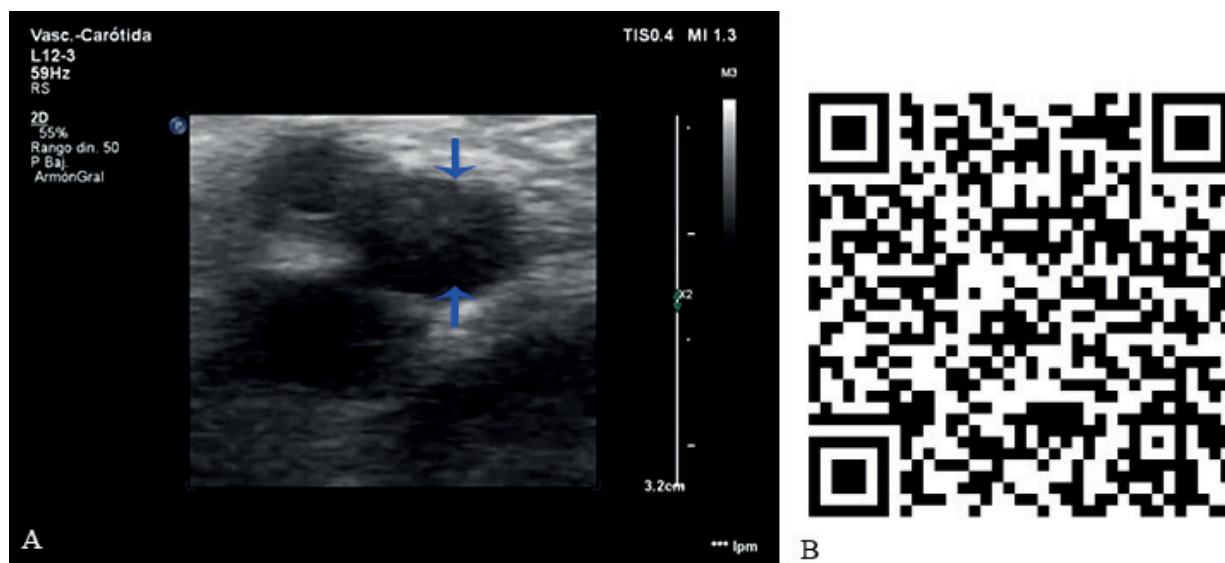


Figura 1. A. Ecografía vascular que evidencia trombo (flechas azules en vena femoral común derecha). B. Código QR para visualización de videos en tiempo real de la ecografía vascular realizada que demuestra la presencia del trombo en vena femoral común derecha.

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. 2020 Feb 15;395(10223):497–506.
2. Drosten C, Günther S, Preiser W, van der Werf S, Brodt H-R, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med*. 2003 May 15;348(20):1967–76.
3. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med*. 2012 Nov 8;367(19):1814–20.
4. Naming the coronavirus disease (COVID-19) and the virus that causes it [Internet]. [cited 2020 Apr 16]. Available from: [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-\(covid-2019\)-and-the-virus-that-causes-it](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it)
5. Ammirati E, Wang DW. SARS-CoV-2 inflames the heart. The importance of awareness of myocardial injury in COVID-19 patients. *Int J Cardiol* [Internet]. 2020 Apr 6 [cited 2020 Apr 16]; Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7134214/>
6. Musa S. Hepatic and gastrointestinal involvement in coronavirus disease 2019 (COVID-19): What do we know till now? *Arab J Gastroenterol*. 2020 Apr 3;
7. Gauna, Mauricio B Juan. Respuesta Inmune Asociada al COVID 19 [Internet]. Bonamí Estudio Creativo; 2020. Available from: <https://fundacionio.com/wp-content/uploads/2020/04/Si%C3%81ndrome-RITAC.pdf.pdf.pdf.pdf.pdf.pdf.pdf.pdf>
8. Gattinoni L, Coppola S, Cressoni M, Busana M, Rossi S, Chiumello D. Covid-19 Does Not Lead to a “Typical” Acute Respiratory Distress Syndrome. *American Journal of Respiratory and Critical Care Medicine* [Internet]. 2020 Mar 30 [cited 2020 Apr 16]; Available from: <https://www.atsjournals.org/doi/10.1164/rccm.202003-0817LE>
9. Pang PS, Collins SP, Gheorghiade M, Butler J. Acute Dyspnea and Decompensated Heart Failure. *Cardiology Clinics*. 2018 Feb 1;36(1):63–72.

10. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. *JAMA*. 2012 Jun 20;307(23):2526–33.
11. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. *Lancet*. 1967 Aug 12;2(7511):319–23.
12. Hussain SN, Simkus G, Roussos C. Respiratory muscle fatigue: a cause of ventilatory failure in septic shock. *Journal of Applied Physiology*. 1985 Jun 1;58(6):2033–40.
13. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. Features, Evaluation and Treatment Coronavirus (COVID-19). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 [cited 2020 Apr 16]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK554776/>
14. Feelders RA, Vreugdenhil G, Eggermont AM, Kuiper-Kramer PA, van Eijk HG, Swaak AJ. Regulation of iron metabolism in the acute-phase response: interferon gamma and tumour necrosis factor alpha induce hypoferrinaemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. *Eur J Clin Invest*. 1998 Jul;28(7):520–7.
15. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet*. 2020 Mar 28;395(10229):1054–62.
16. Marietta M, Ageno W, Artoni A, De Candia E, Gresele P, Marchetti M, et al. COVID-19 and haemostasis: a position paper from Italian Society on Thrombosis and Haemostasis (SISET). *Blood Transfus*. 2020 Apr 8;
17. Municio C, Dominguez-Soto Á, Fuentelsaz-Romero S, Lamana A, Montes N, Cuevas VD, et al. Methotrexate limits inflammation through an A20-dependent cross-tolerance mechanism. *Annals of the Rheumatic Diseases*. 2018 May;77(5):752–9.
18. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost*. 2020 Mar 27;
19. Gauna M, Bernava JL. Recomendaciones diagnosticas y terapeuticas ante la Resuesta Inmune Trombotica Asociada al Covid19 (RITAC). Pautas del Ministerio de Salud de la República Argentina. 2020.
20. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. *Clin Chim Acta*. 2020 Mar 13;506:145–8.
21. Gattinoni L, Chiumello D, Rossi S. COVID-19 pneumonia: ARDS or not? *Critical Care*. 2020 Apr 16;24(1):154.
22. J.B.Moore,C.H.June, Cytokine release syndrome in severe COVID-19. *Science*10.1126/science.abb8925 (2020)
23. Vélez JL, Montalvo M, Irigoyen E. Advanced atrioventricular block and extreme bradycardia as initial symptoms of Covid-19: A case report. *Bionatura*. 2020.05.02.9 Available from: <http://dx.doi.org/10.21931/RB/2020.05.02.9>
24. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020; doi:10.1001/jama.2020.1585.

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REVIEW / ARTÍCULO DE REVISIÓN

The epidemiological plateau of Corona virus in Gulf countries: a descriptive statistics study

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Abstract: The novel SARS-CoV-2 belongs to the beta coronaviruses and causes a severe pandemic disease named as COVID-19. In late December 2019, WHO situation reports on 11 March 2020, declared that COVID-19 a pandemic due to its global spread. All Arab countries have reported COVID-19 cases. The confirmed cases of COVID-19 pandemic in Arab gulf countries were reported in the United Arab Emirates, Iraq, Bahrain, Oman, Qatar, Kuwait, and Saudi Arabia, respectively. The fatality case rates in Gulf Countries are less than 1% in Oman, UAE, Kuwait, Bahrain, and Saudi Arabia, yet it hits 7.5% in Iraq. In this manuscript, we try to interpret the pandemic statistically in gulf countries, especially in Iraq. Additionally, the distribution of COVID-19 confirmed cases based on ABO blood groups were investigated. Epidemiological analyses revealed that a decreased risk of infection was attributed to blood group O compared to non-O blood groups, whereas people with the A and A.B. blood groups showed the highest risk for COVID-19 infection. Besides, high risk for diabetes, cardiovascular disease, blood clotting, and interleukin secretion was also related to blood groups in different orders. Accordingly, patients with a specific blood group that are associate with the above diseases should be under strict medical surveillance when infected with COVID-19 to reduce complications and severity. This study provides further confirmation for the previously reported correlation between the ABO blood groups and the susceptibility to COVID-19 infection.

KeyWords: COVID-19, Blood type, Gulf countries, Epidemic statistics.

Introduction

One of the B- coronavirus was responsible for the last outbreak of pneumonia cases, which recognized in December 2019 in Wuhan, initially the virus named by World Health Organization to a 2019- novel coronavirus (2019-nCoV). Officially WHO called the disease as COVID-19 while the new virus named SARS-CoV-2 by Coronavirus study group. Chinese researchers isolated the virus responsible for COV-19 on 7 January, 2020, and came out to the genome sequence of SARS-CoV-2^{1,2,3}; it is enveloped non- segmented positive-sense RNA virus with 96.2% identity to a bat CoVRaTG13, whereas it shares 79.5% identity to SARS-CoV. Genome sequence analysis suggests that bat is the natural host of virus origin, and it might transmit to humans via unknown hosts. SARS-CoV-2 could use angiotensin-converting enzyme (ACE2) as a receptor that encoded on lung alveolar epithelial cells and enterocytes of the intestine^{4,5,6} just the same for SARS-CoV^{7,8,9}.

Rapid Spreading of COVID-19 across the world led WHO declaring it a pandemic on 11 March, 2020. As an emerging acute respiratory infectious disease, the virus responsible for COVID-19 secreted out of respiratory tract, spread for distance by small droplets through coughing and sneezing beside direct contact among persons for a low infective dose^{10,11}. The epidemiological investigation suggests that the incubation period is almost 1-14 days, mostly 3-7 days; the virus is contagious during the latency period and high transmissible, especially for the elderly and people with serious diseases. Patients with COV-19 presented common symptoms such as fever, malaise, and dry coughs. However, not everyone who was infected shows the symptoms of the disease. The blood groups and their related antigens might be associate with the immune response against COVID-19.

Additionally, ABO antigens may increase the susceptibility to viral infection^{12,13}. Blood groups were distributed among

the human population and varied across populations and geographical regions. The four blood groups were known for their critical role in blood transfusion. Humans received incompatible blood, powerful naturally-occurring anti-A or anti-B antibodies could cause a blood transfusion reaction, as well as many other diseases, are related to blood groups such as diabetes, cardiovascular disease, viral infections, and interleukin production. Many other criteria other than the virus strain itself may have an influence such as season and temperature, sex and age of patients, blood group, sanitation, and alienating strategies as well as the early discovery of infection by COV-19. Early diagnosed infected persons limited the rate of virus transmission to others, controlling the outbreak and decreased the rate fatality of COVID-19 disease.

The statistical situation for the pandemic

Traditional first confirmed infection in Gulf countries

The Neighboring countries belong to Gulf take their containment measures after the first confirmed case of infection to lessen and prevent the incidence of infections with COVID-19; the origin of first diagnosed COVID-19 will present in the table (1) for Gulf countries. Data presented by WHO for confirmed cases of COVID-19 regarding Arab Gulf countries revealed that Iraq came at the third number of cases at 5-March, keeping forth position till 8 April. While Iraq had the fifth and sixth positions in total confirmed cases at mid of April and 19 April, respectively, the infection situation also, revealed that the total number of confirmed cases at 19 April was bellowed other countries that recorded the first case at the end of February 2020 such as Qatar, Kuwait, and United Arab Emirate. On 28 April- 2020, Iraq became the last position among Gulf countries.

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Country	Date for 1 st case	Origin of 1 st case
Iraq	24-2-2020	Iran
Saudi Arabia	2-3-2020	Iran
Qatar	27- 2-2020	Iran
Bahrain	21-2-2020	Iran
United Arab Emirates	23-1-2020	China
Kuwait	24-2-2020	Iran
Oman	24-2-2020	Iran

Table 1. The origin of the first confirmed case of COVID-19 at Arab Gulf region.

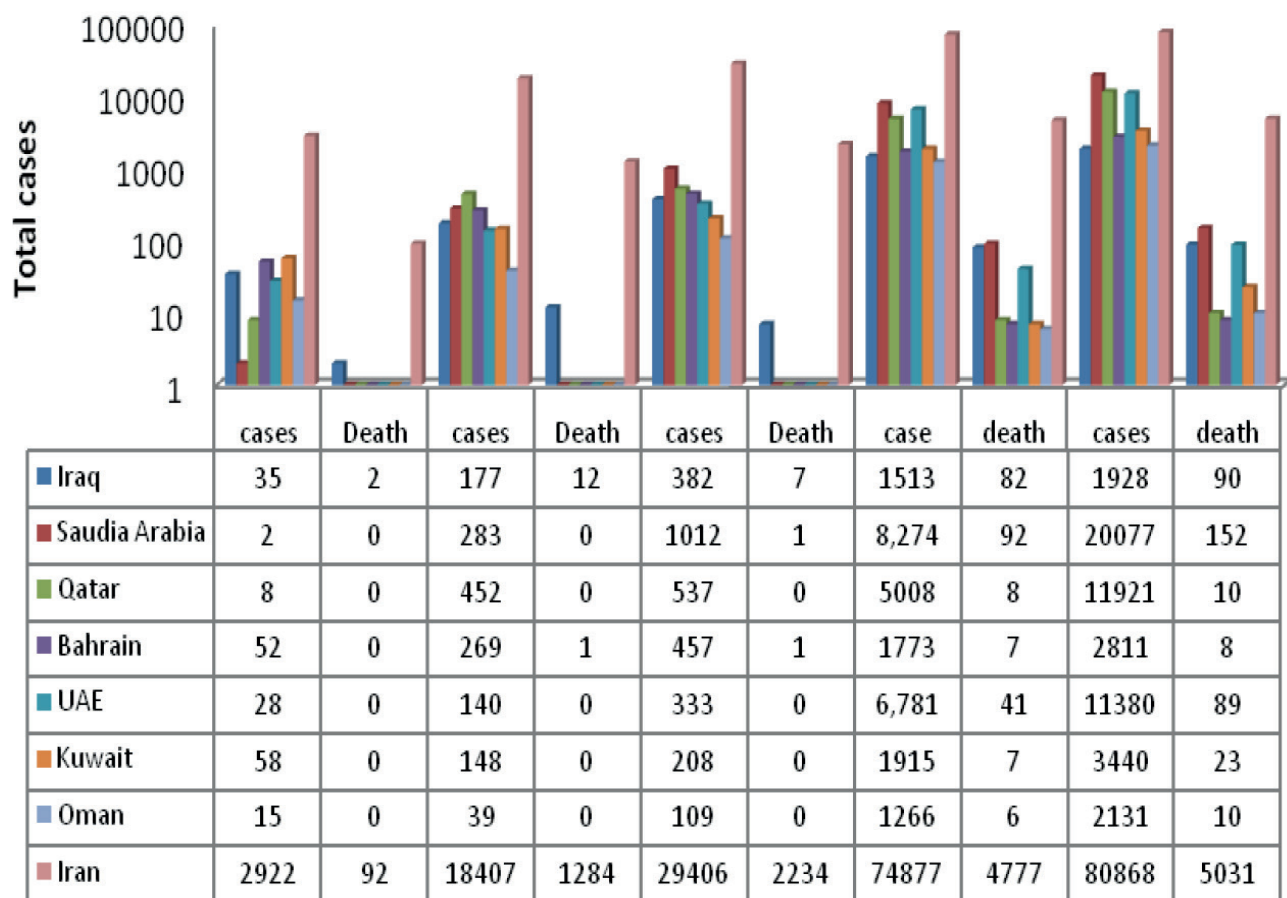


Figure 1. Confirmed cases and Deaths of COVID-19 in Gulf Countries for March- April.

Confirmed cases of COVID-19

COVID-19 regular prevalence data were obtained from the official website between 19 March- April 2020. The charts have been designed to describe the infections in the Gulf area; total cases were analyzed for confirmed infection and deaths in Iraq, Kuwait, Saudi Arabia, UAE, Qatar, and Oman with the first case of infection and its origin. On the other hand, early confirmed the first case in Saudi Arabia began may be the reason for its higher count of cases, as illustrated in figure (1). Many reasons may be attributed to the relatively low incidence of confirmed COVID-19 in the Iraqi population, high temperature (reached to 30-32 in April), prevention of socials aggregation such as Friday prey, religious occasions, closed schools, and Universities. Iraqi hygiene practices were outstanding in streets and houses; they almost wear gloves and masks, wash their hands with soap, shopping with care avoiding aggregation. Other factors may influence infection, such blood group, in which blood

group O is more abundant in the Iraqi population; researchers found that individuals with blood group O are more resistant to infections, including SARS and COVID-19. Also, vaccination strategies for the Iraqi population that taken on during past decades; vaccination of BCG and Measles may induce immune responses against COVID-19 infection.

Data collection

COVID-2019 regular prevalence data were obtained from the official website of (14) between 20 January and 18 April 2020. The charts have been extended to a collection of 18 governorates. Figures indicate a growing tendency against the disease network in the total incidence of COVID-2019. There was a persistent rise in the number of reported cases between one-day cases and cases the previous day. The frequency of newly reported cases of COVID-2019 and descriptive statics is evaluated in a detailed review of the results. Also, the pie

chart shows the proportion of Iraq confirmed cases who acquired COVID-19 overseas by region, including a category for acquired at traveling, which represents the cases acquired while traveling on visits to religious shrines trips.

The traditional infectious disease prediction model

In February 2020, it was first reported that the ongoing global pandemic was transmitted to Iraq, the first recorded cases of COVID-19 infections were identified in Najaf on 22 February 2020. In Iraq, illness is stigmatized, and many Iraqis are worried not to pursue medical treatment and get screened, which may contribute to inadequate numbers of cases. Quarantine has a certain stigma. Although 0.20 percent of the population of the KRG was checked, just 0.05 percent of the rest of the world was evaluated, and the possible disparity in overall positive statistics in the area and the rest of the nation was then illustrated. The first case was reported on 1 March of COVID-19 infection in Iraqi Kurdistan. The first fatality of the epidemic in Iraq is 3 March. It had been confirmed in the district of Sulaymaniyah.

The number of cases has risen to 67 on 4 March. The following day in Babil and Maysan, two new deaths were recorded. On 14 March, two patients died, on 18 March a death count rose to 12, and 10 new cases were identified, out of 105. Fifty new cases were reported on 24 March. The Ministry of Health has reported that there has been a minimum of 75 recoveries and 27 fatalities. On 25 March, the number of recoveries went up to 89, and 30 new cases were reported. The first case identified in the town of Ishaqi was the Saladin Governorate, thus verifying the virus for the first time in all 18 Iraqi provinces. On 5 April, there is a regular increase of 83 new cases in the number of confirmed cases and, thus, a total of 961, with 279 reported cases. On the same day, five new deaths were also confirmed. Both Baghdad, which saw a rise of 27 and Erbil, the capital of the KRG, which saw an increase of 18 cases, was due to the large increase in the incidents. The cases of Erbil is linked to a prohibited funeral meeting held on 21 and 23 March, which will continue to increase with inquiries and checks. On 15 April, a total of 15 new cases, the lowest in a single day since 21 March, were recorded.

Figure 2, showed that among all areas of Iraq, the number of portable infections, which we find is the largest in Baghdad city 22%, and then Najaf by 21%, while Erbil and Basra share by 13%, and Suleimaniya has risen by 11% and Karbala by 6%. As for the other southern Governorates, it was 3-1% in contrast to the Central Governorates and Kurdistan. Everybody had zero levels of Anbar, Nineveh, Salahuddin, and Maysan. Such percentages are due to the religious visits to central and southern governorates and trade crossings in Iraqi Kurdistan provinces that have been listed above.

In Baghdad's highest mortality rate, the rate of recovery expressed in the number of cases, accompanied by Najaf's 23 percent, and Suleimaniya 17 percent, whereas Dohuk also accounted for the lowest mortality level, which was 3 percent, in Kurdistan; That could be attributed to the injured's age, safety or other causes that we may address later. In Karbala, the 7 percent, and the level for in the regions with maximum rates. The Governorates of Anbar, Saladin, Babil Maysan, and Nineveh had zero recovery rates it's the same infection case. (figure 3).

Descriptive statistics of COVID-19 epidemic plateau in Gulf countries

The process of statistical analysis of a sample of data and use it to conclude its population includes the use of descriptive static for COVID- 19 in Gulf countries (table 2). We can test and start investigating causal relationships within infection data and country information with inferential statistics. We pursue useful information or models for data mining, especially in those which can be used to predict the epidemic development of COVID-19.

Descriptive statistics were reported for total Bahrain confirmed cases ($M=233.77$, $SD=406.37$, $min=0$, $mix=1744$) and the confirmed cases of Iran with difference in area and population ($M=1487.03$, $SD=24062.33$, $min.=0$, $max.=79494$) and Iraq with others' countries ($M=241.31$, $SD=433.38$, $min=0$, $max=1482$). The shortened COVID development cases in Oman comprised 8 Golf countries of a small public people ($M=72.02$, $SD=229.97$, $min=0$, $mix=1069$) also and Kuwait the confirmed cases have intermediate descriptive statistics' ($M=187.06$, $SD=373.85$, $min=0$, $mix=1658$). The means for Saudi Arabia

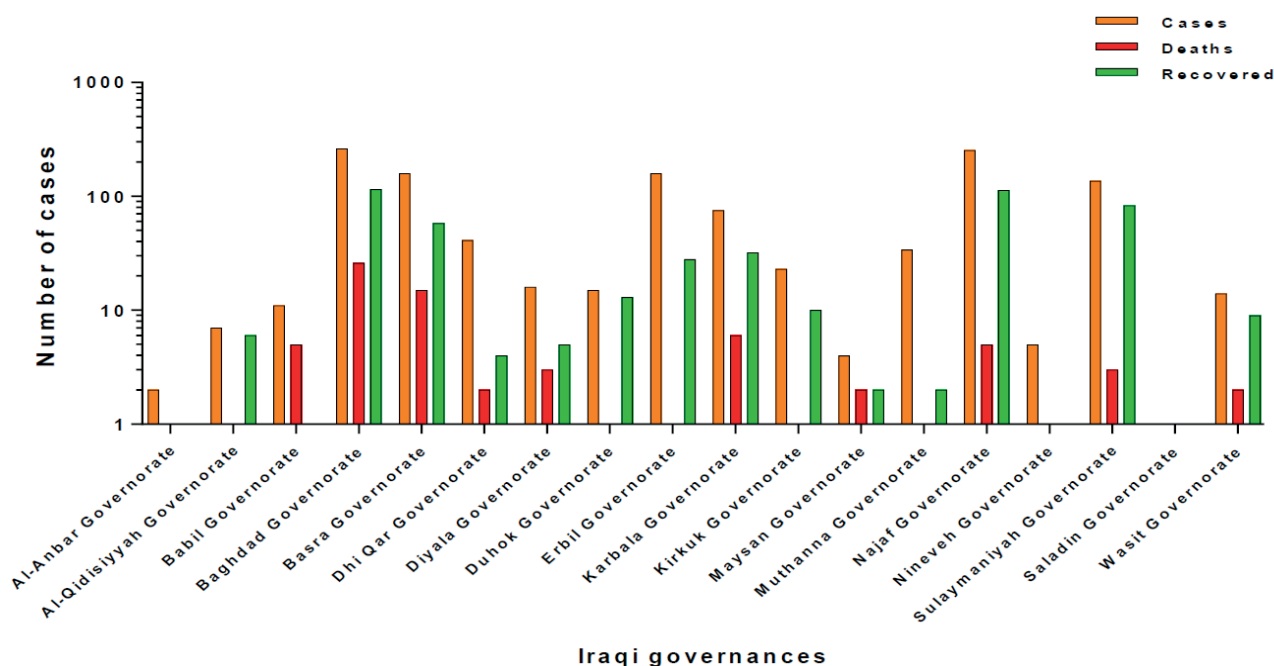


Figure 2. Prevalence graph of confirmed cases of COVID-19 in Iraq.

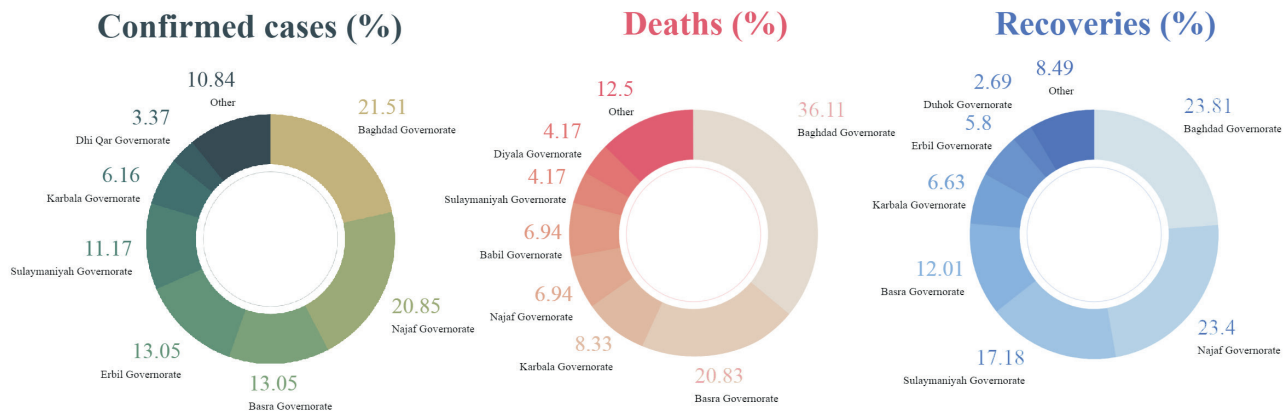


Figure 3. Dounut chart displays the proportion of reported Iraqi COVID-19 confirmed cases, deaths, and recoveries sofar.

were highly infectious in few months (SD=1955.04, M=1678.42, min.=1, max.=7142) for others of Gulf countries have foreigners and expatriates more than 2% (Qatar and UEA), so the max of cases was ≤ 4500 and meant ≤ 450 .

Skewness is the peakedness or flatness in COVID-19 cases that can be predicated the country statement in epidemic growth or stability. Statistics of the asymmetry of vector distribution was strongly distorted in all Gulf countries. Oman > UEA > Qatar > Kuwait > Bahrain > Iraq > Iran>Saudi Arabia became extremely biased countries. The degree of skewness determines how symmetrical the distribution of a variable is. The variance tests the component distribution accuracy. Nevertheless, we think that in every Gulf country, organizing is a grant that can be linked to the similarity of infection types, food, and living habits. Skewness in coved confirmed cases was represented in kurtosis. Kurtosis is the calculation of outliers found in the distribution, the values for asymmetry and kurtosis of ± 2 are considered appropriate, but most Gulf countries were disrupted by regular kurtosis means, Oman > UEA > Qatar > Kuwait > Bahrain > Iraq > Iran>Saudi Arabia.

The current case fatality rate (CFR) of COVID-19

CFR is the ratio between confirmed cases and deaths, the figure 4. the number of confirmed COVID-19 events, and the total number of fatalities is seen in contrast. In every individual illness, there is no specific CFR statistic. The CFR differs from location to position and typically shifts with time. There are significant differences between the CFRs in Gulf countries, which are less than 1% in Oman, UEA, Kuwait, Bahrain, and Saudi Arabia, hitting 7.5% in Iraq and 8% in Iran. The Gulf Countries vary in material capacities in the medical knowledge,

and other professional capabilities of trained administrators and Iraq is the competence of health personnel, who in recent years, have assisted emergencies.

However, it does not imply a fair assessment of the real likelihood that someone would die of COVID-19. Many cases are asymptomatic versus symptomatic, or whether the same test criteria apply to countries. The actual mortality rate remains uncertain without more and more systematic monitoring and reporting standards. To consider and direct decisions taking the CFR variations. CFR will assist us in recognizing the disease's nature and what to do with it since CFR is a very weak metric for a small sample size mortality event. As time goes by, the CFR continues to slip.

Relationship between ABO blood group and COVID-19.

When comparing total cases of COVID - 19 in Gulf countries with the origin of their first case as Iran, which reached to 92542 at 28-April 2020, this may contribute to other factors such as blood group and their associated diseases such as diabetes, hypertension, and cardiovascular diseases. Blood group O is more abundant in Iraqi, Kuwait, Oman, Saudi Arabia, and UAE population; researchers found that individuals with blood group O are more resistant to infections, including SARS and COVID-19 while, blood group A may need more attention to prevent infection¹². This assumption may differ due to antigens of blood group A on different cells that can act as binding adhesion receptors, which allows COVID-19 to adhere and enter the cells via the ACE2 receptor. On the other hand, anti-A and anti-B antibodies that circulate in blood group O persons may be part of the first natural defense mechanism against viral infection.

COVID confirmed cases	Min	Max	Mean	Std. Deviation	Skewness		Kurtosis
					Statistic	Std. Error	Statistic
Bahrain	0	1744	233.77	406.37	2.16	38.92	4.39
Iran	0	79494	1487.03	24062.33	1.52	2294.25	0.91
Iraq	0	1482	241.31	433.38	1.78	41.89	1.81
Kuwait	0	1658	187.06	373.85	2.42	0.37	6.54
Oman	0	1069	72.02	229.97	2.75	22.55	7.3
Saudi Arabia	1	7142	1678.42	1955.04	1.27	291.44	0.74
Qatar	0	4663	485.99	987.03	2.49	95.86	5.76
UEA	0	5825	569.52	1342.92	2.72	131.68	6.59

Table 2. Descriptive Statistics for COVID-19 in Gulf countries.

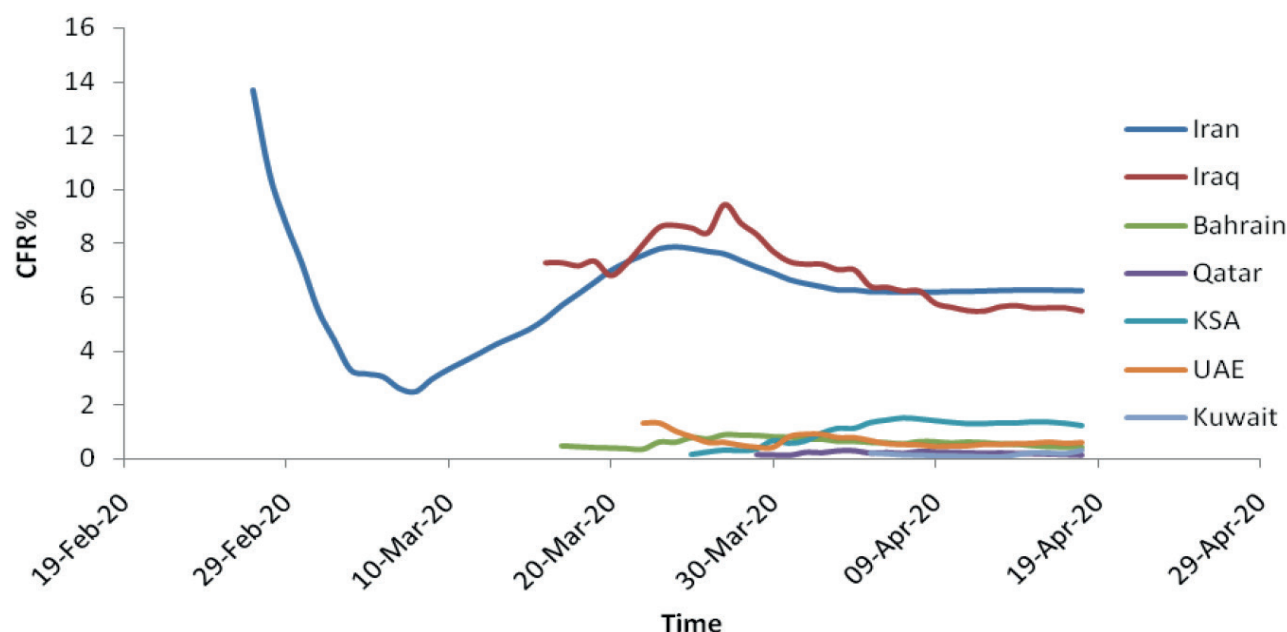


Figure 4. The case fatality rate of the ongoing COVID- 19 pandemics in Gulf countries.

The blood groups consist of two antigens, A and B antigens; they are produced by the ABO gene and are autosomal codominant. The group O phenotype is an autosomal – recessive phenotype due to the homozygous inheritance of two null ABO alleles; group O also express the H antigen, the biosynthetic precursor to A and B antigens. In addition to red blood cells, ABH antigens are widely expressed in many other tissues and secretions, such as intestinal mucosa, kidney, heart endothelium, and other organs. For such reason, the virus, which displays many glycosylated regions, could adhere to another organ such as intestinal mucosa. One of the most attractive areas of research is to find whether blood type and their natural antibodies could reduce the spread or severity of the infection.

On the other hand, many types of research were focused on the role of blood group in infection incidence of many pathogenic bacterial species as Enterotoxigenic *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, as well as many viruses as norovirus, Norwalk virus¹³, and Hepatitis B virus¹⁴. The most accepted theory is that antigens of blood group on different cells can act as binding receptors that allow bacteria and viruses to attach and enter the cells¹⁵. On the other hand, anti-A and anti-B antibodies may be part of the body's natural defense that takes place to prevent infection or limit it.

The original severe acute respiratory syndrome outbreak caused by SARS-CoV in winter of 2002 to 2003 which infected 8000 persons worldwide, with a fatality rate of 10%; SARS-CoV is an enveloped RNA virus which adheres to host cells via viral adhesion glycoprotein the spike protein 210-230 kDa with 23 potential N- glycosylation sites¹⁵. The analysis of glycan structure showed a wide range of structures, including complex N- glycans with 2 to 4 antennae capable of supporting ABH epitopes. The primary host target cells of SARS-CoV are respiratory and gastrointestinal mucosa, so its likely most human isolates express ABH antigens on the S protein and host envelope GSLs; expressing A antigen can be blocked by monoclonal anti-A and human anti-A¹⁶. Recent pandemic COVID-19 is similar in structure to SARS-CoV regarding its spike protein, which is called NSP15; scientists from the U.S. found 89% identity to NSP15 for both viruses. However, spike protein for SARS-CoV-2 is 10-20 times more likely to attach

to human cells, which explain its rapid spread throughout the world.

According to epidemiological study as well as in vitro study, Guillon *et al.*¹⁶ hypothesized that group O persons are more resistant to SARS-CoV due to ABO antibodies and could decrease the rate of infection throughout the population. The same pattern of distribution of blood group¹⁷ O, A, B, AB occurring in the order O > A > B > A.B. were reported in the studies from neighboring countries; Saudi Arabia¹⁸, Kuwait¹⁹, Iran²⁰. Although, protection from infection may be influenced by the ABO antibodies titer, secretor status, and the incidence of group O in population. High titer of anti-A (1:256) gave effective blocking to infection, while the lower titer was ineffective¹⁵. Other factors may affect, such as the environment of the population as industrialized countries tend to be less in ABO-antibodies titer. A non-secretor phenotype would also nullify viral neutralization since viral transmitted from a non-secretor lacks ABH expression. The result of Zhao and others²¹ a significantly increased risk of blood group A for SARS-CoV-2 in comparison to group O. The differences in significant antigens present on the surface of red blood cells and other epithelial cells have significant clinical importance. These antigens have proved the association between ABO blood group and several serious diseases²²; besides infections, the most important is, cardiovascular disorders, diabetes and IL-6, and IL-10, IL-18, which may affect the infection with recent pandemic SARS-CoV-2 (table3).

Many researchers ensured the involvement of ABO blood group and diabetes; it was found that blood type O had a lower risk of type 2 diabetes mellitus²³. Another work found that the B group was more prevalent in diabetic patients^{24,25}.

Hypertension has a significant impact on the health of communities in the whole world; it suspected to link to the ABO blood group via genetic determine at conception and remain fixed for life. Hence, its frequency distribution follows a known pattern governed by gene transmission from generation to generation and varies with the race and geographical distribution of the human being²⁶. There are reports of increased cardiovascular (CV) risks in different blood groups (non-O group), and an increase in B.P. is considered as a common CV risk²⁷.

Disease	Low risk	High risk	Order of risk	Ref.
COVID-19	O	A	A-B-AB-O	7
Other viruses:				
Norwalk virus	B	O	O-A-AB-B	8,9
Hepatitis B virus	B	O	O-A-AB-B	
Diabetes	O	B	B-A-AB-O	20
Hypertension	AB	O	O-A-B-AB	24
	AB	O		25
Hypertension	B	AB	B-O-A-AB	22
Cardiovascular	O	B	B-A-AB-O	23
	AB	O	O-A-B-AB	25
IL-10	AB	O	O-A-B-AB	26
IL-6	AB	O	O-A-B-AB	26

Table 3. Relationship between ABO group risk factors and diseases.

A study that came from Iraq suggested that blood group O is at risk of hypertension and total cholesterol, followed by group A, B, then AB²⁸. Coronary Artery Diseases in Iranian Patients is more prevalent in blood group O comparing with another group²⁹. Other estimation of hypertension gave the percentage as follows B (8.7%) followed by group O (7.6%) group A (3.7%) and group AB (1.9%). Inflammation interleukins are also associated with ABO blood group; ABO antigens O and A2 are associated with increased IL-10 levels and decreased VWF level, and that the ABO antigens also influence the levels of sTF in Patients to have Artery coronal syndrome which is associated with disease outcomes in ACS patients³⁰.

Since the emergence of COVID-19 many types of research were conducted to determine specific comorbidities associated with increased risk of infection and worse outcomes with the development of increased severity of lung injury and mortality, the most common comorbidities in one report were hypertension (30%), diabetes (19%), and coronary heart disease (8%). Diabetes has emerged as an essential risk factor for severe illness and death from COVID-19³¹. Diabetes was present in 19% of COVID-19 cases in Wuhan, China, and non-survivors were significantly more likely to have diabetes than survivors (31% vs. 14%, $P = 0.0051$). While another study found that 27% of patients achieving the primary endpoint of intensive care unit (ICU) admission, mechanical ventilation, or death, compared with 6.1% if none of these complications occurred. Also, Bruce Bode *et al.*³² in the USA diagnosed that diabetes and uncontrolled hyperglycemia frequently occurred among COVID-19 patients who had a particularly high mortality rate reached to 28.8% in diabetes patients who infected with COVID-19.

A Chinese study dealing with immune markers in severe cases of COVID-19 found that baseline IL-6 was significantly increased in patients with severe type, which is ensured by the maximal body temperature during hospitalization and C.T. findings. Baseline IL-6 was also significantly linked to the increase of baseline level of other immune markers, the CRP, lactate dehydrogenase (LDH), ferritin, and D- Dimer³³. The increase of baseline IL-6 level suggests that it may positively

correlate with the cytokine storm syndrome of COVID-19. An early study by Jaques³⁴ demonstrated that a surface viral protein from the SARS virus directly stimulated the production of tumor necrosis factor (TNF)- and interleukin.

Recent pandemic SARS-CoV-2 is similar in structure to SARS-CoV regarding its spike protein, which is called NSP15; scientists from the U.S. found 89% identity to NSP15 for both viruses. However, spike protein for SARS-CoV-2 is 10-20 times more likely to attach to human cells, which explain its rapid spread throughout the world. Proteome analysis derived from online NCBI databases showed existing the following:

- A polyprotein orf1a 7096 amino acid long (QHD43415.1)
- A surface glycoprotein "Spike" (1273 aa - QHD43416.1)
- ORF3a or structural protein E (273 aa - QHD43417.1)
- An envelope protein (ep - 75 aa - QHD43418.1)
- A membrane glycoprotein (MGP - 222 aa - D43419.1)
- ORF6 (61 aa - QHD43420.1)
- ORF7a (121 aa - QHD43421.1)
- ORF8 (121 aa - QHD43422.1)
- A Nucleo capsid phosphoprotein (419 aa - QHD43423.2)
- ORF10 (38 aa - QHI42199.1).

Conclusions

The present research has shown that for the COVID- 19 epidemic, an epidemic spike somewhat of an epidemic height, owing to the length of the height cycle from week to month and beyond. For this, the epidemic spike is emotional rather than a strong epidemic. Accurate enough, as figures and quadratic growth are contained here, are the details necessary to discern between early exponential growths.

It is also noteworthy that the fit isolates of January 20/2020 are a deciding moment for the creation of the gulf breakup among the Gulf States. It has been predicted that growth would not be exponential and that they would get degrees slowly off

in response to demographic changes and interventions. The case fatality rate in the gulf community is already still below 5 percent, but monitoring measures should be optimistic that this will not rise. Significant gaps in our knowledge of epidemiology, community prevalence, and clinical spectrum of infection and disease need urgent definition.

After its diagnosis as a causative agent of pneumonia cases in China, COVID-19 has become a pandemic and affects almost all places in the whole world. Arab countries in the Gulf region recorded their first case of infection from persons who came from Iran. Arab countries became alert for the new pandemic disease and gave serious attention to overcome COVID-19.

Blood group is One of the most critical subjects in controlling COVID-19 and its severity; many serious diseases link to blood group either through antigens located at the erythrocyte or encoding different genes that correlate to diseases. Diseases such as hypertension, diabetes, coronary heart disease, cytokines secretion as well as infection by the virus itself. Knowledge of disease history to a person with COVID-19 allowed proper cure, observation, and treatment to avoid the severity of the infection.

Bibliographic references

1. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395(10224):565–74.
2. Tuite AR, Fisman DN. Reporting, Epidemic Growth, and Reproduction Numbers for the 2019 Novel Coronavirus (2019-nCoV) Epidemic. *Ann Intern Med*. 2020. (February):2019–20. 2.
3. Zhao S, Cao P, Gao D, Zhuang Z, Chong MKC, Cai Y. Epidemic growth and reproduction number for the novel coronavirus disease (COVID-19) outbreak on the Diamond Princess cruise ship from 20 January to 19 February, 2020: A preliminary data-driven analysis. SSRN. 2020. Preprint at: <https://ssrn.com/abstract=3543150>.
4. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B. (2020). Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect*;9(1):386–9.
5. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*. 2004;203(2):631–7.
6. Chen YS, K.; Qian, W. Asians Do Not Exhibit Elevated Expression or Unique Genetic Polymorphisms for ACE2, the Cell-Entry Receptor of SARS-CoV-2. Preprints, 2020, 2020020258. 2020.
7. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*.<https://doi.org/10.1038/s41586-020-2012-7>.
8. Roussel, Y., Giraud-Gatineau, A., Jimeno, M. T., Rolain, J. M., Zandotti, C., Colson, P., & Raoult, D. (2020). SARS-CoV-2: fear versus data. *International journal of antimicrobial agents*, 105947. Advance online publication. <https://doi.org/10.1016/j.ijantimicag.2020.105947>
9. Coleman CM, Sisk JM, Mingo RM, Nelson EA, White JM, Frieman MB. Abelson Kinase Inhibitors Are Potent Inhibitors of Severe Acute Respiratory Syndrome Coronavirus and Middle East Respiratory Syndrome Coronavirus Fusion. *J Virol*. 2016;90(19):8924–8933. Published 2016 12 September. doi:10.1128/JVI.01429-16
10. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa2001316> [Epub ahead of print]..
11. Lee PI, Hsueh PR. (2020). Emerging threats from zoonotic coronaviruses—from SARS and MERS to 2019-nCoV. *J Microbiol Immunol Infect*. <https://doi.org/10.1016/j.jmii.2020.02.001> [Epub ahead of print].
12. Zhao J., Yang Y., Huang H., , Li D. Gu D., Xiangfeng, Lu X, Zhang Z, Liu L., Liu T., Liu Y. He Y., Sun B., Wei M., Yang G. , Wang X., Zhang L. Zhou X.Xing M. Wang P. G. (2020). Relationship between the ABO Blood Group and the COVID-19 Susceptibility.<https://doi.org/10.1101/2020.03.11.20031096>.
13. Hutson A.M., Atmar R. L., Graham D. Y. and ester M. K. (2002). Norwalk virus infection and disease is associated with ABO Histo- Blood group Type. *The Journal of Infectious Diseases*, 185:1335–1337.
14. Jing W, Zhao S, Liu J, et al. ABO blood groups and hepatitis B virus infection: a systematic review and meta-analysis. *BMJ Open* 2020; 10:e034114. doi:10.1136/ bmjopen-2019-034114.
15. Mazda T, Yabe R, NaThalang O, Thammavong T, Tadokoro K. 2007. Differences in ABO antibody levels among blood donors: a comparison between past and present Japanese, Laotian and Thai populations. *Immunohematology*,23:38–42.
16. Guillon P, Clement M, Seville V, Rivain J-G, Chou C-F, Ruvoen-Clouet N, Le Pendu J.(2008). Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. *Glycobiology*18:1085–1093. <http://dx.doi.org/10.1093/glycob/cwn093>.
17. Saleh, S. M. and Abood A. S. (2016). ABO and Rh (D) Blood Groups' Distribution and Gene Frequencies in North Baghdad Population-Iraq. *International Journal of Scientific & Engineering Research*, 7(8): 581-584. <http://www.ijser.org>.
18. Sarhan MA, Saleh KA, Bin-Dajem SM. (2009) Distribution of ABO blood groups and rhesus factor in Southwest Saudi Arabia. *Saudi Med J*;30(1):116–119.
19. Al-Bustan S, El-Zawahri M, Al-Azmi D, Al-Bashir AA. (2002). Allele frequencies and molecular genotyping of the ABO blood group system in a Kuwaiti population. *Int J Hematol*;75(2):147–153.
20. Boskabady MH, Shademan A, Ghamami G, Mazloom R.(2005). Distribution of blood groups among population of the city of Mashhad (North East of Iran). *Pak J Med Sci Q*;21(2):194–198.
21. Zhao J., Yang Y., Huang H., , Li D. Gu D., Xiangfeng, Lu X, Zhang Z, Liu L., Liu T., Liu Y. He Y., Sun B., Wei M., Yang G. , Wang X., Zhang L. Zhou X.Xing M. Wang P. G. (2020). Relationship between the ABO Blood Group and the COVID-19 Susceptibility.<https://doi.org/10.1101/2020.03.11.20031096>.
22. Cooling L. (2015).Blood Groups in Infection and Host Susceptibility. *Clinical Microbiology Reviews*, 28 (3):801- 870.
23. Fagherazzi G, Gusto G, Clavel-Chapelon F, Balkau B, Bonnet F. (2015). ABO and Rhesus blood groups and risk of type 2 diabetes: evidence from the large E3N cohort study. *Diabetologia*; 58(3):519–22. PMID: 25533388
24. Bener A. andYousafzai MT. (2014).The distribution of the ABO blood groups among diabetes mellitus patients in Qatar. *Niger J ClinPract*; 17(5):565-8. PMID: 25244264
25. Moizadeh F, MahdihNajafabady G, Toghiani A. (2005). Type 2 diabetes mellitus and ABO/Rhblood groups. *J Res Med Sci*. 2014; 19(4): 382. PMID: 25097615.
26. Kearney PM, Whelton M, Reynolds K et al. (2005): Global burden of hypertension: Analysis of worldwide data. *Lancet*, 365:217-23.
27. Okeke CO., Okoro US. and Babatunda A. (2018). Variation in activated partial thromboplastin time and prothrombin time in individuals of A, B, AB and O blood groups. *Iraqi J. Hematol*. 7: 85-89.
28. Jasim W.E. (2012). Association of ABO blood group in Iraqis with hypercholesterolemia, hypertension and diabetes mellitus. *EMHJ* 18 (8):888-891.
29. Anvari M. S., Boroumand M. A., BineshEmami, AbbasaliKarimi, Maryam Soleymanzadeh, SeyedHesameddinAbbasi, SoheilSaadat, (2009). ABO Blood Group and Coronary Artery Diseases in Iranian Patients Awaiting Coronary Artery Bypass Graft Surgery: A Review of 10,641 Cases. *Lab medicine*, 40 (9) :528-530.
30. Johansson Å, Alfreðsson J, Eriksson N, Wallentin L, Siegbahn A (2015) Genome-Wide Association Study Identifies That the ABO Blood Group System Influences Interleukin-10 Levels and the Risk of Clinical Events in Patients with Acute Coronary Syndrome. *PLoS ONE* 10(11): e0142518. doi:10.1371/journal.pone.0142518.
31. Hussain, A., Bhowmik, B., & do Vale Moreira, N. C. (2020). COVID-19 and diabetes: Knowledge in progress. *Diabetes research and clinical practice*, 162, 108142. Advance online publication. <https://doi.org/10.1016/j.diabres.2020.108142>

32. Bruce Bode , Valerie Garrett, Jordan Messler, Raymie McFarland, Jennifer Crowe, Robby Booth, David C. Klonoff, M.D.(2020). Glycemic Characteristics and Clinical Outcomes of COVID-19 Patients Hospitalized in the United States.
33. Metha P., McAutey D. F., Brown M., Sanchez E., Tatterall R. and Mansan J. J.(28 March 2020). COVID-19 considers cytokine storm syndrome and immunosuppression. *Lancet*,395(10229).
34. Jacques A. Alexandre Jacques,1 Christian Bleau,1 Claire Turbide,2 Nicole Beauchemin2 and Lucie Lamontagne1 et al. Macrophage interleukin-6 and tumor necrosis factor- α are induced by coronavirus fixation to Toll-like receptor 2/heparansulphate receptors but not carcinoembryonic cell adhesion antigen 1a, *Immunology* (2009) doi: 10.1111/j.1365-2567.2008.02946.x

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REVIEW / ARTÍCULO DE REVISIÓN

Cellulose for the Effective Decontamination of Water Pollution

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Abstract: The widespread industrialization, urbanization, and technological development have triggered the daily release of considerable amounts of pollutants, specifically in aquatic environments. Previous research and work-studies indicate the existence of defined properties, such as low cost, non-toxicity, biodegradability, reusability, and easy synthesis, preparation or extraction, which make a material an ideal agent for the remediation of water or the environment. Therefore, the scientific community has focused on the development and study of several novels, environmentally friendly, and cost-effective materials. Cellulose is the most abundant natural polymer encountered worldwide. Thereby, due to the unique biological properties that this biopolymer possesses, it has emerged as a potential candidate to replace synthetic materials for practical bioremediation of contaminated water. Furthermore, the presence of hydroxyl groups on its surface makes this biopolymer highly malleable, thus significantly enhancing its physicochemical properties by using a wide variety of functional groups and modification methods. The present review describes the different biopolymers useful for remediation of environmental pollution, explores in more detail the characteristics of cellulose and its promising applications in the decontamination of water pollution, and pays special attention to the removal of heavy metal ions, dyes, and hydrophobic organic compounds.

KeyWords: cellulose, water pollution, water decontamination, biopolymers, biodiversity.

Introduction

In the last decade, one of the significant threats concerning the environment is the alarming growth rate of industrialization, urbanization, and technological development¹⁻³. Environmental contamination is commonly classified into the air, water, and soil pollution². Due to the high importance and value of the limited water resources for all the living beings, it is of fundamental interest to explore efficient and safe solutions for the proper decontamination of water resources^{4,5}. Water pollution is defined as the disturbance of the quality and the standard conditions of any aquatic environment by either organic or inorganic foreign matter⁶. Numerous hazardous contaminants are daily released by diverse anthropogenic sources such as industrial procedures, food processing, clothing manufacturing, landfills, plastic production, medicine development, and pesticide usage^{3,7-10}. The most common contaminants present in the different types of pollution (*i.e.*, water, air, and soil) are carbon monoxide, chlorofluorocarbons, heavy metals (arsenic, copper, chromium, lead, cadmium, mercury, and zinc), hydrocarbons, chemical wastes, nitrogen oxides, organic compounds (volatile organic compounds and dioxins), sulfur dioxide, pesticides, and particulates^{11,12}.

Additionally, pollution can lead to the development of multiple diseases negatively influencing human wellness^{13,14}. Hence, environmental pollution harms the ecosystems and the biodiversity of the world. Drinking contaminated water with pesticides or heavy metal ions is related to the development of tumors in almost all organs, immunosuppression, and disruption of the regular hormonal activity, causing damage to the nervous system, as well as the kidney and liver⁹. In the same way, the reason for the appearance of different respiratory, cardiovascular, neurological diseases, adverse effects on the endocrine system, and the neurodevelopment in infants is attributable to the exposure to water pollution^{9,14}.

Current Remediation Technologies for Water Pollution Decontamination

Currently, nanotechnology approaches, different materials,

and novel technologies have been explored and studied by the academia and scientific community to remediate the water contamination^{11,15-23}. The use of various organic and inorganic materials, the implementation of nanotechnology, and the manipulation of particles at the nanoscale have been the most studied and developed decontamination methods in the literature²⁴. Also, it has been reported that porous nanoscale materials (NMs), NPs-TiO₂, Graphene-based NMs, Titanate Nanotubes, Fullerenes, Silica Nanocomposites or Polymeric-based NMs can be used as adsorbents for different contaminants, water decomposition agents or can either enhance the microbial degradation of waste and toxic materials or directly catalyze them^{2,25,26}. Notwithstanding, these innovative methods have been gaining much attention from the academy, and from governmental organizations, NMs have demonstrated to be unstable at standard conditions, present synthesis complexity, and may create adverse toxic byproducts once used². The process of synthesis of NMs is expensive, without mention their lack of renewal or reuse capacity, turning them eventually into new pollutants. Consequently, only a few nanotechnologies approach for remediation of polluted water can be finally marketed²⁷.

Sustainability of Biopolymers

Several studies point out the existence of defined properties that would make a material a suitable agent for the decontamination of water pollution, features such as cost-effectiveness, non-toxicity, biodegradability, biocompatibility, reusability, and ease of synthesis, preparation or extraction¹⁵. These critical properties are all characteristics of natural polymers encountered in the biodiversity, listed in Table 1. Based on their physicochemical and environmentally friendly properties, academia and governmentally organizations have been strongly interested in the development and exploration of biopolymers to find efficient and greener solutions to environmental issues and concerns²⁸.

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Biomaterial	Application	References
Cellulose	Removal of different contaminants (HOCs and VOCs) from water and air pollution	(29,30)
Chitosan	Water purification	(7)
Alginate	Removal of dyes and heavy metals	(31)
Lignin	Metal adsorption	(32)
Silk	Remediation of air pollution	(33)

Table 1. Most common biopolymers and their applications in environmental pollution decontamination.

Chitosan

Chitosan is a linear polysaccharide obtained by the incomplete alkaline deacetylation of chitin. This biopolymer is known to be the second most abundant polysaccharide in Earth after cellulose³⁴. Usually, it is mainly extracted from the exoskeletons of crustaceans, shrimps, and crabs' shells³⁵. The structure of chitosan is composed of b-(1,4)-2-acetamido-2-deoxy-D-glucose and b-(1,4)-2-amino-2-deoxy-D-glucose monomers as building blocks repeats. This biodegradable polymer has a wide range of potential applications, including food preservation, packaging technologies, rice production, and tissue regeneration technologies, among others^{34,36,37}. According to Alaa Jabbar *et al.* (2016), chitosan had been widely used in water purification plants to effectively remove oils, grease, and heavy metals in contaminated water³⁸. Moreover, Zhao *et al.* (2019) proved that carboxylated chitosan composite beads were useful materials for the efficient adsorption and immobilization of Cu²⁺ ions in contaminated water and soil³⁹. Thus, several studies have demonstrated the remediation potential of chitosan for decontamination purposes of the environment⁴⁰.

Alginate

Alginate is an anionic biocopolymer mainly composed of an alternative repeating of blocks of glucuronate and mannuronate. This biomaterial is commonly extracted from brown seaweed (*Phaeophyceae*); its extraction methodology requires the use of strong bases, salts, and acid treatments, such as NaOH, NaCl, and dilute HCl. Based on its promising biological properties (*i.e.*, biocompatibility, low toxicity, relatively low cost, and mild gelation), alginate has been extensively used in different biomedical applications. Diverse studies depicted the use of alginate in wound healing and applications in tissue engineering, as well as its use in drug delivery systems for the release of a variety of bioactive agents⁴¹. In 2018, Wang *et al.* extensively described the utility and application of different alginate-based composites as effective biosorbents for environmental remediation objectives. Thereby, they successfully demonstrated the great potential of these hybrid biomaterials for the removal of dyes, heavy metals, antibiotics, and other pollutants from contaminated water³¹.

Lignin

Lignin is a racemic heteropolymer composed of aromatic subunits derived from phenylalanine. In nature, lignin provides mechanical support to plant cell walls; it is synthesized from the oxidative coupling of three hydroxycinnamyl alcohol monomers

that differ in their methoxylation degree: p-coumaryl, coniferyl, and sinapyl⁴². Currently, lignin is a biomaterial with prevalent importance in the industry. There are many examples that demonstrate how lignin can improve the efficiency of the industrial procedures in the manufacturing of products derived from plant biomass, forage digestibility and, enhancing the process of fabrication of biofuels from the recollection of feedstock until their application or commercialization⁴³. Many studies have been conducted to assess the feasibility of lignin-based gels and green porous lignin-based spheres as potential heavy metal capturing agent for bioremediation. As reported by Parajuli *et al.* in 2005, the application of lignin-catechol complex gels exhibits optimal low cost, simple preparation, and biodegradation properties. Besides, this complex presents excellent adsorption characteristics for the assessed cations, such as Pb²⁺^{44,45}.

Silk

Silk is a natural fiber produced mainly by certain spiders to build their cocoons or webs⁴⁶. Although silk is produced by many insects, only the filaments made by the mulberry silk moth *Bombyx mori* are commercially available owing to their biocompatibility, biodegradability, non-toxicity and adsorption properties⁴⁷. In addition, silk produced by spiders can be used in other commercial applications, for instance, weapon and telescope cross-hairs and other optical instruments⁴⁶. Due to their mechanical qualities of strength, elasticity, softness and other features, such as improved absorbency, an affinity for dyes, and great adaptability to various forms of twisting, natural silk fibers have been recognized as a promising biomaterial suitable for the development of surgical threads and wound dressing, to name a few⁴⁸. Moreover, Wang *et al.* (2016) have reported a human-friendly, high efficient, and lightweight silk nanofiber air filter which exhibits not only a considerable filtration efficiency for submicron and particulate matter but also antibacterial activity demonstrating that this biopolymer is appropriate for remediation of air pollution³³.

Cellulose Applications for the Remediation of the Water Pollution

Cellulose is the most abundant biopolymer available worldwide^{4,49,50}. This biomaterial is a highly ordered crystalline polysaccharide consisting of parallel arrays of β -1,4-linked glucose chains⁴⁹. Several studies suggest that the characteristic cellulose high order structure is the result of basic dimer repeats, known as cellobiose⁵¹⁻⁵³. Also, these dimer repeats are described in the literature as two D-glucopyranose units linked by β -1,4-glycosidic bonds^{4,54-56}. Moreover, cellulose

chemical structure is based on the presence of strong H-bonds between their multiple hydroxyl groups, which confers its unique physicochemical features such as hydrophilicity, chirality, crystallinity, or biodegradability⁵⁷. Furthermore, the highly dense OH-groups on its surface structure allows this natural polymer to be readily functionalized and modified by diverse chemical methods using a variety of functional groups to hence enhance and modify its physicochemical properties. This makes cellulose a versatile and malleable material⁵⁵. As a result, cellulose has been of particular interest due to its low cost, availability, flexibility, secure processing, non-toxic, and biodegradable properties, emerging as one of the most promising substitutes for synthetic and fuel-based materials^{21,50,58}.

Cellulose is a water-insoluble material and a crucial component in the structural maintenance of plant cell walls⁵⁶. Nowadays, there are several well-known sources for cellulose, such as plants, algae, bacteria, or sea animals: Thanks to their abundance and economic viability, plants have been so far the primary source of cellulose extraction^{29,56,59}. Indeed, the most common cellulose sources are wood pulp and cotton linters. Besides, agricultural wastes such as sugarcane bagasse, fruits, grasses, or rice straws, are other sustainable plant sources to produce cellulose. Bacterial cellulose is unique as it offers several advantages over plant-derived cellulose (*i.e.*, unique nanostructure, purity, higher-dimensional stability, higher mechanical strength, and polymerization). Additionally, the marine invertebrate Tunicates are well-known for assembling cellulose. These sea animals use cellulose as a skeletal structure in their leathery mantle tissues, which is produced by enzyme complexes found in the membrane of the tunicate epidermis⁵⁰.

Per the excellent and promising biological properties that biopolymers possess, cellulose has emerged as the perfect decontamination agent for the development of greener and improved environment remediation technologies, as illustrated in Fig. 1. Moreover, Latin America (LATAM) biodiversity plays an essential role as the ideal scenario for the extraction of an abundant unique variety of cellulose fibers^{60,61}. LATAM houses a significant fraction of the world's biodiversity, including the most abundant biomes on Earth. The richness in flora and fauna of the Amazonian biodiversity plays an essential role in the securing of unique, diverse, and eco-friendly native biomaterials mentioned above⁶¹. Despite its small size (250.000 km² or 1.5% of South America extension), Ecuador hosts 18.000-22.000 species of plants, ranking it among one

of the most biodiverse countries in the world^{62,63}. Therefore, numerous studies have focused on the usage of different cellulose-based materials extracted from biodiversity that can effectively adsorb or capture water contaminants^{4,20,29,39,64-66}. Besides, the literature has shed light onto the mechanism and the physicochemical properties related to the performance of these biopolymers, since it is of paramount consideration that the scientific community clearly understands that natural sources play along with the remediation capabilities of cellulose^{32,67,68}.

The most common methods described in the literature for removing contaminants from polluted water include sorption, chemical precipitation, and electrochemical treatment, among others. Of these, the sorption remains one of the most effective and efficient processes^{20,69}. Some cellulose-based adsorbent is commonly used for heavy metal, and dyes removal from wastewater and are listed in Table 2⁷⁰.

As a consequence of the easy modification of cellulose by a vast number of functional groups and via chemical or physical methods, one of the first applications of functionalized cellulose is the remediation of contaminated water from heavy metals and different residues contaminants. For instance, Tursi *et al.* (2018) demonstrated that cellulose fibers with a hydrophobic surface extracted from Spanish Broom (SB) are a suitable material for the fast and significant adsorption of hydrophobic organic compounds (HOCs) present in water pollution. Specifically, this research team modified the surface of SB cellulose fibers by chemical nebulization protocols using 4, 4'-diphenylmethane diisocyanate to remove the hydrophilic surface properties of the polymer. After confirming the functionalization of the cellulose is confirmed by XPS and ATR-FTIR, they performed batch experiments to test the absorption kinetics and capacity of the fibers to remediate polluted water with gasoline petrol. As an outcome, these functionalized SB cellulose fibers could adsorb petroleum hydrocarbons with efficiency higher than 90% within a few minutes, which makes this biomaterial a novel green technology for the remediation of polluted water²⁹.

Gamze *et al.* screened the ability of cellulose graft copolymers to remove heavy metal ions from aqueous solutions. They considered the ion-exchange property of the biomaterial and studied the competitive replacement of Pb²⁺, Cu²⁺, and Cd²⁺ ions. The copolymers used were cellulose-g-polyacrylic acid (cellulose-g-pAA) with different grafting percentages, cellulose-g-p(AA-NMBA) prepared by grafting of acrylic acid (AA) onto cellulose in the presence of the

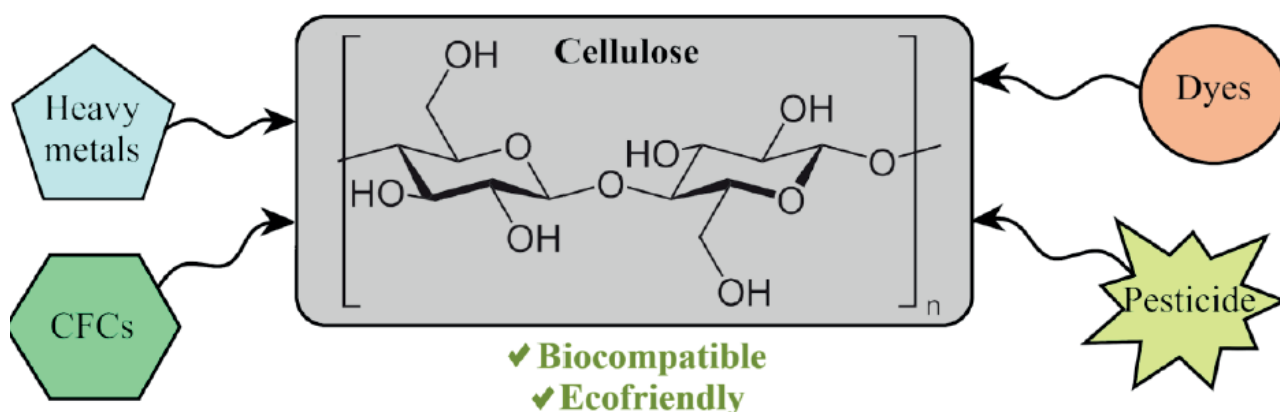


Figure 1. Cellulose is considered one of the best environment remediation agent candidates due to its unique biological properties and economic sustainability.

Cellulose-based adsorbent	Dyes/Heavy metals	Adsorption capacity (mg/g)
Grass waste	Methylene blue	457.64
Jack fruit peel	Basic blue 9	285.71
Spent tea leaves	Methylene blue	300.05
Mosambi (sweet lime) peel	Cr (VI)	250
Cucumber peel	Pb (II)	133.60
Sunflower stalk	Cd (II)	69.80
Orange peel	Cu (II)	63

Table 2. Adsorption capacities of cellulose-based material for heavy metal and dyes removal from wastewater.

crosslinking agent N, N-methylene bisacrylamide (NMBA), cellulose-g-p(AA-AASO3H) prepared by grafting a monomer mixture of AA and 2-acrylamide-2-methyl propane sulfonic acid (AASO3H), and cellulose-g-pAASO3H obtained by grafting of AASO3H onto cellulose. Their results showed that all types of cellulose graft copolymers exhibited a selective removal of the previously mentioned heavy metal ions⁷¹. These findings corroborate the result published by Waly *et al.* who demonstrated that among many copolymers investigated (*e.g.*, glycidyl methacrylate, dimethyl aminoethyl methacrylate, and polyacrylic acid (pAA)), pAA-cotton cellulose-based copolymer was the most efficient one regarding heavy metal removal, specifically Cu²⁺ and Co²⁺, and presented excellent efficiency for capturing basic dyes⁷².

Microcellulose and Nanocellulose for the Remediation of Water Pollution

As detailed above, nano and microscale cellulose can be obtained from a vast assortment of sources. Due to their unique biological properties, these materials have been extensively used for water remediation. Despite presenting similar biological properties, these materials have exhibited significantly different performances when used as decontamination agents. These differences have been largely explained based on their specific surface area/volume (S/V) ratio variation. For instance, cellulose microcrystals (CMC) compared with nanocrystals (CNC) possess a lower S/V ratio, which in turn influences their adsorption capacities and leads to much worse performance. Thus, it is deduced that the smaller the S/V ratio, the lower the percentage of adsorption of water pollutants. Also, comparative analysis between CMC and CNC suspensions showed that CNC suspension presents higher stability, relevant for a more prolonged exposure to the contaminant to be captured⁷³. The study of their morphologies and structures demonstrated that CNC and CMC have similar functional groups and crystalline structures, as confirmed by FTIR and XRD experiments. TGA analysis indicated that the initial degradation temperatures of CMC and CNC are slightly different. These results improve the understanding of how variations in size can affect the physicochemical properties of commercially available CMC and CNC⁷⁴.

Besides the usage of cellulosic materials for the efficient adsorption of pollutants, cellulose nano- and microscale materials can be engineered and explored for the fabrication of filtration membranes for water treatment²⁰. According to Honyang *et al.* (2013), the development of efficient microfiltration membranes was achieved thanks to the integration of ultrafine cellulose nanofibers onto a platform based on a nanoscale layer of polyacrylonitrile (PAN) deposited on a microscale polyethylene terephthalate (PET) film. They proved that the high surface area and large pore density

were critical parameters that could enhance the retention of bacterial (*Escherichia coli*), heavy metals (*e.g.*, Cr⁶⁺ and Pb²⁺), and viruses (MS2 bacteriophage), which constitute the common pollutants encountered in contaminated water samples. The researchers successfully demonstrated that ultrafine cellulose nanofibers could remove toxic contaminants and thus, becoming a promising tool for water purification²¹.

Conclusions

The fast growth rate of industrialization has seriously affected the different water sources of Earth. Furthermore, many organic and inorganic materials, along with the development of nanotechnology-based approaches have been described throughout the literature as non-suitable methods for the decontamination of the environment, since they lack biological and sustainable properties. Thereby, biopolymers extracted from the biodiversity emerged as appropriate remediation agents for the purification and decontamination of water sources due to their intrinsic unique physicochemical properties. Numerous research works detailed in this review have demonstrated that biopolymers are suitable and sustainable materials for the remediation of water pollution.

Cellulose possesses the properties of an ideal biomaterial: It is the most abundant biopolymer on Earth, cheap, non-toxic, biodegradable, biocompatible, and highly versatile. Additionally, the rich flora and fauna of Latin America biodiversity plays a vital role in the securing, synthesis, and development of unique, diverse, and eco-friendly native cellulose-based materials. Finally, different research projects using numerous cellulosic materials have demonstrated its malleability in the field of bioremediation making it an excellent alternative to synthetic polymers, and so proving that biopolymers, but more importantly cellulose, are a green and better solution for the remediation of the environment.

Bibliographic references

1. Muralikrishna VI, Manickam V. Chapter one: Introduction. In: Environmental Management: Science and Engineering for Industry. 2017. p. 1–4.
2. Bhandari G. Environmental Nanotechnology : Applications of Nanoparticles for Bioremediation. Approaches in Bioremediation. 2018;301–15.
3. Inyinbor Adejumo A, Adebisi Babatunde O, Abimbola O, Tabitha A, P OA, A AT. Water Pollution : Effects , Prevention , and Climatic Impact, Water Challenges of an Urbanizing World., IntechOpen. 2018;
4. Bethke K, Palantöken S, Andrei V, Roß M, Raghuwanshi VS, Kettemann F, et al. Functionalized Cellulose for Water Purification, Antimicrobial Applications, and Sensors. Adv Funct Mater. 2018;28(23):1–14.
5. Barakat MA. New trends in removing heavy metals from industrial wastewater. Arab J Chem. 2011;4(4):361–77.

6. Verma R, Dwivedi P. Heavy metal water pollution- A case study. 2013;5(5):98-9.
7. Kumar V, Ioan S. Recent advances in cellulose and chitosan based membranes for water purification : A concise review. *Carbohydr Polym.* 2016;146:148-65.
8. Duruibe J, Ogwuegbu M, Ekwurugwu J. Heavy metal pollution and human biotoxic effects. *Int J Phys Sci.* 2007;2(5):112-8.
9. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metal Toxicity and the Environment. *Exp Suppl.* 2012;101:133-64.
10. Klimont Z, Streets DG, Gupta S, Cofala J, Lixin F, Ichikawa Y. Anthropogenic emissions of non-methane volatile organic compounds in China. *Atmos Environ.* 2002;36(8):1309-22.
11. Taylor P, Yunus IS, Kurniawan A, Adityawarman D, Indarto A. Nanotechnologies in water and air pollution treatment. *Environ Technol Rev.* 2012;(May 2013):37-41.
12. Khin MM, Nair AS, Babu VJ, Murugan R, Ramakrishna S. A review on nanomaterials for environmental remediation. Vol. 5, *Energy and Environmental Science.* 2012. p. 8075-109.
13. Kampa M, Castanas E. Human health effects of air pollution. *Environ Pollut.* 2008;151:362-7.
14. Haseena M, Malik MF, Javed A, Arshad S, Asif N. Water pollution and human health . *Environ Risk Assess Remediat.* 2017;1(3):16-9.
15. Guerra FD, Attia MF, Whitehead DC, Alexis F. Nanotechnology for Environmental Remediation: Materials and Applications. Vol. 23, *Molecules (Basel, Switzerland).* 2018. p. 1-23.
16. Zhang WX. Nanoscale iron particles for environmental remediation: An overview. Vol. 5, *Journal of Nanoparticle Research.* 2003. p. 323-32.
17. Wang D, Pillai SC, Ho SH, Zeng J, Li Y, Dionysiou DD. Plasmonic-based nanomaterials for environmental remediation. Vol. 237, *Applied Catalysis B: Environmental.* 2018. p. 721-41.
18. Bates ME, Grieger KD, Trump BD, Keisler JM, Plourde KJ, Linkov I. Emerging Technologies for Environmental Remediation: Integrating Data and Judgment. *Environ Sci Technol.* 2016;50(1):349-58.
19. Tian Y, Wu M, Liu R, Li Y, Wang D, Tan J, et al. Electrospun membrane of cellulose acetate for heavy metal ion adsorption in water treatment. *Carbohydr Polym.* 2011;83(2):743-8.
20. Carpenter AW, De Lannoy CF, Wiesner MR. Cellulose nanomaterials in water treatment technologies. *Environ Sci Technol.* 2015;49(9):5277-87.
21. Ma H, Burger C, Hsiao BS, Chu B. Ultra-fine cellulose nanofibers: New nano-scale materials for water purification. *J Mater Chem.* 2011;21(21):7507-10.
22. O'Connell DW, Birkinshaw C, O'Dwyer TF. Heavy metal adsorbents prepared from the modification of cellulose: A review. *Bioresour Technol.* 2008;99(15):6709-24.
23. Ahmad S, Pandey A, Pathak VV. Phycoremediation : Algae as Eco-friendly Tools for the Removal of Heavy Metals from Wastewaters. *Bioremediation Ind Waste Environ Saf.* 2020;53-76.
24. Wang S, Sun H, Ang HM, Tadé MO. Adsorptive remediation of environmental pollutants using novel graphene-based nanomaterials. *Chem Eng J.* 2013;
25. Lee LZ, Abbas M, Zaini A, Tang SH. Porous Nanomaterials for Heavy Metal Removal 20. *Handb Ecomater.* 2019;469-94.
26. Elham M. Nanotechnology : Future of Environmental Air Pollution Control. *Macrothink Inst Online J.* 2017;6(2):429-54.
27. Mueller NC, Nowack B. Nanoparticles for Remediation: Solving Big Problems with Little Particles. *Elements.* 2010;6(6):395-400.
28. Klemm D, Heublein B, Fink H, Bohn A. Polymer Science Cellulose: Fascinating Biopolymer and Sustainable Raw Material *Angewandte Chemie Int Ed.* 2005;44(22):3358-93.
29. Tursi A, Beneduci A, Chidichimo F, Vietro N De, Chidichimo G. Remediation of hydrocarbons polluted water by hydrophobic functionalized cellulose. *Chemosphere.* 2018;(201):530-9.
30. Ion VA, Pârvulescu OC, Dobre T. Volatile organic compounds adsorption onto neat and hybrid bacterial cellulose. *Appl Surf Sci.* 2015;335:137-46.
31. Wang B, Wan Y, Zheng Y, Lee X, Liu T, Yu Z, et al. Technology Alginate-based composites for environmental applications : a critical review. *Crit Rev Environ Sci Technol.* 2018;0(0):1-39.
32. Hatakeyama H, Hatakeyama T. Lignin Structure, Properties, and Applications. *Adv Polym Sci.* 2009;1-63.
33. Wang C, Wu S, Jian M, Xie J, Xu L, Yang X, et al. Silk nanofibers as high efficient and lightweight air filter. *Nano Res.* 2016;1:1-8.
34. Aider M. LWT - Food Science and Technology Chitosan application for active bio-based films production and potential in the food industry : Review. *LWT - Food Sci Technol.* 2010;43(6):837-42.
35. Abdou ES, Nagy KSA, Elsabee MZ. Extraction and characterization of chitin and chitosan from local sources. *Bioresour Technol.* 2008;99:1359-67.
36. Khan S, Garg M, Chockalingam S, Gopinath P, Kundu PP. TiO2 doped chitosan/poly (vinyl alcohol) nanocomposite film with enhanced mechanical properties for application in bone tissue regeneration. *International Journal of Biological Macromolecules.* Elsevier B.V; 2019.
37. Toan N Van, Hanh TT. Application of chitosan solutions for rice production in. *African J Biotechnol.* 2013;12(4):382-4.
38. Al-manhel AJ, Al-hilphy ARS, Niamah AK. Extraction of chitosan , characterisation and its use for water purification. *J SAUDI Soc Agric Sci.* 2016;
39. Zhao L, Yang S, Yilihamu A, Ma Q, Shi M, Ouyang B, et al. Adsorptive decontamination of Cu2+-contaminated water and soil by carboxylated graphene oxide/chitosan/cellulose composite beads. *Environ Res.* 2019;108779.
40. Carvalho IC, Borsagli FGLM, Alexandra AP, Caldeira CL, Haas DJ, Lage AP, et al. 3D sponges of chemically functionalized chitosan for potential environmental pollution remediation : biosorbents for anionic dye adsorption and ' antibiotic-free ' antibacterial activity. *Environ Technol.* 2019;0(0):1-21.
41. Yong K, Mooney DJ. Alginate : Properties and biomedical applications. *Prog Polym Sci.* 2012;37(1):106-26.
42. Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. *Annu Rev Plant Biol.* 2003;54:519-46.
43. Vanholme R, Morreel K, Ralph J, Boerjan W. Lignin engineering. *Curr Opin Plant Biol.* 2008;(11):278-85.
44. Parajuli D, Inoue K, Ohto K, Oshima T. Adsorption of heavy metals on crosslinked lignocatechol : a modified lignin gel. *React Funct Polym.* 2005;62:129-39.
45. Li Z, Ge Y, Wan L. Fabrication of a green porous lignin-based sphere for the removal of lead ions from aqueous media. *J Hazard Mater.* 2014;
46. Babu KM. Introduction to silk and sericulture. In: *Silk.* 2019. p. 1-29.
47. Cao Y, Wang B. Biodegradation of Silk Biomaterials. *Int J Mol Sci.* 2009;1514-24.
48. Aigner TB, Desimone E, Scheibel T, Engineering B, Aigner TB. Biomedical Applications of Recombinant Silk-Based Materials. *Adv Mater.* 2018;1-28.
49. Peng B, Yao Z, Wang X, Crombeen M, Gsweene D, Tam KC. Cellulose-based Materials in Wastewater Treatment of Petroleum Industry. *Green Energy Environ.* 2019;
50. George J. Cellulose nanocrystals : synthesis , functional properties , and applications. *Nanotechnology, Sci Appl.* 2015;45-54.
51. Kumar M, Turner S. Plant cellulose synthesis : CESA proteins crossing kingdoms. *Phytochemistry.* 2014;
52. McNamara JT, Morgan JLW. A Molecular Description of Cellulose Biosynthesis. *Annu Rev Biochem.* 2015;84(1):895-921.
53. Maleki SS, Mohammadi K, Ji K. Characterization of Cellulose Synthesis in Plant Cells. *Sci World J.* 2016;2016.
54. Guerra FD, Campbell ML, Attia MF, Whitehead DC, Alexis F. Capture of Aldehyde VOCs Using a Series of Amine-Functionalized Cellulose Nanocrystals. *ChemistrySelect.* 2018;3(20):5495-501.
55. Thakur VK, Thakur MK. Processing and characterization of natural cellulose fibers/thermoset polymer composites. Vol. 109, *Carbohydrate Polymers.* Elsevier Ltd.; 2014. p. 102-17.
56. Nabili A, Fattoum A, Passas R, Elaloui E, Nabili A. EXTRACTION AND CHARACTERIZATION OF CELLULOSE FROM DATE PALM SEEDS (Phoenix dactylifera L .) Laboratory of Pulp , Paper and Graphic Arts Sciences , UMR CNRS 5518 „ Cellul Chem Technol. 2014;50:9-10.

57. Mischnick P, Momcilovic D. Chemical Structure Analysis of Starch and Cellulose Derivatives [Internet]. Vol. 64, *Advances in Carbohydrate Chemistry and Biochemistry - Volume 64*. Elsevier Inc.; 2010. 117–210 p. Available from: [http://dx.doi.org/10.1016/S0065-2318\(10\)64004-8](http://dx.doi.org/10.1016/S0065-2318(10)64004-8)
58. Ateia M, Attia MF, Maroli A, Tharayil N, Alexis F, Whitehead DC, et al. Rapid Removal of Poly- and Perfluorinated Alkyl Substances by Poly(ethylenimine)-Functionalized Cellulose Microcrystals at Environmentally Relevant Conditions. *Environ Sci Technol Lett*. 2018;5(12):764–9.
59. Morán JI, Alvarez VA, Cyras VP, Vázquez A. Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose*. 2008;15(1):149–59.
60. Programme UUNE. Biodiversity in Latin America and the Caribbean. 2016. p. 1–5.
61. Almeida MA. Extraction of Bioactive Compounds from Latin American Plants. 2007;
62. Luque S, Mart G, Echeverr C, Pacha MJ. Chapter 15 Overview of Biodiversity Loss in South America : A Landscape Perspective for Sustainable Forest Management and Conservation in Temperate Forests. In: *Landscape Ecology in Forest Management and Conservation*.
63. Valencia R. Composition and structure of an Andean forest fragment in eastern Ecuador. Biodiversity and conservation of Neotropical montane forests. *Proc. symposium, New York Botanical Garden*, 1993. 1995. p. 239–49.
64. Hokkanen S, Bhatnagar A, Sillanpää M. A review on modification methods to cellulose-based adsorbents to improve adsorption capacity. *Water Res*. 2016;91:156–73.
65. Bismarck A, Aranbefwi-Askargorta I, Springer J, Lampke T, Wielage B, Stamboulis A, et al. Cellulose Fibers ; Surface Properties and the the Water Uptake Behavior. *Polym Compos*. 2002;23(5):872–94.
66. Manjarrez Nevárez L, Ballinas Casarrubias L, Canto OS, Celzard A, Fierro V, Ibarra Gómez R, et al. Biopolymers-based nanocomposites: Membranes from propionated lignin and cellulose for water purification. *Carbohydr Polym*. 2011;86(2):732–41.
67. Rinaudo M. Chitin and chitosan : Properties and applications. *Prog Polym Sci*. 2006;31(7):603–32.
68. Moon RJ, Martini A, Nairn J, Simonsen J, Youngblood J. Cellulose nanomaterials review: Structure, properties and nanocomposites. Vol. 40, *Chemical Society Reviews*. 2011. p. 3941–94.
69. Holant ZR. Biosorption of Heavy Metals. 1995;235–50.
70. Varghese AG, Annie S, Latha PMS. Remediation of heavy metals and dyes from wastewater using cellulose - based adsorbents. *Environ Chem Lett*. 2018;
71. Guclu G, Gurdag G, Ozgumus S. Competitive Removal of Heavy Metal Ions by Cellulose Graft Copolymers. *J Appl Polym Sci*. 2003;90(8):2034–9.
72. Waly A, Aly AS, Hebeish A. Synthesis and Characterization of Cellulose Ion Exchanger . II . Pilot Scale and Utilization in Dye – Heavy Metal Removal. *J Appl Polym Sci*. 1998;68(13):2151–7.
73. Xiong R, Zhang X, Tian D, Zhou Z, Lu C. Comparing microcrystalline with spherical nanocrystalline cellulose from waste cotton fabrics. *Cellulose*. 2012;19(4):1189–98.
74. Bravo, I., Figueroa, F., Swasy, M. I., et al. Cellulose particles capture aldehyde VOC pollutants. *RSC Adv*. 10, 7967–7975 (2020).

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REVIEW / ARTÍCULO DE REVISIÓN

LOX-1 en las afecciones cardiovasculares, perspectivas terapéuticas futuras LOX-1 in cardiovascular diseases, future therapeutic perspectives

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Resumen: El receptor de la lipoproteína de baja densidad oxidado tipo lectina 1 (LOX-1), también conocido como OLR-1, es un receptor *scavenger* (SR) clase E, que media la absorción del colesterol LDL en su forma oxidada, por las células vasculares. LOX-1 está involucrado en la disfunción endotelial, la adhesión de monocitos, la proliferación, migración y apoptosis de las células del músculo liso, la formación de células espumosas, la activación de plaquetas, así como la inestabilidad a nivel del endotelio vascular; todos eventos críticos en la patogénesis de la aterosclerosis. LOX-1 contribuyen a la inestabilidad de la placa aterosclerótica y a las últimas secuelas clínicas de ruptura endotelial e isquemia tisular cardíaca potencialmente mortal. No existe en la actualidad ningún fármaco aprobado o en desarrollo clínico a partir de LOX-1, debido a sus complejos mecanismos biológicos no dilucidados completamente. Se han utilizado diversas terapias con el objetivo de inhibir la acción de LOX-1; medicamentos como: antioxidantes, estatinas, agentes antiinflamatorios naturales, que actúen sobre su expresión, pero todos con eficacia moderada. También se ha evaluado la administración de anticuerpos anti-LOX-1 inhibe la aterosclerosis al disminuir eventos celulares. El diseño de fármacos enfocados en el conocimiento de las vías de señalización de LOX-1 y la aplicación de herramientas biotecnológicas permite el desarrollo de nuevas dianas terapéuticas basadas en la potencialidad que tienen los anticuerpos monoclonales. Con estos antecedentes el receptor LOX-1, representa un objetivo terapéutico atractivo para el tratamiento de enfermedades ateroscleróticas humanas. La evidencia reciente indica que la acción sobre este SR es una posible estrategia para el tratamiento de la enfermedad vascular, explorando en esta revisión su papel y posibles futuras aplicaciones en el diagnóstico y la terapéutica.

Palabras clave: Aterosclerosis; LOX-1 receptor de la lipoproteína de baja densidad oxidado tipo lectina 1; Dislipidemia; Disfunción endotelial; lipoproteína de baja densidad oxidado (ox-LDL); forma soluble LOX-1 (sLOX-1).

Abstract: The low-density oxidized lipoprotein lectin-1 (LOX-1) receptor, also known as OLR-1, is a scavenger receptor (SR) class E, which mediates the absorption of LDL cholesterol in its oxidized form by vascular cells. LOX-1 is involved in endothelial dysfunction, monocyte adhesion, smooth muscle cell proliferation, migration and apoptosis, foam cell formation, platelet activation, and vascular endothelial instability, all critical events in the pathogenesis of atherosclerosis. LOX-1 contributes to atherosclerotic plaque instability and the ultimate clinical sequelae of endothelial rupture and potentially fatal cardiac tissue ischemia. There is currently no drug approved or in clinical development from LOX-1, due to its complex biological mechanisms not fully elucidated. Various therapies have been used to inhibit the action of LOX-1; drugs such as antioxidants, statins, natural anti-inflammatory agents, which act on its expression, but all with moderate efficacy. The administration of anti-LOX-1 antibodies has also been evaluated to inhibit atherosclerosis by decreasing cellular events. The design of drugs focused on the knowledge of LOX-1 signaling pathways and the application of biotechnological tools allow the development of new therapeutic targets based on the potential of monoclonal antibodies. With this background, the LOX-1 receptor represents an attractive therapeutic target for the treatment of human atherosclerotic diseases. Recent evidence indicates that action on this SR is a possible strategy for the treatment of vascular disease, exploring in this review its role and potential future applications in diagnosis and therapy.

Key words: Atherosclerosis; LOX-1 lectin-like OxLDL receptor 1; Dyslipidemia; Endothelial dysfunction; oxidized low-density lipoprotein; Soluble LOX-1.

Introducción

Antecedentes

Las enfermedades cardiovasculares (ECVs) representan alrededor del 30% de defunciones a nivel global, siendo la principal causa de muerte en la población en general¹, consecuencia de tres afecciones subyacentes: el ataque cardíaco, el accidente cerebrovascular y enfermedad vascular periférica².

La aterosclerosis es la base fisiológica de las enfermedades cardiovasculares más peligrosas³. Se desarrolla en la pared vascular de las arterias y arteriolas, y se describe por una acumulación local de colesterol, inflamación no resuelta, muerte celular y fibrosis⁴. Es un proceso dinámico gradual con

modificaciones estructurales multifocales, que da lugar al desarrollo de placas ateroscleróticas⁵. Estas placas se asocian a disfunción y activación endotelial^{6,7,8}. Todo esto ocasiona una restricción del flujo, riesgo de rotura, ataque cardíaco o una isquemia a nivel vascular⁹.

El factor de riesgo más importante para la aterosclerosis son los niveles elevados de colesterol LDL y su apolipoproteína B (apoB) 100, principal proteína estructural de colesterol-LDL¹⁰ y específicamente su forma oxidada (ox-LDL)¹¹. Esta variante oxidada del colesterol LDL es mucho más importante en la aterogénesis que el colesterol LDL nativo no modificado¹².

La forma ox-LDL aumenta con la disfunción endotelial¹³ y

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su presencia en la pared arterial conduce a la aterogénesis¹⁴. Esta lipoproteína en su forma oxidada, ejerce diversos efectos sobre células endoteliales, plaquetas, macrófagos, fibroblastos y células del músculo liso (SMC), a través de la glucoproteína de transmembrana, el receptor de la lipoproteína de baja densidad oxidado tipo lectina 1 (LOX-1)¹⁵.

La evidencia actual demuestra que ox-LDL inicia y sostiene la aterogénesis por activación LOX-1¹⁶ y a nivel molecular la internalización de ox-LDL a la íntima de los vasos sanguíneos, se provoca por este receptor LOX-1¹⁷, que establece una fuerte asociación sistema ligando-receptor¹⁶. LOX-1 juega un rol crítico en la disfunción y daño endotelial, permitiendo el inicio y la progresión del proceso aterosclerótico y ha sido propuesto como una diana terapéutica para el desarrollo de una molécula recombinante dirigida al tratamiento de la aterosclerosis¹⁸.

El receptor 1 de lipoproteína de baja densidad oxidada tipo lectina (LOX-1) se descubrió por primera vez como un receptor vascular para partículas de lipoproteína modificada¹⁹. Desde entonces, los estudios *in vitro/in vivo* han demostrado su asociación con patologías como: aterosclerosis, artritis, hipertensión, hiperlipidemias y diabetes²⁰. Sin embargo, no existe en la actualidad ningún medicamento aprobado ni en ensayos clínicos a partir de LOX-1, debido a los complejos mecanismos fisiopatológicos e inmunológicos en los que se encuentra inmersa esta molécula.

Conocemos que la terapia basada en LOX-1 reduce la aterogénesis y anomalías celulares asociadas y que su inhibición, mediante el uso de anticuerpos, podría influir sobre la lesión miocárdica²¹. Toda la evidencia obtenida lo ha convertido en un objetivo potencialmente terapéutico para la aterosclerosis y las enfermedades cardiovasculares, debido a dos propiedades importantes: (1) Es un receptor de transmembrana, y los sitios de unión para ox-LDL y otros ligandos han sido bien caracterizados²²; (2) los mecanismos y las vías de señalización mediadas por LOX-1 son bien conocidos²³.

Existen avances en la fisiopatología de las enfermedades cardiovasculares en las últimas décadas²⁴⁻²⁶. Varios medicamentos con impacto en los factores de riesgo coronario y la cardiopatía isquémica han sido estudiados inhibiendo la función de LOX-1²⁷.

Se requieren sistemas diagnósticos sólidos y nuevas terapias emergentes para las enfermedades cardiovasculares²⁸. Tenemos las bases para la formulación de nuevas moléculas, a través de la aplicación de las herramientas de la biotecnología molecular, que disminuyan los altos índices de las enfermedades cardiovasculares; y LOX-1 por sus particularidades y

funciones biológicas, pudiera tener un impacto favorable sobre las terapias emergentes.

Desarrollo

El receptor de la lipoproteína de baja densidad oxidado tipo lectina (LOX-1), es el principal responsable de la unión, internalización y degradación de la ox-LDL en células endoteliales²⁹. Tiene un papel protagonista en el desarrollo de la aterosclerosis y la enfermedad cardiovascular³⁰.

LOX-1 es un receptor *scavenger* (SR) de clase E, glicoproteína de membrana tipo II, que une carbohidratos dependiente Ca^{2+} ³¹. Perteneciente a la familia de receptores de lectina tipo C³², se clonó por primera vez 15 e identificó como un receptor vascular endotelial de ox-LDL³³.

El gen de esta proteína (gen OLR1) se encuentra ubicado en el cromosoma humano 12³⁴ y su producto es un homodímero de 50 kDa³⁵ conformado por 273 aminoácidos que consta de cuatro dominios: un dominio de lectina extracelular tipo C-terminal principal responsable de su funcionalidad con 131 residuos (143-273), un dominio cuello de 82 aminoácidos (61-142), un solo dominio transmembrana o transcelular de 27 aminoácidos (34-61) y una cola citoplasmática N-terminal corta de 34 aminoácidos (1-34) (ver Figura 1)³⁶.

LOX-1 se comporta como un SR en la superficie celular y participa en la unión, endocitosis y degradación proteolítica de ox-LDL. Sin embargo, no comparte ninguna homología con otros SR³⁷. LOX-1 es sintetizado como un precursor (pre-LOX1) glicosilado con residuos de manosa, ligados al extremo N-terminal (40 kDa). Posteriormente, es procesado como una glicoproteína madura³⁸. Se identificó un sitio de glucosilación potencial en su forma recombinante en asparagina 139 (Asn-139)³⁹. Similar a otros receptores con un único dominio transmembrana, LOX-1 puede ser escindido por proteasas y secretado en forma soluble (sLOX-1)⁴⁰, a través de la escisión proteolítica de su dominio extracelular²⁸.

El receptor LOX-1 promueve la activación y disfunción endotelial al mediar la absorción de LDL oxidada e inducir la señalización pro-aterogénica⁴¹. Se han descrito moduladores de las respuestas mediadas por LOX-1 pero, se sabe poco sobre ellos⁴². Se ha descrito la proteólisis como un mecanismo regulador del receptor que se controla a través de su interacción con metaloproteasas (ADAM10) y la degradación lisosómica⁴³. Los autores consideraron la proteólisis intramembrana por SPPL2a/b como un nuevo mecanismo ateroprotector por medio de la regulación negativa de la señalización de LOX-1.

Por medio de la escisión proteolítica de LOX-1 también se

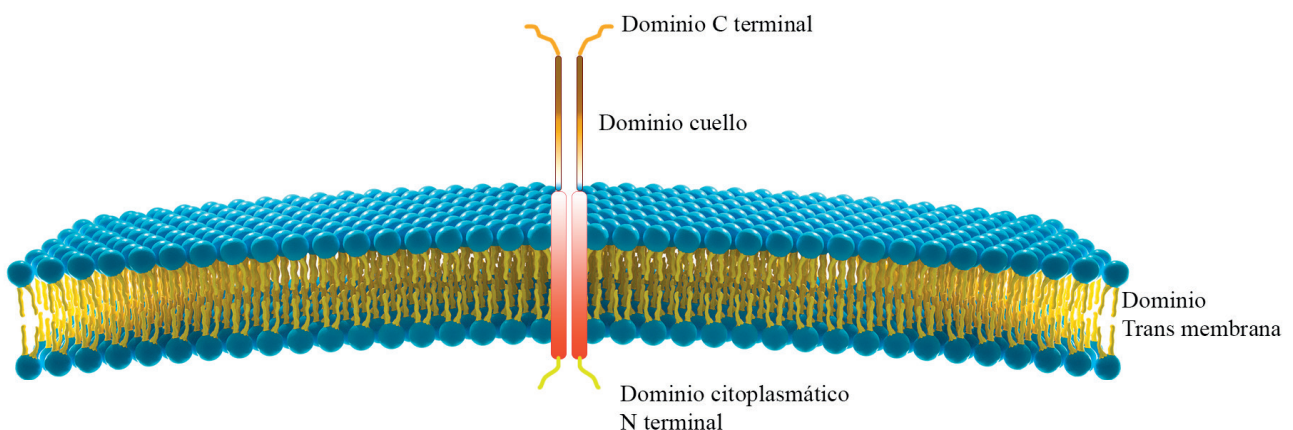


Figura 1. Receptor de la lipoproteína de baja densidad oxidado tipo lectina 1.

libera la forma soluble de este receptor (sLOX-1)²⁸, la cual se expresa en la enfermedad cardiovascular⁴⁴. Sin embargo, las enzimas proteolíticas responsables de esto han seguido siendo controversiales (43)⁴⁵. La función de los fragmentos de escisión individuales y el impacto de la proteólisis en la señalización de LOX-1 no están definidos hasta la fecha.

En cuanto a la expresión de LOX-1, fue reportada por primera vez en células endoteliales de aorta de bovino¹⁵. Se caracterizó como el receptor responsable del reconocimiento, internalización, y degradación de la LDL oxidada. Posteriormente, identificaron LOX-1 en células endoteliales de aorta humana⁴⁶.

En el contexto actual conocemos que LOX-1 se expresa principalmente en células endoteliales y también en muchas otras células, incluidas monocitos / macrófagos, células de músculo liso (SMC)⁴⁴, cardiomiocitos⁴⁷, fibroblastos⁴⁸, adipocitos⁴⁹, células epiteliales de las vías respiratorias⁵⁰, células dendríticas⁵¹ y plaquetas⁵². Lo más importante, es que LOX-1 se expresa también en células derivadas de ateroma y se observa en gran abundancia en lesiones ateroscleróticas de animales y humanas²⁸.

Algunos estudios^{33,53,54} han demostrado que la expresión basal de LOX-1 es baja, pero se produce una regulación positiva significativa durante varios estados patológicos que incluyen diabetes, hipertensión y aterosclerosis, debido a la presencia de estímulos inflamatorios y oxidativos y esto provoca un incremento de su expresión⁵⁵.

La expresión *in vivo* de LOX-1 es mucho mayor de que lo que se observa *in vitro*, es inducida por ciertos activadores proinflamatorios como; Angiotensina II, Factor de necrosis tumoral alfa (TNF- α), Interferón gamma (IFN- γ) entre otros⁴⁰. También pueden aumentar su expresión los estímulos oxidativos como la propia LDL oxidada, lisofosfatidilcolina, productos de glicación avanzada⁵⁶.

Las tres principales hipótesis de aterosclerosis: modificación oxidativa, respuesta a la lesión y retención; convergieron en las funciones versátiles de LOX-1 (Yoshimoto *et al.*, 2011), aumentando su potencialidad como atractiva diana terapéutica.

La evidencia directa de la participación de LOX-1 en la aterogénesis se ha obtenido utilizando modelos animales transgénicos, knock-out de Apolipoproteína E (LOX-1tg / ApoE^{-/-} y transferencia de genes adenovirales^{57,58,59}.

El receptor se encuentra involucrado en una serie de vías de traducción de señales al unirse a una gran variedad de ligandos incluyendo lipoproteínas modificadas, proteína C-reactiva (CRP) y fibronectina. LOX-1 activado está implicado en múltiples vías de transducción de señales que influyen en el inicio y la progresión de la placa de aterosclerosis. Estas vías de señalización incluyen proteínas quinasas activadas por mitógeno (MAP)⁶⁰ y se generan especies reactivas de oxígeno (ROS), que estimulan el factor nuclear la vía NF- κ B⁶¹. El incremento constante de ROS, debido al proceso inflamatorio, da lugar a la movilización de otras vías de señalización intracelular que impulsan a NF- κ B, aumentando aún más la expresión de LOX-1²⁷. De esta manera, el receptor induce la expresión de moléculas de adhesión y citocinas proinflamatorias y promueve la aterogénesis. También está involucrado en la muerte celular programada (apoptosis) del músculo liso y las células endoteliales; y ejerce una regulación positiva de la actividad caspasa-3 y 9⁴⁴. Otra función fisiopatológica de LOX-1, incluye la activación de la enzima arginasa II (ARG2), que conduce a la supresión de los niveles de óxido nítrico (NO) y al aumento del tono vascular⁶². Finalmente, LOX-1 está implicada en la dismi-

nución de la estabilidad de la placa aterosclerótica a través de una mayor activación de metaloproteasas⁶³.

Al contribuir en múltiples vías de señalización, LOX-1 se convierte en un objetivo terapéutico potencial. Todo esto conlleva a plantear la posibilidad de considerarlo como un predictor diagnóstico y objetivo terapéutico de la enfermedad cardiovascular.

Un tipo de terapia que se ha estudiado mucho es el beneficio de eliminar LOX-1, que provino del estudio de Hinagata *et al.* en un modelo de rata de hiperplasia neointimal en una lesión arterial⁶⁴. Mehta y col. mostraron más adelante que la delección del gen LOX-1 se asoció con una disminución del estrés oxidativo, la respuesta inflamatoria, la degradación de NO y la aterosclerosis⁶⁵.

Medicamentos como la aspirina⁶⁶, los bloqueadores betaadrenérgicos⁶⁷ los inhibidores de la reductasa HMG CoA (estatinas)²⁰ y los bloqueadores del sistema renina-angiotensina (RAS) son fármacos en los que se han evaluado sus efectos con relación a la una posible inhibición de LOX-1²⁷.

También se ha postulado el uso de sLOX-1 como biomarcador diagnóstico o predictivo de la patología vascular, aunque se necesitan estudios prospectivos y a gran escala para establecer su papel de sLOX-1⁶⁸. Los niveles elevados de sLOX-1 están presentes en la hipertensión, diabetes mellitus II; y estos factores de riesgo coexisten en pacientes con enfermedades cardiovasculares, por lo que la interpretación es difícil^{69,70}.

Existen avances en la fisiopatología de las enfermedades cardiovasculares y la cardiopatía isquémica en las últimas décadas²⁴. Hasta la fecha no se ha probado ningún tratamiento eficaz de supresión de LOX-1 en humanos.

LOX-1 se continúa investigando como un biomarcador y factor contribuyente a la enfermedad aterosclerótica. Gran parte de las evaluaciones terapéuticas preclínicas de anticuerpos dirigidos contra LOX-1 también se han centrado en esta patología (tabla 1).

Actualmente, los moduladores sintéticos de LOX-1 se están desarrollando en base a técnicas de interferencia de ARN, diseños de fármacos basados en estructuras y el uso de anticuerpos monoclonales^{71,72}. En este momento, existen varias estrategias de direccionamiento que inhiben a LOX-1, ya sea mediante el bloqueo directo de la función de la proteína, utilizando anticuerpos monoclonales, o la modulación de la producción, empleando oligonucleótidos anti-sentido⁷³. Estas investigaciones están en uso clínico, lo que indica que pudieran ser opciones viable para atacar a LOX-1 en humanos²³. No obstante, el desarrollo de anticuerpos monoclonales contra LOX-1 constituye un desafío, debido al dominio de lectina de tipo C altamente conservado de LOX-1 entre las especies de mamíferos¹⁸.

Un ejemplo de esto lo constituye los iPCSK9⁵⁴, anticuerpos monoclonales que se unen a la pro-proteína convertasa subtilisina / kexina tipo 9 (PCSK9), importante regulador del metabolismo de las lipoproteínas de baja densidad (LDL), e impiden en parte la degradación de los receptores de LDL y por lo tanto aumentan la expresión de la PCSK9, que regula positivamente la captación de ox-LDL mediada por LOX-1²⁵.

En resumen, las estrategias de focalización contra LOX-1 en el diagnóstico y tratamiento de la enfermedad aterosclerótica están comenzando a surgir²³. Estas estrategias se encuentran en las primeras etapas de desarrollo y tienen un potencial significativo, pero es necesario descubrir las funciones exactas de LOX-1 para establecer si la selección clínica de este receptor sería efectiva y segura en el futuro.

Modelo	Ruta	Dosis	Duración	Principales conclusiones	Ref
Traumatismo de la arteria carótida rata <i>Wild-type</i>	IV	10 mg/kg cada 3 días	14 días	Reducción de la hiperplasia íntima	64
Análisis de la vasculatura renal en rata obesa <i>wild-type</i>	IV	2 µg cada 7 días	15 semanas	Reduce la densidad vascular y la fibrosis renal	74
Daño por reperfusión en isquemia miocárdica en ratas <i>wild-type</i>	IV	10 mg/kg dosis única	Solo una vez	Reducción del infarto de miocardio	75
Dieta occidental en murinos <i>ApoE knockout</i>	—	—	—	Hipertrofia ventricular reducida	76
Ratas hipertensas propensas al derrame cerebral	IV	10 mg/kg cada 4 días	4 días	Reducción de los lípidos mesentéricos intravasculares	21
Ratones knockouts <i>ApoE</i> emisiones motoras	IP	1.6 µg cada 2 días	7 días	Reduce la expresión de marcadores de stress endotelial	77
Artritis inducida por Zymosan en ratas <i>wild-type</i>	IV	2 mg/kg dosis única	1 día	Reducción de la hinchazón articular, erosión del cartilago	78
Ratas <i>wild-type</i> inyectadas IA con oxLDL	IA	20 µg una vez al día	7 días	Reducción de la inflamación de las articulaciones, sinovial hiperplasia	79
Ratas <i>Wild-type</i> inyectadas con endotoxinas bacterianas	IV	10 mg/kg dosis única	una vez al día	Reducción de la leucopenia, mejora de la supervivencia en respuesta a la sepsis	23
Ratones <i>Wild-type</i> inyectados con LPS	IV	100 µg/kg dosis única	una vez al día	Disminución de la apoptosis, expresión de marcadores de inflamación	80
BKSdb ratones administrados con anti-LOX-1	IP	0.16 µg cada 2 días	6 semanas	Reduce la neuropatía diabética	81
Crías de ratas <i>Wild-type</i> con ligadura de la arteria carótida	IP	60 µg/kg dos veces al día	3 días	Reducción del volumen hipóxico En la encefalopatía	82

Tabla 1. Evaluaciones terapéuticas preclínicas de anticuerpos dirigidos contra LOX-1.

Conclusiones

Actualmente existen más datos que se han ido sumando al concepto fisiopatológico de un estado hiperinflamatorio con una actividad trombótica elevada en el COVID 19, hasta hace pocos meses no se conocía sobre estos mecanismos, y aunque no han podido ser del todo descritos consideramos que la tormenta de citoquinas y la activación exagerada de macrófagos juegan un rol importante en el desarrollo de trombosis.

El caso reportado pondera la solicitud de ferritina y dímero D como biomarcadores, ya que pueden anticipar el deterioro y evolución a cuadros clínicos agresivos que evolucionen rápidamente a la muerte.

Referencias bibliográficas

1. oms. Cardiovascular diseases (CVDs). (2017). Available at: [https://www.who.int/es/news-room/fact-sheets/detail/cardiovascular-diseases-\(cvds\)](https://www.who.int/es/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)). (Accessed: 14th October 2019)
2. Mozaffarian D et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation* 131, (2015).
3. Rahman, M. S. & Woollard, K. Atherosclerosis. 121–144 (2017). doi:10.1007/978-3-319-57613-8
4. Kavurma, M. M., Rayner, K. J. & Karunakaran, D. The walking dead: Macrophage inflammation and death in atherosclerosis. *Curr. Opin. Lipidol.* 28, 91–98 (2017).
5. Yahagi, K. et al. Pathophysiology of native coronary, vein graft, and in-stent atherosclerosis. *Nat. Rev. Cardiol.* 13, 79–98 (2016).

6. Skälén, K. et al. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature* 417, 750–754 (2002).
7. oshida, H., Kondratenko, N., Green, S., Steinberg, D. & Quehenberger, O. Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavenger receptor. *Biochem. J.* 334, 9–13 (1998).
8. Takei, A., Huang, Y. & Lopes-Virella, M. F. Expression of adhesion molecules by human endothelial cells exposed to oxidized low density lipoprotein. Influences of degree of oxidation and location of oxidized LDL. *Atherosclerosis* 154, 79–86 (2001).
9. Lubrano, V. & Balzan, S. Roles of LOX-1 in microvascular dysfunction. *Microvasc. Res.* 105, 132–140 (2016).
10. Baigent, C. et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *Lancet* 366, 1267–1278 (2005).
11. Fraley, A. E. & Tsimikas, S. Clinical applications of circulating oxidized low-density lipoprotein biomarkers in cardiovascular disease. *Curr. Opin. Lipidol.* 17, 502–509 (2006).
12. Winkhofer-Roob, B. M., Faustmann, G. & Roob, J. M. Low-density lipoprotein oxidation biomarkers in human health and disease and effects of bioactive compounds. *Free Radical Biology and Medicine* (2017). doi:10.1016/j.freeradbiomed.2017.04.345
13. Segrest, J. P., Jones, M. K., De Loof, H. & Dashti, N. Structure of apolipoprotein B-100 in low density lipoproteins. *J. Lipid Res.* 42, 1346–1367 (2001).
14. Li, D. & Mehta, J. L. Oxidized LDL, a critical factor in atherogenesis. *Cardiovascular Research* 68, 353–354 (2005).
15. Sawamura, T. et al. An endothelial receptor for oxidized low-density lipoprotein. *Nature* 386, 73–77 (1997).

16. Shaw, D. J., Seese, R., Ponnambalam, S. & Ajjan, R. The role of lectin-like oxidized low-density lipoprotein receptor-1 in vascular pathology. *Diabetes Vasc. Dis. Res.* 11, 410–418 (2014).
17. Di Pietro, N., Formoso, G. & Pandolfi, A. Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis. *Vascul. Pharmacol.* 84, 1–7 (2016).
18. Sugiyama, D. et al. The Relationship between Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Ligands Containing Apolipoprotein B and the Cardio-Ankle Vascular Index in Healthy Community Inhabitants: The KOBE Study. *J. Atheroscler. Thromb.* 22, 499–508 (2015).
19. Aoyama, T. et al. Structure and chromosomal assignment of the human lectin-like oxidized low-density-lipoprotein receptor-1 (LOX-1) gene. *Biochem. J.* 339, 177–184 (1999).
20. Cheung, B. M. Y., Lauder, I. J., Lau, C.-P. & Kumana, C. R. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. *Br. J. Clin. Pharmacol.* 57, 640–651 (2004).
21. Nishizuka, T. et al. Procyanidins are potent inhibitors of LOX-1: A new player in the French Paradox. *Proc. Japan Acad. Ser. B Phys. Biol. Sci.* 87, 104–113 (2011).
22. Singh, S. & Gautam, A. S. Upregulated LOX-1 Receptor: Key Player of the Pathogenesis of Atherosclerosis. *Curr. Atheroscler. Rep.* 21, (2019).
23. De Siqueira, J. et al. Clinical and Preclinical Use of LOX-1-Specific Antibodies in Diagnostics and Therapeutics. *J. Cardiovasc. Transl. Res.* 8, 458–465 (2015).
24. Jiang, J., Yan, M., Mehta, J. L. & Hu, C. Angiogenesis is a link between atherosclerosis and tumorigenesis: Role of LOX-1. *Cardiovasc. Drugs Ther.* 25, 461–468 (2011).
25. Ding, Z. et al. PCSK9 regulates expression of scavenger receptors and ox-LDL uptake in macrophages. *Cardiovasc. Res.* 114, 1145–1153 (2018).
26. Liu, S. & Ding, Z. LOX-1 biology and targeting LOX-1 in cardiovascular diseases. *Cardiovasc. Disord. Med.* 1, (2016).
27. Kattoor, A. J., Goel, A. & Mehta, J. L. LOX-1: Regulation, Signaling and Its Role in Atherosclerosis. *Antioxidants* 8, 218 (2019).
28. Kataoka, H. et al. Expression of lectinlike oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation* 99, 3110–7 (1999).
29. Kattoor, A. J., Pothineni, N. V. K., Palagiri, D. & Mehta, J. L. Oxidative Stress in Atherosclerosis. (2017). doi:10.1007/s11883-017-0678-6
30. Lü, J. & Mehta, J. L. LOX-1: A critical player in the genesis and progression of myocardial ischemia. *Cardiovasc. Drugs Ther.* 25, 431–440 (2011).
31. Pothineni, N. V. K. et al. LOX-1 in Atherosclerosis and Myocardial Ischemia: Biology, Genetics, and Modulation. *J. Am. Coll. Cardiol.* 69, 2759–2768 (2017).
32. Kume, N. & Kita, T. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in atherogenesis. *Trends Cardiovasc. Med.* 11, 22–5 (2001).
33. Stancel, N. et al. Interplay between CRP, Atherogenic LDL, And LOX-1 and its potential role in the pathogenesis of atherosclerosis. *Clin. Chem.* 62, 320–327 (2016).
34. Yamanaka, S., Zhang, X. Y., Miura, K., Kim, S. & Iwao, H. The human gene encoding the lectin-type oxidized LDL receptor (OLR1) is a novel member of the natural killer gene complex with a unique expression profile. *Genomics* (1998). doi:10.1006/geno.1998.5561
35. Xie, Q. et al. Human Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Functions as a Dimer in Living Cells. *DNA Cell Biol.* 23, 111–117 (2004).
36. Ishigaki, T., Ohki, I., Utsunomiya-Tate, N. & Tate, S. I. Chimeric structural stabilities in the coiled-coil structure of the NECK domain in human lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1). *J. Biochem.* 141, 855–866 (2007).
37. Xu, S. et al. LOX-1 in atherosclerosis: biological functions and pharmacological modifiers. *Cell. Mol. Life Sci.* 70, 2859–2872 (2013).
38. Kume, N. & Kita, T. Roles of lectin-like oxidized LDL receptor-1 and its soluble forms in atherogenesis. *Curr. Opin. Lipidol.* 12, 419–423 (2001).
39. Qian, Y. et al. Site-specific N-glycosylation identification of recombinant human lectin-like oxidized low density lipoprotein receptor-1 (LOX-1). *Glycoconj. J.* 29, 399–409 (2012).
40. Chen, M., Masaki, T. & Sawamura, T. LOX-1, the receptor for oxidized low-density lipoprotein identified from endothelial cells: Implications in endothelial dysfunction and atherosclerosis. *Pharmacology and Therapeutics* 95, 89–100 (2002).
41. Akhmedov, A. et al. Endothelial overexpression of LOX-1 increases plaque formation and promotes atherosclerosis in vivo. *Eur. Heart J.* 35, 2839–2848 (2014).
42. Mentrup, T., Häslér, R., Fluhrer, R., Saftig, P. & Schroder, B. A Cell-Based Assay Reveals Nuclear Translocation of Intracellular Domains Released by SPPL Proteases. *Traffic* 16, 871–892 (2015).
43. Mentrup, T. et al. Atherogenic LOX-1 signaling is controlled by SP-PL2-mediated intramembrane proteolysis. *J. Exp. Med.* 216, 807–830 (2019).
44. Kataoka, H. et al. Oxidized LDL modulates Bax/Bcl-2 through the lectinlike Ox-LDL receptor-1 in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 21, 955–60 (2001).
45. Dong, Y. et al. Activation of protease calpain by oxidized and glycated LDL increases the degradation of endothelial nitric oxide synthase. *J. Cell. Mol. Med.* 13, 2899–910 (2009).
46. Moriawaki, H. et al. Ligand specificity of LOX-1, a novel endothelial receptor for oxidized low density lipoprotein. *Arterioscler. Thromb. Vasc. Biol.* 18, 1541–7 (1998).
47. Iwai-Kanai, E. et al. Activation of lectin-like oxidized low-density lipoprotein receptor-1 induces apoptosis in cultured neonatal rat cardiac myocytes. *Circulation* 104, 2948–2954 (2001).
48. Hu, C. et al. Modulation of angiotensin II-mediated hypertension and cardiac remodeling by lectin-like oxidized low-density lipoprotein receptor-1 deletion. *Hypertension* 52, 556–562 (2008).
49. Chui, P. C., Guan, H. P., Lehrke, M. & Lazar, M. A. PPAR γ regulates adipocyte cholesterol metabolism via oxidized LDL receptor 1. *J. Clin. Invest.* 115, 2244–2256 (2005).
50. Dieudonné, A. et al. Scavenger receptors in human airway epithelial cells: Role in response to double-stranded RNA. *PLoS One* 7, (2012).
51. Parlato, S. et al. LOX-1 as a natural IFN- γ -mediated signal for apoptotic cell uptake and antigen presentation in dendritic cells. *Blood* 115, 1554–1563 (2010).
52. Chen, M. et al. Activation-dependent surface expression of LOX-1 in human platelets. *Biochem. Biophys. Res. Commun.* 282, 153–158 (2001).
53. Hofmann, A., Brunssen, C. & Morawietz, H. Contribution of lectin-like oxidized low-density lipoprotein receptor-1 and LOX-1 modulating compounds to vascular diseases. *Vascul. Pharmacol.* 107, 1–11 (2018).
54. Ding, Z. et al. Cross-talk between LOX-1 and PCSK9 in vascular tissues. *Cardiovasc. Res.* 107, 556–67 (2015).
55. Zuniga, F. A. et al. Role of lectin-like oxidized low density lipoprotein-1 in fetoplacental vascular dysfunction in preeclampsia. *BioMed Research International* 2014, (2014).
56. Oka, K. et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates phagocytosis of aged/apoptotic cells in endothelial cells. *Proc. Natl. Acad. Sci.* 95, 9535–9540 (1998).
57. Marwali, M. R. et al. Modulation of ADP-induced platelet activation by aspirin and pravastatin: Role of lectin-like oxidized low-density lipoprotein receptor-1, nitric oxide, oxidative stress, and inside-out integrin signaling. *J. Pharmacol. Exp. Ther.* 322, 1324–1332 (2007).
58. Hu, C. et al. LOX-1 deletion decreases collagen accumulation in atherosclerotic plaque in low-density lipoprotein receptor knock-out mice fed a high-cholesterol diet. *Cardiovasc. Res.* 79, 287–293 (2008).
59. Inoue, K., Arai, Y., Kurihara, H., Kita, T. & Sawamura, T. Overexpression of lectin-like oxidized low-density lipoprotein receptor-1 induces intramyocardial vasculopathy in apolipoprotein E-null mice. *Circ. Res.* 97, 176–184 (2005).
60. Wang, R., Ding, G., Liang, W., Chen, C. & Yang, H. Role of LOX-1 and ROS in oxidized low-density lipoprotein induced epithelial-mesenchymal transition of NRK52E. *Lipids Health Dis.* 9, (2010).
61. Balzan, S. & Lubrano, V. LOX-1 receptor: A potential link in atherosclerosis and cancer. *Life Sci.* 198, 79–86 (2018).

62. Ryoo, S. et al. OxLDL-dependent activation of arginase II is dependent on the LOX-1 receptor and downstream RhoA signaling. *Atherosclerosis* 214, 279–287 (2011).
63. Li, D. et al. Expression of lectin-like oxidized low-density lipoprotein receptors during ischemia-reperfusion and its role in determination of apoptosis and left ventricular dysfunction. *J. Am. Coll. Cardiol.* 41, 1048–1055 (2003).
64. Hinagata, J. I. et al. Oxidized LDL receptor LOX-1 is involved in neointimal hyperplasia after balloon arterial injury in a rat model. *Cardiovasc. Res.* 69, 263–271 (2006).
65. Mehta, J. L. et al. Deletion of LOX-1 reduces atherogenesis in LDLR knockout mice fed high cholesterol diet. *Circ. Res.* 100, 1634–1642 (2007).
66. Mehta, J. L., Chen, J., Yu, F. & Li, D. Y. Aspirin inhibits ox-LDL-mediated LOX-1 expression and metalloproteinase-1 in human coronary endothelial cells. *Cardiovasc. Res.* 64, 243–249 (2004).
67. Li, D. et al. Statins modulate oxidized low-density lipoprotein-mediated adhesion molecule expression in human coronary artery endothelial cells: Role of LOX-1. *J. Pharmacol. Exp. Ther.* 302, 601–605 (2002).
68. Civelek, S. et al. Soluble Lectin-Like Oxidized LDL Receptor 1 as a Possible Mediator of Endothelial Dysfunction in Patients With Metabolic Syndrome. *J. Clin. Lab. Anal.* 29, 184–90 (2015).
69. Takanabe-Mori, R. et al. Lectin-like oxidized low-density lipoprotein receptor-1 plays an important role in vascular inflammation in current smokers. *J. Atheroscler. Thromb.* 20, 585–90 (2013).
70. Yavuzer, S. et al. Endothelial damage in white coat hypertension: role of lectin-like oxidized low-density lipoprotein-1. *J. Hum. Hypertens.* 29, 92–8 (2015).
71. Yang, X., Wan, M., Cheng, Z., Wang, Z. & Wu, Q. Tofacitinib inhibits ox-LDL-induced adhesion of THP-1 monocytes to endothelial cells. *Artif. Cells, Nanomedicine Biotechnol.* 47, 2775–2782 (2019).
72. Lekuona, I. PCSK9 Inhibitors: From Innovation to Sustainable Clinical Application. *Rev. Esp. Cardiol.* 71, 996–998 (2018).
73. Pirillo, A., Norata, G. D. & Catapano, A. L. LOX-1, OxLDL, and atherosclerosis. *Mediators Inflamm.* 2013, (2013).
74. Dominguez, J. H. et al. Anti-LOX-1 therapy in rats with diabetes and dyslipidemia: Ablation of renal vascular and epithelial manifestations. *Am. J. Physiol. - Ren. Physiol.* 294, (2008).
75. Li, D. et al. LOX-1 mediates oxidized low-density lipoprotein-induced expression of matrix metalloproteinases in human coronary artery endothelial cells. *Circulation* 107, 612–7 (2003).
76. Kampschulte, M. et al. Western diet in ApoE-LDLR double-deficient mouse model of atherosclerosis leads to hepatic steatosis, fibrosis, and tumorigenesis. *Lab. Invest.* 94, 1273–1282 (2014).
77. Lund, A. K. et al. The oxidized low-density lipoprotein receptor mediates vascular effects of inhaled vehicle emissions. *Am. J. Respir. Crit. Care Med.* 184, 82–91 (2011).
78. Nakagawa, T. et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates leukocyte infiltration and articular cartilage destruction in rat zymosan-induced arthritis. *Arthritis Rheum.* 46, 2486–2494 (2002).
79. Rosenbaum, M. A., Miyazaki, K. & Graham, L. M. Hypercholesterolemia and oxidative stress inhibit endothelial cell healing after arterial injury. *J. Vasc. Surg.* 55, 489–496 (2012).
80. Zhang, B., Ramesh, G., Uematsu, S., Akira, S. & Reeves, W. B. TLR4 signaling mediates inflammation and tissue injury in nephrotoxicity. *J. Am. Soc. Nephrol.* 19, 923–932 (2008).
81. Schaeffer, D. F. et al. LOX-1 augments oxLDL uptake by lysoPC-stimulated murine macrophages but is not required for oxLDL clearance from plasma. *J. Lipid Res.* 50, 1676–1684 (2009).
82. Chang, Z. et al. Accelerated atherogenesis in completely ligated common carotid artery of apolipoprotein E-deficient mice. *Oncotarget* 8, 110289–110299 (2017).

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NEWS AND VIEWS

Nanoparticles functionalized for target delivery of siRNA in Lung cancerous cells

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Abstract: In the present medical field, scientists have designed new techniques to improve human health. These techniques focus on molecular level controls. Small interference RNA is an example of genetic control. This, together with Dicer and Argonaute 2 do not allow the transcription process to synthesize new protein. By using this mechanism, it is possible to control some diseases that are responsible for metastasis, such as lung cancer. Some experiments were conducted to prove the effectiveness of this technique. However, the problem is how to introduce the double-strand RNA to the cells. The issue was solved using nano-carriers as NP's. This paper aims at making a brief review of lung cancer, siRNA mechanism, and some different targeting techniques.

KeyWords: - siRNA, dsRNA, lung cancer, AGO2-Dicer complex, nanoparticles.

Introduction

Nowadays, Lung cancer remains the first cause of cancer death in the world¹. It is estimated that just in 2012, approximately 1.8 million people were diagnosed with lung cancer, leaving a more incredible figure of 1.6 million deaths, producing more deaths than breast, colon, and prostate cancer together. And they are commonly affecting male smokers². Besides, another of the big enemies that we need to fight is metastasis, which is the cause of more than 90% of cancer deaths in the world. Metastasis is a multistep process by which cancerous cells colonize distant parts of the body (concerning the original site of the tumor growing) and also by which malignant cells became resistant or immune to cell apoptosis³. It is essential to understand that day by day, and more people are going to increase these figures. Probably one of the main reasons for these alarming phenomena is due to the current procedures that are used to treat lung cancer patients, such as surgery, chemotherapy, and oral drugs, which remain ineffective or poorly effective. A few years Fire and Mello discovered RNA interference (RNAi), which has shown great potential in the treatment of a vast range of diseases in an innovative way, and within those diseases metastatic cancer. RNAi action processes could be defined as simple as the selective silencing of targeted genes⁴. Some problems that this relatively novel technique presents are related to the stability of the RNAi strands when those are inside the body and their capacity to bind the desired genes to avoid their expression correctly. In this way, many biological and artificial vectors were to achieve the "perfect carrier," a carrier that could overcome these barriers. Nanotechnology was one of the fields that many scientists around the world chose to develop it due to its huge range of possibilities of components, combinations and functionalization that could be performed to modify the properties of the final nanoparticles, their chemical stability and their relative low cost⁵. Here we present some of the most interesting procedures that combine these two technologies (RNAi or siRNA + Nanoparticles) to change the way that we deal with and how we treat lung cancer.

Lung Cancer

Cancer occurs when specific healthy cells begin to grow out of control. Cancer alters cell metabolism⁶. Commonly cancer cells develop due to damage in the DNA. Although this

damage can be controlled by cells specialized, in cancer cells, damaged DNA cannot be repaired, and these cells do not die. Generally, cancer cells look like a solid tumor, except in the case of leukemia. When these tumors are advanced, they enter the metastasis phase⁷. Metastasis is a complex process where tumor cells replicate out of control, thus expanding to other organs. Due to their functional structure, high vascularity, and high levels of oxygen lungs are perfect for developing metastasis because they provide a nutrient-rich environment⁸.

Cancer is the leading cause of death in the 21st century. Lung cancer is the most frequently represented because it causes 18.4 % deaths out of 9.6 million, mainly in males¹. The relation between smoking and cancer was first established in 1930 when clinicians identified this uncommon disease⁹. At present, the causes are: genetic or environmental. The genetic causes are DNA mutations and parental inheritance⁷. Environmental carcinogens agents are smoking of cigarettes, air pollution, and occupational exposure¹⁰. The smoking intensity and average life are directly associated; therefore, this increases lung cancer².

Moreover, exposure to asbestos and radon also increases this possibility. There are two types of lung cancer: non-cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC)¹¹. Thus, it is essential to distinguish these types and the consequent treatment they need. Currently, there are some lung cancer treatments such as surgery resection, chemotherapy, immunotherapy, hormonal therapy, and radiotherapy⁷. Surgical resection is accepted for treating lung metastasis. However, it is not too useful because it only allows 5-year survival for patients⁸.

In the present, a new type of treatment had been developed. It is called targeted therapy inhibitor, for example, Gefitinib. Gefitinib is an inhibitor that targets tyrosine kinase to epidermal growth factor receptor (EGFR). Another targeted therapy use liposomes with arginine. It is used to encapsulate human double-minute 2 (HDM2) gene to specific siRNA and low-density polyethylene (LPD) nanoparticles for delivery siRNA to lung cancer cells¹¹. This alters the major cell-signaling and regulatory pathways¹⁰. Recent advances in cancer treatment are the use of small interfering RNAs (siRNAs)⁷. These molecules do not allow the replication process; therefore there is no synthesis of a new protein.

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siRNA mechanism

Small interference RNA (siRNA), or also called post-transcriptional gene silencing (PTGS), is a biological mechanism that silences genes that affect the DNA replication process¹². siRNA is a type of RNA interference with 21-23 nucleotides^{13,14}. The phenomenon was discovered in the late 1980s in plants by Napoli *et al.*^{14,15}. Later, similar events were found in *Caenorhabditis elegans*¹⁶. They concluded that silencing the mechanism occurs because of mRNA degradation¹⁷.

Furthermore, the functions of siRNA are protected from viruses and defense against transposable or transposons genes¹⁶. In mammal cells, the primary purpose is endogenous protection against cancer cells. This process differs in plants and mammals' cells. The first one is a transcriptional and post-transcriptional process, while the second one is only post-transcriptional¹³. Therefore genetic silencing is a novel method to limit transcription or active a sequence to degradation process¹⁸.

Gene expression is possible to interrupt a genetic speech with a process called genetic silencing¹⁹. It starts with the degradation of exogenous double-strand RNA (dsRNA) into siRNA by an enzymatic complex. siRNA hybrids the complementary regions of mRNA. When dsRNA is introduced into the cells, it interacts with Dicer. Dicer is an enzyme that cleavages the long double-stranded RNAs into shorter fragment (siRNA)¹⁷. These short fragments have an extreme nucleotide 3' and 5' free¹³. These extremes are recognized by Argonaute 2 (AGO2), forming AGO2-RISC-Complex enzyme^{14,20}. When this complex is built, siRNA is transformed into a single strand that guides the enzyme complex to mRNA. AGO2-RISC-Complex interacts with mRNA and cleavage it by Ago2^{21,22}. Finally, mRNA is degraded; thus, replication process is not allowed¹⁴. (Figure 1)

siRNA is a molecular mechanism that shows the potential for the treatment of a disease such as cancer²³. This technique

is emerging as innovative nucleic acid treatment¹¹. In contrast, traditional chemotherapy is widely accepted in the fight against metastasis. However, chemotherapy is harmful and toxic for the body giving the patient a lower quality of life⁸. Besides, chemotherapy affects not only malignant cells but also surrounding cells. Therefore, there is severe damage to the patient's body.

siRNA has high specificity, high efficiency, and low toxicity. Thus, it is a potential treatment for targeted genetic silencing⁸. However, siRNA is unable to enter into the cells without a transporter or nanocarriers. These transporters can be natural or synthetic¹². Unaffected carriers can be viruses and bacteria, while synthetic transporters can be a self-assembled nanoparticle that is introduced in the tumor by intravenous administration²⁴. The characteristics mentioned above make siRNA an efficient technology for oncology therapies because it allows us to silence specific genes, thereby stopping the protein synthesis¹².

siRNA studies for cancer treatment

As described above, siRNA opened a huge door in the fight against cancer because of its role in the regulation (down-regulation) of the gene expression through sequence-specific post-transcriptional gene silencing^{25,26}. Nonetheless, some of the main issues that siRNA silencing presents are: 1) The limited half-life of the uncovered strand of siRNA into the body due to the action of RNases and their low chemical stability in biological environments²⁶. 2) The fact that systemic delivery of siRNA could produce adverse effects on the patient as liver toxicity and activation of immune responses. 3) The fact that it is practically impossible for the siRNA strands to enter the cell through diffusion due to its relatively large size and its negative charge²⁷.

All these points made evident the necessity to develop a drug carrier that efficiently distributed the siRNA strands into

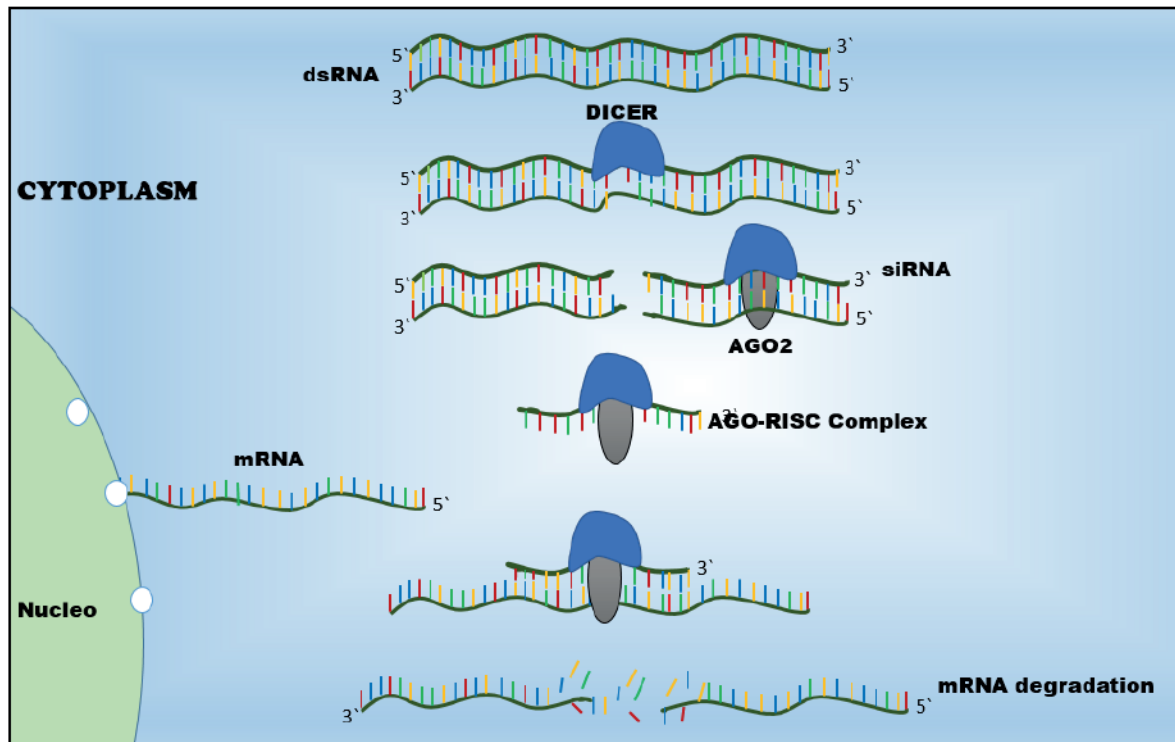


Figure 1. Gene silencing mechanism of siRNA. dsRNA is processed by Dicer into siRNA. After dsRNA is broken, Argonaute 2 interacts with it forming AGO-RISC Complex. siRNA guides these enzymes to mRNA because it has a site that recognizes mRNA. Later, AGO2 cuts mRNA, which finally is degraded, so there is not protein synthesis.

the cancerous cells avoiding these barriers and minimizing the toxicity and the adverse effects in the patients²⁸. There is where nanoparticles, liposomes, nanoplateforms, and nanotechnology, in general, came to have an essential role in the improvement of siRNA and its potential to affect cancer^{26,27}. Since many target genes have been identified in lung cancer, until 2015, six clinical trials were undertaken with nanoparticle-based siRNA delivery systems²⁹.

For this article, more than seven different cancer-targeting techniques were analyzed. These targeting techniques go from single gene targeting to more specialized techniques such as the work performed by Yang *et al.* in 2011 with a system that wants to affect a combination of ways to reduce tumor growth:

- Murine Double Minute 2 (MDM2) known as the significant suppressor of p53 function in cancerous cells³⁰.
- The transcription factor *c-myc* that controls many essential functions within the cell as the cell cycle, the proliferation and sensitization to apoptotic stimuli³¹.
- Vascular Endothelial Growth Factor (VEGF) a growth factor that has been shown to play an essential role in the tumor angiogenesis, and metastasis³².

At the end of their investigation, Yang *et al.* reported

- A reduction in the tumor load of 20/30 (%),
- Prolonged survival of ~27%, and that
- ~11% of the tumoral cells treated underwent apoptosis.

We also have the reports of Han *et al.* that synthesize Self-Assembled nanoparticles by the use of cationic bovine serum albumin (CBSA) with a significant reduction in lung metastasis, an increase in the proapoptotic activity and a notable decrease in cell proliferation³³. As in the case of these three examples, all the procedures analyzed show positive results in the reduction or elimination of cancerous cells in the experiments carried out in vitro and in vivo, as can be seen in Table 1.

A remarkable job was performed by Davis *et al.* from 2008 to 2013 when the first official clinical trial was done by targeting the subunit M2 of Ribonucleic Reductase (RRM2) to reduce the growth potential of the cancer cells³⁴. The hearing focused on solid tumors was probed in 24 patients and reported a decrease in the RRM2 mRNA up to 76%. Despite these encouraging results, these techniques need to be improved and refined for future applications.

Drug or Experiment	Type of siRNA delivery	Target	Delivery Method	Results
CALAA-01	Cyclodextrin-based polymer(CDP)	RRM2	Iv. injection	A decrease in the RRM2 mRNA up to 76%
siRNA/RGD gold Np's	Arginine-glycine-aspartic acid (RGD) gold nanoparticles	$\alpha v\beta 3$ integrin	Intratracheal instillation	A decrease in the incidence and severity of the tumor clones, tumor regression, ~80% increase in survival.
Nano-based inhalation co-delivery of anticancer drugs.	Lutein hormone-releasing hormone (LHRH)-modified mesoporous silica nanoparticles (MSN)	BCL2 and MRP1	Inhalation	58% of MRP1 silencing 56% of BCL2 silencing 50% and 35% downregulation in pump and nonpump cellular resistance respectively
CBSA Np's	Cationic bovine serum albumin	Not specified	Iv. injection	-Significant reduction in lung metastasis, -Increase in the proapoptotic activity -Notable reduction in cell proliferation
LPC Np's	Lipid/Calcium/Phosphate (LCP)	MDM2, c-myc, VEGF	Iv. injection	-Reduction on the tumor load of 20-30% -Prolonged survival of ~27. % - ~11% of the tumoral cells treated underwent apoptosis
Cationic nano-Lipoplexes	Ethylphosphocholine-based lipoplexes	Mcl 1	Intratracheal Injection	-Reduction of tumor nodules in the lung tissue
Ph responsive Np's	Poly (methacryloyloxy ethyl phosphorylcholine)-block-poly (diisopropanolamine ethyl methacrylate) (PDMA-b-PDPA)	MDM2	Tail vein injection	-Good biocompatibility. -Significant induction of cell death - Inhibition of cell proliferation

Table 1. A summary of the procedures analyzed where specified: the drug or experiment used, type of siRNA delivery, target, delivery method, and the result obtained.

Conclusions

RNA interference therapy is used to silence a selective gene expression. Thanks to this, it is possible to control the not healthy growth of tumor cells. dsRNA is introduced as exogenous genes that interact with Dicer getting siRNA. When AGO2 is linked, it forms AGO-RISC-Complex. siRNA server as a guide to the complex for attaching to mRNA. Finally, mRNA is degraded. As a result, the new protein cannot be synthesized. From the current review, we can conclude that although there is still entirely to investigate, siRNA is a reasonably powerful technique in the war against cancer and many other diseases for which we do not know a cure today. Nanotechnology also does not fail to present opportunities to promote innovative techniques for the development of biomedicine and health care.

Conflict of interests

The authors declare no conflict of interest, financial or otherwise.

Bibliographic references

- Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA. Cancer J. Clin.* (2018) doi:10.3322/caac.21492.
- Nasim, F., Sabath, B. F. & Eapen, G. A. Lung Cancer. *Medical Clinics of North America* vol. 103 463–473 (2019).
- Mehlen, P. & Puisieux, A. Metastasis: A question of life or death. *Nature Reviews Cancer* (2006) doi:10.1038/nrc1886.
- Davis, M. E. et al. Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* 464, 1067–1070 (2010).
- Lohcharoenkal, W., Wang, L., Chen, Y. C. & Rojanasakul, Y. Protein nanoparticles as drug delivery carriers for cancer therapy. *BioMed Research International* (2014) doi:10.1155/2014/180549.
- Beger, R. A Review of Applications of Metabolomics in Cancer. *Metabolites* (2013) doi:10.3390/metabo3030552.
- Sudhakar, A. History of Cancer, Ancient and Modern Treatment Methods. *J. Cancer Sci. Ther.* (2009) doi:10.4172/1948-5956.100000e2.
- Yang, Y., Li, J., Liu, F. & Huang, L. Systemic delivery of siRNA via LCP nanoparticle efficiently inhibits lung metastasis. *Mol. Ther.* 20, 609–615 (2012).
- Witschi, H. A short history of lung cancer. *Toxicol. Sci.* (2001) doi:10.1093/toxsci/64.1.4.
- Molina, J. R., Yang, P., Cassivi, S. D., Schild, S. E. & Adjei, A. A. Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. in *Mayo Clinic Proceedings* vol. 83 584–594 (Elsevier Ltd, 2008).
- Oh, Y. K. & Park, T. G. siRNA delivery systems for cancer treatment. *Advanced Drug Delivery Reviews* (2009) doi:10.1016/j.addr.2009.04.018.
- van den Brand, D., Mertens, V., Massuger, L. F. A. G. & Brock, R. siRNA in ovarian cancer – Delivery strategies and targets for therapy. *Journal of Controlled Release* (2018) doi:10.1016/j.jconrel.2018.05.012.
- Ozcan, G., Ozpolat, B., Coleman, R. L., Sood, A. K. & Lopez-Berestein, G. Preclinical and clinical development of siRNA-based therapeutics. *Advanced Drug Delivery Reviews* (2015) doi:10.1016/j.addr.2015.01.007.
- Bernstein, E., Caudy, A. A., Hammond, S. M. & Hannon, G. J. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* (2001) doi:10.1038/35053110.
- Napoli, C., Lemieux, C. & Jorgensen, R. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell* (1990).
- Sen, G. L. & Blau, H. M. A brief history of RNAi: The silence of the genes. *FASEB Journal* (2006) doi:10.1096/fj.06-6014rev.
- Fire, A. et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* (1998) doi:10.1038/35888.
- Agrawal, N. et al. RNA Interference: Biology, Mechanism, and Applications. *Microbiol. Mol. Biol. Rev.* (2003) doi:10.1128/mmbr.67.4.657-685.2003.
- Baum, J. A. & Roberts, J. K. Progress Towards RNAi-Mediated Insect Pest Management. in *Advances in Insect Physiology* (2014). doi:10.1016/B978-0-12-800197-4.00005-1.
- Whangbo, J. S. & Hunter, C. P. Environmental RNA interference. *Trends in Genetics* (2008) doi:10.1016/j.tig.2008.03.007.
- Castel, S. E. & Martienssen, R. A. RNA interference in the nucleus: Roles for small RNAs in transcription, epigenetics and beyond. *Nature Reviews Genetics* (2013) doi:10.1038/nrg3355.
- Sarkies, P. & Miska, E. A. Small RNAs break out: The molecular cell biology of mobile small RNAs. *Nature Reviews Molecular Cell Biology* (2014) doi:10.1038/nrm3840.
- Xu, X. et al. Ultra-pH-Responsive and Tumor-Penetrating Nanoplat-form for Targeted siRNA Delivery with Robust Anti-Cancer Efficacy. *Angew. Chemie - Int. Ed.* 55, 7091–7094 (2016).
- Li, S. D., Chen, Y. C., Hackett, M. J. & Huang, L. Tumor-targeted delivery of siRNA by self-assembled nanoparticles. *Mol. Ther.* 16, 163–169 (2008).
- Conde, J. et al. In vivo tumor targeting via nanoparticle-mediated therapeutic siRNA coupled to inflammatory response in lung cancer mouse models. *Biomaterials* 34, 7744–7753 (2013).
- Wall, N. R., Shi, Y. & Shi, Y. For personal use. Only reproduce with permission from The Lancet. www.thelancet.com.
- Shim, G. et al. Enhanced intrapulmonary delivery of anticancer siRNA for lung cancer therapy using cationic ethylphosphocholine-based nanolipoplexes. *Mol. Ther.* 21, 816–824 (2013).
- Li, J., Yang, Y. & Huang, L. Calcium phosphate nanoparticles with an asymmetric lipid bilayer coating for siRNA delivery to the tumor. *J. Control. Release* 158, 108–114 (2012).
- Fujita, Y., Kuwano, K. & Ochiya, T. Development of small RNA delivery systems for lung cancer therapy. *International Journal of Molecular Sciences* vol. 16 5254–5270 (2015).
- Haupt, Y., Maya, R., Kazaz, A. & Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* (1997) doi:10.1038/387296a0.
- Miller, D. M., Thomas, S. D., Islam, A., Muench, D. & Sedoris, K. c-Myc and cancer metabolism. *Clinical Cancer Research* vol. 18 5546–5553 (2012).
- Holash, J. et al. VEGF-Trap: A VEGF blocker with potent antitumor effects. www.pnas.org/cgi/doi/10.1073/pnas.172398299 (2002).
- Han, J., Wang, Q., Zhang, Z., Gong, T. & Sun, X. Cationic bovine serum albumin based self-assembled nanoparticles as siRNA delivery vector for treating lung metastatic cancer. *Small* 10, 524–535 (2014).
- Heidel, J. D. et al. Potent siRNA inhibitors of ribonucleotide reductase subunit RRM2 reduce cell proliferation In vitro and In vivo. *Clin. Cancer Res.* 13, 2207–2215 (2007).

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NEWS AND VIEWS

Diagnostic tests for Coronavirus Disease 2019. What happens behind the assays?

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Abstract: Pandemic caused by Coronavirus Disease 2019 (COVID-19) shows a plethora of clinical manifestations from the absence of symptoms to the development of pneumonia and even death. Nowadays, the number of new infections estimated to stem from a single COVID-19 case is between 2 and 3. For this reason, a rapid diagnosis will allow the massive screening of the population and the isolation of carriers and asymptomatic people. However, selecting an appropriate diagnostic test might be highly relevant, depending on the prevalence of the illness and the population to be tested. This communication has as purpose to describe the methodological tests employed to the COVID-19 diagnosis and analyze the pros and cons of them.

KeyWords: COVID-19, diagnosis, immunoassays, q-RT-PCR.

COVID-19 Diagnostic Tests

Coronavirus Disease 2019 (COVID-19) is caused by a novel coronavirus, which is closely related to two bat-derived severe acute respiratory syndromes (SARS)-like coronaviruses¹. Patients may undergo different manifestations from asymptomatic carriers to developing interstitial pneumonia, multi-organ failure, and death². This illness emerged in December 2019 in Wuhan, China, from where it spread worldwide (Singhal 2020), infecting over 4 000 000 people and causing the death of around 290 000 people³.

Hitherto, many research groups are working to develop an accurate diagnostic method. In particular, they have focused on two principal approaches: (i) molecular and (ii) immunoassays, which aim to be the primary diagnostic alternatives in both developed and developing countries⁴ (Figure 1).

Regarding molecular analysis, approaches based on quantitative Reverse Transcription Polymerase Chain Reaction (q-RT-PCR) have been designed. Some q-RT-PCR protocols analyze two genes. The amplification of one gene is interpreted as a positive screening, while the presence of the second one is interpreted as a confirmatory result. On the other hand,

other methodologies examine three or more genes, and the test is interpreted as positive only when the three genes are detected⁵.

The United States Centers for Disease Control (CDC) q-RT-PCR detects specific viral SARS-CoV-2 genes of the viral nucleocapsid (N1 and N2) while the methodology of the World Health Organization (WHO) targets the SARS-CoV-2-RNA-dependant RNA polymerase (RdRP) and envelope (E) genes. Both of them use a cycle threshold of less than 40 as the criterion for positivity^{6,7}.

According to the Guidelines of the Korean Society for Laboratory Medicine and the Korea Centers for Disease Prevention and Control the q-RT-PCR must be carried out to (i) confirm patients' release from quarantine, (ii) screen asymptomatic people related to COVID-19 patients and (iii) make a differential diagnosis among COVID-19 and other respiratory syndromes⁵.

Although q-RT-PCR is considered as the confirmatory diagnostic test, the principal disadvantage of this method is the high number of false-negative results. The causes of this

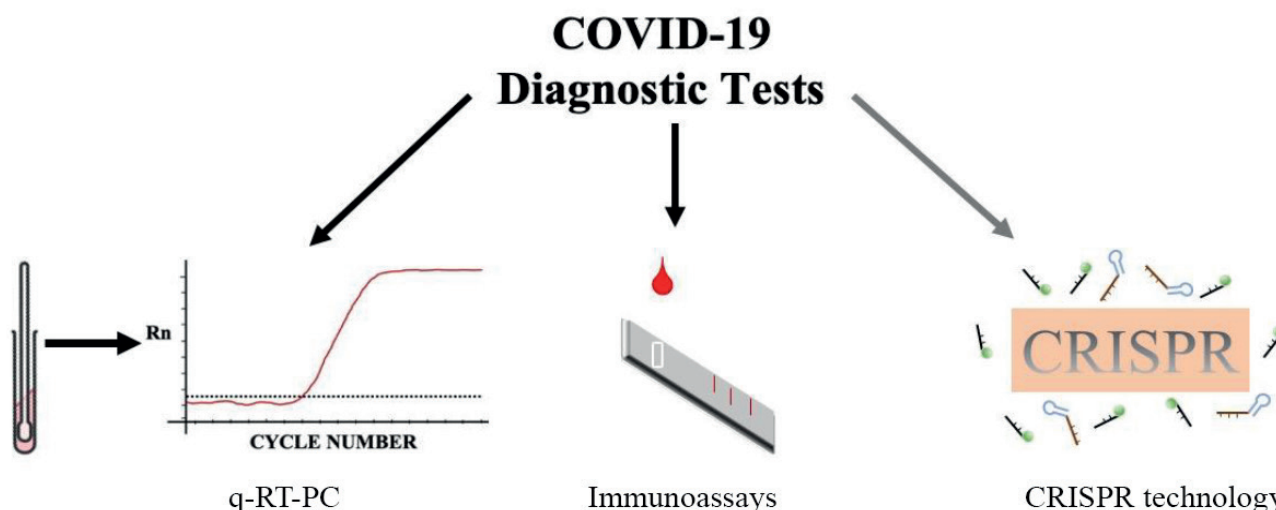


Figure 1. COVID-19 diagnostic tests. Quantitative Reverse Transcription Polymerase Chain Reaction (q-RT-PCR) is considered the gold standard to diagnose COVID-19. Several immunoassays have been developed; some of them are based on the lateral flow principle these tests is being used as rapid screening tests. Nowadays, Cluster Regularly Interspaced Short Palindromic Repeats (CRISPR) based tests are emerging as a recent alternative, although they have not been certified as an in vitro diagnostic test yet. For more details, see the text.

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inconvenience might be: (i) poor specimen quality, (ii) improper samples handling or transported, (iii) a viral genetic mutation, (iv) presence of PCR inhibitors, or even (v) samples with low viral loads⁵.

On the other hand, considering the immunogenic response of S and nucleocapsid viral proteins that trigger immunological response associated to immunoglobulins production from 17 and 23 days after disease onset⁸, with IgM and IgG seroconversion within 20 days after symptoms⁹ different immunological approaches based on lateral flow, ELISA, and chemiluminescence have been developed as diagnostic immunoassays¹⁰.

Previous studies describe diverse results on the sensitivity and specificity of lateral flow tests. For example, Li *et al.* (2020) analyzed the accuracy of this serologic test in 397 SARS-CoV-2 patients and 128 healthy people confirmed by q-RT-PCR. The results showed that the sensitivity and specificity values for the immunoassay were 88.66% and 90.63%, respectively¹¹. These results were similar to those reported by Castro *et al.* (2020). These researchers, in a meta-analysis carried out in Brazil that had a purpose of setting the accuracy of available lateral flow tests to diagnose COVID-19 in that country, found sensitivity values between 55% and 100% and specificity between 94% and 100%¹².

However, the applicability of these tests depends on the prevalence of the disease. In a high-prevalence location with more than 300 COVID-19 cases among 12000 inhabitants, 49 patients were randomly selected and were evaluated using a lateral flow immunoassay IgM/IgG vs. the q-RT-PCR. The results showed only 8 q-RT-PCR positive tests were positive to the immunoassay (sensitivity: 36.4%), and from 27 q-RT-PCR negative samples, 24 were detected as negative by the immunoassay (specificity: 88.9%)¹³.

Other immunological methodologies, such as ELISA and chemiluminescence, have similar accuracy to the lateral flow immunoassays. For example, Adams *et al.* (2020) reported the SARS-CoV-2 IgM/IgG ELISA sensitivity and specificity values of 85% and 100%, respectively¹⁴. Alike, IgM/IgG titers measured among 43 COVID-19 patients and 33 health people by chemiluminescence showed a sensitivity of 48.1% and 88.9% and specificity of 100% and 90% for each immunoglobulin, respectively¹⁵.

Another alternative to COVID-19 diagnosis might be CRISPR (Cluster Regularly Interspaced Short Palindromic Repeats) technology. Gootenberg *et al.* (2017) previously reported the SHERLOCK system (Specific High-Sensitivity Enzymatic Reporter UnLOCKing) as a virus CRISPR-based diagnostic platform which takes advantage of the unspecific catalytic activity of the Cas13a enzyme releasing a fluorescent RNA reporter previous an isothermal amplification¹⁶. This system has been improved recently by a lateral readout platform, which guarantees a quantitative and rapid detection of specific nucleic acids¹⁷.

Until now SHERLOCK system has not been tested on biological samples. However, a modification of this platform termed DETECTR (DNA endonuclease-targeted CRISPR trans reporter)¹⁸ was probed on samples, although its use in diagnosis has not yet been approved by the U.S. Food and Drug Administration. Recently, Broughton and co-workers (2019) described a modification of this system based on CRISPR-Cas12 lateral flow assay as a visual and faster alternative to diagnose COVID-19¹⁹.

Conclusions

Immunoassays might be an alternative for the rapid diagnosis of COVID-19 as a complement to viral nucleic acid detection specially among carriers, asymptomatic, symptomatic patients and health sector workers²⁰. However, one possible disadvantage of the immunoassays is the fallen of the IgG titers at 8 weeks post symptoms onset, although these titers remain above the detection threshold¹⁴ being possible to detect anti-SARS-CoV-2 IgG up to 50 days from symptoms onset^{21,22}. On the other hand, previous studies on 11 patients diagnosed with pneumonia due to coronavirus at day 240 after symptoms onset showed that all patients were still positive to SARS-CoV anti-nucleocapsid IgG²³. SARS-CoV-2 could have different IgG kinetics than anti-nucleocapsid IgG of SARS-CoV.

Considering the highly variable performance of lateral flow immunoassay devices¹⁴ it is urgently needed to address studies to analyze the diagnostic yields of the immunoassays for COVID-19 diagnosis. Against this background, new molecular technologies based on editing gene tools might be a feasible, cheap, and rapid alternative to the existing COVID-19 diagnostic systems¹⁹.

Bibliographic references

1. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* [Internet]. 2020;395(10224):565–74. Available from: [http://dx.doi.org/10.1016/S0140-6736\(20\)30251-8](http://dx.doi.org/10.1016/S0140-6736(20)30251-8)
2. Infantino M, Damiani A, Gobbi FL, Grossi V, Lari B, Macchia D, et al. Serological Assays for SARS-CoV-2 Infectious Disease: Benefits, Limitations and Perspectives. Vol. 22, *The Israel Medical Association journal* : IMAJ. 2020. p. 203–10.
3. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* [Internet]. 2020;20(5):533–4. Available from: [http://dx.doi.org/10.1016/S1473-3099\(20\)30120-1](http://dx.doi.org/10.1016/S1473-3099(20)30120-1)
4. C. Bachelet V. Do we know the diagnostic properties of the tests used in COVID-19? A rapid review of recently published literature. *Medwave* [Internet]. 2020;20(03):e7891–e7891. Available from: <https://www.medwave.cl/link.cgi/English/Reviews/GeneralReviews/7891.act>
5. Hong KH, Lee SW, Kim TS, Huh HJ, Lee J, Kim SY, et al. Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea. *Ann Lab Med*. 2020;40(5):351–60.
6. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 -nCoV by RT-PCR. 2019;(December):1.
7. Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, et al. Diagnostic Testing for Severe Acute Respiratory Syndrome-Related Coronavirus-2. *Ann Intern Med*. 2020;(April).
8. Qu J, Wu C, Li X, Zhang G, Jiang Z, Li X, et al. Profile of IgG and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020;caa489.
9. Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* [Internet]. 2020;1–4. Available from: <http://www.nature.com/articles/s41591-020-0897-1>
10. Vashist SK. In vitro diagnostic assays for COVID-19: Recent advances and emerging trends. *Diagnostics*. 2020;10(4).
11. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol*. 2020;(February).
12. Castro R, Luz PM, Wakimoto MD, Veloso VG, Grinsztejn B, Perazzo H. COVID-19: a meta-analysis of diagnostic test accuracy of commercial assays registered in Brazil. *Brazilian J Infect Dis* [Internet]. 2020;(x x):1–9. Available from: <https://doi.org/10.1016/j.bjid.2020.04.003>

13. Döhla M, Boesecke C, Schulte B, Diegmann C, Sib E, Richter E, et al. Rapid point-of-care testing for SARS-CoV-2 in a community screening setting shows low sensitivity. *Public Health*. 2020;182:170–2.
14. Adams E, Anand R, Andersson M, Auckland K, Baillie J, Barnes E, et al. Evaluation of antibody testing for SARS-CoV-2 using ELISA and lateral flow immunoassays. *medRxiv*. 2020;1–24.
15. Jin Y, Wang M, Zuo Z, Fan C, Ye F, Cai Z, et al. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. *Int J Infect Dis* [Internet]. 2020;94:49–52. Available from: <https://doi.org/10.1016/j.ijid.2020.03.065>
16. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* (80-). 2017;356(6336):438–42.
17. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a and Csm6. *Science* (80-). 2018;360(6387):439–44.
18. Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* (80-). 2018;360(6387):436–9.
19. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, et al. CRISPR – Cas12-based detection of SARS-CoV-2. 2019;
20. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. *J Med Virol* [Internet]. 2020;0–1. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/32104917>
21. Wang B, Wang L, Kong X, Geng J, Xiao D, Ma C, et al. Long-term Coexistence of SARS-CoV-2 with Antibody Response in COVID-19 Patients. *J Med Virol*. 2020;2019:0–1.
22. Lee Y-L, Liao C-H, Liu P-Y, Cheng C-Y, Chung M-Y, Liu C-E, et al. Dynamics of anti-SARS-Cov-2 IgM and IgG antibodies among COVID-19 patients. *J Infect* [Internet]. 2020; Available from: <https://doi.org/10.1016/j.jinf.2020.04.019>
23. Woo PCY, Lau SKP, Wong BHL, Chan KH, Chu CM, Tsoi HW, et al. Longitudinal profile of immunoglobulin G (IgG), IgM, and IgA antibodies against the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in patients with pneumonia due to the SARS coronavirus. *Clin Diagn Lab Immunol*. 2004;11(4):665–8.

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NEWS AND VIEWS

Infodemic during an epidemic

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"We're not just fighting an epidemic; we're fighting an infodemic," were the words of Tedros Adhanom Ghebreyesus, director-general of the World Health Organization (WHO), referring to the continually circulating false news about the Covid-19 pandemic crisis. WHO explains that infodemics are an excessive amount of both real and inaccurate information about a problem, making it challenging to identify a solution, they create confusion in the population by spreading erroneous information, misinforming with apparently real data. The amount of time and other essential resources that can be affected by this problem make it a priority to combat¹.

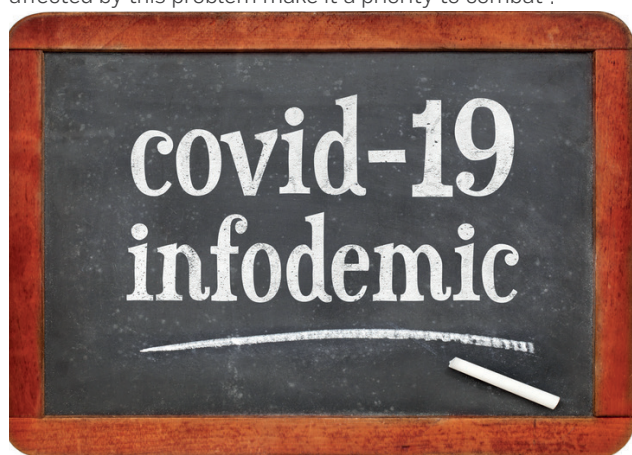


Figura 1. Covid-19 infodemic - coronavirus pandemic information overload concept

The existence of social networks and the internet is for better or for worse is one of the differentiating factors between this pandemic and the previous ones. From the manipulation of information for ideological purposes or material gain to simple jokes in the form of memes or chains on social networks, this disinformation can have substantial repercussions on the population by generating panic². The clickbait nourishes many of these publications to its rapid spread reaching large sectors of the population in a matter of hours. This erroneous information presented by some media or shared on social networks has covered everything related to the virus from where it was generated to how it is spread. For this reason, pages like the World Health Organization have devoted themselves to destroying myths and correcting erroneous information such as those that claim that sunlight, warm weather, and drinking water can affect the coronavirus³.

An excellent way to avoid this problem and get reliable information is according to Neil Walsh, chief of the Cybercrime and Anti-Money Laundering Section of the United Nations Office on Drugs and Crime (UNODC), is to visit the pages of official sources and instructed on the subject as the World Health Organization (WHO) and the United Nations¹.

To combat disinformation, UNESCO provided in its Media and Information Literacy Curriculum for teachers, a series of actions to consider when consuming information⁴.

- Verify the veracity of the sources in cases when the information comes from non-formal news media.
- Search for sources within any information we review.
- Know which are the main channels and providers of truthful information.
- Verify the information with other reputable media sources.
- Do not share or promote information without first checking its sources.

It is important to note that the vast majority of fake news generators can make it credible by including fake quotes, images, figures, data, and including the names of public personalities related to the topic³. Critical thinking and analysis are of utmost importance in the face of this problem. The need to compare sources and go to official sources of information is essential to avoid further damage.

Bionatura journal recommends the following information sources that have been proven truthful during the current health crisis in addition to those already mentioned before:

Open-Access Data and Computational Resources to Address COVID-19: <https://datascience.nih.gov/covid-19-open-access-resources>

Centers for Disease Control and Prevention: <https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html>

Covid19.21wecan.com <https://covid19.21wecan.com/covid19en/index.shtml>

Bibliographic references

1. UN. UN tackles 'infodemic' of misinformation and cybercrime in COVID-19 crisis | United Nations. <https://www.un.org/en/un-coronavirus-communications-team/un-tackling-'infodemic'-misinformation-and-cybercrime-covid-19> (2020).
2. Robson, D. Why smart people believe coronavirus myths. BBC (2020).
3. Naughton, J. Fake news about Covid-19 can be as dangerous as the virus. The Guardian (2020).
4. Tips to avoid online misinformation during the coronavirus crisis. <https://www.bbva.com/en/tips-to-avoid-online-misinformation-during-the-coronavirus-crisis/>.

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NEWS AND VIEWS

COVID-19 and Mental Health

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The World Health Organization (WHO) defines health as the state of perfect (complete) physical, mental and social well-being, and not just the absence of disease¹.

In epidemic and pandemic situations, such as the COVID-19 pandemic that we are currently going through, all these spheres, physical, mental, and social, are affected, which threatens the lives of many people and significantly increases the number of sick and deceased people. The general fear of contagion worsens the existing situation and leads to a psychosocial impact that will, at some point, exceed the ability of health personnel to confront and manage the affected population. It is considered that the increase in the incidence of mental disorders and emotional manifestations is directly related to the magnitude of the epidemic and the level of vulnerability of the country affected. However, not all of the mental problems that occur can be classified as a mental health disorder, as many of these are normal reactions to an abnormal situation².

The tremendous psychological impact that the consequences of the COVID-19 pandemic has is already being widely discussed, predicting another pandemic; a pandemic of mental disorders, which comes with a wave of depression, anxiety, and post-traumatic stress that is going to affect society after this crisis. The confinement measures are taken to slow down the transmission of the coronavirus, the harsh situation for the sick and health personnel, as well as the loss of loved ones, is testing our mental health, individually and collectively, constituting a tremendous psychological challenge for health professionals and the population in general. This incredible impact is going to occur since our social, work, and daily life has been turned upside down, which in many cases has exposed us to situations of solitude and has changed our way of socializing, working and enjoying life. When this confinement

is over, we will not return to the normality we are used to³.

Common mental disorders (CMD), according to the WHO, comprise depressive and anxiety disorders and are a leading cause of disability worldwide. The WHO also estimates that more than 300 million people suffer from depression (4.4% of the world population), and almost the same number of people are affected by anxiety disorders; furthermore, there is also significant comorbidity between the both of them. Depressive disorders include major depressive disorder and dysthymia, while anxiety disorders include generalized anxiety disorder (GAD), panic disorder, phobias, social anxiety disorder, obsessive-compulsive disorder (OCD), and post-traumatic stress disorder (PTSD)^{4,5}.

In 2013, the Mental health action plan 2013-2020⁶, was published by the WHO, whose targets are mentioned in Table 1, which will be affected as a consequence of the COVID-19 pandemic. (Table 1 and figure 1)

In a recent study, conducted in China, the country where the first cases of coronavirus appeared and from which the disease spread to the rest of the world, a nationwide survey was conducted to evaluate mental health. The findings reported a wide variety of psychological problems such as panic disorders, anxiety, and depression. In the case of anxiety and depression, the most affected groups were the female sex and the older population. Additionally, high levels of stress were reported in younger adults⁷. Another study carried out during the outbreak, but on a smaller scale, used a different survey to evaluate mental health in Spain. The results showed that the most affected groups were the youth and patients with chronic diseases, who's discomfort worsened when the confinement began⁸.

In our country, we still don't have official data on the psychological/psychiatric disturbances produced and

Global target 1.1	By 2020, 80% of nations will develop or updated their mental health policies/plans following international and regional human rights instruments.
Global target 1.2	By 2020, 50% of nations will develop or update their mental health legislation by international and regional human rights instruments
Global target 2	By 2020, they should have increased the coverage for services for severe mental disorders by 20% worldwide.
Global target 3.1	By 2020, 80% of nations will have at least two national multisectoral mental health promotion and prevention programs in operation.
Global target 3.2	By 2020, the nation's suicide rate will have been reduced by 10%.
Global target 4	By 2020, 80% of nations will calculate and report a basic set of mental health indicators, every 2 years systematically through their national health and social information system.

Table 1. Targets of the comprehensive mental health action plan 2013-2020 (taken from WHO. Mental Health Action Plan 2013-2020, Geneva 2013.)

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Figura 1. Recommendations on how to cope with stress related to the pandemic by the World Health Organization

worsened due to the COVID-19 pandemic. However, all the social problems that are triggered by confinement, mobility restrictions, loss of jobs, business closures, among others, are factors that have affected all countries worldwide and will continue to do so for as long as this painful situation lasts.

In conclusion, we can then emphasize that maintaining adequate Mental Health is an essential aspect for the excellent individual and collective performance of all people, in their different activities. It remains for the state of each country, in addition to ensuring the biological well-being of the population, to create programs that will maintain the psychological and social well-being of inhabitants, so that the future doesn't come with more misfortunes.

Bibliographic references

1. OPS Argentina. Acerca de la OPS. Buenos Aires. [Internet] Updated in march 18, 2020. Available from: https://www.paho.org/arg/index.php?option=com_content&view=article&id=28:preguntas-frecuentes&Itemid=142
2. Sandoval-Obando E. Recomendaciones de Salud Mental en Contexto de Pandemia COVID-19. Centro de Comunicación de las Ciencias. 2020. Available from: https://www.researchgate.net/publication/340133585_RECOMENDACIONES_SALUD_MENTAL_EN_CONTEXTO_PANDEMIA_COVID19/link/5e7a804492851cdfca2f5b13/download
3. Rodríguez-Cahill C. Cinco retos psicológicos de la crisis del COVID-19. JONNPR. 2020;5(6):nnn-nn. DOI: 10.19230/jonnpr.3662. Available from: <https://revistas.proeditio.com/jonnpr/article/view/3662/PDF3662>
4. Roberts T, Miguel-Esponda G, Krupchanka D, Shidhaye R, Patel V, Rathod S. BMC Psychiatry. 2018. 18:262. doi.org/10.1186/s12888-018-1837-1. Available from: <https://bmcp psychiatry.biomedcentral.com/track/pdf/10.1186/s12888-018-1837-1>
5. OMS. Depresión. [Internet] Updated january 30, 2020. Available from: <https://www.who.int/es/news-room/fact-sheets/detail/depression>
6. WHO. Mental health action plan 2013-2020. Geneva 2013. [Internet]. Available from: https://apps.who.int/iris/bitstream/handle/10665/89966/9789241506021_eng.pdf?sequence=1
7. Qiu J, Shen B, Zhao M, Wang Z, Xie B, Xu Y. A nationwide survey of psychological distress among Chinese people in the COVID-19 epidemic: implications and policy recommendations. General Psychiatry. 2020;33:e100213. doi:10.1136/gpsych-2020-100213. Available from: <https://gpsych.bmj.com/content/gpsych/33/2/e100213.full.pdf>
8. Ozamiz-Etxebarria N, Dosil-Santamaria M, Picaza-Gorrochategui M, Idoiaga-Mondragon N. Niveles de estrés, ansiedad y depresión en la primera fase del brote del COVID-19 en una muestra recogida en el norte de España. Cad. Saúde Pública 2020; 36(4):e00054020. doi: 10.1590/0102-311X00054020. Available from: <https://blog.scielo.org/wp-content/uploads/2020/04/1678-4464-csp-36-04-e00054020.pdf>



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A photograph of three female students in white lab coats working in a laboratory. One student is looking through a microscope, while the others look on. The background shows lab equipment and a whiteboard.

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