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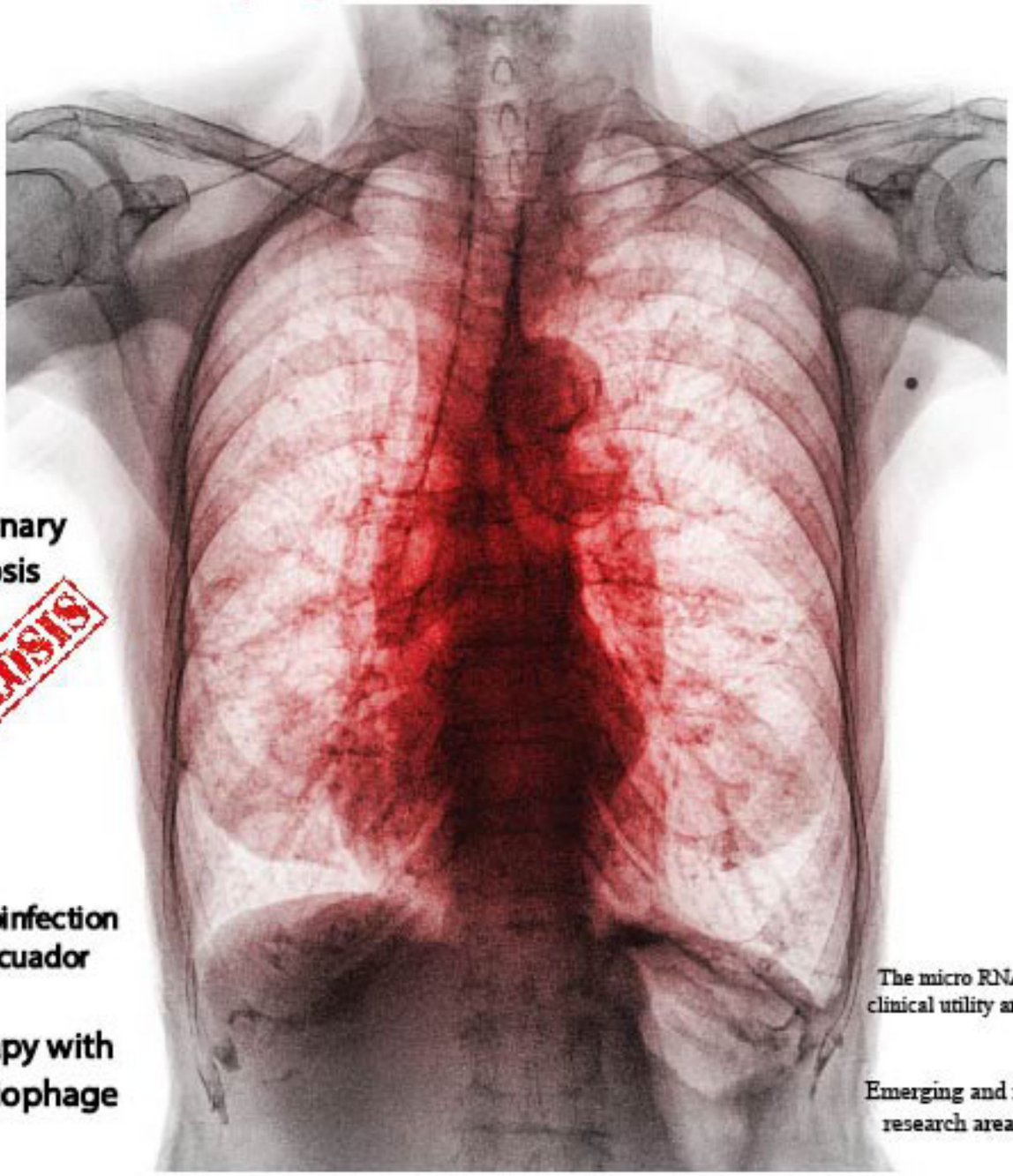
Anthropologists Respond to The Lancet EAT Commission



Extrapulmonary tuberculosis

TUBERCULOSIS

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The micro RNAs in human pathology: clinical utility and translational approach

Emerging and inter/trans-disciplinary research areas related to Chemistry

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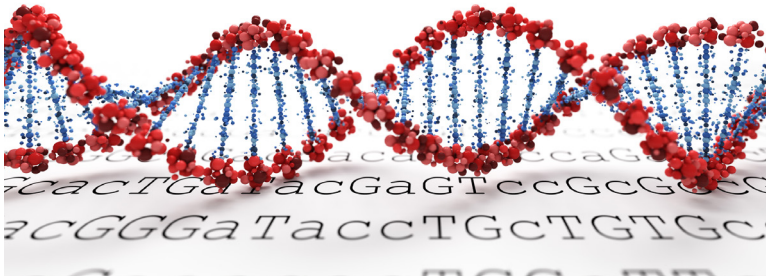

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Bionatura

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ÍNDICE / INDEX

EDITORIAL

Emerging and inter/trans-disciplinary research areas related to Chemistry 1021

Hortensia M. Rodríguez Cabrera

LETTER TO EDITOR / CARTA AL EDITOR

Anthropologists Respond to The Lancet EAT Commission 1023

The Nutrire CoLab

RESEARCH / INVESTIGACIÓN

Overview of Tuberculosis Coinfection with HIV in Ecuador 2010-2015 1025

Ariel Torres, María Cedeño, Rosa Pinargote, Martha Fors

The Impact of Temperature-Dependent Sex Determination on the Population Dynamics of Green Sea Turtles (*Chelonia mydas*) 1029

Candy Herrera, Evelyn Guerra, Victoria Penalver, Andrea Rosas, Yingying Wei, Jack Pringle, Baltazar Espinoza, Baojun Song

Morphological study of different varieties of rice traits influencing nitrogen and water uptake efficiency 1039

Raghad S. Mouhamad

Laboratory scale evaluation of Effective Microorganisms in the control of odor of organic waste from a market in the city of Riobamba, Ecuador 1044

Cristina Calderón-Tapia, Abigail Montero-Calderón, María Núñez-Moreno, Esteban Pazmiño-Arias

Evaluation of Gene Variants in TGFB1, SERPINF1 and MEPE in a Spanish Family Affected by Otosclerosis and Tinnitus 1050

Francisco J. Álvarez, Santiago Álvarez, Jesús Alonso, Pedro García

CASE REPORTS / REPORTE DE CASO

Evaluación de Nimotuzumab para el tratamiento de cáncer de cabeza y cuello: Meta-análisis de ensayos controlados. 1056
Evaluation of Nimotuzumab for the treatment of head and neck cancer: Meta-analysis of controlled trials

Carmen Viada, Aliz M. Vega, Mayte Robaina, Aliuska Frías, Mabel Álvarez, Yanela Santiesteban, Yuliannis Santiesteban, Lázara García, Braulio Mestre, Marta Osorio, Leslie Pérez, Amparo Macias, Tania Crombet, Mayra Ramos

Sindrome de Touraine-Solente-Gole. (Paquidermoperiostosis primaria). Reporte de dos casos 1063
Touraine-Solente-Gole Syndrome. (Primary paquidermoperiostosis). Two case report

Martha Mengana Medina, Adonis Frómeta Guerra, Eduardo Enrique Fuentes Liens, Sandra Amalia Sánchez Figueredo

REVIEW / ARTÍCULO DE REVISIÓN

Extrapulmonary tuberculosis 1066

Valarezo-Sevilla Diego, Restrepo-Rodas Gabriela, Sarzosa-Terán Vanessa

A new area of application and research in bio-processes: Biotechnologies in civil construction 1072

A. Barberán , D. Chávez , A. Cajas , MC Egas , M. Criollo , J. Pineda , JM. País-Chanfrau , LE. Trujillo

NEWS AND VIEWS / NOTICIAS Y OPINIONES

Phage therapy with mycobacteriophage as an alternative against antibiotic resistance produced by Mycobacterium tuberculosis 1078

Pamela Rodríguez H., Angie Changuán C, and Lizbeth X. Quiroz

Los micro ARNs en patología humana: utilidad clínica y enfoque traslacional 1082
The micro RNAs in human pathology: clinical utility and translational approach

Jorge Luis Vélez, Pablo Morocho, Mario Montalvo, Santiago Aguayo, Pablo Andrés Vélez, Gustavo Velarde, Fernando Jara, César Paz y Miño

An overview of synthetic biology 1088

Maria Belén Paredes, Maria Eugenia Sulen

EDITORIAL

Emerging and inter/trans-disciplinary research areas related to Chemistry

Hortensia M. Rodríguez Cabrera

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Undoubtedly, Chemistry is a central science because it serves as a support and contributes to the development of many other disciplines, such as biology, geology, physics, paleontology, etc. Just by observing the environment that surrounds us, we can conclude that the world is composed of an infinite number of inert or living components, and all of them are composed of chemical substances of a broad range of complexity.

On the other hand, Chemistry has a direct relationship with the economic development of a country, due to most industries are based on chemical processes or materials. The impact of chemistry in different activities is high, in construction, ceramics, and steel, in the food, Energy or Petrochemical industry, in textile design, in Cosmetics and fragrances, in Agrochemicals and fertilizers, Pharmaceutical products, Water treatment, in Companies specialized in Environmental Chemistry, Manufacture of plastics and rubbers, quality and safety processes, in the mining industry, and so forth.

In the last decade, the areas very well defined for different first-generation disciplines have expanded blurring their borders and have led to the new generation of subjects where Chemistry occupies a predominant place. Materials Science and Technology, Theoretical and Computational Chemistry; and Medicinal Chemistry and Molecular Pharmacology, are examples of emerging and inter/trans-disciplinary research areas related to Chemistry as the core, in which the existing boundaries between the different areas of knowledge involved, blur interrelated in a multi / inter and transdisciplinary way.

Materials Science and Technology is an inter/transdisciplinary field involving research in a broad and diverse range of topics related to the design, synthesis, characterization, modeling, and use of materials, which have natural or incorporated properties and functions that add value to specific applications. Research projects in Materials Science connect the disciplines of science with design and engineering fields (electronics, electrical, chemical, civil, environmental, mechanical, aerospace, etc.), with areas such as geology, architecture, biology, medicine, and health professions. In this sense, the research activity of Materials Science highlights the need to connect different fields from their scientific and technological knowledge, indicating that education in Materials Science also requires an integrated trans-disciplinary approach[1].

Theoretical and Computational Chemistry involves the development of theories, programs (algorithms), and computational technology, allowing us today the careful study of the electronic structure of systems whose sizes made them prohibitive 40 years ago. The greater difficulty in the theoretical study of Chemistry is that, on all scales, it is a problem of many bodies whose solution and understanding remain an unsolved and fascinating scientific challenge. The results obtained through theoretical and computational chemistry have been used not only in the essential aspects of theoretical chemistry, but also in fields such as pharmacology, molecular biology, and organic or inorganic chemistry, to mention a few. This field has emerged as a useful tool that allows researchers to shorten research times, but also a better understanding of the phenomena involved[2].

Research in Medicinal Chemistry and Molecular Pharma-

cology involves various scientific disciplines and implies collaboration between researchers in the development of new drugs. Researchers in the area focus on the discovery and development of active principles and are concerned about the isolation of medicinal agents found in nature (plants, corals, etc.), as well as the creation of new synthetic compounds with potential biological activity. Medicinal Chemistry refers to the discovery, development, identification, and interpretation of the mode of action of biologically active compounds at the molecular level. It includes bioactive compounds in general. It also studies the identification and synthesis of metabolic products of drugs and related compounds. All this allows the development of products with high added value and well-defined pharmacological characteristics that constitute the basis of the drugs. This type of research is carried out with a multidisciplinary team of scientists, including chemists, biologists, toxicologists, pharmacologists, theoretical chemists, microbiologists, and biopharmaceuticals[3].

Ecuador is a country with great potential in the industry where the role of a chemist should be fundamental. The School of Chemical Sciences and Engineering of Yachay TECH is determined to open in the closer future a Master's Program of Research in Chemical Sciences, seeking to have in Ecuador a fourth level program of research capable of covering, from Chemistry as a center, the three transversal and integrating areas mentioned before, that include Materials Science and Technology, Theoretical and Computational Chemistry; and Medicinal Chemistry and Molecular Pharmacology. For this, we will offer an objective project, with a program of high academic quality focuses on research, but above all, we were seeking to be competitive at national, regional, and global levels.

We must not forget that science is expensive but, a change, doing quality science, leads to a more critical and formative University and, therefore, to the next generation of citizens with more and better resources and professional training, which will be reflected in the future development of the country.

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LETTER TO EDITOR / CARTA AL EDITOR

Anthropologists Respond to The Lancet EAT Commission

The Nutrire CoLab, listed in alphabetical order: Diana Burnett; Megan A. Carney; Lauren Carruth; Sarah Chard; Maggie Dickinson; Alyshia Gálvez; Hanna Garth; Jessica Hardin; Adele Hite; Heather Howard; Lenore Manderson; Emily Mendenhall; Abril Saldaña-Tejeda; Dana Simmons; Natali Valdez; Emily Vasquez; Megan Warin; Emily Yates-Doerr

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The Lancet Commissions are widely known as aspirational pieces, providing the mechanisms for consortia and networks of researchers to organize, collate, interrogate and publish around a range of subjects. Although the Commissions are predominantly led by biomedical scientists and cognate public health professionals, many address social science questions and involve social science expertise. Medical anthropologist David Napier was lead author of the *Lancet* Commission on Culture and Health (2014), for example, and all commissions on global health (https://www.thelancet.com/global-health/commissions) address questions of social structure, everyday life, the social determinants of health, and global inequalities.

Founded in Stockholm in 2013 (and funded by the Stordalen Foundation, Stockholm Resilience Center and the Wellcome Trust), the EAT Foundation published *Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems* in January 2019. Led by Walter Willett (Professor of Epidemiology and Nutrition at Harvard Chan School of Public Health) and co-authored by 36 scientists from 16 countries around the world, the Commission aimed to use scientific targets to address how to feed the world within environmental limits. Concerned about the critical role of the food system in climate change, deforestation, biodiversity loss, water consumption and poor health, the Commission combined medical and environmental science knowledge to deliver a unified framework to quantify a sustainable food system for the future.

The final report brings to its readers -- public health professionals, policymakers across sectors, academics, journalists, the public -- a refreshing conversation about how to improve the health of populations and the sustainability of the planet[1].The authors propose multiple strategies to improve people's health through transformed global food systems. These strategies include defining a "healthy reference diet" for all populations to follow, re-orientating agricultural priorities away from producing high quantities of monocultural agriculture, applying a coordinated global food governance system, and halving food loss and waste.

Yet, the narrow way in which the EAT-Lancet Commission describes strategies to tackle broken food systems and poor population health requires revision. For instance, the Commission frames premature death as primarily a consequence of individual dietary and lifestyle choices, repeating the term "healthy diets" nearly 100 times. Interventions aimed at changing individuals' behaviors, and so addressing such choices, fail to address the more fundamental challenges of structural inequalities. The Commission promotes the language of sustainability, but the repeated and dominant focus on "healthy diets" as a means to achieve this "frame[s] health as an issue of personal responsibility and deflects societal responsibility for restructuring economic, political and food systems."[2]. Moreover, the Commission overlooks the socio-cultural practices of the people who will be eating these healthy diets, and the complexities of nourishment that are at the heart of kinship, social life, and caregiving. We encourage those who read the Commission's report to consider individual and structural factors in conversation, and so to focus on what it means to nourish populations.

By prioritizing and promoting "healthy diets" over other ways of nourishing, the Commission defines the problem as one of individual behavior and education rather than inequality within food systems and across societies. This is a critical misstep which, if enacted, would exacerbate the very problems the strategies seek to address[3–6]. For example, since the beginning of Spanish colonization in Mexico, European foods were presented as morally and nutritionally superior to traditional foods. European foods became crucial to the colonial enterprise, continuously disrupting Indigenous and traditional foodways. These histories, which play a central role in the now far-reaching spread of chronic disease[7,8], demonstrate how ineffective and potentially harmful diet-focused interventions can be[2,4]. In arguing for the urgency of a "universal, healthy reference diet" (447), the Commission may repeat this pattern under the guise of environmental sustainability.

Nutrient supplementation, as suggested by the Commission, represents another commonly misplaced intervention. Public health nutrition fortification campaigns have not effectively reduced global rates of stunting, and randomized trials of nutrient supplements consistently demonstrate that supplemental feeding alone will not make people taller and healthier[9]. Nutrition research increasingly points to recurrent infectious diseases, which are shaped in large part by infrastructures that include water systems and universal health coverage, as a key determinant of severe and acute malnutrition[8].

Diseases associated with malnutrition and obesity often reflect intergenerational histories of poverty and dispossession and resulting stress and trauma[8,10]. Focusing on what foods and how many calories people consume erases environmental and economic exposures that shape diets and health across the life course[8]. This focus also assumes that different kinds of foods, including fresh foods, are readily available and affordable. This is not always the case. Further, access to fresh foods is but one piece of a larger problem[8,10–12].

Shifting attention and the language of policy responses from "healthy diets" to nourishment, which stems from the Latin word *nutrire* -- to feed and to cherish -- encompasses both food and care. Nourishment better captures ways to think empirically about how food environments are shaped, constrained, and confined. It draws attention to cultural factors and how these vary in different local contexts[5]. Concern for nourishment also insists upon holding corporations that shape the global food system accountable by addressing the political and economic foundations of food environments[2,5,10,12].

Ultimately individuals have little control over why they eat what they do[6,13]. The concept of nourishment directs public attention towards sustainability in food, soil, air, water, bodies, and communities. This approach is in direct opposition to consumption-oriented economic development. This shift deprioritizes interventions and innovations that target individual behavioral change, instead pushing to change governmental and corporate policy to ensure people have the support and resources they need to nourish their loved ones.

Shifting attention and the language of policy responses from “healthy diets” to nourishment, which stems from the Latin word nutrire -- to feed and to cherish -- encompasses both food and care. (Photo by Gastón Saldaña).

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RESEARCH / INVESTIGACIÓN

Overview of Tuberculosis Coinfection with HIV in Ecuador 2010-2015

Ariel Torres[1], María Cedeño[2], Rosa Pinargote[3], Martha Fors[4]

DOI. 10.21931/RB/2020.05.01.3

Abstract: To describe the behavior of Tuberculosis/Human Immunodeficiency Virus co-infection in a cohort of people affected by sensitive Tuberculosis in Ecuador from 01 January 2010 to 31 December 2015. Design: Secondary analysis and descriptive study of patients with TB/HIV in the study period. Results: The percentage of coinfected persons reached 11% in the whole period of study, with a range from 8.4% to 12.7%. Male sex shows the highest incidence rate, representing 76.7% at the rate of 1 man for every 3.3 women. The population with the highest incidence of patients is economically active; the age group of 25-34 years reaches 40.1%. The coastal zone of the country reports more than 75% of the coinfected patients. Conclusion: Increased HIV/AIDS screening should be increased for Tuberculosis, with particular emphasis on male sex and enhance the actions in the coastal provinces.

Key words: Tuberculosis, Human Immunodeficiency Virus, co-infection.

Introduction

Tuberculosis is one of the ten leading causes of mortality in the world. In 2015, 10.4 million people became ill with tuberculosis, and 1.8 million died from tuberculosis (including 0.4 million people with HIV). More than 95% of tuberculosis deaths occur in low- and middle-income countries. It is estimated that one million children became ill of tuberculosis in 2015 and that 170,000 children died due to this cause (excluding children with HIV). Tuberculosis is one of the leading causes of death in HIV-positive people: by 2015, 35% of HIV-related deaths were due to tuberculosis[1]. Global tuberculosis control faces significant challenges today. In general, intensive efforts are still needed to make quality care accessible to all, regardless of gender, age, type of illness, social environment and ability to pay. Co-infection with Mycobacterium tuberculosis and HIV (TB/HIV), especially in Africa, and multidrug-resistant (MR) tuberculosis, and extensively resistant in all regions, requires more complex and demanding control activity. Various risk groups require special attention. These challenges must be addressed by national tuberculosis programs with care tailored to each need. HIV is the main reason that tuberculosis control goals are not achieved in areas where HIV infection is prevalent. Tuberculosis, at the same time, is the most important cause of mortality among people living with HIV/AIDS[2]. In 2015, 268,500 people with TB were estimated in the region of the Americas, with an incidence rate of 27.1 x 100,000, but 218,700 people with TB were notified, an incidence rate of 22.1 x 100,000, with a notification gap of 49,774 patients. Of the reported cases, 21,885 cases corresponded to TB/HIV Coinfection (9.49%). 81.8% of TB patients were aware of their HIV status, 12% of those who knew their HIV status were infected with HIV (TB/HIV). A total of 31,700 cases of TB associated with HIV were estimated; 6,000 people with HIV developed TB, of which 58.4% of patients with TB/HIV received Antiretroviral therapy (ART). The estimated number of deaths from TB was 18,500, of which 5,900 people died from TB/HIV[3].

In 2015, the WHO estimate in Ecuador was 8,400 new cases of TB (51.6/100 a thousand inhabitants), including those with TB/HIV Coinfection, however, the National Health System (NHS) diagnosed and reported 5,215 cases (32.03/100 a thousand inhabitants), and this represents 62.08% of the estimated, evidencing a gap between notification and estimation. In TB/HIV Coinfection, 545 cases were reported, representing 10.45% of TB cases, information obtained from the official database of the National Strategy for Tuberculosis Control and Prevention in Ecuador[4]. In 2014, the mortality rate reported by the National Institute of Statistics and Censuses (INEC) was 2.59 per ten thousand inhabitants.

Materials and methods

Study population and methods

A secondary analysis of the database of patients with sensitive tuberculosis and HIVinfected was performed. We included six years (2010-2015) in this analysis, and the total of patients reported during these years. The source of tables and figures was the Information System of the National Strategy for the Prevention and Control of Tuberculosis, collected by the Ministry of Public Health of Ecuador.

Ethics approval and consent to participate

The study did not need to be approved by the clinical research ethics committee since we worked with a de-identified dataset. We followed the STROBE checklist to report this work.

Results

The frequency of cases of sensitive TB, reflected in Figure 1, shows a progressive increment up to the year 2012, with a declination of the number of subjects with the disease in the last years. Similar behavior is evidenced in coinfected patients who maintain a rising curve until 2013, and from this year, a diminution of the number of cases with this condition.

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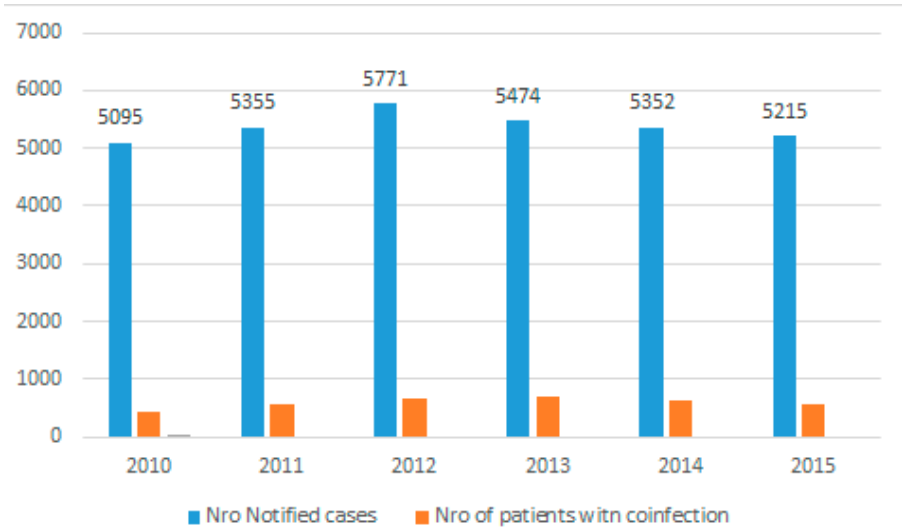

Figure 1. Incidence of cases of Tuberculosis and Co-infected TB/VIH, Ecuador 2010-2015.

Figure 2 shows the number and percentage of coinfected patients with sensitive TB. Ranges go between 8.4 and 12.7, being 2013 the year with the highest rate of coinfected cases. In the 6 years of study, coinfected represented 11% of the sensitive TB cohort.

During the six years (Figure 3), male sex was the most prevalent, with a percentage of 76.7 and at a rate of 3.3 men per woman. Men in economically active age represent the highest percentage of affected persons reaching more than 80%, the age group between 25-34 reported the highest rate (41%). With a frequency of 152 cases, the age group of 0-14 years said 4.3%.

Ecuador has three main geographical areas: the mountainous area (sierra), the coastal zone and the amazonian region. Figure 4 shows the frequency of coinfected subjects by geographic area, the coastal zone with only four provinces of the country reports more than 75% of coinfection TB/ HIV.

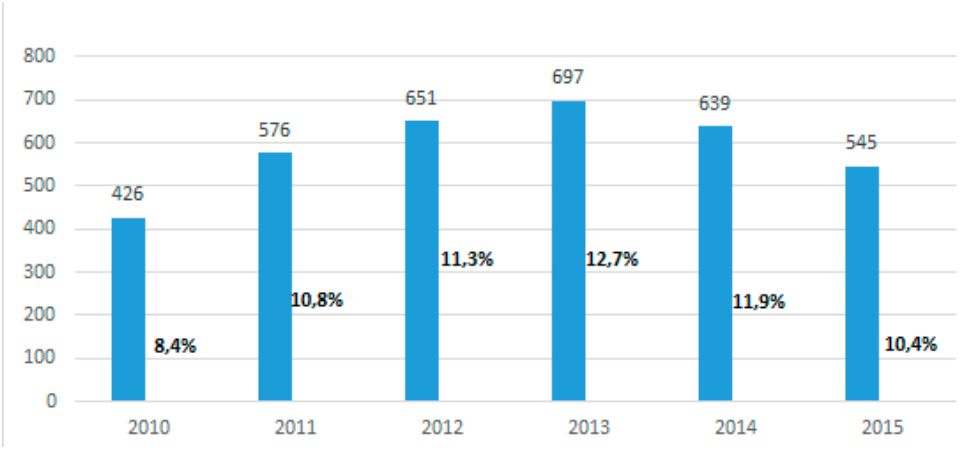

Figure 2. Frequency and percentage of cases with Coinfection in sensitive TB cohorts, Ecuador 2010-2015.

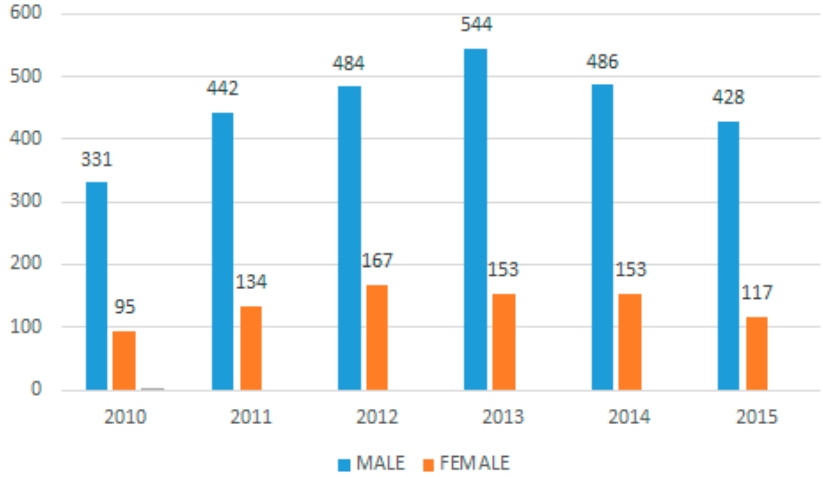

Figure 3. TB/VIH infection by sex, Ecuador 2010-2015.

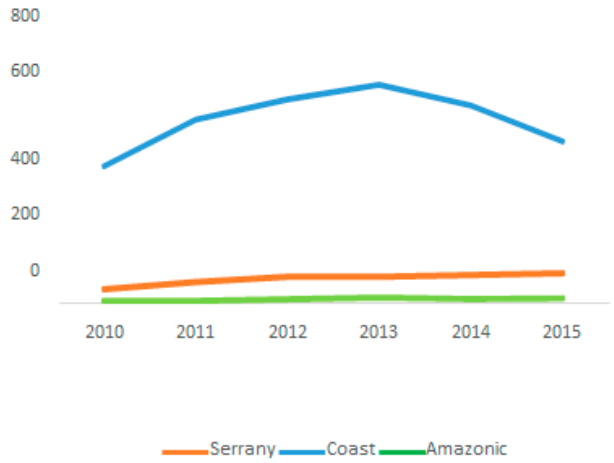

Figure 4. Number of cases according to geographical areas.

Discussion

The contribution of the Region of the Americas to the global burden of tuberculosis is only 4% of all forms of the disease. It is the second-largest region in the world and with the highest estimated incidence of TB/HIV coinfection. Estimates for 2009 showed around 24,000 cases of TB/HIV. This occurs in a region where significant progress has been made in the prevention and control of tuberculosis, as well as to access to antiretroviral treatment, and where in the same year was found that only 6% of the estimated cases of TB/HIV were reported. Undoubtedly, the lack of familiarity of clinicians with the manifestations of TB in the person with HIV, the diagnostic difficulties of both pulmonary and extrapulmonary forms, as well as the complexity of concomitant treatment contributes to unacceptable morbidity and mortality attributable to coinfection TB/HIV. This requires coordinated action for diagnosis, care and treatment. Collaboration between tuberculosis and HIV programs for the integration of care and treatment of both infections is a strategy that improves the diagnosis, treatment, and prognosis of patients with both diseases[5].

Tuberculosis is the most common co-infection in HIV and the risk of presenting it in HIV-negative patients are 5-10%; however, in those HIV-positive, the risk is 50%. One-third of the increase in people with tuberculosis on the planet is attributed to the spread of HIV. One in three people who die from AIDS has TB, and 8-10% of all HIV-related deaths are TB-related. In some localities, tuberculosis is the cause of death for up to 50% of people with AIDS[6].

In Ecuador, the frequency of cases of sensitive TB, as shown in Figure 1, does not show a progressive linear trend; nevertheless, a gradual increase is observed before 2012. Similar behavior is evidenced in coinfected patients who maintain an upward curve until 2013. In 2011, according to data from the World Health Organization (WHO), 8.7 million new cases of TB occurred, of which 13% were coinfected with HIV[7]. Ecuador shows coinfection rates very similar to those of the global trend. Countries in the region such as Peru had a 4.4% of coinfected cases with TB and HIV in 2014, and in 2006 a co-infection of less than 2% was reported, with screening coverage in patients who initiated primary anti-tuberculosis treatment tripled in the last 3 years, from a coverage of 20% for 2012 to a coverage of 73%[8]. According to data from the National Tuberculosis Control and Prevention Strategy in Ecuador, there is a progressive increase in HIV/AIDS screening for tuberculosis patients, starting in 2010 with 66% and by the end of 2015 reaching a screening rate above 90%. The ratio between male and female[9] ranged from 1.7 in 2005 to 2.3 in 2009 around the world. Despite the evident predominance of males in coinfected people, WHO/PAHO points out that TB has been a predominantly male disease.

Nowadays, due to the high rates of HIV infection in women, TB is more frequent in women than in men in many countries with high HIV prevalence, so the prevention, diagnosis, and appropriate treatment of TB in women is a priority. Because of the greater intimacy of contact, TB can spread more rapidly in families. Because women are often the caretakers of children, the sick, and the elderly, the fact that they can become ill with TB can have a double impact: on themselves and the most vulnerable members of the family because it reduces their ability to care for them[10].

The average age of coinfected in a study in Colombia was 36.7, the analysis in the six years of research in Ecuador, shows that the affected group aged 25-34 reported the highest percentage (41%). Significantly, the population in the economically active age represents the highest percentage of affected people, reaching more than 80%. The behavior of coinfection by geographical areas shows that the coast with only 4 provinces report more than 75% of TB/HIV coinfected subjects led by the region of Guayas with 60.8%. A similar trend has this province in terms of rates of incidence and prevalence of cases of Sexual Transmitted Infections (STI) and HIV/AIDS according to data and reports issued by the National STI-HIV/AIDS Prevention and Control Strategy of Ecuador.

Conclusions

Although the country has shown significant progress in reducing tuberculosis mortality by more than 50%, with a decreasing progressive curve, starting in 2006 with a rate of 5.77 and at the end of 2012, the rate reached 2.76; Tuberculosis continues to be one of the leading causes of mortality in patients with HIV/AIDS. It is important to note that another significant achievement of the country is to ensure that 100% of coinfected people have started antiretroviral therapy, a factor that undoubtedly contributed to the reduction of the mortality rate.

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Consent for publication

Not applicable.

Data availability

Data will be available on request at the National Strategy for Control and Prevention of Tuberculosis in the National Directorate of Prevention and Control Strategies of the Ministry of Public Health of Ecuador.

Competing interest

The authors declare that there are no competing interests.

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Authors contributions

AT participated in the design of the study, collected data, interpreted the data, as well as drafted the manuscript. MC participated in the design of the study, collected data, and interpreted data. RP participated in the design of the study and collection of data. AT, MC, RP, MF made substantial contributions to conception and design and participated in the data collection. All authors reviewed and approved the final manuscript

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RESEARCH / INVESTIGACIÓN

The Impact of Temperature-Dependent Sex Determination on the Population Dynamics of Green Sea Turtles (Chelonia mydas)

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Abstract: The sex of the turtles is determined by the incubation temperature of the eggs during the mid-trimester of development. In green sea turtles (*Chelonia mydas*), recent studies show that sex ratios are changing, producing a female-biased sex ratio within the population. We developed a novel continuous model to analyze the dynamics of the green sea turtle population long-term. We determine the safe operating space for the proportion of eggs that become male at which the population of green sea turtle can exist without going to extinction. When the proportion of male eggs leaves this range the overall turtles' population collapses. Additionally, we examined how temperature changes affect the sex ratios of the green sea turtle population.

Key words: Green sea turtle, Sex ratio, Temperature-dependent sex determination, Population dynamics.

Introduction

In this paper, we examine the sex ratio of sea turtles in relation to population collapse. We are particularly concerned with the population of green sea turtles (*Chelonia mydas*), in which temperature-dependent sex determination has been observed. Temperature-dependent sex determination (TSD) is a process where the temperature of an embryo's environment results in the production of sex hormones that dictate the embryo's sex development. This is an environmental sex determination system (ESD), meaning that the sex is determined depending on factors derived from the physical and biotic environments like temperature[1,2]. Every reptile species that exhibit TSD has a thermosensitive period during which the embryo sex is developed. For turtles, this period has been observed to take place during the mid-trimester of the embryo incubation period[3].

Within the realm of TSD, there are three different possible patterns that a species uses for sex determination: FM-pattern, MF-pattern, and FMF-pattern. In the FM-pattern, female eggs are developed in low hatching temperatures while male eggs occur in high hatching temperatures; the MF-pattern is the inverse of the FM-pattern. The FMF-pattern dictates that female eggs develop in high and low temperatures while a medium range of temperature results in male eggs. Turtle embryos follow the MF-pattern, so at lower temperatures, the result is a mostly male hatchling population, and at higher temperatures, a primarily female hatchling population exists[4]. The temperature at which the sex differentiates is around 29.4◦C[2,5]. When the mean temperature of the nest during the thermosensitive period is at 29.4◦C, known as pivotal temperature, then we see an even distribution of male and female hatchlings occur. Whereas, when the mean temperature is above the vital temperature, then the hatchling sex population will be mostly female, and below it, will result in a primarily male population[2,5,6]. In recent years a disproportionate ratio of female to male turtle eggs has been observed in several different studies and has also been predicted to possibly lead to the extinction of sea turtles in the future[5,7,8].

Since female sea turtles lay their eggs in chambers that they dig into the sand of their nesting beaches, it is reasonable to look into the factors that could be directly affecting these sand temperatures to understand why there is an imbalance in the ratio of female to male turtles. To determine these factors, researchers have proposed multiple hypotheses. One such hypothesis credits climate change with potentially leading to rising sand temperatures. This comes from the fact that global air temperatures are projected to increase, and there is a strong relationship between air temperatures and sand temperatures[9–11]. Another possible explanation for potential increases in temperature is an accumulation of microplastic on and within the beach sediment[12].

This problem naturally lends itself to investigation via a sex-structured population model. An overview of the history of sex-structured models was described by Iannelli *et al.*[13]. The discussion of sex-structured models first arose after it was realized that the one-sex stable population theory was insufficient in answering questions about the existence and interrelations of the sexes. It was found that trying to apply a one-sex model for both genders at the same time resulted in contradictory results. Kuczynski[14] first noted this inconsistency. These inconsistencies between male and female reproduction rates were further noted and explored by Karmel[15], making him the first to observe many of the ideas of two-sex population modeling. Whilst Karmel[15] introduced many of the two-sex population ideas, Kendall[16] brought the first significant dynamic model. The work done by Kendall[16] on two-sex populations more accurately accounted for the dynamics of observed systems leading to further research-based on his work[13]. Although Kendall's work has inspired several further studies into sex-structured models, the majority of published population models are unstructured concerning sex[17]. Some of the papers that use sex-structured models are Lee *et al.*[18] and Mignatti *et al.*[19]. In both articles, the sex-structure modeling

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framework was utilized because it was noticed that the sexual dimorphism was critical in understanding the dynamics of the population as a whole.

In this paper, we developed a system of ordinary differential equations (ODE's) model for general green sea turtles focusing on the impacts of the TSD on the overall population dynamics to determine a male to female sex ratio that leads to extinction.

Methods

2.1 Model Development

The life cycle of green sea turtles starts on land when they emerge from their nests as hatchlings and immediately travel to the sea. Upon entering the ocean, the hatchlings are relatively unseen until they reappear as juveniles in the open sea[20]. This period between the hatchling and juvenile stage is composed of several years and is the life-stage that remains relatively unknown[20]. Thus, scientists have coined the term "lost years" to describe this stage of life[20,21]. Since these two first stages of the life cycle of the turtles do not carry relevant information for the development of the model, a simplified life cycle can be seen in Figure (1), which shows the "egg" stage as each individual that has not yet reached sexual maturity and the adult stage as only the sexually mature adults.

For green sea turtles, the mean age of sexual maturity is estimated to be within the range of 40-60 years[22–24]. When sea turtles reach sexual maturity, females and males return to their natal beaches to mate and nest. Male sea turtles usually breed every year or every two years, whereas female sea turtles generally breed every 2 to 5 years[25,26]. During mating season, sea turtles have multiple mates and lay several clutches of eggs at approximately 2-week intervals[27]. This scheme of the life cycle focuses mainly on the relationship between adults during the mating period. This interaction eventually will result in the production of eggs that can later develop into either female or male mature sea turtles. Also, it shows how the death of the adults and the movement out of the reproductive stage decreases the population of adult sea turtles, while the end of eggs and juveniles reduces the people of the "eggs" class.

On the other hand, the successful mating of adults will increase the number of eggs deposited every season. Each clutch can be divided into female and male eggs depending on the incubation temperatures[7]. If eggs reach maturity, this will increase the number of adults in the population.

To describe the long-term behavior of the green sea turtle population, we study the dynamics of the community with a continuous model. The main objective is to figure out the sex ratio that would ultimately lead the overall population of green sea turtles to extinction. We chose the continuous model to be able to witness the change within the turtle's entire life cycle (60-70 years)[24] and for the whole population over time. Modeling the distinctive aspects of the reproductive biology and nesting behavior of green sea turtles poses a significant challenge. The mating process is a complex interaction between male and female sea turtles that involves a variety of factors that could strongly affect the birth rate of the population. To clearly understand the principles involved in this interaction, we consider the mating process as a functional response, similar to continuous-time predator-prey models[28].

Such as in predator-prey models, we can conveniently classify most of our variables involved in the mating process: (1) density of female population, (2) density of male population, (3) behavioral response of females during mating process, (4) characteristics of the males, e.g., searching efficiency[29]. To represent the successful number of mating interactions between males and females per unit time, which will eventually result in the production of eggs, we used the Holling type II

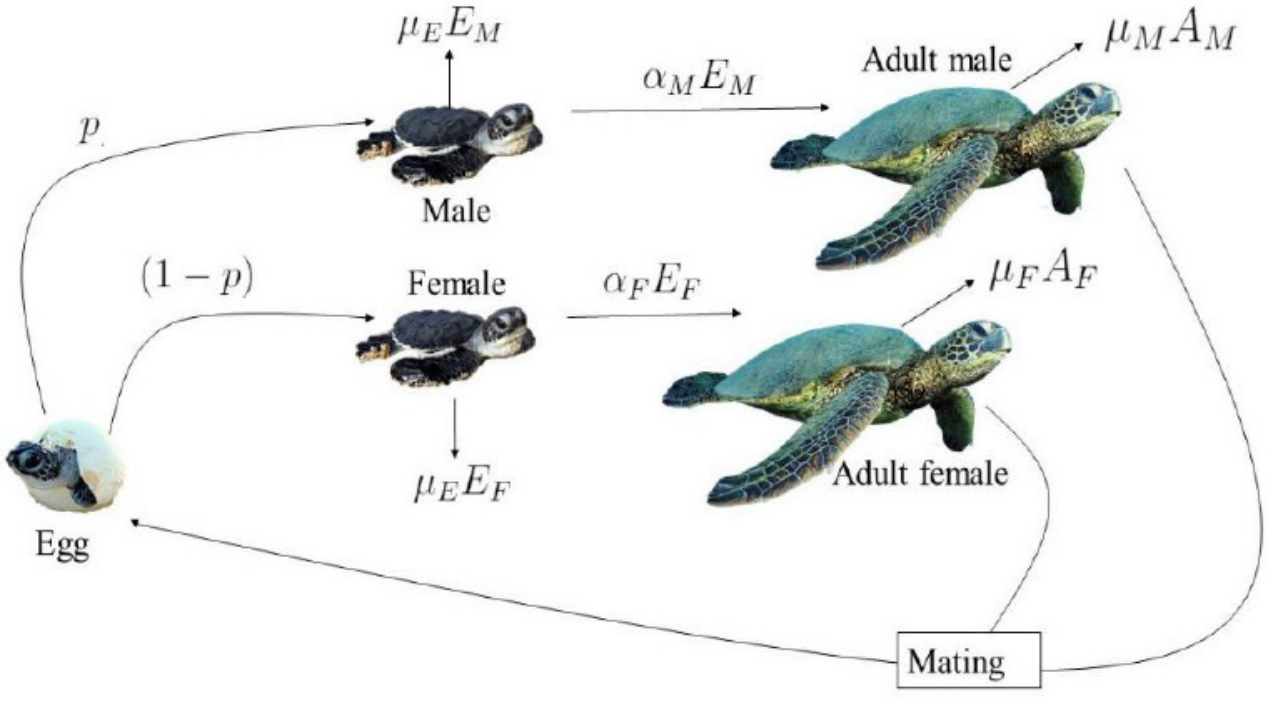

Figure 1. Compartmental diagram of the simplified life cycle of green marine turtle (*Chelonia mydas*). The eggs with undefined sex will develop either in male or female, depending on the incubation temperature. Once hatchlings hatch, they will mature and will reach sexual maturity. After mating, new offspring are produced.

functional response[29,30]. In predator-prey models, researchers suggest that ratio-dependence is suitable when the predator must seek its prey and consequently compete for food[31]. In modeling the process of mating interactions between green sea turtles, we have to consider that male sea turtles have to search for females, and that male turtles will compete with one another to mate with a female[32]. Moreover, we have to consider some aspects, such as coupling time, associated with each female being found by a male and the fact that not every male will interact with every female. Thus, it made more sense to use a ratio-dependent system to produce more accurate results[31].

In our model, we focus on the proportion of eggs allocated to males and females in the population, which is influenced by the temperature they are exposed to during the incubation period. Since the juvenile stage does not provide relevant information to the main objective of this investigation, the population is divided into two groups: eggs and adults, where "eggs" refers to the pre-reproductive life stages of the turtles. We denote the egg population and adult population for males and females as $A_M(t)$, $A_F(t)$, and $E_M(t)$ and $E_F(t)$ at time t.

of male eggs in (1c) and female eggs in (1d) produced per successful interaction per unit of time is represented as the product of the successful number of interactions between males and females per unit of time $(bA_FA_M)/(A_F+\alpha A_M)$, the maximum average number of eggs found in a clutch (r), the proportion of male (p) or female ($1-p$) eggs and the saturation constraint $(1-((A_M+A_F)/K))$. The expression $(bA_FA_M)/(A_F+\alpha A_M)$, is derived from the product of a ratio-dependence Holling type II functional response and the adult male population. The saturation constraint shows that when the adult population approaches the carrying capacity (K) , then the egg per-capita approaches zero.

The parameters α_M and α_F are maturity rates that ultimately show us the number of male and female hatchlings that survive onto the reproductive stage, while μ_M and μ_F tell us the leaving rates for reproductive males and females. These leaving rates for adult female and male turtles in our model symbolize either one of two things, the death rate of the sexually mature turtles, or the rate at which they move onto the post-reproductive stage. The parameter μ_E defines the

$$\frac{dA_M}{dt} = \alpha_M E_M - \mu_M A_M, \tag{1a}$$

$$\frac{dA_F}{dt} = \alpha_F E_F - \mu_F A_F, \tag{1b}$$

$$\frac{dE_M}{dt} = -\alpha_M E_M + pr\left(1 - \frac{A_M+A_F}{K}\right)\left(\frac{bA_FA_M}{A_F+\alpha A_M}\right) - \mu_E E_M, \tag{1c}$$

$$\frac{dE_F}{dt} = -\alpha_F E_F + (1-p)r\left(1 - \frac{A_M+A_F}{K}\right)\left(\frac{bA_FA_M}{A_F+\alpha A_M}\right) - \mu_E E_F. \tag{1d}$$

Formula 1.

The initial values, A_M^0, A_F^0, E_M^0 , E_F^0 are all $\in [0, K]$, and all the parameters are $\in \mathbb{R}^+$.

2.2 Model Description

The first two differential equations (1a) and (1b) in our system show the total number of eggs that will reach adulthood after passing the hatchling and juvenile stage, which is denoted by $\alpha_M E_M$ for males and $\alpha_F E_F$ for females. They also show the total number of adults that leave the sexual reproductive stage, which includes those who are dying and those who are reaching the post-reproductive stage, denoted by $\mu_M A_M$ for males and $\mu_F A_F$ for females. The last two differential equations (1c) and (1d) show the flow of turtles leaving the "egg" stage and entering to the sexual maturity stage. The total number death rate of the pre-reproductive sea turtles (hatchlings and juveniles). b represents the copulation rate and a is the half saturation constant. The combined duration of courtship and copulation is considered to be handling time (t_h), and the rate at which a male finds a female is considered the searching efficiency (c). We denote the copulation rate as $\beta=1/t_h$ and the half saturation constant as $\alpha=1/ct_h$.The carrying capacity, K, tells us the maximum population size of green sea turtles that the environment can sustain indefinitely. The description and units for our parameters are described in Table (1).

Parameter	Description	Estimates	Citations
α_M	Maturity rate of eggs that become adult females	0.022 ± 0.004 yr^{-1}	22,33
α_F	Maturity rate of eggs that become adult females	0.029 ± 0.05 yr^{-1}	33,34
μ_M	Leaving rate for adult males	0.05 ± 0.09 yr^{-1}	24
μ_F	Leaving rate for adult females	0.04 ± 0.07 yr^{-1}	24
μ_E	Death rate for eggs	0.36 ± 0.33 yr^{-1}	33,35,36
p	Proportion of eggs that become male	N/A	
$1-p$	Proportion of eggs that become female	N/A	
r	Average number of eggs per successful interaction	117 ± 38	37,38
b	Copulation rate	180 ± 20 yr^{-1}	39
a	Half saturation constant	N/A	
K	Carrying capacity of adults	N/A	

Table 1. Parameters description and values.

Analysis

3.1 Equilibrium

By continuously extending the vector field of system (1) to the origin, then the point (0,0,0,0) is a biologically meaningful equilibrium, which contributes to explain the dynamics of the model. Since the vector field is not differentiable at the origin, one cannot determine its stability with a linearization approach. However, we used a different approach to show that (0,0,0,0) is globally attractive when $rb<\mu=min\{\mu_M,\mu_F,\mu_E\}$ (See subsection 3.3)

3.2 Existence

In expression (2), the parameter r is the average number of eggs, while the product of our interaction rate (b) and the average reproductive lifespan of males gives us the maximum number of copulations in a male's lifetime. When we multiply this by the proportion of male eggs that survive until adulthood, we get J^{-1}, which gives the ratio of male death rate to maximum theoretical egg production. In expression (3), we have the product of the maximum number of eggs, searching efficiency, the average reproductive lifespan of female turtles, and the number of female eggs that reach adulthood. Thus, D^{-1} is the ratio of female death rate to maximum theoretical egg production.

The system (1) also has a unique positive equilibrium,

$$I^*\left(A_M^*, \frac{H}{G}A_M^*, \frac{\mu_M}{\alpha_M}A_M^*, \frac{H\mu_F}{G\alpha_F}A_M^*\right),$$

with

$$A_M^* = \frac{(K-Q)G}{H+G}.$$

For simplification we let $H = \frac{(\mu_M\mu_E+\mu_M\alpha_M)(p-1)}{p\alpha_M}$, $G = \frac{\mu_F\mu_E+\mu_F\alpha_M}{\alpha_F}$, and $Q = \frac{(\mu_E+\alpha_M)\mu_M(H+a)K}{\alpha_M prbH}$.

Specific existence conditions and proof of existence conditions for both equilibrium points will be discussed in the next subsection.

The positive equilibrium I^* biologically exists if $K > Q$ which is equivalent to the demographic basic reproductive number $\left(R_0^d\right)$ is greater than one.

$$R_0^d = \frac{rbp\left(\frac{\alpha_M}{\alpha_M+\mu_E}\right)\frac{1}{\mu_M}(1-p)\left(\frac{\alpha_F}{\alpha_F+\mu_E}\right)\frac{1}{\mu_F}}{ap\left(\frac{\alpha_M}{\alpha_M+\mu_E}\right)\frac{1}{\mu_M}+(1-p)\left(\frac{\alpha_F}{\alpha_F+\mu_E}\right)\frac{1}{\mu_F}} > 1$$

The same condition can be analyzed in terms of p, given as a result,

$$p^2 + (D - J - 1)p + J < 0,$$

where $J = \frac{(\mu_E+\alpha_M)\mu_M}{\alpha_M br}$ and $D = \frac{(\mu_E+\alpha_F)a\mu_F}{\alpha_F br}$. Taking the inverse of J and D we can analyze the biological meaning of those expressions.

$$J^{-1} = br\left(\frac{1}{\mu_M}\right)\left(\frac{\alpha_M}{\alpha_M+\mu_E}\right) \quad (2)$$

$$D^{-1} = \frac{b}{a}r\left(\frac{1}{\mu_F}\right)\left(\frac{\alpha_F}{\alpha_F+\mu_E}\right) \quad (3)$$

Solving quadratic expression $p^2 + (D - J - 1)p + J = 0$, we get

$$p_1 = \frac{J-D+1-\sqrt{\Delta}}{2}, \qquad p_2 = \frac{J-D+1+\sqrt{\Delta}}{2}.$$

with $\Delta = (D - J - 1)^2 - 4J$. The existence condition for the positive equilibrium point I^* is

$$\Delta > 0, \text{ and } p_1 < p < p_2. \tag{4}$$

When this condition is met, all dependent variables are positive, and the equilibrium point exists. However, when that existence condition is not met, all dependent variables must be less than or equal to zero. We have already established that all of the dependent variables are non-negative, suggesting that they must be zero. Therefore, the existence condition for the point (0,0,0,0) can be derived by negating the existence condition for the positive equilibrium point I^*. When the condition for (0,0,0,0) is met, the population decays to zero toward extinction.

Total turtle population is defined as

$$N = E_M + A_M + E_F + A_F$$

The changes in the population through time then will be

$$\frac{dN}{dt} = \frac{dE_M}{dt} + \frac{dA_M}{dt} + \frac{dE_F}{dt} + \frac{dA_M}{dt}$$

$$\frac{dN}{dt} = rbA_M\left(1 - \frac{A_M + A_F}{K}\right)\left(\frac{A_F}{A_F + aA_M}\right) - \mu_E E_M - \mu_M A_M - \mu_E E_F - \mu_F A_F$$

Based on our assumptions, we know that the total amount of adult females and males turtles is less than the environmental capacity of turtles, that is $A_M + A_F < K$.

Letting $\mu = min\{\mu_E, \mu_M, \mu_F\}$, then we obtain,

$$\frac{dN}{dt} \leq rbA_M - \mu(E_M + A_M + E_F + A_F) \tag{5}$$

$$\frac{dN}{dt} \leq rbA_M - \mu N$$

$$\frac{dN}{dt} \leq rbN - \mu N$$

$$\frac{dN}{dt} \leq N(rb - \mu)$$

$$\int_0^t \frac{dN}{N} \leq \int_0^\tau (rb - \mu)dt$$

3.3 Global stability condition for extinction

The total population of green sea turtles at any time t is given by the following expression

$$N(t) \leq N_0 e^{(rb-\mu)t} \tag{6}$$

If $rb-\mu<0$, then $N(t)$ approaches 0 as t approaches ∞. This shows that when the condition $rb-\mu<0$ is met, the zero-equilibrium point is globally asymptotically attractive. Therefore, the turtle population goes to extinction.

In the global stability condition of (0,0,0,0), r is the average number of eggs per successful interaction, b is the interaction rate, and μ is the minimum mortality between egg mortality μ_E, adult male mortality μ_M, and adult female mortality μ_F. Therefore, rb is the birth rate of eggs. So $rb-\mu<0$ means the birth rate is less than the minimum mortality, which leads to extinction of the turtle population.

Results

The following simulations were developed by using parameter estimations as seen in Table (1). All parameter values in Table (1) are kept constant except for values of p.

For system (1), the parameter p was defined as the proportion of eggs that become male as determined by incubation temperature (T). To examine p as a function of incubation temperature $p(T)$, we use the following function derived in a previously published paper by Girondot[40]:

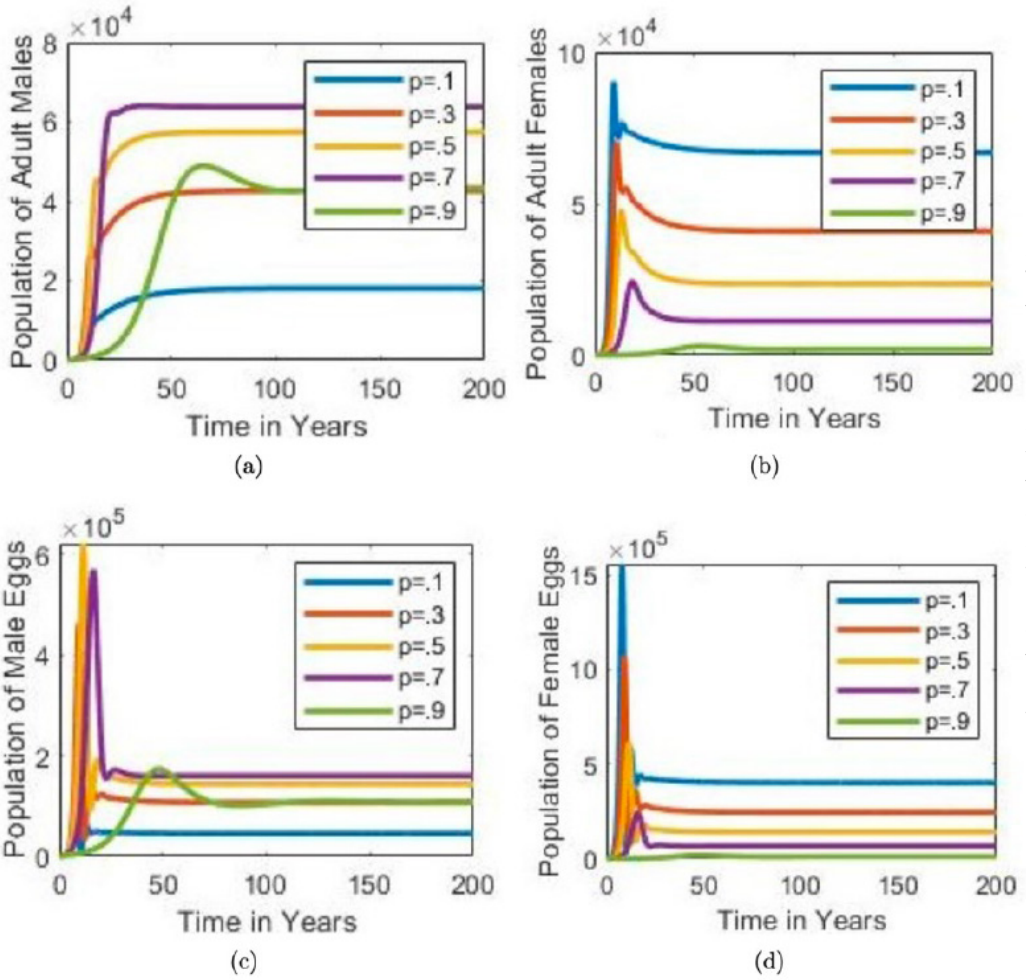

Figure 2. The population of individuals in the adult and egg stages over time. Variations in the population are due to changes in the proportion of male eggs (*p*). When the equilibrium point *(I*)* is locally asymptotically stable; for any deviation of p , the population numbers change until they reach a steady-state. Because we are examining values of that are within our existence condition for the proportion of males, our population remains viable.

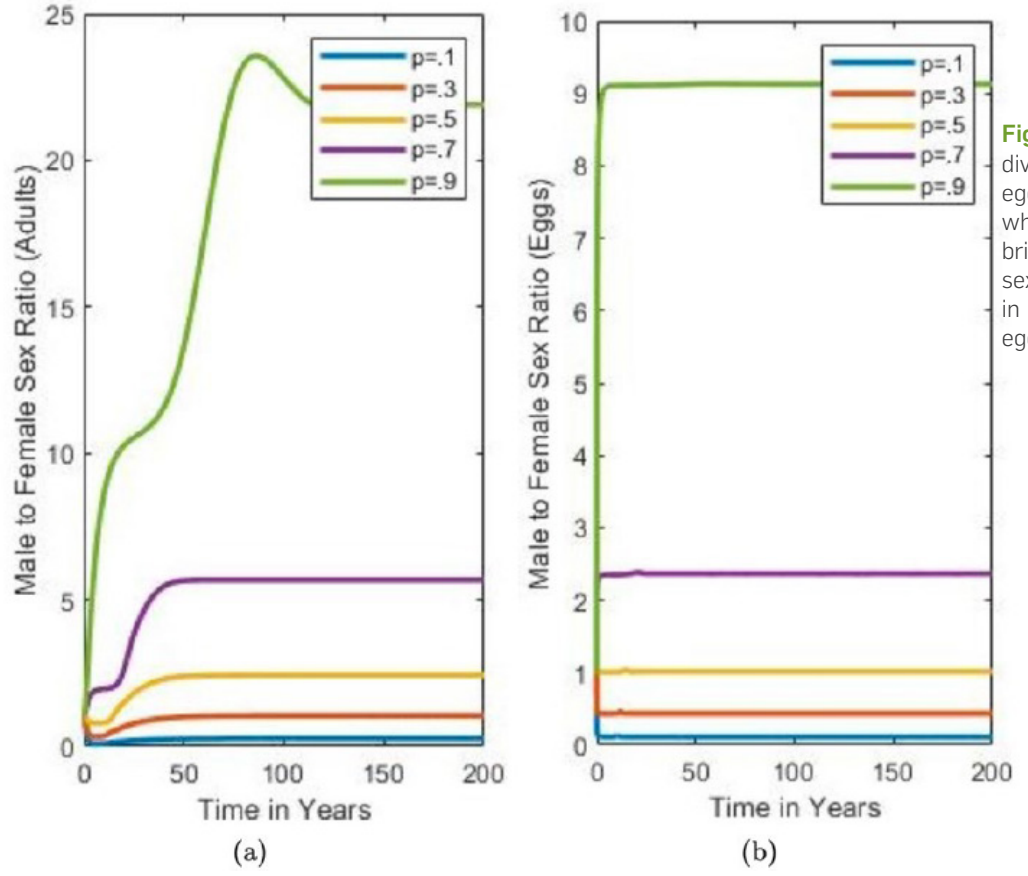

Figure 3. Sex ratios of individuals in adult (3(a)) and egg (3(b)) stages overtime when the interior equilibrium exists. Variation in sex ratios is due to changes in the proportion of male eggs (*p*).

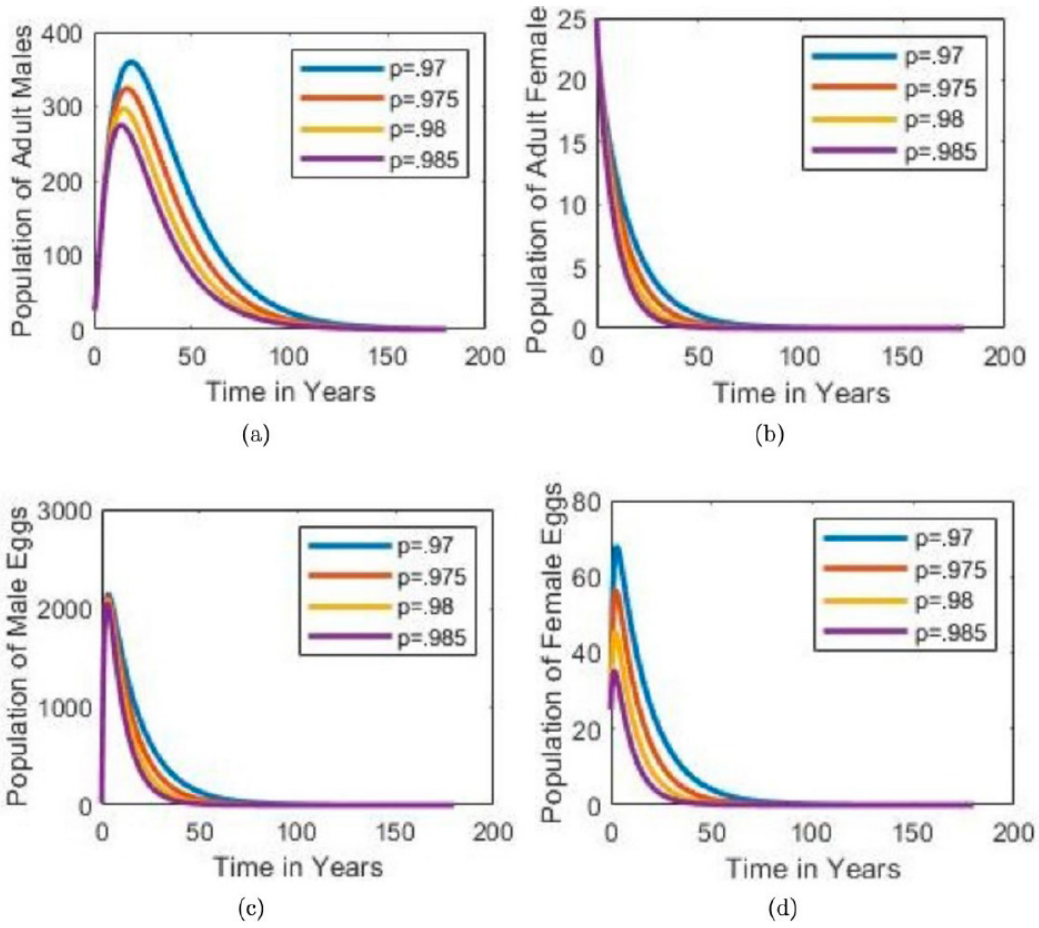

Figure 4. The population of individuals over time for variations of outside of the existence condition for p. When the existence condition is not satisfied, the interior equilibrium (I^*) does not exist and system (1) only has an equilibrium point at (0.0.0.0.). For values outside of the existence condition, the adult and egg populations approach zero towards extinction.

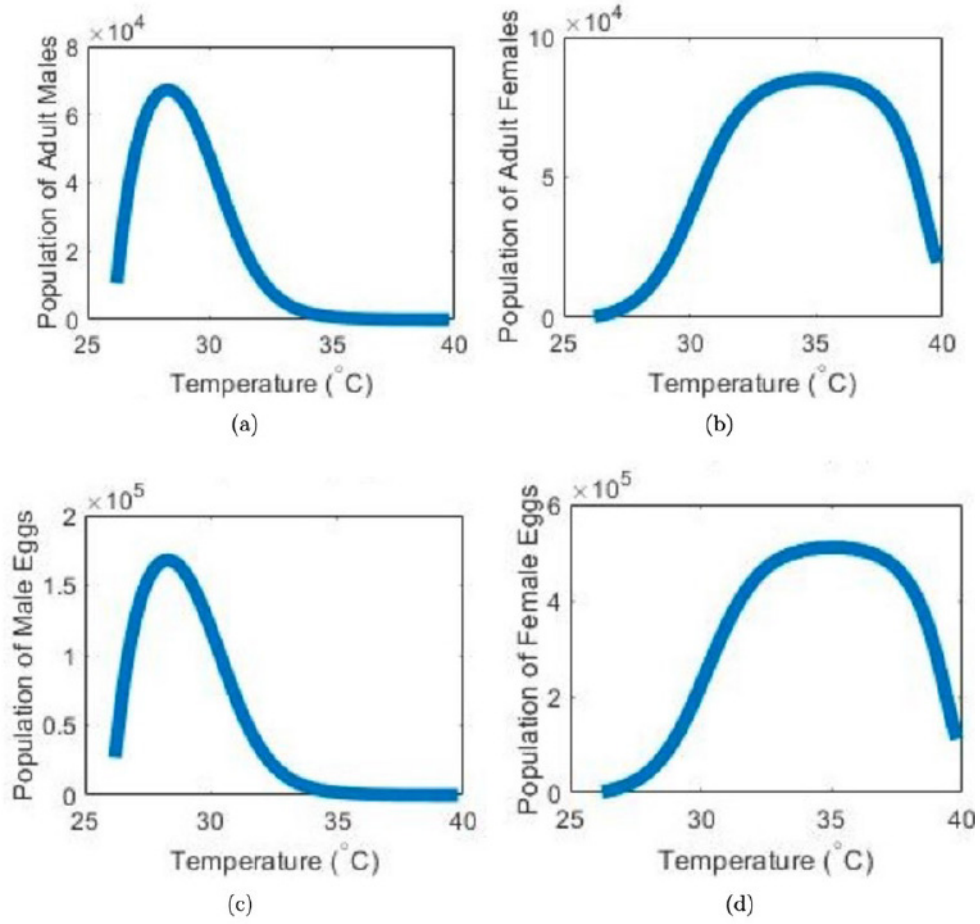

Figure 5. Population of adults and eggs as daily mean temperature increases. The changes in the population are due to variations in the proportion on male eggs caused by temperature $p(T)$.

$$p(T)=1/(1+e^{-1/S(\beta-T)}) \quad (7)$$

In this particular equation the pivotal temperature is represented by β and is in the range of 29.34 ± 0.17. The shape of transition from masculinizing to feminizing is given by *S* and is in the range of 1.01 ± 0.24. The estimates and their standard deviations were found in the same paper from which the expression was taken[40].

We only analyzed our system for values of p that satisfy the equilibrium existence condition. In other words, since p is a function of *T*, we analyzed our system for temperatures that allowed $p(T)$ to still be within the equilibrium condition (8). Since our system is now analyzed with the new $p(T)$ function, the graphs in Figure (5) reflect how temperature changes lead to different proportions of males and how those proportions lead to different population numbers for the different stages.

Discussion

$$ln\left(\frac{1}{p_2}-1\right)S+\beta<T<ln\left(\frac{1}{p_1}-1\right)S+\beta \quad (8)$$

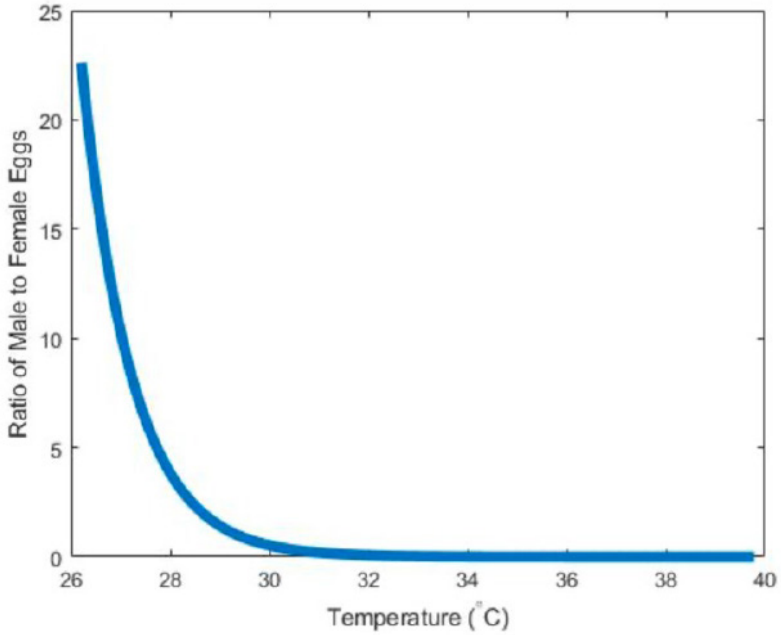

Figure 6. Taking the ratio of (5(c)) and (5(d)), we obtain the changes in the male to the female sex ratio of eggs as daily mean temperature increases. The graph reflects that as temperatures increase, the sex ratio decreases because the proportion of male eggs becomes smaller than the proportion of female eggs.

According to the International Union for Conservation of Nature (UICN) Red List Threatened Species[41], Green sea turtle is considered as an endangered species and its population is continuously decreasing. This population decline is due to many factors like illegal trade of eggs, turtle-shell trade, plastic, and other marine debris, ocean pollution, and global warming[42-45]. Most studies focus on how these factors harm the adult population and have determined that we should maintain current efforts to reduce mortality of long juvenile and adult stages to keep a sustainable population[46]. Our concern, in contrast, focuses on some aspects at the earliest stages of the life cycle that can harm the adult population long-term. Due to this, we studied how the temperature at the mid-trimester of the incubation period impacts the sex ratios of the population. Fluctuations in the incubation temperature can cause a female or male-biased sex ratios in the population. With this model, we analyzed how the sex ratio is decisive for the population growth and how this is affected by the temperature. We determined a safe operating space at which temperature can alter the sex ratios without taking the population to collapse. It has been shown that different populations of sea turtles have female-biased offspring production due to temperature increases during the incubation period[47,48]. Regardless of that, our results show that the proportion of female eggs can go to extreme values without having a severe impact on the turtle population. However, our numerical results depend completely on the values that our parameters take since many of these are not found in the literature; most of them had to be estimated affecting the accuracy of the results. To avoid this, more fieldwork is necessary to strengthen the parameter estimations.

A system of ordinary differential equations was used to represent the life cycle of the turtle population. The emphasis of our study is the mechanics of sex structure on population stability. A closed-form analytic condition for the temperature to ensure species persistence was found. The previous literature relating to this subject has been relatively sparse, but our results seem to be in general in accordance with prior publications[49]. At the moment, the lack of studies on the subject has left us without any current contrary findings.

Since we simplified our model to denote all pre-reproductive stages as the egg stage and the reproductive stage as the adult stage, we think that this might propose limitations on the details of each sub-stage in the life cycle, as well as including the post-reproductive stage. A dearth of information has been reported with regard to the searching efficiency and coupling time of green sea turtles. We believe that having inaccurate parameter estimations and a simplified model probably caused our condition on and our condition on to be so large, thus giving us a broad range where highly skewed proportions are viable. We also based our model in a very general sense, rather

than focusing on a specific area such as a beach or ocean. Male sea turtles often never come back to land after they are born and females only come to lay their eggs, so much is unknown about green sea turtles, thus proposing further limitations for us to study population dynamics, this suggests uncertainties in our mathematical model. The well-mixed, homogeneous assumption has far more severe limitations based on our understanding of sea turtle biology than in other cases. Green sea turtles both male and female return, mate, and lay their eggs at their natal beaches. A discrete-time model of a single beach or a discrete-time model that takes into spatial dynamics would be far more appropriate. The limitations of our continuous-time approach cannot be stated until these other methods are explored.

Our model can be used as a basis for a more complex model involving details about each stage in the life cycle of males and females and determine which stage is the most critical for the survival of the population. It can also be used in future research to include the thermodynamics of microplastic in the sediment and see how it is directly affecting the sea turtle population sex-ratio. We believe that one can incorporate climate change into the model to show that many species and ecosystems are being affected by it. We also think it would be very useful to create a proportion of male eggs as a time-dependent function of temperature $p(T(t))$ to replace $p(T)$. First, the random variation from mean daily temperatures in June and July would be the most appropriate complication added to the model. Secondly, it would be essential to include a general trend of climate change into the stochastic yearly variation as this is an observable trend. If we could find better information about the impact of microplastic proliferation on beaches on soil thermodynamics, then we could begin to include some heuristic to capture this effect. Lastly, a much more complicated model might be interested in studying how cyclical climate variations like El Niño on the long-term stability of turtle populations. It will be essential in the future to have broad trans-disciplinary collaborations to properly account for the edaphoclimatic factors that impact the turtles TSD. Lastly, it will be necessary for further models to include egg death induced by extreme temperatures[49].

We found that there is a limited safe operating space of temperature for green sea turtle sustainability, as seen from Equation (8). If this condition is not met, then the total population approaches extinction. However, it is essential to note that this threshold is substantial, so the probability that the temperature would be outside of this range is unlikely. Based on our simulations in Figure (5), we found that the proportion of eggs that become male can decrease dramatically without a severe impact on turtle populations. When the balance of males p approaches zero, the population of females can still survive in high numbers. Highly skewed sex ratios do not necessarily mean that the population is no longer viable because males will breed with many females, suggesting that just a few males are sufficient to father multiple clutches[50].

Moreover, male sea turtles breed twice as frequently as female sea turtles, meaning females can continue to find mates even when males are low in numbers[49]. However, when the male proportion reaches zero, then the whole population of Green sea turtles will go extinct. Our research indicates that it is necessary to urge policymakers and environmental managers about the importance of monitoring beach temperatures to ensure the continued existence of Green sea turtles. If temperatures get too high and exceed our threshold, then the population will go extinct, thus hurting the two ecosystems that they are a part of.

Conclusions

Expression (8) give us a safe operator space of temperature where green sea turtle population is sustainable. When temperature increases and exceeds the boundaries of this condition, then the overall population will decline until extinction. According to our simulations the probability that temperature reaches values outside the range is unlikely. A highly skewed population towards females do not necessarily mean that the population will be no longer viable because males are able to breed with many females, suggesting that just few males are sufficient to father multiple clutches[50]. However, these results could be due to an inexact parameter estimation, since the majority of these are combination of other parameters that are not easily found or are not present in the literature. Our research indicates that it is necessary more field work to obtain highly reliable parameters. Additionally, it is essential to impulse policy makers and environmental managers about the value of supervising beach temperatures to safeguard the existence of the green sea turtle population.

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RESEARCH / INVESTIGACIÓN

Morphological study of different varieties of rice traits influencing nitrogen and water uptake efficiency

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Abstract: This research aimed at establishing the morphology of the root and sizes under various irrigation cultures. The comparison was made for root to shoot ratio under the traditional culture of flooding. We hypothesize that, due to limited root system development under aerobic conditions, rice is poorly adapted to different environments. In the meantime, there has to be an increase in demand for grain and output per area, as newly planted areas are scarce. This study discusses the latest theoretical physiological, metabolic and genetic factors affecting nitrogen intake and use in different N processes. It covers the root system's position and nitrate- ammonium assimilation and its relationship with Nitrogen Use Efficiency (NUE) and Water Use Efficiency (WUE), were discussed. Phenotyping and molecular breeding techniques concerning N and water regimes and genetic diversity were also evaluated and simulated.

Key words: QTLs, NUE, WUE, Morphology, Irrigation culture.

Introduction

Rice root is a fasciculate system and sense at the herbaceous plant, chill, and water deficiencies sensitive[1,2], indices, the essential role of the root is extracted and absorption of dissolved minerals and water from the flooded region[3,4]. Recently studies report about the characteristics of root for assessed and attributed the relationships genetic expression and root physiology and morphology[5,6]. The reactions of nitrogen (N) to organic growth and associated characteristics can, therefore, reflect the genetic traits inherited from ancestral to cultivated species[7,8,46]. The features to improve root for uptake N catch associated with profundity, density[9,10]. The regular plant irrigation, including alternative wetting and mild soil drying drainage, could improve root tip cell ultra-structure; enhance root thickness size and cytokinin accumulation in root[11]. Root architecture traits have been related to water and N uptake such as the ammonium (NH_4^+) transport systems at physiological levels, exhibit linear kinetics[12,13]. Ammonium: nitrate (NH_4^+: NO_3^-) ratio arrived at 1:100 in agriculture land, with low focus NH4+ uptake from plant roots highest rates[14]. Unusually, perhaps the root plasma film negative electrical polarization in this way its high fondness to the NH_4^+ more than NO_3^- [15,16]. Without a doubt, rice root has NH_4^+ tolerant respiratory increment, and show neither a tight electrochemical angle for NH_4^+ efflux over the plasma layer[17]. The efflux components of transport NH_4^+, because of the intense cytosolic concentration of NH_4^+ found under NH_4^+ conditions, are often energy-consuming due to inward plant plasma membrane[18]. Because of the enormous size of NH4 +, the energy consumption in respiratory oxygen could increase[19,20]. But deep root systems extract more water from dee phrenic soil layers as a result of changes in as similar partitioning, where roots grow more (number and depth) during vegetative growth. Partitioning between different shooting components is seldom hindered[21]. aerobic rice farming is a disruptive technology that aims to reduce water consumption, but the vulnerability of rice to aerobic conditions has restricted its progress[22]. Total root biomass variability was mainly due to the individual root growth for aerobic culture. In aerobics, the stomach closing was distinct at the vegetative stage although the soil water production was close to the field power, in part due to poor rooting, vigor[23]. Nitrogen fertilizer as an Ammonium or nitrate is associated with a release of Green House Gases (GHGs) arrived at 10- 20% from N fertilizer uses in the world[24]. Alternative agronomic approaches can be established for minimizing the use of N-fertilizers through the production and adoption of NUE varieties[25], the use of molecular breeding inputs, agronomy and nutrient modeling, genetic variation[26]. The usability of NO3-N seems, therefore, to be impaired in the NUE as well, representing the condition of nature, two carriers family nitrate 1/peptide carrier family (NPF) and 2 families of nitrate carriers (NRT2) were response on uptake and translocation of nitrate in rice[27]. It is still NUE is only about 50-fold[28], which indicates that there is a big challenge to develop rice plants with a strong NUE capacity[29]. For understanding the pathway regular of N movement in rice root and increasing NUE results from Quality of N-use (NUtE) & quality of N-uptake (NUpE) by increase lateral roots to absorbed more surface area for N uptake[30]. Script enzymes lead to physiology and quantitative genetics in hereditary strategy enhanced proteins that connect N translocations of quantitative traits loci (QTLs)[31]. Obara *et al.*[32] found in the study. Several QTLs were mapped to chromosome regions containing GS2 in rice for agronomic features related to the use and yield of N.

Water management had the association between the root morphological characteristics and the productivity of water use in rained lowlands, deep water, marshland, and rain-fed uplands and irrigated grain[33]. They review the findings of many studies of SRI supporters. It is more important for poor and marginalized farmers because it tends to boost yields in terms of increased radically-based physiological productivity[34]. Nature rice plants become likely to develop a variety of climatic conditions, including sustained aerobic, drought (radical or irregular)[47], poor soil quality, and floods[35]. Hence, the classification among root epigenetic regulation characteristics desirable for versatility to the unique number of conditions encountered by rice grains, and even the hereditary territories accountable for such plasticity characteristics, can encourage the choice for large tolerance of rice genotypes to variable conditions to maintain reliable output[36].

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Materials and methods

Case 1#

Simulated results of the study by Matsunami *et al.*[36] for four Indica rice varieties (Puluik Arang, Badari Dhan, Shwe Nang Gyi, and Ratul), after transplanting varieties were identified under flooded condition (soil water potentials of -0.02 Megapascals (MPa)) 43% [w/w], and unflooded soil type (probable soil water; -0.10 MPa), 33% [w/w]. Water consumption became assessed about three times every day. Therefore, shooting and root biomass were evaluated at three weeks by extracting all the root and shoot and drying at 80 ° C for more than three days and weighing. Root statistical analysis: measurement of the firing ratio in SAS software (version 6.12, SAS Institute, Cary, NC, USA) and graphed for each case at the excel system 2010.

Case 2#

This simulated Qun *et al.*[41] survey analyzing two elite check rice (CK) cultivars (SY-63 and HD-5) and super rice (IIY-084 and WYJ-24) varieties and water quality. The two cultivars and varieties mature in pots with three soil moisture levels after 11 days from transplantation to maturity, three water specifications have been placed by regulating water distribution in well-watered (WW) (0) kilopascal (kPa), mild water deficit (MWD) (–15±5) kPa and severe water deficit (SWD) (–30±5) kPa. Four pots of each the root and shoot dry weight (gpot-1) measurement has been dried at 70 ° C to continue in the oven during drought. Root: shoot ratio was calculated using the formulas Root/Shoot and Painted on the adoption of the program of Excel 2010.

Case 3#

Chu *et al.*[44] Two representative cultivars (Chunyou927 (CY-927) and Yongyou538 (YYY-537)) and two representative JIR cultivars (Xiushui09 (XS-09)) and Zhejing99(ZJ-99)) have been searched for in the field of alternative wetters and severe drying experiments (AWSD), Watered up to 30 kPa of surface capacity and constant flooding with 2-3 cm of precipitation, CF. Precipitation is continually flooded. Upon physiological maturity, the dry matter of each root and shoot was estimated at a constant weight upon drying at 70 ° C and then weighed and measured by Root: shooting ratio equations for IJHR cultivars in addition to the root of the IJHR: efficiency of shooting and specifications for water were regulated by two irrigation systems based on a mean-± standard error at P = 0.05 and graphically based on excel 2010.

Results and Discussion

WUE's interaction with root systems

The high-water absorption potential in Figure.1 results in Puluik Arang are more excellent than Badari Dhan for the conservation of root: shoot ratio, while Badari Dhan was significant among the cultivars Ratul and Shwe Nang Gyi. In Puluik Arang, the results were outstanding. Puluik Arang showed no significant root variations under the unflooded condition: shooting between the cultivars (Badari Dhan, Shwe Nang Gyi and Ratul).

Such results confirm the physiologic and morphological characteristics of spark-and-rain cultivar Puluik Arang and Badari Dhan which are prone to unsafe conditions and are thus associated with water absorption and which therefore adds significant bio-mass renewability under a flooded environment. Research showed that genetic variation in root function was essential to better water intake under stressful conditions[50].

At root: the root ratio was reduced under the flood conditions compared with flooded areas, irrespective of the cultivar. Similar results have been documented in other experiments under soil moisture conditions and were generally limited even when water is mild or the soil is saturated[37,38].

The findings in figure 2 indicate the same actions as root: corresponding shooting ratio for both crops. When water surpluses rose, significantly below the SWD, moderately below MWD and lowest below the WW, the cultivars decreased considerably and no noticeable difference between the four varieties under WW.

The findings indicate either MWD or SWD: the root ratio decreased as water deficit increased to deal with soil water deficits and is better at growing rice cultivars. The root ratio decreased. No significant difference in moisture treatment between four cultivars: fire ratio showing the greater photosynthetic potential of the root and the snorkel, especially in soil deficits. The expectation is that small root biomass can sustain

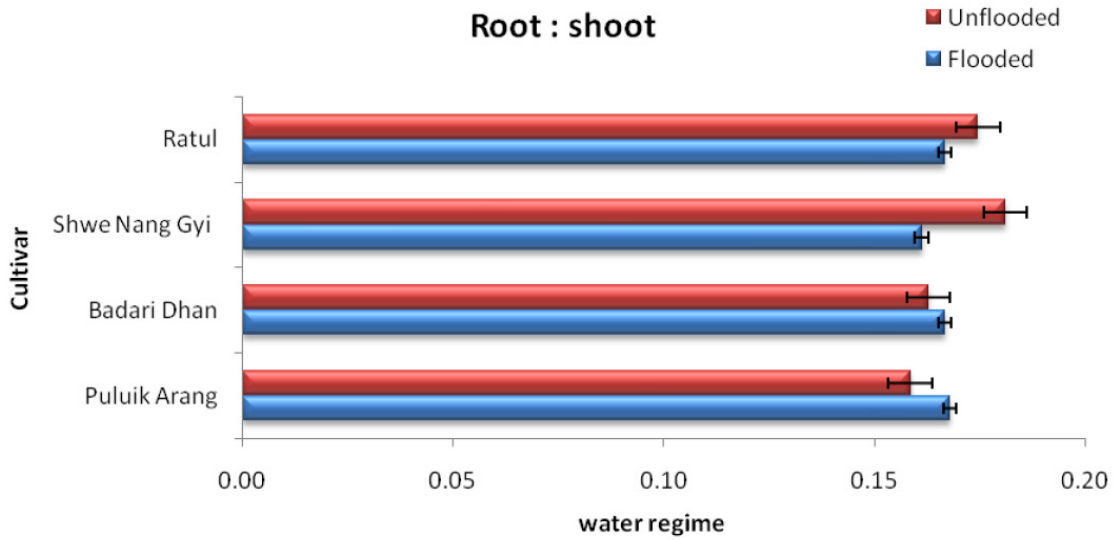

Figure 1. Mean± Standard Error of Root: Shoot ratio of four rice cultivars subjected to flooded and unflooded conditions. (Figure design depended[36] results at $p < 0.05$ according to Turkey's test).

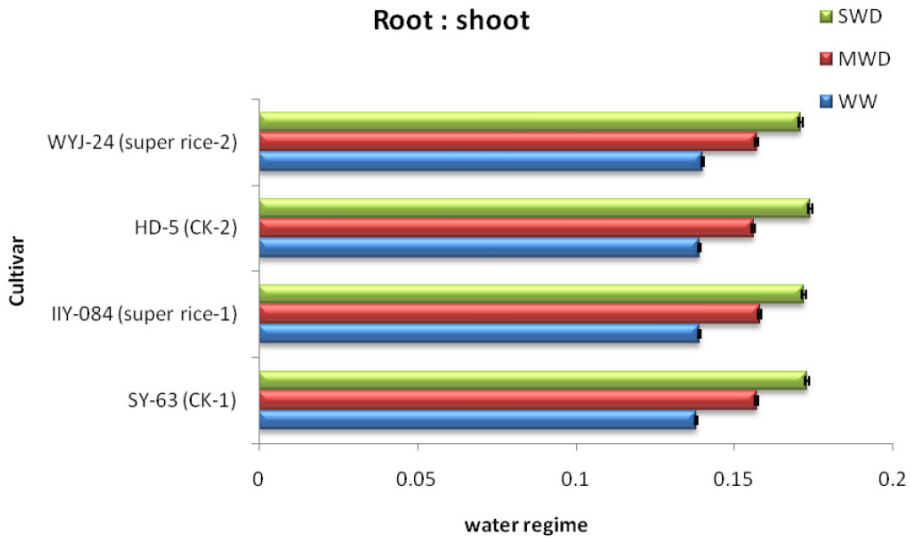

Figure 2. Mean± Standard Error of Root: Shoot ratio of four rice cultivars subjected to well-watered (WW), moderate water deficit (MWD), and severe water deficit (SWD) conditions. (Figure design depended on[41] results at $p < 0.05$ according to Turkey's test)

a strong biomass production over the field, while a deep root distribution will maximize land moisture capture and thus hold plant water in the high condition under dry conditions[39,40].

Results in figure 3 show that the AWSD irrigation system was highly significantly increased root: shoot ratio among cultivars relative to CF, with a more significant reduction in CY-927> YY-538> XS-09> ZI-99 varieties consecutively, but also better root: shoot ratio performance and highly under the CF irrigation regime compared with the XS-09 cultivars compared with other cultivars. Larger root: association of shoot with the deeper distribution of root and photosynthesis of plants with IJHR cultivar AWSD irrigation system. The experimental variations were due to changes in the hydrological component of the soil and the pacing of irrigated techniques. Better grains production and WUE for AWMD is mainly due to reduced vegetative residual growth and improved stability of canopy and increased root growth in hormone levels. Increases abscisic acid concentration and cytokinin levels usually during soil drying and decreased carbon transfer from tissue to vegetative grain during rewatering[37,38].

NUE's interaction with root systems

In figure 4 simulated, the analyze the root-shooting and root-soil relationships are the underlying role for higher seed production, the root-sourced hormonal roles in regulating crop development and growth, and the effect of soil moisture and nutrient distribution on root morphology and metabolism. The overall root productivity was significantly lower than in flooded plants due to the reduction in root abundance in the soil. Due to the significant reduction in the number of preventive plants, the role of fast root growth in soil water absorption and, hence, air perspiration survival, the weak ratio of root to shoot and disadvantaged root production in the surface layer of aerobic culture. The root morphology under upland rice included some portion of an ideotype to improve N capture for potential NO_3^- passage in the shaped profile at lower depths, but root appropriation varies unambiguously with soil conditions, supplement availability, and mechanical impedance. Upland rice's root morphology is an ideotype for optimizing N collection, extracting nitrate from lower root profiles, but its root distribution varies widely with the soil characteristics, fertilizer

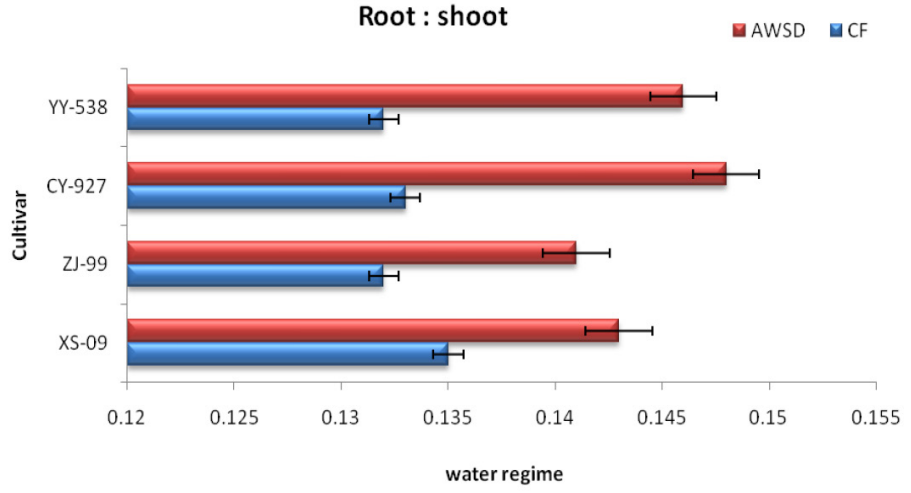

Figure 3. Mean± Standard Error of Root: Shoot ratio of four rice cultivars subjected to alternate wetting and severe drying (AWSD) and constant flooding (CF) conditions.
(Figure design depended on[44] results at $p < 0.05$ according to Turkey's test)

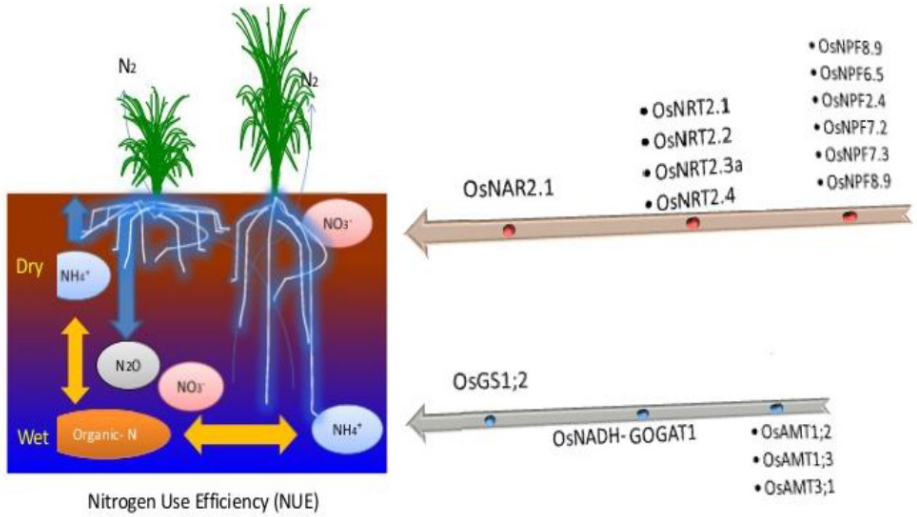

Figure 4. Schematic description of identified and predicted roles of NRT and NPF family rice nitrate carriers, AMT family ammonium carriers, and GOGAT nitrogen assimilation proteins[32,46].

efficiency and mechanical impedance. NRT, NAR (OsNAR 2.1, 2.2), OsNRT, has functions for upregulating the transcription of N-use-related genes for both NO3 and NH_4^+, and also for floral genes, for the most part of the NO3-transport families (OsNRT 2.1; 2.2, 2.3 and 2.4). NPF family members described to date were low-affinity NH4 transporters, with the especially that OsNPF family (8.9; 6.5; 2.4; 7.2 and 7.3) demonstrated dual-affinity nitrate transportation behavior associated with increased NO3- absorption and root-to-shoot transportation. OsNPF6.5, called an OsNPF8.9 putative mRNA splicing drug, has a major impact on both NUE and yield OsNPF8.9, expressed predominantly in root Skin and epidermis were cloned to cause N absorbance. Location of OsNPF4.1 in rice panicle elongation and OsNPF8.20 (OsPTR9) role in the absorption of NH4 +, lateral root development and decreased kernel yield were shown to work. The NUE-related QTLs and glutamine synthetase 1 (OsGS1;2), OsAMT(1;2, 1;3, and 3;1) and Glutamine oxoglutarate aminotransferase1 (OsNADH-GOGAT1) are affinity with NH4 transporters[32,43,44,45]. Here we noted that OsNRT (OsNPFs), a member of the rice (Oryza sativa) nitrate transporter family 1/peptide transporter, is involved in regulating N intake and yield, Gene over-expression in different cultivars appeared not to affect root size or WUE, being a good candidate gene for optimizing NUE and yield has been significantly improved, these conclusions mean that under stress conditions root uptake increases area uptake[48,49], root: shoot ratio has finally been correlated with grain uptake N.

Conclusions

NH_4^+ and NO_3^- is a major driving factor for growing crop yields, but with considerable flowering delays, prolonging the maturation and thus increasing the risk of yield losses. Therefore, characteristics that use high N levels without slowing ripening are highly desirable for plant breeding. The root morphology for upland and flood rice is part of an ideotype to optimize N aggregation, absorb nitrate at lower root profile depths, but the root distribution varies widely with soil capital, fertilizer quality, and irrigation system. Gene over-expression in different cultivars appeared not to affect yield or NUE or WUE, being a functional candidate gene for optimizing NUE and return has been significantly improved. In rice, these conclusions mean that under stress conditions root uptake increases area uptake, root: shoot ratio has finally been correlated with grain uptake N.

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RESEARCH / INVESTIGACIÓN

Laboratory scale evaluation of Effective Microorganisms in the control of odor of organic waste from a market in the city of Riobamba, Ecuador

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Abstract: Inadequate waste management and poor storage conditions are a problem that still affects the population and environment. In the city of Riobamba (Chimborazo, Ecuador) some people feel affected by odor pollution generated by the accumulation of waste in landfills near to markets, corners, and public places. For minimizing this problem, the present work analyzed the potential odor reduction of organic waste from a market located in Riobamba, using Effective Microorganisms: *Lactobacillus plantarum*, *Rhodopseudomonas palustris*, *Streptomyces albus*, and *Aspergillus oryzae*. Four combinations of cocktails were formed subsequent to evaluating the antagonism of microbial strains. The odor sensory evaluation was carried out by 40 people using the American Society of Heating, Refrigeration and Air Conditioning Engineers odor scale, biochemical oxygen demand, chemical oxygen demand, temperature, pH, conductivity, turbidity, and color were measured in the treatment which reached imperceptible odor intensity. In this way, the cocktail formed by the four strains of Effective Microorganisms presents a reduction in the values of the physicochemical parameters of the leachate compared to the sample without the microorganisms, and furthermore, that cocktail controls bad smell produced by the decomposition of organic matter. Therefore, the application of Effective Microorganism opens up a possibility for the treatment of organic waste within local garbage collection stations.

KeyWords: Odor, decomposition, control, Effective Microorganisms.

Introduction

Solid waste is the biggest problem of environmental impact worldwide; they affect the soil and air quality by the gases produced at their decomposition. Additionally, they transform water when they are deposited into it or dragged by rain. Organic waste resulting from animal, agricultural, and industrial production is the primary source of pollution in several countries[1].

In 2014, approximately 11203.24 tons of solid waste were collected daily in Ecuador; of that, 62% was organic waste, 25% was a recyclable inorganic waste, and 13% was non-reusable hazardous waste[2,3]. In 2010, an average of 150 tons of solid waste per day was generated in the city of Riobamba (Chimborazo, Ecuador) by 225.74 habitants[4]. The biggest problem in Riobamba is odor pollution, due to the accumulation of waste in landfills located in markets, corners, and public places.

The decomposition of organic waste is a severe problem due to the large amount produced, also, air pollution due to bad odors has been increasing in recent years. In Spain for example, 25% of the population feels affected by this problem; in Ecuador, 26.76% of the population indicates having issues due to inadequate environmental odors[5].

Some gases are generated as a result of waste rot: acetic acid, acetaldehyde, ammonia, amines, mercaptans, phenol, toluene, sulfuric acid, and other sulfur compounds[6].

Additionally, the incorrect way of waste storage can generate the production of pathogens, which present a high risk to the health of the population, and mainly to people who handle the waste for final disposal[7].

Effective Microorganisms (EM) are cultures of mixed organisms that degrade organic matter and allow its use for plants, improve soil characteristics and conditions for agriculture. The EM was formulated as a microbial cocktail using: photosynthetic bacteria, lactic acid bacteria, yeasts, fungi, and actinomycetes. In the process of rotting organic matter, the EM produces organic acids that are not usually in the soil, such as lactic acid, acetic acid, amino acids, malic acid, and vitamins that could be absorbed by plants[8].

This research aimed to formulate a microbial cocktail that reduces odors caused by the decomposition of organic waste. Four strains of microorganisms were chosen to obtain an EM consortium: *Lactobacillus plantarum*, *Rhodopseudomona palustris*, *Streptomyces albus*, and *Aspergillus oryzae* to check the ability of the microbial consortium to reduce the substances that produce bad odors from the waste by removing pathogenic microorganisms through competitive exclusion.

Materials and methods

This study was carried out in the Molecular Biology-Genetics and Microbiology laboratory at Science Faculty, Escuela Superior Politécnica de Chimborazo (ESPOCH), Ecuador. The culture collection is belonging to Plantsphere Laboratories, Quito, Ecuador (Table 1).

Antagonism test

A confrontation was made between the four microbial strains in Petri dishes with PDA medium at 28°C for seven days. The microbial suspensions were prepared in concentrations of $1x10^4$ CFU mL^{-1}, in saline solution for *L. plantarum* and *R. palustris*, and tween 80 0.1% for *S. albus*. Then 5mm diameter discs of *A. oryzae* were taken, using sterile punches[9]. Three essays of the antagonistic effect and three controls were performed with the fungus.

The inhibition rate was determined by I = [(C-T) /C] x 100. Where, C is the radius of the mycelium of the control, T is the radius of the mycelium.

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Microorganisms	Code	Treatment	Provenance
Lactobacillus plantarum	PSL 40215	Enriched in TSB broth, seeded in MRS medium, 24 hours, 30°C	Probiotics
Rhodopseudomonas palustris	PSL 40460	Seeded in PDA medium, 5 days, 28°C	Organic substrates and dairy products
Streptomyces albus	PSL 40123	Seeded in PDA medium, 7 days, 28°C	Organic material
Aspergillus oryzae	PSL 50127	Seeded in PDA medium, 5 days, 28°C	Rice seed

Table 1. Summary of the methods for microbial strains activation.

Treatment design

Four treatments (T1 to T4) and control treatment (T5) was performed with three repetitions each, with concentrations described in Table 2[10].

To determine a concentration of microorganism to work, the spore counts of *A. oryzae* and *S. albus* strains was performed in triplicate using a Neubauer chamber. Once the required concentration was found, the inoculums were stored at 5°C until the preparation of the EM cocktails.

Additionally, the inoculum of *L. plantarum* and *R. palustris* was suspended into 10 mL of saline solution, homogenized in a vortex for 5 minutes, and successive dilutions were made until 10^{-5}. To define a concentration, each dilution was counted by triplicate in a cell counting chamber[11].

Strains / Treatment	*R. palustris* CFU mL^{-1}	*L. plantarum* CFU mL^{-1}	*A. oryzae* spores mL^{-1}	*S. albus* spores mL^{-1}
Treatment 1 (T1)	$1x10^4$	$1x10^4$	$1x10^4$	$1x10^4$
Treatment 2 (T2)	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^4$
Treatment 3 (T3)	$1x10^6$	$1x10^6$	$1x10^4$	$1x10^6$
Treatment 4 (T4)	$1x10^6$	$1x10^6$	$1x10^6$	$1x10^6$
Control (T5)	-	-	-	-

Table 2. Treatment design.

Treatment Formulation

The cocktails were prepared with: 90% distilled water, 5% sterile molasses, and 5% of microbial inoculums (1.25% of each EM inoculum); they were incubated at 28°C for 24 hours. For each treatment, the ratio of cocktail to organic waste was 1:1000. The biomass used as a substrate was obtained from the organic waste of markets from Riobamba. The substrate was chopped for obtaining a homogeneous mixture of 200 Kg m^{-3}.

The presence of the microbial strains was checked, before and after the assays, by observation under the microscope: fungal staining using lactophenol blue dye[12] and the Gram staining technique was used for bacteria[13].

Odoriferous evaluation of cocktails

A group of 40 heterogeneous people from 19 to 23 years old was selected. An eight days assay was performed[14]. The microbial cocktails were scattered on biomass on days: one, three, and six; and the odor evaluation was performed on days: one, three, six and eight. On day one, the organic waste was placed into experimental devices; the panel checked in each treatment if the smell was homogeneous or if it was in advanced decomposition. The scale used for the analysis of the tests is shown in Table 3 and is proposed by the American Society of Heating Refrigerating and Air Conditioning Engineers (ASHRAE). Additionally, some parameters as temperature, pH and conductivity were measured for each treatment into experimental devices[15].

VALUE	CORRESPONDS TO
0	No odor
1	Slight
2	Moderate
3	Strong

Table 3. Relative odor force scale.

Leachate Analysis

The leachates from the treatment with the highest efficiency on odoriferous evaluation and the control treatment were collected for determining: biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), turbidity, color, temperature, pH, and conductivity (Table 4). These analyses were performed in the Water Quality laboratory at Science Faculty, ESPOCH, Ecuador.

Results and Discussion

Antagonistic activity

The inhibition percentage associated with: *L. plantarum*, *R. palustris*, *S. albus*, and *A. oryzae*, ranged from 4% to 7.8%. The low antagonistic degree of *A. oryzae*, equal to 1, suggests that the fungus can invade ¼ of the surface of other microorganisms without damaging it. Additionally, the results show no formation of inhibition halos among the four microbial cul-

Parameters	Methods and references
BOD$_5$	Digester Hach BODTrak™ 11 for 5 days[16].
COD	Volumetric method using a distillation equipment[17].
Turbidity	Hach RATIO XR Turbidimeter in a scale of 1 – 2 000 NTU units[18].
Color	DR 2800 photometer at a wavelength of 465 nm in platinum cobalt units (PCU)[19].
Temperature, conductivity and pH	Consort™ C562 multiparameter equipment[15].

Table 4. Methods for monitoring leachate.

tures, so its use as a single consortium of microorganisms is recommended due to the symbiotic effect presented. Therefore fungi, actinomycetes, and bacteria can co-exist in a mixed culture[8]; and they can be included in a biological treatment system for odor abatement[20].

Evaluation of the growth of the culture in the microbial consortium

The four microbial cultures, which formed the initially mixed consortium, were remained after the treatments (Figure 1). Despite the notorious presence of *A. oryzae*, the growth of *R. palustris*, *L. plantarum*, and *S. albus*, was not inhibited.

The efficiency of a biological treatment system for odour reduction depends on its heterotrophic microbial consortium[20]. *Lactobacillus plantarum*, *Streptomyces albus*, and *Aspergillus oryzae* are heterotrophic microorganisms while *Rhodopseudomonas palustris* has a versatile metabolism; for that reason, when they were placed into organic waste and molasses as substrate, they had nutrients necessary for gain energy[21] and growing after the treatments.

Odor analysis

The application of EM consortium had a significant effect ($p<0.05$) on the differentiation of odor levels from organic waste, in fact, the panel perceived odor variations during the period of treatment until day eight. The use of pure cultures in biological treatment system for odor reduction in air, like they used in the mixed consortium for T1 to T4, ensures the early action against the potential pathogens that cause possible emissions of odour[22]; likewise, Fan, *et al.*[23], determined the reduction of time for elimination of pungent odor coming from the decomposition of organic matter on home scale organic waste composting, so the unpleasant smells of compost with EM varied to earthy smell on week five compared to control treatment (without EM) which generated earthy smell on week seven.

According to the sensory procedure performed[14], T4 can be considered as an effective way to odor control of organic waste. The parameter "Strong odor" was: 25%, T1; 17.5%, T2; 2.5%, T3; and 0%, T4; as shown in Figure 2. The threshold level of olfactory identification for some malodorous compounds is: 42 ppm, acetone; 17 ppm, ammonia, and 0.00041 ppm, hydrogen sulphide[21]. In this way, T4 could have eliminated the perception for unpleasant smell compared to control T5 which kept the parameter "Strong odor" on 80% of the panel.

By comparison, the T4 contained the highest concentration of EM: ~10^6 CFU mL^{-1} of L. plantarum and *R. palustris*, and

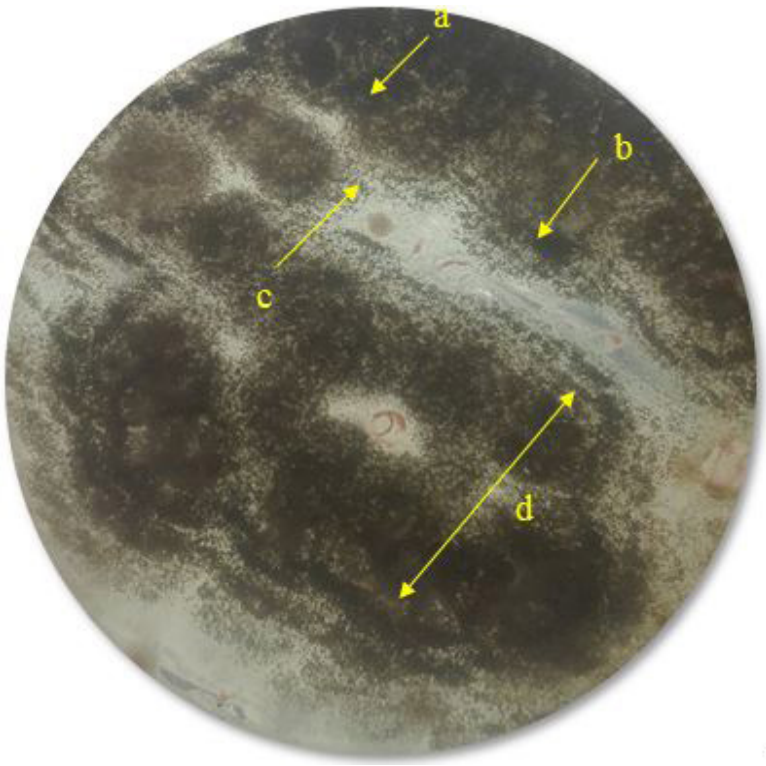

Figure 1. The consortium of microorganism: a) *S. albus*; b) *L. plantarum*; c) *R. palustris*; and d) *A. oryzae*.

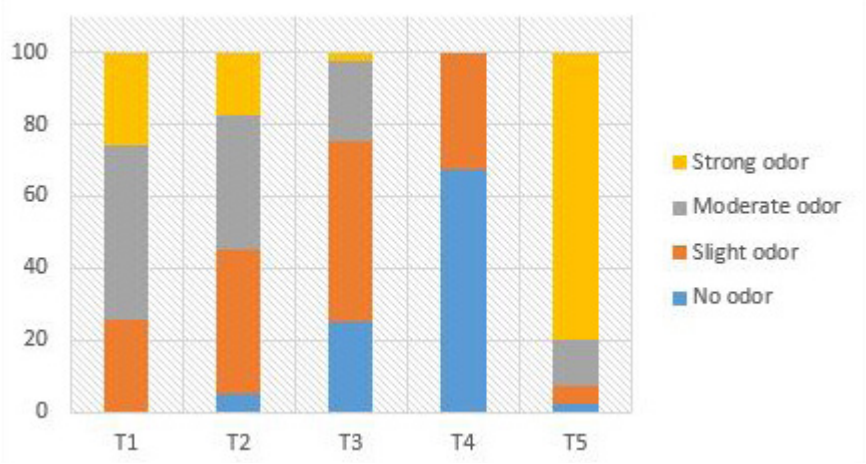

Figure 2. Odor levels by treatments with capable microorganisms (T1 to T4) and control without EM (T5).

~10^6 spores mL^{-1} of *S. albus* and *A. oryzae*. Namasivayam and Kirithiga[24] verified that native microorganism increased when EM consortium also increased, they used high concentrations of compost with EM (12.1x10^6 CFU g^{-1} of bacteria, 21.3x10^5 CFU g^{-1} of actinomycetes, and 15.1x10^4 CFU g^{-1} of yeast and mold) for improving the soil nitrogen, phosphorus, and potassium levels.

Effect of temperature and pH

The temperature had a gradual increase in all treatments (Figure 3), but the treatment without EM presented the lowest temperature (23°C) at the end of the assay.

Changes in the temperature of the composting of organic wastes are closely related to microbial activity[25]; in this way, each increase of 10°C in the medium, is directly related to the microbial metabolic rate.

The highest temperature (33°C) was reached at T4 on day 6. This behavior is similar to the presented by Song, *et al.*[25], for the decomposition of organic waste, where a higher temperature was observed in the treatment with a microbial consortium instead of those without microbial inoculation.

On the other hand, the tests with EM had a pH equal to 6 on day 8, while the maximum pH reached by the control treatment was balanced to 5 at the same time (Figure 4). The pH range suggested[26] to carry out an appropriate degradation of organic matter with EM consortium is between 6 to 8.5; considering that in the initial phase of decomposition, the pH decreases during the first days; then it has a gradual increase until reaching values of 8.16 (at day 15) and 7.90 (at day 30).

The variation in pH can be related to the production of odors since acidification, neutralization, and alkalization of pH in composting processes are closely related to microbial activity through the release of ammonia and the conversion of organic acids into CO_2[25]. Likewise, Miller, Macauley and Harper[27], identified that a pH between 8 to 9 leads to the loss of nitrogen through the volatilization of ammonia, which is a compound identified as causing the bad smell in compost.

Leachate analysis

The values of BOD_5, COD, turbidity, and conductivity obtained for T4 against T5, were as shown in Table 5. In leachates generated from vegetable waste in composting processes in laboratory[28], COD concentration varies between ranges from 18 to 68 g L^{-1}, and for BOD5 between 10 and 46 g L^{-1}; in this way, the COD values obtained for the leachates of T4 and T5 are within the typical range, while the BOD5 is below the lower limit.

In order to assess the level of contamination caused by

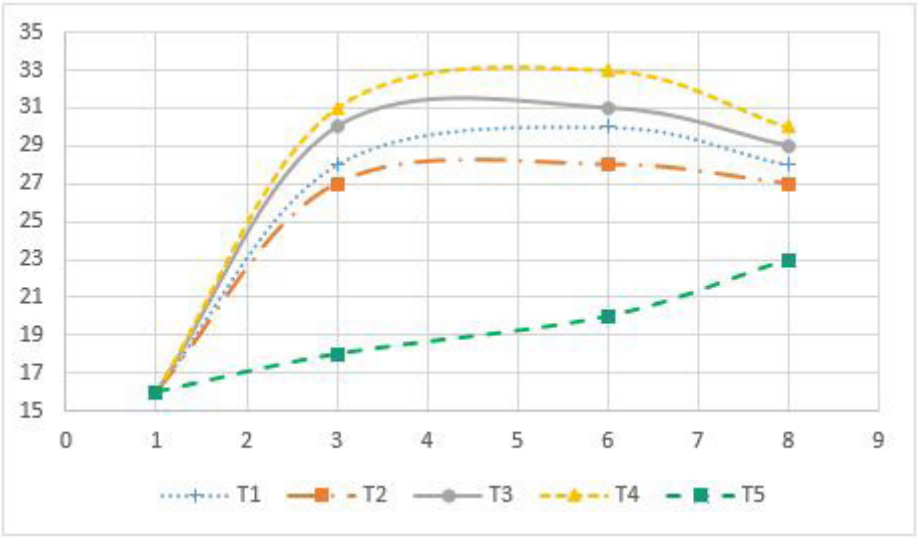

Figure 3. Temperature variations (°C) for treatments with EM (T1 to T4) and control without EM (T5).

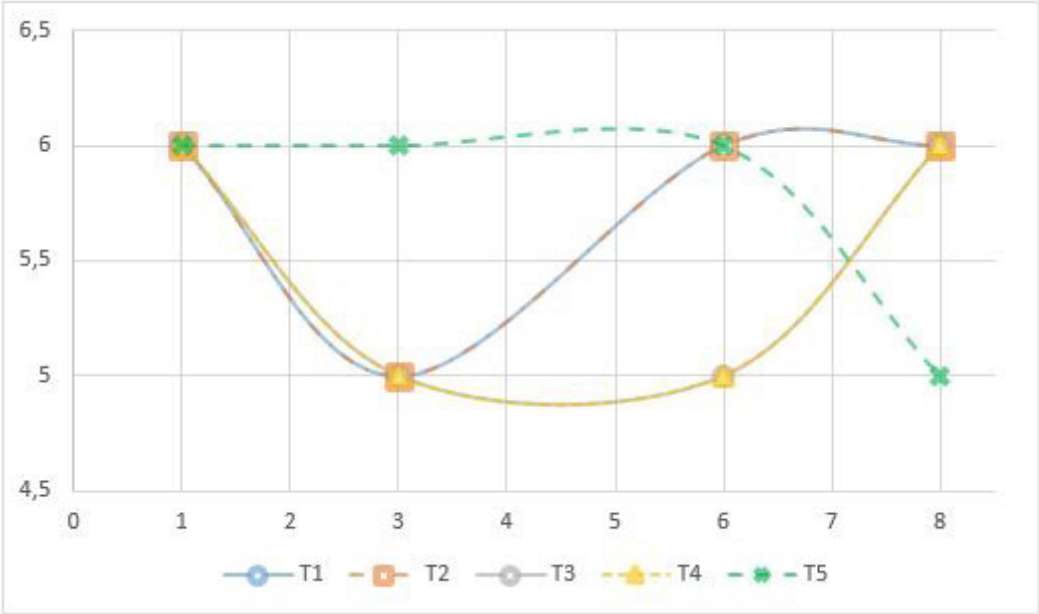

Figure 4. pH variations for treatments with EM (T1 to T4) and control without EM (T5).

organic matter, it is necessary to calculate the ratio of BOD/COD to elucidate the biodegradability of the leachate[28]. In this sense, the leachate generated by the treatment with EM is moderately biodegradable (ratio T4 BOD/COD = 0.25) compared to the treatment without inoculated microbial consortium, which has leaching with low biodegradability (ratio T5 BOD/COD = 0.16).

For the conductivity, the values of T4 and T5 (from 1.42 to 82.6 mS cm^{-1}) are adjusted to the typical range for leachates of degradation processes from vegetable waste obtained in the laboratory[28]. Besides, T4 showed a 30% reduction in color units (232.34 PCU) compared to the treatment without the application of EM.

Parameters	T4	T5
BOD_5 (g L^{-1})	7.63	8.34
COD (g L^{-1})	30.67	53.33
Turbidity (NTU)	4547	8300
Conductivity (mS cm^{-1})	1.82	3.75

Table 5. Analysis of organic waste leached from T4 (with EM) and T5 (control).

Conclusions

The application of the microbial consortium formed by: *Lactobacillus plantarum*, *Rhodopseudomonas palustris*, *Streptomyces albus*, and *Aspergillus oryzae* had reduced odors produced by the decomposition of residues from a market of Riobamba, Ecuador. The present study was conducted only on organic wastes (legumes peel, vegetables and fruit leaves); for that reason, it should be considered as a preliminary study for the control of odors from another kind of waste. The ME concentrations used (~ 10^6 CFU mL^{-1} and ~ 10^6 spores mL^{-1}) achieved a reduction in the perception of strong odor according to the panel. The species of ME used in the microbial consortium were observed at the beginning and at the end of the treatments, which proves their symbiotic action within the biological deodorization treatment system. During the treatments, the measurement of pH and temperature was necessary for its use as operating parameters that guarantee the biological activity of the microorganisms.

Finally, the parameters: BOD_5, COD, turbidity, color, and conductivity of the treatment inoculated with *L. plantarum*, *R. palustris*, *S. albus*, and *A. oryzae* were lower compared to the values of the treatment without EM inoculated; that demonstrates the microbiological action in the purification of leachates.

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RESEARCH / INVESTIGACIÓN

Evaluation of Gene Variants in TGFB1, SERPINF1 and MEPE in a Spanish Family Affected by Otosclerosis and Tinnitus

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Abstract: Otosclerosis (OTSC) is a common type of deafness affecting up to 0.4 % of Caucasians. Its familial form is inherited in an autosomal dominant fashion, although to this date, no definitive cause for OTSC has been found. In the development of OTSC, three recent genetic association studies have suggested the participation of particular point mutations and small indels in the *TGFB1*, *SERPINF1*, and *MEPE* genes. Consequently, replicative studies are needed to confirm the role of the proposed mutations in OTSC patients. The goal of this study was to test the presence of the candidate variants described in the genes *TGFB1*, *SERPINF1*, and *MEPE* in a new case of familial OTSC with seven affected individuals. DNA was extracted from saliva samples of a Spanish family with several members affected by OTSC. PCR amplified target regions of some candidate genes, and the products were purified, Sanger-sequenced, and analyzed *in silico*. The family subject of the study did not carry the candidate variants for OTSC described in the genes *TGFB1*, *SERPINF1*, and *MEPE*, although it cannot be ruled out the involvement of other mutations in genes related to their same signaling pathways. This result highlights the importance of performing replicative studies for complex diseases, such as OTCS, in families of diverse origins. Additionally, a significant association of subjective tinnitus with OTSC has been found in this family, although the link between the two pathologies should be studied further.

KeyWords: Otosclerosis (OTSC), Hearing Loss (HL), Tinnitus, *TGFB1*, *SERPINF1*, *MEPE*.

Introduction

Otosclerosis (OTSC) is a complex conductive hearing loss that affects 0.3-0.4% of people of Caucasian origin and is rare among Blacks, Asians, and Native Americans[1,2,3]. The disease begins around the age of 30 of average, although it can also affect individuals from the first to the fifth decade of age[4]. Most patients (70-80%) suffer bilateral OTSC, and women are more affected than men in a ratio of up to 2:1[5]. The disease is caused by an abnormal bone remodeling of the otic capsule, which in normal conditions does not undergo remodeling after development. This might, over time, create a deficit in the hearing threshold of air conduction, but not in bone conduction of the sound transmission[6]. Although an audiometric analysis currently determines this, the most reliable diagnosis for OTSC is the stapes surgery, which is the first-line treatment for OTSC patients. This involves the replacement of the defective stapes bone with a micro-prosthetic device[7,8].

The etiology of OTSC is not well understood; however, it is deemed to be both genetic and environmental. The environmental risk factors include measles virus infection, use of oral contraceptives, and low sodium fluoride content in drinking water, all of them still controversial[3]. Regarding the genetic factors, studies on large families with many affected individuals have revealed that OTSC has an autosomal dominant mode of inheritance with a reduced penetrance of about 40% (reviewed in[9,3]). Linkage analysis studies on large families have identified 8 monogenic loci associated with the disease (*OTSC1-5*, *OTSC7-8* and *OTSC10*)[10,11,12,13,14,15,16,17]. To date, no otosclerosis-causing genes have yet been identified in these loci[3,18].

Recent gene expression analysis and genetic association studies have suggested candidate genes for OTSC mapped outside the 8 loci identified by linkage analysis. As an example, several studies have revealed a role for the TGF beta pathway in OTSC. Particularly, the Transforming Growth Factor beta1 protein, coded by the gene *TGFB1*, has been suggested to play a role in the development of the disease[9,19,20,21,22,23,24]. Particularly, a recent genetic association study of several gene variants of *TGFB1*, found the c.-509C ("wild type") allele associated with normal hearing, while the c.-509T allele was associated with OTSC[24]. Another candidate gene that has been recently suggested as causative of OTSC is the *Serpin Family F Member 1* (*SERPINF1*), coding for a potent inhibitor of angiogenesis and a neurotrophic factor. Ziff *et al.* (2016) found a primary *SERPINF1* transcript expressed in the stapes bone, (isoform 2 or *SERPINF1-012)* and six additional rare variants in isoform 1 (*SERPINF1-001*), three of them predicted to be deleterious for its function and also to affect the expression of isoform 2 transcripts[25].

The most recent candidate gene to cause OTSC is *MEPE*, which codes for an extracellular matrix protein belonging to the SIBLING family of secreted phosphoproteins. A functional MEPE protein participates in bone homeostasis, preventing the maturation of osteoclasts and inhibiting bone mineralization[26]. Schrauwen et al. (2019) found an association of *MEPE* and OTSC in a study of a Turkish family affected by hereditary congenital facial paresis (HCFP) and mixed hearing loss (HL). Exome sequencing revealed variants predicted to produce truncated MEPE proteins that would increase bone remodeling in the otic capsule, compared to normal developmental conditions[27].

Tinnitus is an additional hearing condition affecting about 50% of OTSC patients[9,28] and up to 15% of the global population[29,30]. The hearing of phantom sounds characterizes it, usually localized to one or both ears, but can also be felt centrally within the head. Tinnitus can be a very distressing, even disabling condition for its continuous noise perceived as

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a buzzing, hissing, beeping or ringing, and it becomes chronic when lasting more than a year[31,32]. Tinnitus can be classified as objective tinnitus when the body generates the sound, and the examiner can hear it or as subjective tinnitus, more commonly found, if there is not a specific sound source within the body. Its etiology can be environmental, due to exposure to ototoxic drugs, head trauma, noise exposure or infections. Recently, twin studies have determined a possible genetic component with a heritability of 0.68[33,34].

In the present study, a new pedigree representing four generations of a Spanish family with a history of OTSC has been constructed. DNA samples from the core family of the proband were used to test the involvement of recently proposed gene variants of *TGFB1*, *SERPINF1*, and *MEPE* in the development of OTSC in that family. Besides, information about tinnitus causes -a condition of significant distress for many subjects of the family- was gathered, and interesting associations between the two conditions were found.

Methods

Subjects

Participants belonging to a Spanish family with several members affected by OTSC were recruited with informed consent. Every effort was made to keep the family name confidential. All procedures were approved by the Ethics Committee of the University of León (ETICA-ULE-023-2019) and in agreement with the Helsinki Declaration (JAMA 2000)[35]. Confirmation of diagnosis was initially achieved by documented evidence of stapes surgery. Additional OTSC cases were confirmed by audiometry.

Construction of the family pedigree

Information about HL and tinnitus was gathered through interviews in the period 2017-2019. An online survey was also conducted to gather information concerning the incidence of tinnitus in the family. HL among deceased individuals was confirmed when several relatives commented positively on their condition. To confirm OTSC, clinical records of both living and deceased individuals who underwent stapes corrective surgery were obtained from the same hospital they were treated upon formal request. Most of the individuals of Generation IV or V have not been depicted because either they were not coming to age as to participate in the study, they were adults without HL symptoms or their parents were not affected by HL. Individuals II:8 and III:3 underwent corrective surgery, but we were not able to find their medical history. The family pedigree chart was created with PowerPoint (Microsoft©).

Audiometry

Standard pure tone audiometry at frequencies of 0.25-8 MHz and tympanometry plus the examination by an ear, nose, and throat (ENT) specialist were performed to confirm OTSC in individuals of the family who reported any hearing problem and in siblings of individuals with confirmed OTSC.

DNA extraction

Participating individuals were asked to provide a saliva sample. These were obtained after a one-minute mouth rinse with saline solution and kept cold till the time of genomic DNA isolation. This was achieved by standard phenol/chloroform method as in Lum and Le Marchand (1998)[36], with few modifications. The quality of the DNA was confirmed in a Nanodrop apparatus (Thermo Fisher).

DNA amplification and sequencing

Target sequences of the genes *SERPINF1*, *TGFB1* and *MEPE* were amplified by PCR with primers shown in Supplementary Table 1. PCR amplified DNA sequences from both affected individuals and controls from genomic DNA and the sequence of the amplicons determined by the Sanger method and capillary electrophoresis (MegaBACE 500, Amersham Biosciences) with the same primers used for DNA amplification. DNA alignments were performed in MEGA (Molecular Evolutionary Genetics Analysis) software (https://www.megasoftware.net/)[37].

Individual	Age	Affected Ear	Sym/Diag	Age at surgery
II:5	D	L,R	NA	39L/56R
III:4	75	L,R	16	28R
III:5	72	L,R	20	34L/35R
III:7	61	L	25	No surgery
III:10	74	L,R	NA	56L
IV:9	42	R	26	37R
IV:10	41	L	32	41L

Table 1. Age, affected ear and age of earliest symptoms, diagnosis and surgery of OTSC in affected individuals. D: deceased; NA: not available; L: left ear; R: right ear. Sym/Diag: age of earliest symptoms or OTSC diagnosis.

Statistical analysis

To test the independence between the OTSC and tinnitus conditions, a 2 x 2 contingency table was generated sorting selected individuals of the pedigree represented in Figure 1 into each of the following four groups: having or not OTSC, and suffering or not tinnitus. Individuals with undetermined hearing loss conditions were not included in any of the groups. Finally, a two-tailed Fisher exact probability test was applied.

Results

Pedigree analysis

The pedigree of this study (Figure 1) contains 73 individuals of a Spanish family, including seven confirmed cases of OTSC. Not all Generation IV subjects are depicted. Generation V has been omitted because either the immediate ascendants did not suffer HL or because they were too young to be included in the study. Table 1 summarizes relevant information from individuals of the family with confirmed OTSC.

The proband of the study (III:5, arrow in Fig. 1) was a 72-year old Spanish woman who was diagnosed with bilateral OTSC at the age of 20, three years before childbirth. She had (failed) stapes surgery in both ears. She was diagnosed also with osteoarthritis, and never took oral contraceptives. Her two youngest sons (IV:9 and IV:10) were diagnosed with unilateral OTSC and underwent a stapedectomy. Subject III:4 suffers from Paget's disease. The medical history of subject II:5 confirmed stapedectomy. That meant individual I:2 or his spouse must have been carriers of the mutation(s) that caused otosclerosis in some of their descendants.

It was found a high prevalence of chronic subjective tinnitus in the family. Hence, participants were asked to answer a detailed questionnaire on their tinnitus experience, as in Lan-

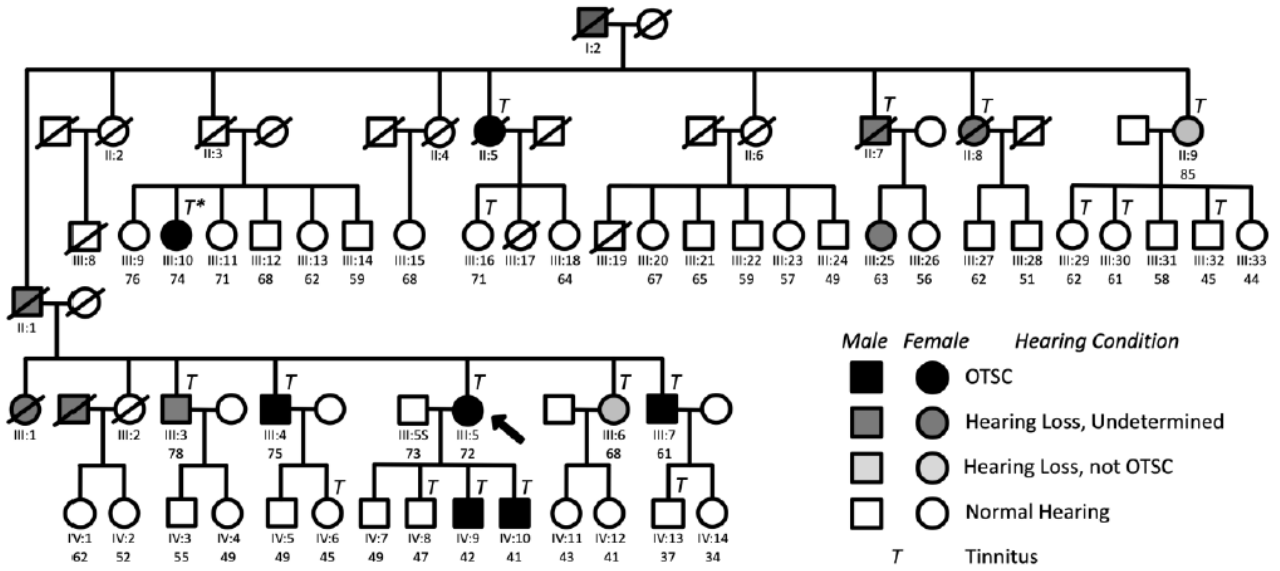

Figure 1. Genealogy of the family subject of the study showing the segregation of OTSC and chronic subjective tinnitus. See legend for conditions. An asterisk in individual III:10 indicates that her tinnitus condition disappeared upon stapes surgery. Arrow, proband.

gguth *et al.* (2006)[38]. The results of the survey are provided in Supplementary Table 2. As shown in Figure 1, all OTSC-affected members of the family suffered from chronic subjective tinnitus. Also, reports from the interviews indicate that the tinnitus experienced by OTSC-affected subjects was severe. Interestingly, tinnitus was also present in non-affected individuals II:9, III:6, III:16, III:29, III:30, III:32, IV:6, IV:8, and IV:13 of the family. The tinnitus in individual III:10 disappeared after corrective surgery.

Genotype analysis of TGFB1, SERPINF1, and MEPE

In the present study, the contribution of previously described mutations in the genes *TGFB1*, *SERPINF1*, and *MEPE* was analyzed. Genomic DNA samples of the core family of the proband were used because they comprise both confirmed OTSC cases and controls. We worked on the assumption that variations found on the core family of the proband would be representative of those affecting close relatives within the family.

TGFB1

The *TGFB1* allele c.-509C has been associated with normal hearing and c.-509T with OTSC[24]. In the present study, we decided to determine whether SNP c.-509C > T was present in individuals affected by OTSC using genomic DNA from the core family of the proband. As shown in Figure 2, there were no differences between the sequences of affected and unaffected individuals. All members of the family carry the c.-509C allele. To note, it is the Reference Sequence Gene that has a T in that position.

SERPINF1

In a recent study, Ziff *et al.* (2016) 25 described three variants in the 5' UTR of *SERPINF1-012* (c.-202_-200 del TCG, c.-161G > C and c.-1G > C), reportedly affecting the expression of the transcript in the otic capsule. In our work we PCR-amplified and Sanger-sequenced the region comprising those variants. Figure 3 shows that none of the variants are present in the OTSC-affected individuals of the core family of the proband.

MEPE

The most recent candidate in the literature to cause OTSC is *MEPE*[27]. Variants of this gene were found first in a Turkish family affected by HCFP and HL and later in a larger pool of OTSC subjects. In the present study none of the family members have HCFP. In our work, we amplified exons 2-4 of *MEPE* and adjacent regions in seven different PCR reactions using the same primers as in Schrauwen *et al.* (2019)[27] and DNA from individuals of the core family of the proband to look for the published gene variants -c.199_202 del GAAA (M2); c.49_54+-delinsCA (M3); c.184G > T (M4); c. 496 dup (M5); c.617del G (M6) and c.679dup (M7)- and also to look for new variants. As shown in Figure 4, none of the published gene variants nor any other polymorphism were found to affect exclusively OTSC subjects.

Association of OTSC with tinnitus

To find out whether OTSC and tinnitus were associated, individuals of the pedigree were sorted into four categories: not suffering tinnitus nor OTSC[48] not suffering tinnitus but su-

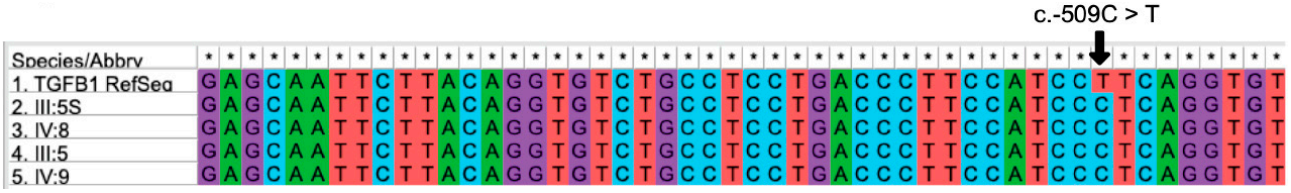

Figure 2. Genotypes of the core family of the proband for the gene *TGFB1*. Subjects III:5S and IV:8, unaffected; III:5 and IV:9, affected. Arrows indicate the sites of the variants found in earlier studies. Image is a screen capture of alignments performed in MEGA[37].

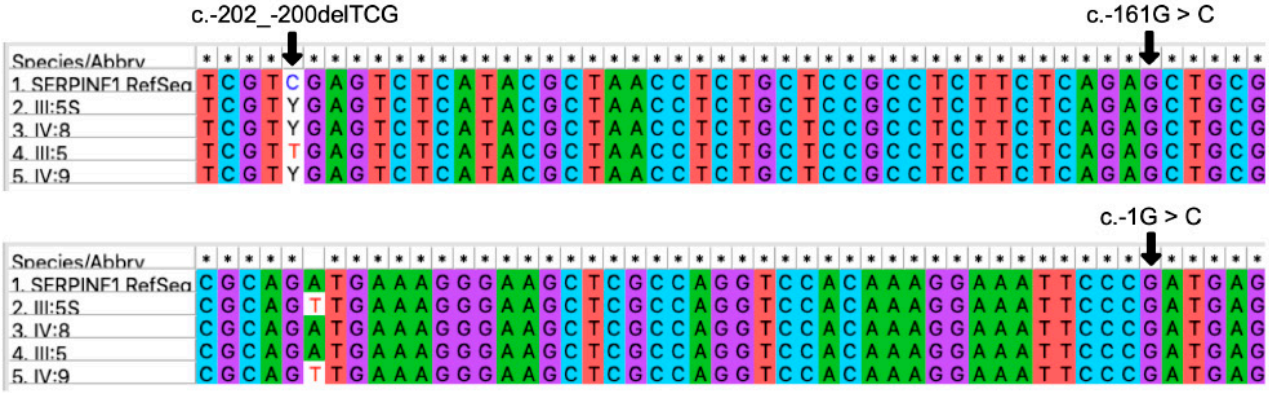

Figure 3. Genotypes of the core family of the proband for the 5'UTR region of the gene *SERPINF1*, isoform 2. Subjects III:5S and IV:8, unaffected; III:5 and IV:9, affected. Arrows indicate the sites of the variants found in earlier studies. Individuals III:5S, IV:8 and IV:9 are heterozygous (C/T) for the position c.-202. Images are screen captures of alignments performed in MEGA[37].

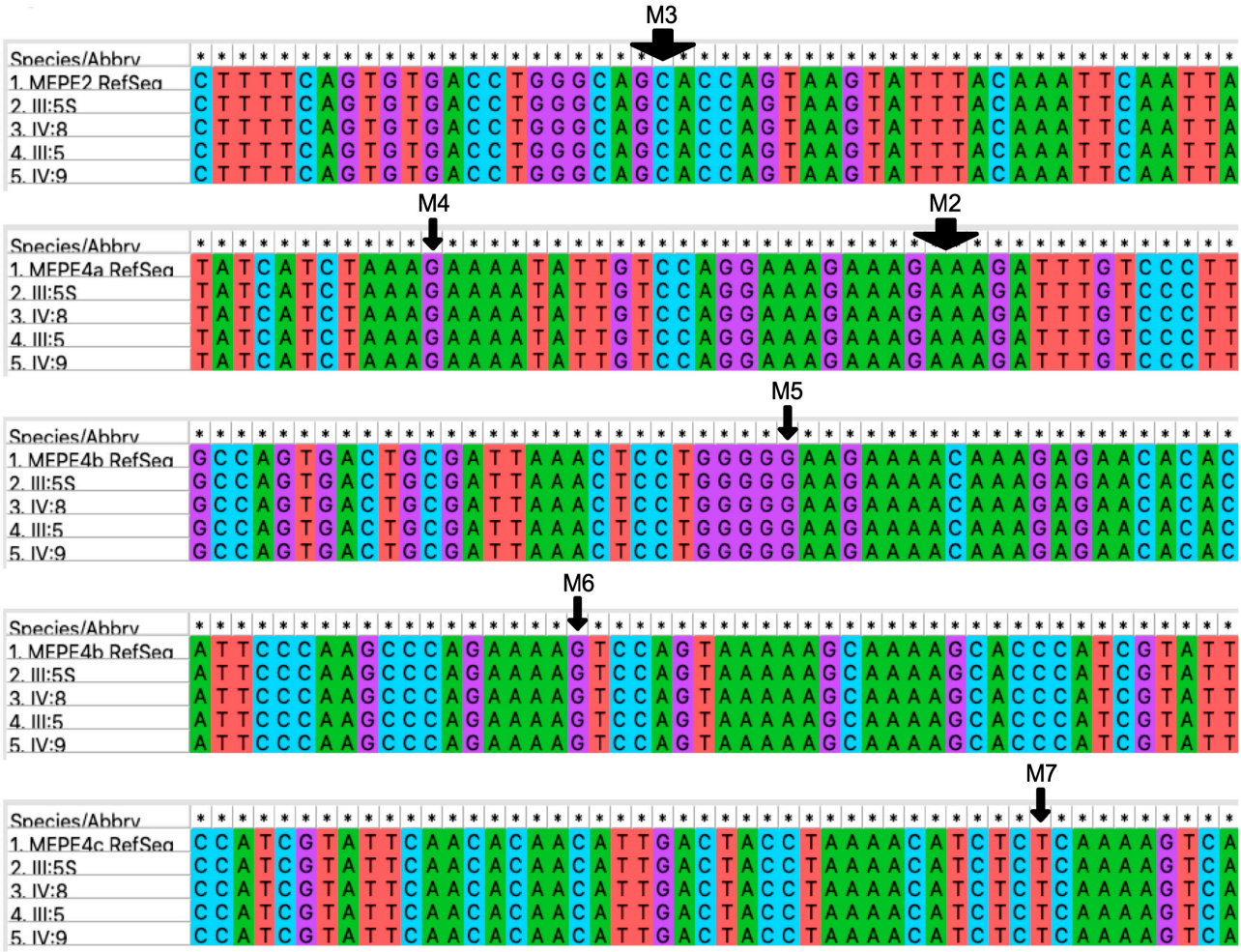

Figure 4. Genotypes of the core family of the proband for *MEPE*. Arrows indicate the sites of variants named M2-M7 as in Schrauwen (2018). Subjects III:5S and IV:8, unaffected; III:5 and IV:9, affected. Images are screen captures of alignments performed in MEGA[37].

ffering OTSC (0); suffering tinnitus but not OTSC[9] and suffering tinnitus and OTSC 7. Individuals with undetermined HL were not included in any of the groups. A two-tailed Fisher exact probability test indicated a very low probability that the two conditions were independent ($p < 0.0001$).

Discussion

In the present study, a novel familial case of OTSC has been presented and used it as a tool to assess the contribution of gene variants of *TGFB1*, *SERPINF1*, and *MEPE*. These genes were selected because they had been recently associated with OTSC in previous reports[24,25,27]. Also, they are also involved in different routes to increase bone remodeling and bone deposition in the otic capsule compared to normal conditions. Despite the available knowledge and having used the same primers and conditions for the Sanger sequencing of the genes, the genetic analysis did not find a role for those variants. This does not rule out the possibility that mutations affecting other members of the pathways to which the genes *TGFB1*,

SERPINF1, and *MEPE* belong could be involved. For instance, the TGFbeta pathway has been suggested for more than a decade as involved in bone regulation at the otic capsule and it is possible that variants in other members of the TGFbeta family of cytokines like BMP2 and BMP4 could be implicated and the subject of future replicative studies[23]. In the case of SERPINF1, the present work is another unsuccessful attempt at replicating the findings of Ziff *et al.* (2016)[25], after another research group did not find a role for the same gene variants[39].

The origin of the families participating in genetic studies may be a factor to take into account in familial OTSC cases. It cannot be excluded from the possibility that different disease-causing variants in the same genes or signaling pathways may act in families of diverse ethnic origin. One precedent is the study of Rodríguez *et al.* (2004)[40], which did not find a role in a Spanish family with a familial case of OTSC for *COL1A1* and *COL1A2*, previously associated to OTSC in a family from Iowa, USA[3,41]. Two other cases involving families from very different geographical locations as tools for replication studies of HL genes -although not for OTSC- analyzed the presence of the A1555G mtDNA mutation combined with suspected exposure to aminoglycosides[42,43]. That mutation had been previously determined in Arab-Israeli populations[44] and was not found in Spanish families.

Reports of chronic subjective tinnitus were found in many members of the family subject of this study. Tinnitus usually affects about half of OTSC patients[9,28], and the symptoms disappear in many patients after stapes surgery -reviewed in Haider *et al.* (2018)[45]. In the present study, 100% of OTSC-affected subjects suffered from severe forms of subjective tinnitus and only in one case (III:10) it disappeared after stapes surgery. Recently, it has been suggested a genetic basis for tinnitus[33,34]. A statistical test indicated a powerful association between OTSC and tinnitus in the family subject of the study.

In conclusion, the present work has investigated the role of variants putative of causing OTSC in the genes *TGFB1*, *SERPINF1* and *MEPE*, previously reported by others. Although it was not found a purpose for those mutations, further work must be done to analyze the role of variants affecting other genes belonging to the same signaling pathways. Finally, it was found a strong association of clinical OTSC with severe cases of subjective tinnitus and also many examples of tinnitus in the absence of OTSC symptoms, all within the same family. This represents an excellent opportunity to further investigate the segregation of OTSC and tinnitus with the use of genomic tools such as whole-exome sequencing of affected individuals. Finding the causative genes for OTSC and tinnitus will surely streamline the design of novel, innovative research, and therapeutic approaches for the benefit of a significant number of affected individuals.

Conclusions

The present work has investigated the role of variants putative of causing OTSC in the genes *TGFB1*, *SERPINF1* and *MEPE*, previously reported by others. Although it was not found a role for those mutations, further work must be done to analyze the role of variants affecting other genes belonging to the same signaling pathways. Finally, it was found a strong association of clinical OTSC with severe cases of subjective tinnitus and also many cases of tinnitus in the absence of OTSC symptoms, all within the same family. This represents a great opportunity to further investigate the segregation of OTSC and tinnitus with the use of genomic tools such as whole-exome sequencing of affected individuals. Finding the causative genes for OTSC and tinnitus will surely streamline the design of novel, innovative research and therapeutic approaches for the benefit of a great number of affected individuals.

Supplementary Material

The following information will be supplemental to this article: sequences of primers utilized in this study and Table 3 of self-reports on tinnitus.

Conflict of Interest

The authors declare no conflict of interest.

Contributions

Study design: F.J.A., P.G. Fieldwork: F.J.A., S.A. Data Collection, and Analysis: F.J.A., S.A., J.A. Sample processing: F.J.A. Data analysis: F.J.A., P.G., J.A. Scientific writing: F.J.A., P.G.

Ethical approval

"All procedures performed in studies involving human participants were following the ethical standards of the institutional Research Ethics Committee of the University of León (ETICA-ULE-023-2019) and with the Helsinki declaration of 1964 and its later amendments or comparable ethical standards."

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CASE REPORTS / REPORTE DE CASO

Evaluación de Nimotuzumab para el tratamiento de cáncer de cabeza y cuello: Meta-análisis de ensayos controlados

Evaluation of Nimotuzumab for the treatment of head and neck cancer: Meta-analysis of controlled trials

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Resumen: Nimotuzumab, anticuerpo monoclonal humanizado, dirigido contra el receptor del factor de crecimiento epidérmico: proteína altamente expresada en tumores malignos de origen epitelial. Ha sido registrado para tumores de cabeza y cuello desde el año 2002. El objetivo de este artículo fue determinar la efectividad del Nimotuzumab en el cáncer de cabeza y cuello a través de la técnica de meta-análisis combinado. Se efectuó una búsqueda en PubMed, en revista indexada con las palabras "Nimotuzumab", "cabeza y cuello", se detectaron 48 artículos publicados por autores cubanos y extranjeros entre el 1 de abril de 2005 y el 31 de julio del 2019, en los que se describen los resultados de los estudios clínicos realizados con el anticuerpo monoclonal Nimotuzumab. Se describen siete ensayos clínicos realizados en Cuba de 2005–2019 con Nimotuzumab; tres Fase I/II (con 14, 10 y 10 pacientes respectivamente), un Fase II/III con 106 pacientes, un Fase II con 37 pacientes, dos Fase IV (con 386 y 225 pacientes cada uno) y un estudio promovido por el investigador con 17 pacientes. De estos estudios fueron seleccionados por el diagrama de flujo PRISMA los tres ensayos controlados. El meta-análisis consistió en la construcción del gráfico de Forest Plot, el análisis de sensibilidad y el análisis acumulado. El meta-análisis arroja resultados favorables al Nimotuzumab, sin heterogeneidad (I^2=0%). El análisis de sensibilidad revela que el ensayo que más difiere de los demás es el Fase II/III. El análisis acumulativo indica que después del segundo ensayo ya existen evidencias suficientes.

Palabras clave: Receptor del factor de crecimiento epidérmico, nimotuzumab, anticuerpo monoclonal humanizado, tumores de cabeza y cuello.

Abstract: Nimotuzumab, humanized monoclonal antibody, directed against the epidermal growth factor receptor: highly expressed protein in malignant tumors of epithelial origin. It has been registered for head and neck tumors since 2002. To determine the effectiveness of Nimotuzumab in head and neck cancer through the combined meta-analysis technique. A search was conducted in PubMed, in an indexed magazine with the words "Nimotuzumab", "head and neck," 48 articles published by Cuban and foreign authors were detected between April 1, 2005, and July 31, 2019, in which the results of clinical studies conducted with the monoclonal antibody Nimotuzumab are described. Seven clinical trials conducted in Cuba from 2005-2019 with Nimotuzumab are described; three Phase I / II (with 14, 10 and 10 patients respectively), a Phase II / III with 106 patients, a Phase II with 37 patients, two Phase IV (with 386 and 225 patients each) and a study promoted by the Researcher with 17 patients. From these studies, the three controlled trials were selected by the PRISMA flow chart. The meta-analysis consisted of the construction of the Forest Plot graph, the sensitivity analysis and the cumulative analysis. The meta-analysis shows favorable results for Nimotuzumab, without heterogeneity (I^2 = 0%). The sensitivity analysis reveals that the test that differs most from the others is Phase II / III. The cumulative analysis indicates that after the second trial, there is already sufficient evidence.

Key words: Epidermal growth factor receptor, nimotuzumab, humanized monoclonal antibody, head and neck tumors.

Introducción

El receptor del factor de crecimiento epidérmico (EGFR) es una proteína transmembranal relacionada con la proliferación y maduración celular, fundamentalmente en células de origen epitelial: piel, mucosa intestinal e hígado[1]. Su activación depende de varios ligandos entre ellos: el factor de crecimiento epidérmico (EGF), que contribuyen a la activación de la cascada de señalización del sistema EGF-EGFR, una vez que ocurre la dimerización de dos receptores y la fosforilación de la porción transmembranal.

Ese sistema se ha estudiado ampliamente, y es muy atractivo como blanco para la terapia del cáncer. Se asocia con la proliferación anárquica, la inmortalización celular, la inhibición de la apoptosis, la neoangiogénesis y la metástasis: signos de mal pronóstico que provocan resistencia a los tratamientos oncológicos convencionales, como la radioterapia, la quimioterapia y la hormonoterapia[2-4].

La inmunoterapia pasiva con AcMs es de las más efectivas contra el EGFR[5]. Actualmente existen varios AcM contra ese receptor, registrados para el tratamiento de algunos tumores sólidos de origen epitelial: Cetuximab® (AcM quimérico), nimotuzumab (AcM humanizado) y Panitumumab® (AcM humano)[6].

Nimotuzumab es la denominación genérica internacional del AcM humanizado cubana, que se conoce alternativamente a nivel mundial con otras marcas como Theraloc® (registrada

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para la Unión Europea), TheraCIM® (registrada para Canadá, Indonesia y otros países asiáticos), CIMAher® (registrada para Cuba y América Latina) y BIOMAb-EGFR® (registrada del producto producido en la India).

En este artículo se resumen las evidencias clínicas más recientes que avalan su uso.

Mecanismos de acción y farmacología clínica

Nimotuzumab es un AcM humanizado, inmunoglobina de isotipo IgG1, obtenido por tecnología de ADN recombinante y producido en líneas de células de mamífero (mieloma murino NSO)[7,8]. Reconoce al EGFR con una afinidad intermedia de 10-9 M[7], contiene las regiones hipervariables (CDR) de origen murino (ior egf/r3) y los marcos de las regiones variables y de las regiones constantes de las cadenas pesada y ligera de origen humano[7].

Su unión bloquea la interacción de los dos principales ligandos del EGFR: el EGF y el TGF-α. Esto inhibe la actividad tirosina quinasa del receptor y arresta el ciclo celular en la fase G1-S, con un marcado efecto anti-proliferativo[8,9,10]. Además ejerce un efecto antiangiogénico y proapoptótico en aquellos tumores que sobrexpresan el EGFR[9-12], y reduce el número de células CD133+: células madres tumorales, responsables de la radiorresistencia entre potras características funcionales[13].

Eficacia clínica del Nimotuzumab

Han concluido más de 10 ensayos clínicos para su evaluación en varios tumores, como pruebas de concepto, pruebas de eficacia y de eficacia terapéutica, que avalaron su registro sanitario en el tratamiento de tumores de cabeza y cuello en estadios avanzados, en combinación con radioterapia, quimioterapia o ambas.

También se ha evaluado el empleo del nimotuzumab en combinación con citostáticos, con agentes alquilantes (ciclofosfamida, platino, carboplatino), alcaloides de la vinca (vinblastina, vinorelbina, etopósido), inhibidores de topoisomerasa (irinotecan), antibióticos citostáticos (adriamicina, mitoxantrone), antimetabolitos (metotrexato, 5-fluouracilo) y taxanos (docetaxel), entre otros[18, 19,22-24]. Los pacientes que se han tratado con tales combinaciones las han tolerado muy bien. Seguidamente se detallan los principales resultados clínicos en las enfermedades para las que se ha registrado.

Tumores avanzados de cabeza y cuello

Los ensayos (fases I y II) para la evaluación de las dosis y el efecto terapéutico del nimotuzumab en tumores avanzados de cabeza y cuello, efectuados en Cuba y Canadá, demostraron que, si se combina con radioterapia, incrementa el porcentaje de respuesta objetiva entre 70 y 100 %, en comparación con la respuesta objetiva de la terapia radiactiva, que es de 30 a 40 %[10,25].

Para confirmar la eficacia del nimotuzumab en pacientes con tumores irresecables avanzados de cabeza y cuello de nuevo diagnóstico, se efectuaron tres estudios comparativos, controlados y aleatorizados[16-18,26].

En un primer ensayo en Cuba, se evaluó la combinación de nimotuzumab con radioterapia, frente a la radioterapia más placebo. La tasa de respuesta en los pacientes que recibieron nimotuzumab y radioterapia fue de 59.5 %, significativamente superior al valor en aquellos que recibieron radioterapia y placebo, que fue de 34.2 % (prueba exacta de Fisher, p = 0.038). Hubo un incremento significativo de la supervivencia de los sujetos tratados con nimotuzumab (Mediana: 12.5 meses), en comparación con el grupo que recibió placebo (Mediana: 9.5 meses), según la prueba estadística de Harrington-Fleming ($p < 0.05$). En el subgrupo de pacientes con alta expresión de EGFR (3+), de peor pronóstico, hubo un beneficio aún mayor en aquellos que recibieron nimotuzumab: 19.6 meses de supervivencia, en contraste con el grupo que solo recibió radioterapia: 6.4 meses ($p < 0.05$)[16].

En el segundo ensayo, aleatorizado, efectuado en la India, se evaluó el efecto del nimotuzumab combinado con radioterapia y quimioradioterapia (QRT). La tasa de respuesta objetiva en los grupos que recibieron nimotuzumab en combinación con radioterapia fue del 76 %, y del 100 % en los que recibieron nimotuzumab en combinación con QRT. En los otros dos grupos de pacientes que recibieron solo radioterapia y QRT, la tasa de respuesta objetiva fue del 40 y 76 %, respectivamente. Tales diferencias fueron estadísticamente significativas. La adición del AcM a la radioterapia y a la QRT también incrementó significativamente la supervivencia, que se estimó en una Mediana de 49.4 meses en comparación con 16.4 meses, en aquellos que solo recibieron radioterapia y la QRT convencional (riesgo de muerte, HR: 0.517)[17,18]. La tasa de supervivencia a 48 meses, fue significativamente superior en los dos grupos de pacientes: 47 % en el que recibió nimotuzumab y QRT, frente a 21 % en el grupo que recibió QRT. Mientras que la tasa de supervivencia en el grupo que recibió nimotuzumab y radioterapia fue de 34 %, en comparación con el grupo de pacientes tratados con radioterapia convencional, que fue del 13 %[27].

Un tercer ensayo en pacientes con tumores avanzados de nasofaringe, en China, evaluó el empleo del nimotuzumab en combinación con radioterapia. La tasa de repuesta objetiva a las 17 semanas del tratamiento fue del 90.6 %; y del 51.5 %, en el grupo tratado solo con radioterapia. Posteriormente se estudió la evolución de los pacientes, y el grupo de pacientes que recibió el tratamiento combinado de nimotuzumab y radioterapia tuvo una supervivencia de 3 años: 84.3 %; en comparación con el grupo tratado solo con radioterapia, que fue 77.6 % ($p < 0.05$ %)[26].

Experiencia pos comercial en la indicación de tumores avanzados de cabeza y cuello

Se realizó un estudio clínico observacional, en Cuba, para evaluar el tratamiento combinado de nimotuzumab y radioterapia, y de nimotuzumab y QRT, en pacientes con tumor avanzado de cabeza y cuello. En este estudio se estimó una tasa de supervivencia a 48 meses del 62 %[28]. En un análisis de supervivencia según el número de dosis de nimotuzumab recibidas, se observó que los pacientes que recibieron solamente el tratamiento de inducción (6 dosis) alcanzaron una Mediana de supervivencia de 12.3 meses[29]. Ese tiempo fue equivalente al descrito en estudios previos a su registro, en los que se aplicó un régimen terapéutico idéntico, de solo 6 dosis en combinación con radioterapia (12.5 meses y 14.4 meses)[16,18]. En los pacientes a quienes se les continuó el tratamiento del monoclonal como mantenimiento más allá de 6 dosis no se alcanzó la Mediana[29]. Este resultado sirvió de fundamento para proponer la extensión de la posología del nimotuzumab como mantenimiento.

Un estudio posterior al registro (fase IV), en Cuba, evalúa la terapia combinada del AcM con radioterapia y quimioterapia, así como la terapia de mantenimiento. En el análisis final se estimó una mediana de supervivencia para el grupo de nimotuzumab y radioterapia de 24.9 meses, mientras que la combinación del AcM con QRT concurrente fue de 32.5 meses (comunicación personal con Piedra P, Nimomeeting 2012).

Otro estudio posterior al registro (fase IV), en Cuba, evalúa la terapia combinada del AcM con radioterapia y quimio-

terapia, así como la terapia de mantenimiento. En el análisis final se estimó una mediana de supervivencia para el grupo de nimotuzumab y radioterapia de 12.62 meses, mientras que la combinación del AcM con QRT concurrente fue de 24.39 meses (Vega A, Nimomeeting 2018).

En términos de supervivencia, estos estudios en población abierta confirman la efectividad de la combinación de nimotuzumab con la radioterapia convencional en pacientes con tumores avanzados de cabeza y cuello; y sustentan que como terapia de mantenimiento contribuye también al beneficio clínico.

Materiales y métodos

En la Tabla 1 se dispone de 10 ensayos clínicos terminados, de estos se rechazan 5 por ser ensayos fase I/II, que tienen como objetivo definir las condiciones de experimentación, y 3 por ser fase IV, o sea no tener un brazo de contraste o control y uno controlado con quimioterapia solamente. En la Tabla 2 se presentan 4 estudios clínicos en curso. Este proceso es rectorado por los criterios PRISMA que pueden ser consultados en[15], ver Figura 1. Se incluyen finalmente 3 ensayos controlados: EC055 Fase II/III de Cuba y Fase II y Fase III de India. Al final se obtiene que el tamaño de la población total de estos cuatro ensayos es de 688 pacientes[49-55].

Número de estudio Ubicación	Fase	Dosis de Nimotuzumab	Agente (s) de estudio	Número de Pacientes Tratamiento/Dosis
IIC RD EC-040 (Cuba)	I/II	50, 100, 200, or 400 mg	RT + nimotuzumab	3/50 mg 4/100 mg 3/200 mg 4/400 mg
IIC RD EC-046 (Cuba)	I/II	200 or 400 mg	RT + nimotuzumab	5/200 mg 5/400 mg
IIC RD EC-076 (Cuba)	I	200 or 400 mg	RT + nimotuzumab	5/200 mg 5/400 mg
YMB-1000-004 (Canada)	I/II	100 or 200 mg	RT + nimotuzumab	14/100 mg 17/200 mg
IIC RD EC-055 (Cuba)	II/III	200 mg	RT ± nimotuzumab	54/RT + nimotuzumab, 52/RT + placebo
hR3/SCCHN/001/IND (India)	II	200 mg	QT ± RT ± nimotuzumab	23/ RT + cisplatin + nimotuzumab, 23/RT + cisplatin, 23/RT + nimotuzumab, 23/RT alone
CLG16/BIO009/SCCHN/h-R3/2006 (India)	IV	200 mg	QT/RT + nimotuzumab; QT + nimotuzumab, RT + nimotuzumab, Nimotuzumab alone	105/QT/RT+nimotuzumab 25/RT + nimotuzumab, 19/QT + nimotuzumab, 1/nimotuzumab alone
IIC RD EC-0113 (Cuba)	IV	200 mg	QT/RT + nimotuzumab, QT + nimotuzumab, RT + nimotuzumab, Nimotuzumab alone	152/QT/RT+nimotuzumab 58/QT + nimotuzumab, 45/RT + nimotuzumab, 131/nimotuzumab alone
M9/CIMAB/AEC-04 (Cuba)	IV	200 mg	QT/RT + nimotuzumab	105/QT/RT+nimotuzumab 32/QT + nimotuzumab, 20/RT + nimotuzumab, 68/nimotuzumab alone
PL 013 (Cuba)	I	200 mg	QT+RT+nimotuzumab	17 nimo+QT+RT

Tabla 1. Ensayos clínicos terminados con Nimotuzumab en tumores avanzados de cabeza y cuello.
Leyenda: RT: Radioterapia, QT: Quimioterapia, QT+RT: Quimioradioterapia
IIC RD-EC040 (RPCEC00000071), IIC RD-EC0113 (RPCEC00000089), PL013 N-QRT-CC (RPCEC00000241), M9/CIMAB/AEC-4 (RPCEC00000145).

Número de estudio Ubicación	Fase	Dosis de Nimotuzumab	Terapia Combinada	Incluidos /No. de Pacientes a ser Incluidos
IB/NCCS-01 (Singapore)	I	200 mg	QT/RT + nimotuzumab	37/37
IIC RD EC-0137 (Cuba)	II	200 mg	QT/RT ± nimotuzumab	35/172
IHN01 (Singapore)	III	200 mg	QT/RT ± nimotuzumab	360/420
Nimotuzumab/SCC HN/2010 (India)	III	200 mg	QT/RT + nimotuzumab	468/536

Tabla 2. Ensayos clínicos en curso con Nimotuzumab en tumores avanzados de cabeza y cuello.

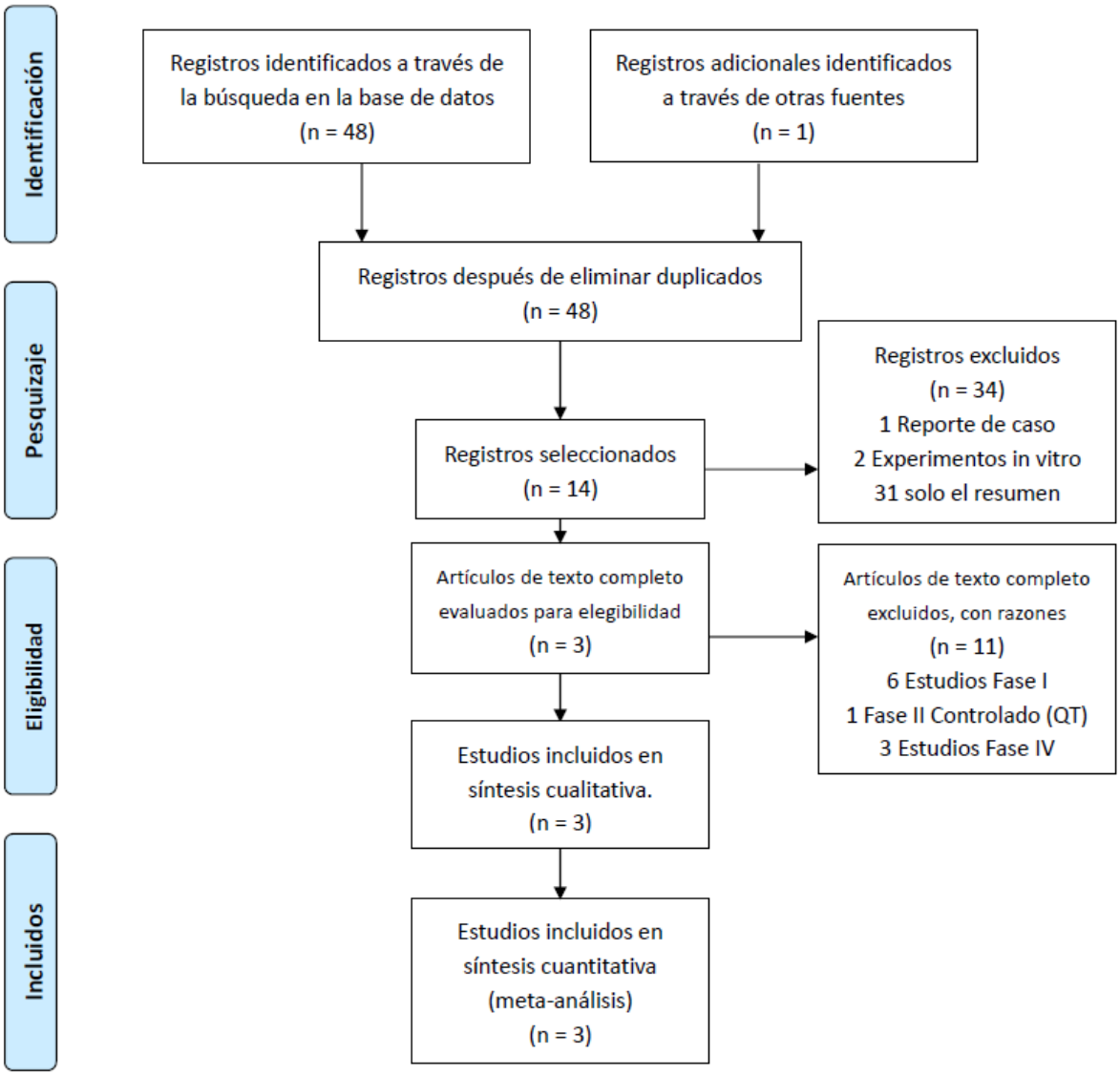

Figura 1. Diagrama de Flujo PRISMA para el proceso de selección de los estudios con Nimotuzumab.

Resultados y discusión

En esta sección se resumirán los resultados obtenidos de la aplicación de los modelos a cada ensayo de forma independiente, y posteriormente se unirán buscando el tamaño de efecto combinado. Para realizar el meta-análisis se utilizó el sistema Review Manager 5.3 Versión: 5.3.5 y los métodos planteados[15]. Se asume el modelo de efectos fijos, pues se dispone de los datos individuales de cada ensayo[17].

En la Figura 2 se muestran los resultados de HR y su intervalo de confianza del 95%. Hay dos indicadores de que el efecto combinado es favorable a la combinación Nimotuzu-

mab+QRT: el p-valor, que se encuentra por debajo de 0.05, y el z-valor, que es mayor en módulo que 1.96 que es el valor crítico de admisión, o sea el valor que marca la región donde el efecto no es significativo. Se muestran los resultados del Forest Plot, de la prueba de heterogeneidad el valor de I^2 y su intervalo de confianza que corrobora los resultados anteriores.

El ensayo de mayor peso 61.5% es el EC055, por la consistencia de los datos. Se chequeó la heterogeneidad según los criterios Cochrane[15]. Se puede concluir parcialmente que el efecto es favorable al Nimotuzumab, pues el diamante (que representa el efecto combinado y sus intervalos de confianza) no atraviesa la hipótesis nula.

En la Figura 3 se muestra el análisis de sensibilidad, necesario para revelar cuál es el estudio más discordante. En la misma aparece el HR y su intervalo de confianza al 95% omitiendo cada uno de los ensayos, así como la estimación combinada de todos los estudios a la vez. Se presentan los resultados del Forest Plot que corrobora los resultados anteriores.

En esta salida de Review Manager 5.3 Versión: 5.3.5 es posible observar que el estudio que más difiere de los demás es el EC055, pues es el que tiene mayor peso (61.5%).

En la Figura 4 se muestra el análisis acumulativo, necesario para revelar cuál es el estudio más discordante. En la misma aparece el HR y su intervalo de confianza al 95% añadiendo cada uno de los ensayos, así como la estimación combinada de todos los estudios a la vez. Se presentan los resultados del Forest Plot que corrobora los resultados anteriores.

En esta salida de Review Manager 5.3 Versión: 5.3.5 es posible observar que cuando se incluye toda la evidencia acumulada a favor del Nimotuzumab+QRT es cuando el p-valor es más pequeño y el rombo se separa más del HR=1.

Por último, se puede apreciar que a través del gráfico Funnel Plot que prácticamente no existe sesgo de publicación, pues el último estudio incluido ya se encuentra publicado[37]. Figura 5.

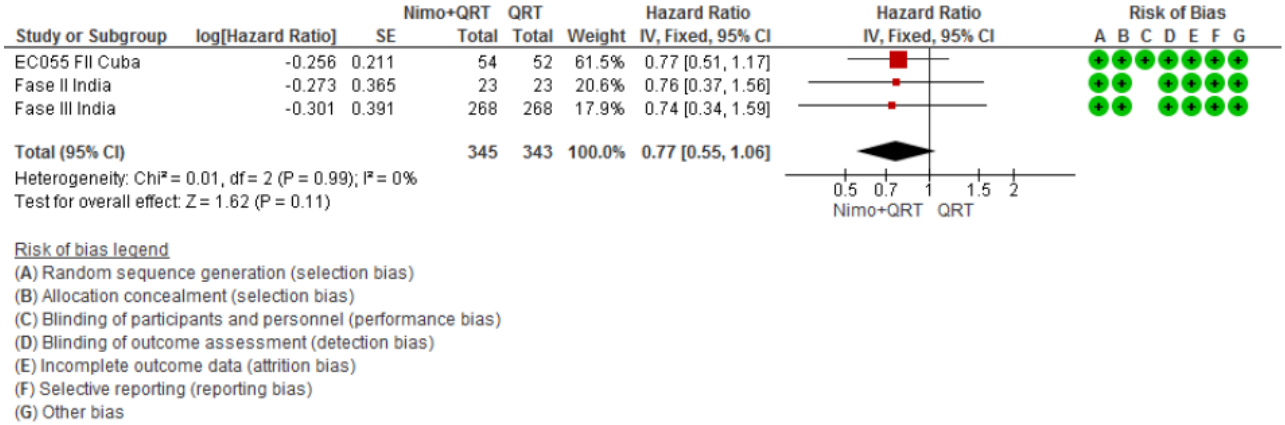

Figura 2. Forest plot para el modelo de efectos fijos.

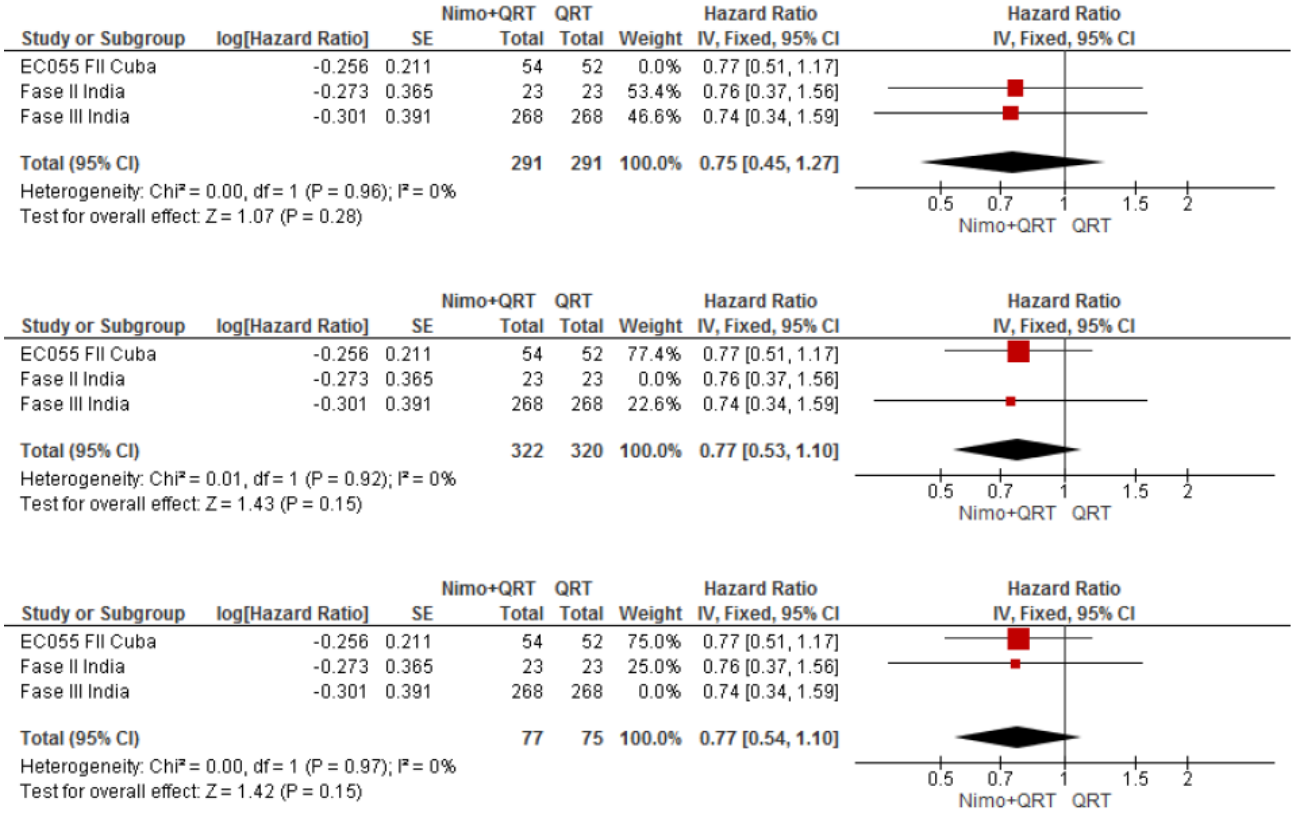

Figura 3. Análisis de sensibilidad.

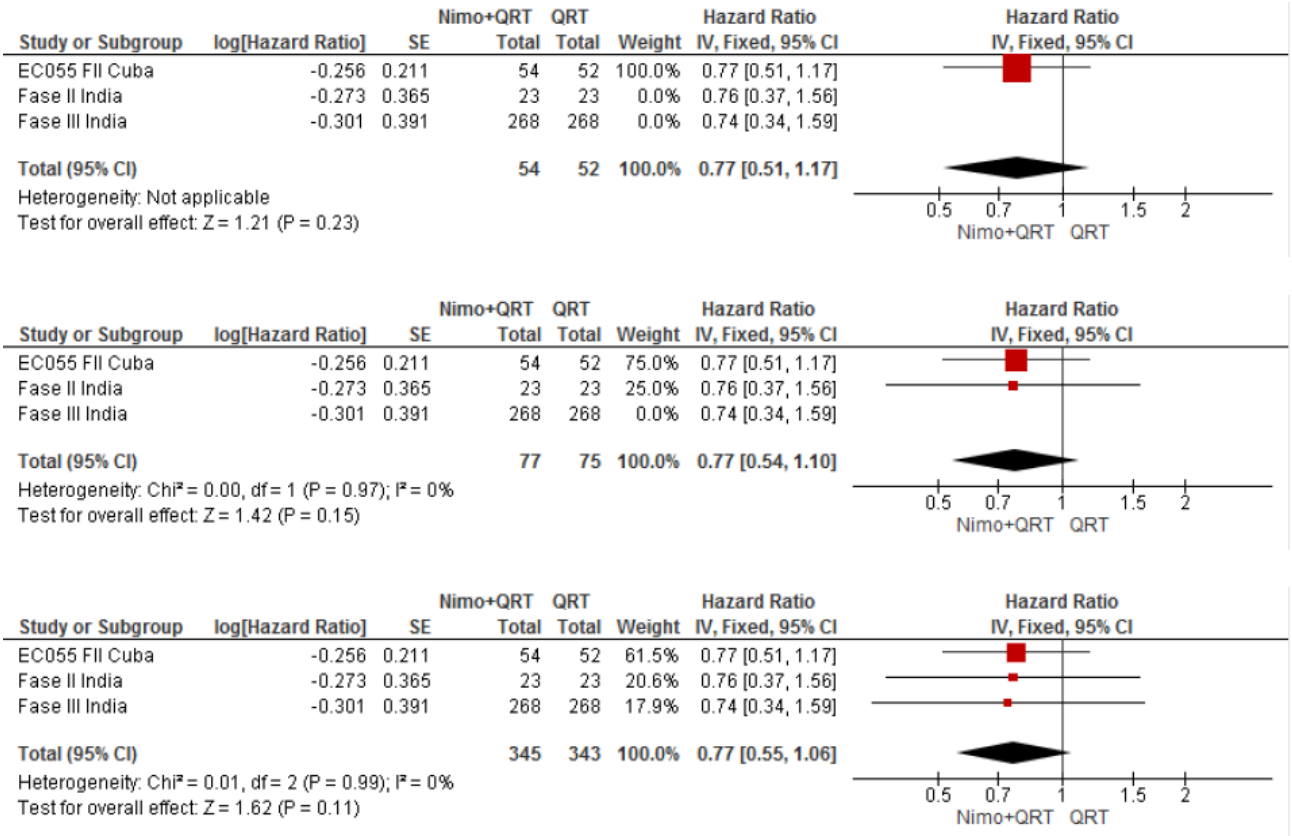

Figura 4. Análisis acumulativo.

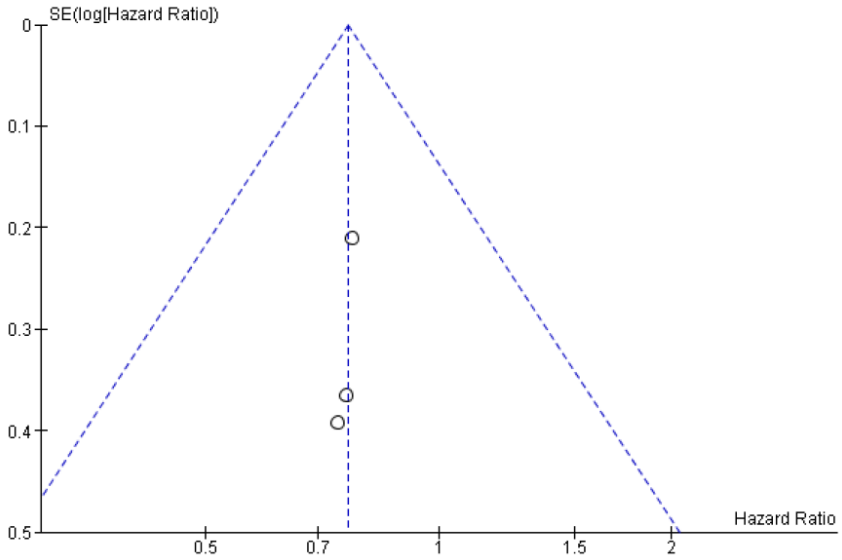

Figura 5. Sesgo de Publicación.

Conclusiones

El nimotuzumab es un novedoso AcM humanizado con efecto antitumoral, que trasciende por el aumento de la supervivencia de los pacientes con tumores avanzados de cabeza y cuello, demostrado por estudios controlados y en la práctica médica. Su perfil de seguridad supera los de otros anticuerpos monoclonales anti-EGFR, lo cual favorece su uso combinado con otras terapias convencionales, su uso como medicamento de mantenimiento prolongado y en poblaciones vulnerables como ancianos y niños.

Sus potencialidades terapéuticas en tumores de origen epitelial, garantizan la continuidad de los actuales estudios de seguridad y eficacia en varias enfermedades.

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CASE REPORTS / REPORTE DE CASO

Sindrome de Touraine-Solente-Gole. (Paquidermoperiostosis primaria). Reporte de dos casos

Touraine-Solente-Gole Syndrome. (Primary paquidermoperiostosis). Two case report

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Resumen: Se presentan dos casos en una misma familia (hermanos) de 30 y 16 años de edad con diagnóstico de paquidermoperiostosis primaria o Síndrome de Touraine-Solente-Goulé (TSG), afección infrecuente, caracterizada por paquidermia, periostosis y paquidactilia, que puede ser idiopática, con inicio en la pubertad, de origen genético, transmisión autosómica dominante. Estos hermanos se presentan a la consulta de medicina interna de del hospital Carlos Manuel de Céspedes quejándose de dolores articulares con manifestaciones cutáneas y osteoarticulares, Inter consultándose con el servicio de dermatología planteándose el diagnóstico de este raro síndrome y realizando estudios pertinentes y luego de su conclusión se hace el reporte clínico para su publicación por ser el primer caso reportado en nuestra provincia.

Palabras clave: Paquidermostosis, genodermatosis, artropatía, paquidermia, periostitis, paquidactilia.

Abstract: Two cases occur in the same family (siblings) of 30 and 16 years of age with a diagnosis of primary pachydermoperiostosis or Touraine-Solente-Goulé Syndrome (TSG), an uncommon condition, characterized by pachydermia, periostosis and pachydactyly, which can be idiopathic, with onset at puberty, of genetic origin, autosomal dominant transmission. These brothers come to the internal medicine office of the Carlos Manuel de Céspedes hospital complaining of joint pain with skin and osteoarticular manifestations, consulting with the dermatology service considering the diagnosis of this rare syndrome and conducting relevant studies and after its conclusion makes the clinical report for publication because it is the first case reported in our province.

Key words: Pachydermostosis, genodermatosis, arthropathy, pachydermia, periostitis,pachydactyly.

Introducción

La paquidermoperiostosis primaria o idiopática es una genodermatosis rara que se presenta una osteoartropatía hipertrófica de origen genético, con un carácter autosómico dominante y penetración variable con predominio en hombres, aunque no se ha encontrado aún el cromosoma responsable y se detectan antecedentes familiares entre el 25-38 % de los pacientes[1,2].

Este síndrome fue descrito por primera vez por Nikoleus Fredreich en 1868 como "hiperostosis de todo el esqueleto" los primeros casos reportados fueron los hermanos Hagner, que tenían características típicas del mismo[3].

La paquidermoperiostosis secundaria, su otra forma clínica, se debe a varias etiologías como enfermedades pulmonares, adenocarcinomas, carcinoma epidermoide bronquial, mesotelioma pleural abscesos pulmonares, carcinomas esofágicos y gástricos, la hipertrofia gástrica con desarrollo de ulcera gástrica (Enfermedad de Menetrier); los carcinomas tímicos, osteosarcomas, leucemia mieloide y el pénfigo paraneoplasico constituyen otras causas[2].

Los criterios de diagnóstico para la TSG son los siguientes: criterios principales: paquidermia, periostosis y paquidactilia, los criterios menores: hiperhidrosis, artralgia, gastritis hipertrófica, ptosis, derrame articular, seborrea, acné y enrojecimiento[4].

Ambas formas clínicas de la enfermedad se diferencian por la edad de aparición; la primaria es propia de la pubertad y juventud, la secundaria es más frecuente entre los 30 a 50 años de edad[3].

La patogenia de este síndrome aún no se conoce bien. Muchas investigaciones en todo el mundo han informado que los altos niveles de factor de crecimiento endotelial vascular (VEGF) en estos pacientes inducen hiperplasia vascular, formación de hueso nuevo, edema y, por lo tanto, el VEGF se postula como el factor de acoplamiento osteogénico-angiogénico prototípico en la patogenia de TSG y mielofibrosis[4-8].

Caso clínico

Caso 1

Se describe el caso de un paciente masculino de 30 años de edad, mestizo, universitario, con antecedentes patológicos personales de buena salud aparente.

A los 15 años de edad, comenzó a presentar un aumento marcado de los pliegues en las arrugas de la frente, trastornos en la sudoración y la secreción sebácea, dolores articulares y aumento ostensible de pies y manos; los cuales notaba eran mayores a los del resto de sus compañeros. Las uñas estaban agrandadas y protuyentes (en vidrio de reloj). Presentó un desarrollo psicosocial dentro de límites normales.

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Examen físico

Se constató facie de angustia de la Paquidermoperiostosis dada por: engrosamiento notable de la piel (demopaquia), con la presencia de surcos y arrugas profundos a nivel de la frente, en cuya localización se observaron surcos gruesos de piel en número de 3 a 4; con un espesor de 2,5 a 3 cms, estos hallazgos son compatibles con la llamada ¨*cutis vértices gyrata* en la región de la frente, donde las arrugas adquieren una presentación cerebriforme, el llamado ¨cutis paquidermis, con paquimenia; moderada hiperhidrosis más marcada en pies y manos y sebocitosis notable en la cara, (figura 1). Estas arrugas fueron disminuyendo en la misma medida de la evolución de la enfermedad.

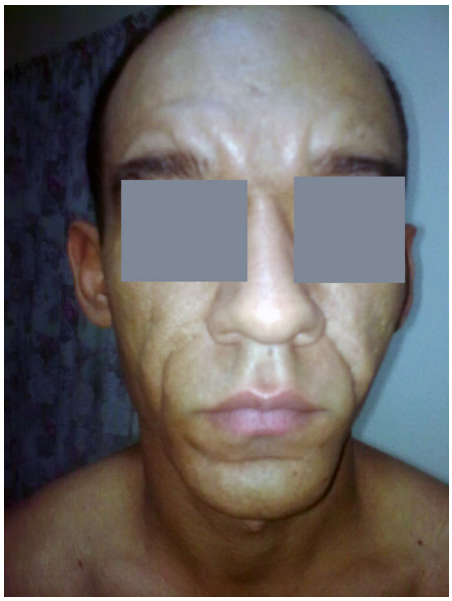

Figura 1. Facie de angustia, *cutis vértice gyrata*.

Al examen físico sistema Osteromioarticular (SOMA), se identificó limitación y dolor a la movilización de la articulación de la rodilla, aumento del tamaño y volumen de los pies y las manos; paquiacria, con engrosamiento cilíndrico notable de los dedos y paquidactilia.

En la piel de los pies y las manos, presentaba una hiperqueratosis moderada, conocida como la queratodermia palmo-plantar, (figura 2)

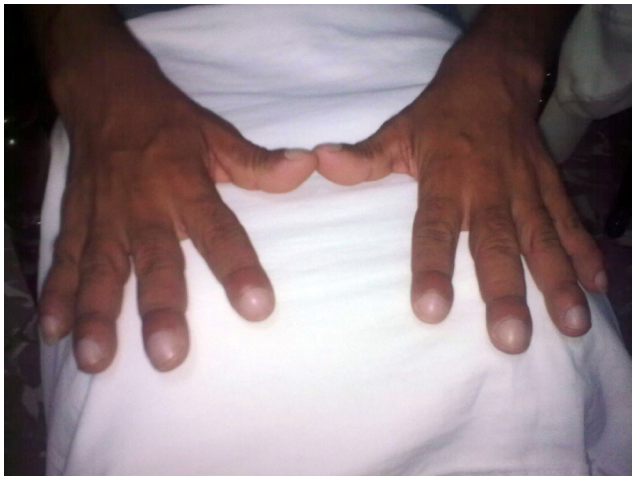

Figura 2. Uñas gruesas en vidrio de reloj, aumento de tamaño de los dedos de las manos y aumento cilíndrico notable de los dedos de las manos.

Exámenes complementarios

Para corroborar el diagnóstico se efectuaron estudios de laboratorio, radiológicos y psicológicos, encaminados a confirmar los criterios clínicos precedentes y descartar otras entidades nosológicas. Se realizaron laboratorio como hemograma completo, glucemia, prueba de tolerancia a la glucosa (P.T.G), colesterol, calcio, fósforo, y conteo de eosinófilos, los cuales mostraron resultados dentro de límites normales.

La tomografía axial computadorizada (TAC) de la silla turca no mostró alteraciones de esa estructura anatómica. En la radiografía de cráneo se evidenció engrosamiento moderado del periostio a nivel cefálico, bóveda craneal gruesa, ausencia de prognatismo y deformaciones faciales.

La proliferación del periostio, con contorno duplicado del hueso y aumentado del grosor cortical de la diáfisis ósea, fueron los hallazgos de la radiografía de la tibia, (figura 3). En la de la mano se encontró engrosamiento de los huesos de la falange, con proliferación periostal, que produce pérdida de la curvatura normal epifisiaria, adquiriendo aspectos de ¨cilindros.

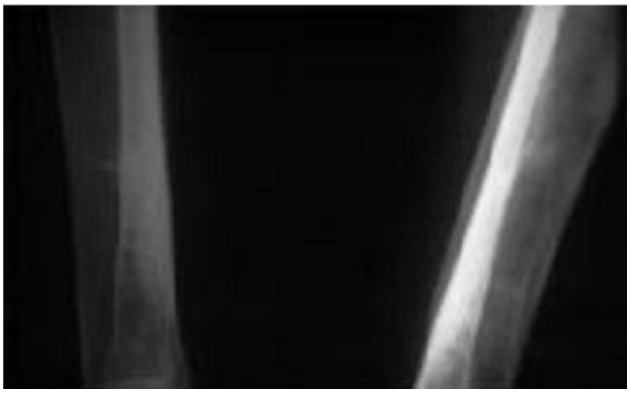

Figura 3. Detalles radiográficos de tibia del caso 1

Caso 2

Se presenta el caso de un paciente masculino de 16 años de edad, raza mestiza, de nivel preuniversitario, con antecedentes patológicos familiares del hermano descrito en el caso 1.

A 15 años de edad aproximadamente, comenzó a presentar un aumento marcado de los pliegues en las arrugas de la frente al igual que su hermano; sudaba mucho y el cutis muy grasiento, dolores articulares y aumento de pies y manos, las uñas algo agrandadas y protuyentes (en vidrio de reloj) y el desarrollo psicosocial dentro de límites normales.

Examen físico

Al examen físico se observó la facie de angustia de la Paquidermoperiostosis, con engrosamiento notable de la piel (demopaquia) y presencia de surcos y arrugas profundos a nivel de la frente. En esa localización encontraron surcos gruesos de piel en número de 3 a 4; con un espesor de 2,5 a 3 cms, constituyendo la llamada ¨cutis verticis gyrata¨, (figura 4); moderada hiperhidrosis y sebocitosis notable en cara. Manos de gran tamaño y dedos cilíndricos, (figura 5).

Comentarios

El síndrome de Touraine Solente Gole (TSG) o Paquidermoperiostosis (PDP) o osteoartropatía hipertrófica primaria (HOA, por sus siglas en inglés) es un trastorno autosómico dominante caracterizado por una tríada de paquidermia (engrosamiento de la piel), cambios esqueléticos (periostosis) y acropachia (clubbing digital)[6].

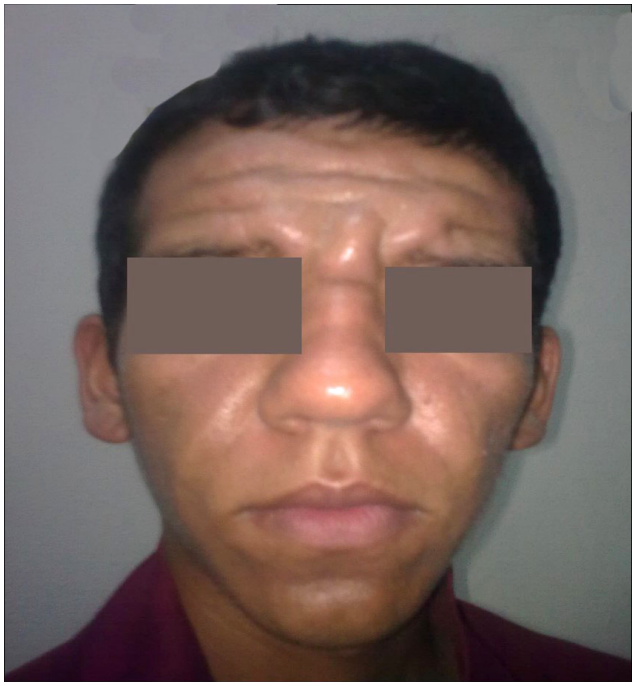

Figura 4. *Cutis vértici gyrata* caso 2.

La presencia de las manifestaciones cutáneas como aparecieron en estos dos casos son las más representativas de la enfermedad, que nos orienta a plantear el síndrome, dada por paquidermia, periostosis y paquidactilia, así como la típica Facie de angustia y el cutis vértice gyrata, alteraciones que estaban presentes en los pacientes estudiados.

El Paquidermostosis primaria, no cursa con manifestaciones sistémica como sucede en la secundaria que se asocia a múltiples cuadros, y en pocos casos existe historia familiar de la misma, suele aparecer en la infancia y la adolescencia, progresa durante varios años y luego se estabiliza[6]. El estudio de este síndrome aún es controvertido debido a su complejidad y amplios aspectos clínicos[9-11]. También hay casos reportados de asociación con blefaroptosis no muy común en estos casos[4].

Conclusiones

Podemos señalar que los dos casos descritos se enmarcan, tanto desde el punto de vista clínico como en el de los exámenes complementarios, dentro de los parámetros establecidos internacionalmente para esta enfermedad. Por lo que podríamos concluir que estamos frente a dos pacientes con forma completa de Paquidermoperiostosis primaria TGS, entidades raras y primeros casos descrito en la provincia de Granma de Cuba.

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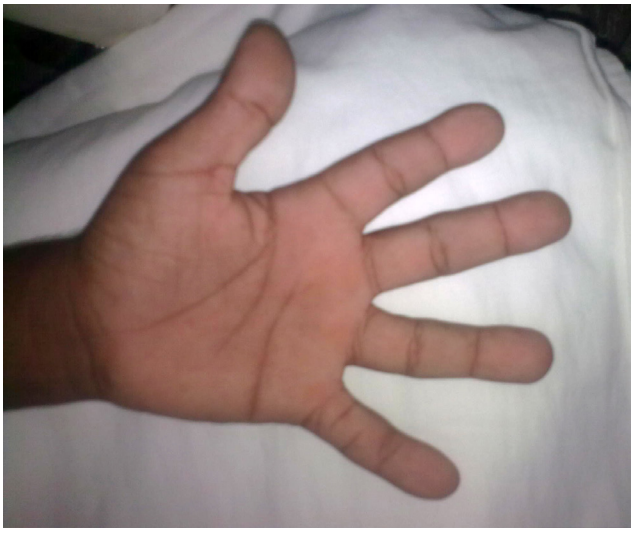

Figura 5. Aumento de tamaño de la mano y aspecto cilíndrico de los dedos.

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REVIEW / ARTÍCULO DE REVISIÓN

Extrapulmonary tuberculosis

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Abstract: Extrapulmonary tuberculosis can be confused with other pathologies because of the variety of symptoms it generates according to the affected organ. So, extrapulmonary tuberculosis must always be taken into account by medical staff within the differential diagnosis. In this paper, a review of the literature on extrapulmonary tuberculosis is carried out with emphasis on the most frequently affected organs.

KeyWords: Extrapulmonary tuberculosis, HIV, social stigma, differential diagnosis.

Introduction

Tuberculosis is an infectious disease caused by the Mycobacterium tuberculosis bacteria and is transmitted from person to person through the air. It is enough for a person to inhale a few bacilli to become infected. It is estimated that a quarter of the world's population has latent tuberculosis, this term applied to people infected by the bacillus but who have not yet become ill or can transmit the infection. Infected people have a lifetime risk of 5-15% of getting sick with tuberculosis; on the other hand, immunosuppressed people, for example, patients with HIV, malnutrition, diabetes, and smokers are at a much higher risk of becoming ill[1].

Tuberculosis is one of the ten leading causes of mortality in the world. In 2017, 10 million people got infected with tuberculosis, and 1.6 million died from this disease (including 0.3 million people with HIV). Which is why tuberculosis is also considered as one of the leading causes of death among people with HIV. Within the pediatric population, it is estimated that in 2017 one million children got tuberculosis and 230,000 children died due to this cause (including children with HIV-associated tuberculosis). Tuberculosis is an infectious disease with a very high mortality rate; it causes around 4500 deaths every day. Communities bear the heaviest burden with socio-economic problems, those who work and live in high-risk environments and the poorest and most marginalized[2].

The region of the Americas has significantly reduced new cases and deaths from tuberculosis in the last 25 years. Despite this, it is estimated that almost 270,000 people contracted the disease in 2015 and that nearly 50,000 do not know they have it yet. In the Americas, the population with a higher risk of infection are people with HIV, homeless, slum dwellers, deprived of liberty and people with addiction problems, communities that generally have limited access to health care and, if they do, they are not always diagnosed with tuberculosis when they suffer from it[3].

The countries of the region are working to make the Americas the first area in the world to achieve the elimination of tuberculosis as a public health problem and currently, 15 countries have a low incidence of tuberculosis (Figure 1)[4].

In 2015, the estimate by the World Health Organization for Ecuador was 8,400 new cases of tuberculosis (51.6 / 100 thousand inhabitants), including those with Tuberculosis / HIV coinfection. However, the National Health System (SNS) comprising the Comprehensive Public Health Network (RPIS) and the Complementary Network (RC) diagnosed and notified 5,215 cases (32.03 / 100 thousand inhabitants), fulfilling 62.08% of the estimated. Of the reported cases, 5,097 correspond to new and relapsed cases, and 118 previously treated cases (Ministry of Public Health 2017)[5].

Tuberculosis is described as the leading cause of death of infectious origin worldwide, therefore it is considered a global health problem; Taking this situation into account, we have reviewed the literature on extrapulmonary tuberculosis with emphasis on the most frequently affected organs.

Generalities of extrapulmonary tuberculosis

In general, the development of extrapulmonary tuberculosis is associated with immune deficiencies, genetic susceptibility, and unknown factors. Risk factors for extrapulmonary tuberculosis are immunosuppression, long-term illnesses such as HIV, children under 5, old age (over 60), female sex, race, and ethnicity (Asian or African origin). However, the large difference in the incidence of extrapulmonary tuberculosis between countries suggests that there are several associated factors such as; the increase of immigrant population that have a frequent delay in medical treatment, sub-registration, and genetic variations of the population and tuberculous mycobacteria (for example from Africa to Europe). Extrapulmonary tuberculosis has a high mortality rate, especially when considering the severity of some forms such as miliary or disseminated, and the prevalence of HIV[6].

Early detection and treatment have been identified as essential elements in the Strategy of the World Health Organization (WHO) to end tuberculosis, a disease that remains a global health problem. Some of the challenges in the fight against tuberculosis are the inequality in access to medical care, resistance to multiple medications, co-infection with human immunodeficiency virus (HIV), the presence of a large group of latent infections and the delay in diagnosis.

Nonetheless, in high- and low-income countries, the delay in presentation, diagnosis and treatment of pulmonary tuberculosis is well documented. Which is why it is important to acknowledge that the rapid onset of tuberculosis treatment is not only crucial for the prognosis of the individual patient, since the delay in diagnosis has been associated with more serious clinical presentations, but also for the people who surround him because it carries a higher risk of transmission of Mycobacterium tuberculosis. Although the overall incidence of tuberculosis decreases in many regions, the increasing

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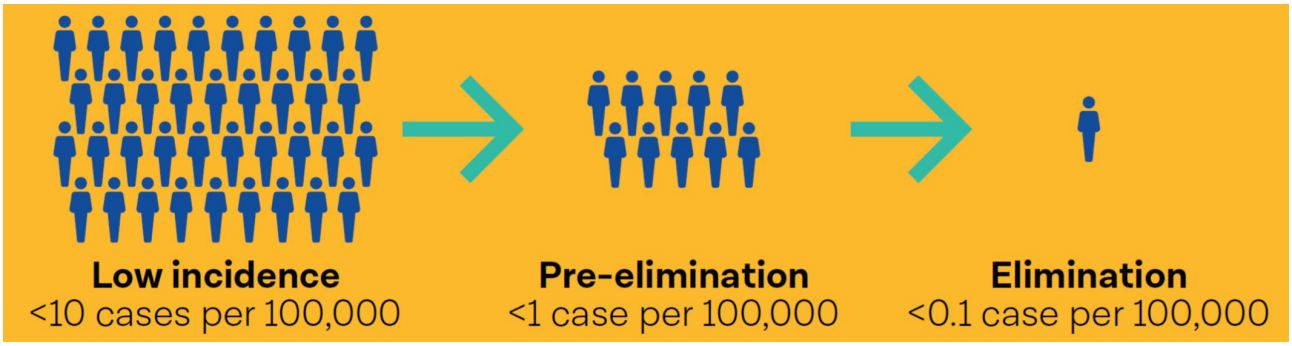

Figure 1. Road to the elimination of tuberculosis (taken from PAHO. Tuberculosis in the Americas 201.

proportion of extrapulmonary tuberculosis is often neglected, consequently, a limited number of studies contain complete information on delays in the diagnosis of extrapulmonary tuberculosis[7].

In some cases, the social stigma related to this highly transmissible pathology limits patients' access to health units. And in the cases that can overcome the social stigma, other factors such as physical, economic and geographic inaccessibility to health services (especially in rural areas) may play a role in late diagnosis and treatment[8].

On average in the world, pulmonary tuberculosis represents 85% of clinical forms of tuberculosis, while extrapulmonary tuberculosis represents the remaining 15%. The most common types of extrapulmonary tuberculosis in adults include lymphatic, pleural, bone, meningeal, genitourinary and peritoneal tuberculosis. Nevertheless, the prevalence of extrapulmonary tuberculosis and its predominant forms varies from country to country. For example, Ethiopia has reported an incidence of 32%, which makes it the third country in the world, after India and Pakistan, with the highest rate of extrapulmonary tuberculosis; a number that has remained high over the years despite the country's population size.

Global efforts to control tuberculosis have largely ignored extrapulmonary tuberculosis. This is because extrapulmonary tuberculosis is generally considered non-infectious and, as such, has no consequences on the global epidemic. However, recent data from northwestern England has shown that the prevalence of active tuberculosis disease among domestic contacts of extrapulmonary tuberculosis was high (440 per 100,000 contacts examined), which indicates that cases of extrapulmonary tuberculosis have a significant impact on tuberculosis transmission. It should also be contemplated that the slower annual decline rate of extrapulmonary tuberculosis compared to pulmonary tuberculosis could delay progress towards the END-TB targets set by the World Health Organization[9].

The pediatric population, especially those under 3 years, have an incidence of up to 40% of developing tuberculosis after primary infection. Tuberculosis has a non-specific clinical presentation with a variety of imaging characteristics depending on the organs involved, which is why it often mimics other diseases. The most common site of tuberculosis in pediatric patients is the lungs (about 80%). The rest of the cases involve extrapulmonary sites: lymph nodes (67%), meninges (13%), pleura (6%), miliary dissemination (5%) and the musculoskeletal system (4%), with abdominal, renal and cutaneous involvement being less common [2]. Immunocompromised children, infants and adolescents have a higher risk of developing extrapulmonary tuberculosis. Pediatric extrapulmonary tuberculosis has an insidious onset without constitutional signs and symptoms in 72% of patients[10].

The following can be considered as clinical clues to cause suspicion of extrapulmonary tuberculosis[11]: Ascites with lymphocyte predominance and negative bacterial cultures; Chronic lymphadenopathy (especially cervical); CSF lymphocytic pleocytosis with elevated protein and low glucose; Differential diagnosis of Crohn's disease and amebiasis; Exudative pleural effusion with a predominance of lymphocytes, negative bacterial cultures and pleural thickening; HIV infection; Joint inflammation (monoarticular) with negative bacterial cultures; Persistent sterile pyuria; A country of origin that has endemic tuberculosis; Unexplained pericardial effusion, constrictive pericarditis, or pericardial calcification; Vertebral osteomyelitis that affects the thoracic spine.

Lymph node tuberculosis

Lymph node tuberculosis or tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis. According to its location, it is divided into intrathoracic and extrathoracic (also called peripheral lymphadenitis). Its most common location (the neck) has been known since ancient times as scrofula. The different routes of infection vary depending on which lymphatic group is affected. Cervical involvement is produced by direct contact of the bacilli with the Waldeyer ring and through it by dissemination and involvement of the adjacent ganglionic chains. It usually presents with unilateral, multiple and matt swelling of the neck in young adults, being a diagnosis and therapeutic challenge because it mimics other pathological processes, and it has an inconsistent physical performance and laboratory findings. The incidence of mycobacterial lymphadenitis has increased in parallel with the incidence of mycobacterial infection and HIV worldwide; so, it is important to differentiate tuberculous cervical lymphadenitis from non-tuberculous mycobacterial lymphadenitis, because their treatment protocols are different[12,13].

In the peripheral ganglionic form, the most frequent pathogenic mechanism is the reactivation of a primary pulmonary tuberculous infection, previously disseminated by the hematogenous route. Abdominal lymph node tuberculosis is also described by Mycobacterium bovis, secondary to ingestion of raw cow's milk, in less developed countries[12].

Since tuberculosis is commonly considered a chronic lung disease, most studies of tuberculosis focus on the lungs, while lymph nodes are almost always represented only as antigen-presenting and immunological activation sites. Nonetheless, lymph nodes are among the most frequently infected sites of Mycobacterium tuberculosis, aside from the lungs. The effects of Mycobacterium tuberculosis infection and how lymph nodes respond to infection by this bacterium is currently unknown, as a consequence, many experimental studies have been conducted in macaques, which have found that general lymph nodes are not effective killers of Mycobacterium tuberculosis. In addition, the studies showed that the infection destroyed the

lymph node structure and this, in turn, was associated with the increased bacterial load. After a brief course of antituberculous therapy, the reduction of the bacterial load was lower in the lymph nodes compared to the pulmonary granulomas. So, it was concluded that the lymph nodes, apart from being sites of antigen presentation and immune activation, can also be niches of growth and persistence of Mycobacterium tuberculosis[14].

In lymphatic tuberculosis, the low culture rate of Mycobacterium tuberculosis forces the clinician to treat the disease without a drug sensitivity test of the evolving organism. This lack of evidence to base the choice of the drug leads to three important problems. First, there is an equally significant proportion of patients with Mycobacterium tuberculosis bacilli resistant to drugs in extrapulmonary tuberculosis as in pulmonary tuberculosis. Second, lymphatic tuberculosis usually responds slowly to tuberculosis treatment and the patient's condition may deteriorate paradoxically during therapy. Third, in extrapulmonary tuberculosis, there are no documented standard guidelines that stipulate the optimal duration of treatment or outcome like in pulmonary tuberculosis. Therefore, even though it is recommended that 6 months of standard tuberculosis treatment is adequate for most cases of lymphatic tuberculosis, many doctors fear the recurrence of tuberculosis in actual clinical practice, especially in cases treated without a drug resistance tests[15].

In a study conducted in Denmark, tuberculous lymphadenitis was the most frequent manifestation of extrapulmonary tuberculosis; with a predominance in young immigrants and less frequently in vulnerable Danish patients. The majority of Mycobacterium tuberculosis isolated in this population harbored unique genotypes that suggest that tuberculous lymphadenitis could be associated with the reactivation of latent tuberculosis infection. Consequently, if this is really an association, future targeting of tuberculous lymphadenitis in risk groups could potentially reduce the number of cases in this country, with the vision of eliminating tuberculosis in the future[16].

Extrapulmonary tuberculosis is most often diagnosed in peripheral lymph nodes. Due to the appearance of a lateral mass of the neck, the differential diagnosis may include neoplastic, infectious or immunological diseases, sarcoidosis, abscesses, tuberculosis, brucellosis, syphilis, toxoplasmosis, cat scratch disease and fungal infections. The presence of draining sinuses with purulent material suggests infectious and suppurative conditions such as pyogenic abscess, or other infectious processes[17].

In another study conducted in the United Kingdom, it was shown that cervical tuberculous lymphadenitis was a condition that affected almost exclusively residents of the country that were born abroad in high-risk countries. The majority of patients were between 20 and 50 years old, generally in good health and had often resided in the United Kingdom for several years before the presentation. It was also found that the lymph nodes of the posterior triangle were more frequently involved and that constitutional symptoms occurred in a minority of patients[18].

Extrapulmonary tuberculosis, although not as common as pulmonary tuberculosis, also exists in the United States. Their guidelines state that to reduce the differential diagnoses of a patient with isolated cervical or diffuse lymphadenopathy, an investigation into the country of origin and travel history should be made. Endoscopic ultrasound, fine needle aspiration, and fine needle biopsy are useful tools for the diagnosis of lymphatic tuberculosis, especially if the abdominal lymph nodes are the most accessible. The therapeutic approach is effective in most patients and surgical intervention can be avoided[19].

The well-established treatment for patients with drug-susceptible tuberculosis lymphadenitis includes a 6-month regimen with isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) during an intensive phase of 2 months, followed by a continuation phase of 4 months of treatment with INH and RIF. For HIV-TB coinfection, it has been suggested that the treatment protocol should include an antiretroviral therapy (ART) regimen. After 6 months of treatment, the reduction in lymph node size has been seen in less than 5 mm in 83% of patients. However, a paradoxical improvement reaction has also been observed in 20-30% of tuberculosis lymphadenitis. Surgical excision of ulcers and / or paranasal sinuses of the affected lymph nodes has been required to improve the effectiveness of the treatment when patients have little or no response to pharmacological treatment. Some variants have been reported in the duration of the continuation phase, because of cases with a poor response or because of episodes of drug resistance. As shown in a study, the 9-month regimen was recommended when treatment responses were delayed. It was reported that cure rates ranged from 89% to 94% when the duration of the regimen increased from 6 to 9 months, yet some patients may maintain persistent evidence of tuberculosis or develop relapses or recurrences after completing treatment[20].

According to some findings, the diagnosis of tuberculous lymphadenitis requires a multifaceted approach that involves microbiology, pathology, radiology and other clinical specialties for the evaluation of suspicious cases. Laboratory findings should always be interpreted together with clinical pictures to consider a patient for an accurate diagnosis. The addition of culture for M. tuberculosis to the diagnostic algorithm together with fine-needle aspiration cytology and Ziehl-Neelsen staining helps to clarify the diagnostic dilemma with a high level of accuracy in cases of suspicion and may be useful in reducing tuberculosis burden. Still, more studies are needed to investigate the immune mechanism and its correlation with M. tuberculosis, so that patients that are negative for tuberculous lymphadenitis in fine-needle cytology but are positive for culture, can be diagnosed and treated with precision[21].

Abdominal tuberculosis

The most common pathogenesis of both abdominal/peritoneal tuberculosis and tuberculous meningitis/encephalitis is the hematogenous spread of primary pulmonary tuberculosis. All of these diseases are forms of disseminated tuberculosis; however, only 15 to 25% of patients with abdominal tuberculosis and 40% of patients with tuberculosis meningitis have concomitant pulmonary tuberculosis. Extrapulmonary tuberculosis occurs most frequently months after pulmonary tuberculosis[22].

Risk factors for abdominal/peritoneal tuberculosis specifically include alcoholism, liver disease, and cirrhosis, continuous ambulatory peritoneal dialysis for chronic renal failure, diabetes mellitus and Bacillus Calmette-Guérin therapy for superficial bladder carcinoma[22].

Abdominal tuberculosis is an important form of extrapulmonary involvement, which is especially difficult to diagnose first due to the difficulty in tissue acquisition and second because it closely mimics certain conditions such as Crohn's disease and malignant neoplasia. In addition, the sequelae of intestinal tuberculosis, such as pain resulting from

intestinal stenosis or peritoneal adhesions can be confused with the activity of the ongoing disease, which persuades doctors to prescribe a treatment of longer duration[23].

Tuberculosis of the digestive system has many important relationships for its development. These relationships include some conditions, such as low socioeconomic status, immunosuppression status, as well as HIV infections, alcoholism or drug addiction. The symptoms presented in this disease are nonspecific, because the clinical picture is similar to that of other diseases, so the histopathological study remains the reference pattern for its diagnosis. In the literature, several mechanisms have been proposed by which tuberculosis spreads to the abdominal cavity, including the hematogenous, lymphatic or ingestion of mycobacteria[24].

Pleural tuberculosis

Pleural tuberculosis should be suspected in any patient with unilateral pleural effusion of any size. It typically presents as an acute condition with fever (75%), chest pain with a pleuritic characteristic (50-75%) and non-productive cough (70-75%). More than two-thirds of patients are symptomatic for less than 1 month and the rest are symptomatic less than 1 week. Additional symptoms of presentation may be night sweats (50%), chills, weakness, dyspnea (50%), and weight loss (25-85%)[25].

In a study conducted in Italy, it was reported that 28% of cases of pleural tuberculosis were diagnosed as a result of thoracoscopy sampling. Spontaneous resolution of pleural tuberculosis is a well-known phenomenon, but 65% of patients can progress to pulmonary or extrapulmonary tuberculosis within 5 years. The usefulness of thoracoscopy for the diagnosis and treatment of pleural tuberculosis has already been previously reported, emphasizing that it is minimally invasive and can be performed under local anesthesia. Other diagnostic tests such as adenosine deaminase (ADA), interferon (IFN) -γ, interleukin-2, tumor necrosis factor-α, nucleic acid amplification tests (NAATs) and interferon-γ release assays are used to diagnose tuberculous pleural effusion but are only suggestive of the diagnosis, do not identify resistant mycobacteria and cannot guide treatment[26].

You can also use a real-time polymerase chain reaction test for tuberculosis, which consists of a real-time amplification for the qualitative detection of the M. tuberculosis complex in biological materials (the result is obtained in an average of 3.5 days). The DNA is extracted from the samples; amplified and detected by fluorescent probes specific for M. tuberculosis[27].

Currently, there is no diagnostic method with high sensitivity and specificity, low cost, ease and speed to perform it and that is widely available in areas where pleural tuberculosis prevails. Although it is usually observed in middle-aged men, with a time of evolution less than a month, such as a pleural exudate type exudate with a predominance of lymphocytes, the diagnosis of certainty has its limitations. Therefore, the clinical, epidemiology, Imaging studies, pathological anatomy and indirect laboratory tests, such as the determination of adenosine deaminase (ADA) levels, are a valuable contribution to the diagnosis[28].

Despite the availability of countless diagnostic tests, and accurate test for pleural tuberculosis is still missing. The existing definition and criteria used for the classification of patients with pleural tuberculosis are not uniform; therefore, in the absence of a uniform case definition, it is a challenge to correctly evaluate and compare the performance of newly developed pleural tuberculosis diagnostic tests[29].

Genitourinary tuberculosis

Renal involvement in tuberculosis may be part of a disseminated infection or a localized genitourinary disease. Urogenital tuberculosis accounts for 27% of extrapulmonary cases, even though renal involvement due to tuberculosis infection is underdiagnosed in most health centers. Most patients with renal tuberculosis have sterile pyuria (in the absence of common bacterial infection), which can be accompanied by microscopic hematuria[30].

Genitourinary tuberculosis occurs due to hematogenous dissemination of tubercle bacilli, it can cause complications such as ureteral stenosis, chronic pyelonephritis and papillary necrosis, which can lead to impaired renal function. Tuberculous bacilli have a predilection for the upper and lower poles of the kidney and can form granulomas within the kidney that can remain indolent for many years. Image findings of renal tuberculosis result from the combination of papillary necrosis and parenchymal destruction[31].

Since there is a high rate of false negatives (20%), a single urine culture-negative for mycobacteria does not mean the absence of genitourinary tuberculosis, so it is advisable to take at least 3 samples of the middle stream of the first-morning urine to be able to isolate the organism[32].

For about 10% of patients with urogenital tuberculosis, the diagnosis is possible and is based on suggestive clinical, laboratory, and radiological findings, without microbiology or histological confirmation. The identification of the bacillus of tuberculosis in urine is achieved through Ziehl-Neelsen staining or by urine culture in Lowenstein-Jensen. The first one is fast, with 96.7% specificity but only 42.1 to 52.1% sensitivity. The culture is the gold standard for urogenital tuberculosis, with a tenderness that varies widely, from 10.7 to 90%, and the results may take 6 to 8 weeks to obtain. Since bacilluria is sporadic and weak, three to six urine samples are required[33].

Renal tuberculosis with few clinical symptoms may be associated with several factors, one of them is the widespread use of broad-spectrum antibiotics; particularly fluoroquinolones. The symptoms may be masked by a nonspecific infection, calculus or tumor. It has also been described that doctors failed to identify or suspect renal tuberculosis in patients that did not show symptoms such as urinary frequency, urgency and dysuria, but showed back pain and hydronephrosis. Young doctors with little experience often settle for a diagnosis of kidney stones or tumor, while neglecting the possibility of renal tuberculosis[34].

Fighting a global health problem

The diagnosis of extrapulmonary tuberculosis is a challenge and many patients start an empirical treatment against tuberculosis without a laboratory-confirmed diagnosis. Therefore, controlling the response to treatment is important to ensure a correct diagnosis and proper management of the disease, yet the definition of a satisfactory response to treatment in extrapulmonary tuberculosis remains unclear[35].

It is recommended to use the same antibiotic regimen with a duration of 6 months. When there is the involvement of the central nervous system, the regimen should be prolonged to 12 months, and to in the case of tuberculous spondylitis with neurological involvement it should last 9 months. Mainly because in these patients, a shorter regimen has been associated with an increased risk of relapse. The treatment guideline is 2 months with rifampicin, isoniazid, pyrazinamide, and ethambutol, followed by 4 months of rifampicin and isoniazid. Once the sensitivity to first-line drugs is identified by

antibiogram, ethambutol can be removed[36].

Ensuring the proper management of household contacts of patients with tuberculosis is a logistical challenge. The cascade of attention for contact management presents many opportunities to get blocked: contacts may not be identified, evaluation procedures may not be available, therapy may not be prescribed, initiated or completed. Certain barriers have been consistently informed from various settings some of them is that contacts may face difficulties in traveling to health facilities for evaluation, that there are knowledge gaps between health workers and patients, and that there is low acceptability of taking medications among people who do not feel sick[37].

National tuberculosis control programs in several countries have patient education programs that make emphasize only the cardinal symptoms of pulmonary tuberculosis.

Consequently, patients may not know about extrapulmonary tuberculosis, which is why it has been observed that many patients with extrapulmonary tuberculosis have advanced disease. Therefore, it is necessary to understand patients with extrapulmonary tuberculosis and their behavior to develop a strategy that improves diagnosis and treatment. The determination of extrapulmonary tuberculosis has always been a challenge for health care providers, and management generally requires more resources and more clinical experience than other diseases. In endemic tuberculosis sites with limited resources, the lack of availability of laboratory facilities in peripheral health centers often delays the diagnosis of extrapulmonary tuberculosis. Also, the increasing incidence rates and sparse literature on the various forms of extrapulmonary tuberculosis are grounds of concern that should be taken into account when designing significant diagnostic and treatment guidelines for national tuberculosis control programs[38].

Conclusions

Extrapulmonary tuberculosis accounts for an essential percentage of patients with mycobacterial infection. Because many organs may be involved, extrapulmonary tuberculosis can present with a diversity of symptoms that can be confused with other pathologies. If the deceptive clinical presentation is associated with the lack of knowledge about this disease, an inevitable delay in both the diagnosis and the treatment results. Control programs must be improved by taking into account extrapulmonary tuberculosis as part of the public health problem.

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REVIEW / ARTÍCULO DE REVISIÓN

A new area of application and research in bio-processes: Biotechnologies in civil construction

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Abstract: Construction Biotechnology is a new scientific and engineering discipline that has been developing exponentially during the last decade. The main directions of this discipline are 1- the selection of adequate microorganisms, 2- development of micro-processed construction bioprocesses as well as 3- the development of new biotechnologies to produce construction biomaterials. Products resulting in construction biotechnologies are low-cost, sustainable, and environmentally friendly microbial biocements for the improvement of the construction terrain. The bioagents used in construction biotechnologies are pure or enrichment cultures of native microorganisms or microorganisms isolated and activated from the soil. Biotechnologically produced construction materials and microbial mediated construction technologies have many advantages compared to conventional construction materials and processes. The current technological landscape offers an objective vision and perspective of how microbes are used in the construction industry as additives for cement and concrete so that these new technologies be used in different provinces of Ecuador. In that sense, the current situation of cement and concrete production in Ecuador is briefly described to have an overview of the applicability of the new methods based on biogenic materials and the environmental advantages of the creation of construction biomaterials over conventional production.

KeyWords: Cement, concrete, biomaterials, biogenic, biotechnological, microorganisms.

Introduction

Construction Biotechnology is a new scientific discipline, named in such a way due to its analogy with medical, environmental, agricultural and food biotechnology. This modern science successfully combines the application of scientific knowledge about engineering methods for the production of construction biomaterials as well as the use of bioprocesses in the construction industry[1–3].

The use of construction biomaterials is one of these novels, friendly and sustainable alternatives since the raw material used mostly is renewable biological resources, e.g. agricultural biomass residues and recently also waste microorganisms resulted from other industries have gained popularity for its production[3].

The industry-related with the conventional production of construction materials consumes a large amount of energy being harmful to the environment. The power consumed represents between 20 and 40% of the total production cost; however, there are novel alternatives that reduce up to 10% of the energy used in conventional manufacturing[1,4].

Different biotechnological products and biotechnologies applied to civil engineering are being developed in that direction (figure 1). The reduction of the environmental impact of the conventional production of construction materials together with a decrease in production costs, use of waste in secondary processes, increased quality and useful life of the materials obtained. These issues, among others, constitute the main advantages of this technology.

The use of biologically based products has increased at a steady pace in the last decade. It is estimated that by 2020 the global market based on bioproducts reaches $ 250 billion and that by 2030 a third of the materials that will be produced will come from biological resources[5]. This study raises the possibility of implementing bio-cements and bio-concrete in Ecuador based on economic reviews and its possible application in several areas where there is a higher demand for construction. Provinces of Pichincha and Guayas, due to their characteristics of great cement producers, could be the main areas to study these environmentally friendly materials.

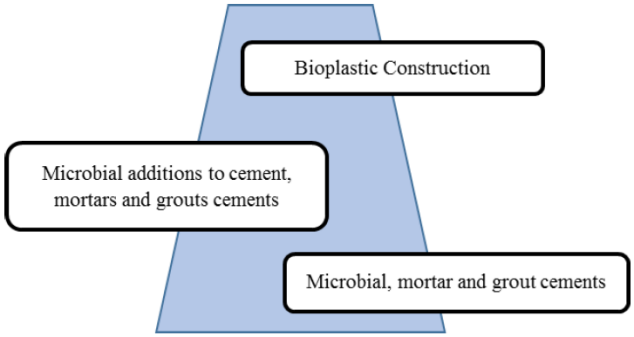

Figure 1. Overview of Biotechnology strategies used for construction materials.

Current situation of convencional cement and concrete production in ecuador

In 2010, the net profitability of the conventional cement production market was 31.56%, billing 600 million dollars, adding the real estate boom and the works carried out by the government, which undoubtedly boosted the production and consumption of this item as seen in figure 2.

Figure 3 shows that the provinces of Pichincha and Guayas had the highest cement consumption at that time due to the sizeable real estate growth. It should be taken into account, and as a curious fact, the number of competitors in this branch is quite low in the country since there are only four companies immersed in this area, being two of them private multinationals Holcim and Lafarge, and the public companies, Chimborazo and Guapán.

As another curious fact, the cost of 1 cement sack was about $8-9.00 US, one of the lowest prices in the market compared to other countries in the region like Bolivia, Colombia, or Venezuela.

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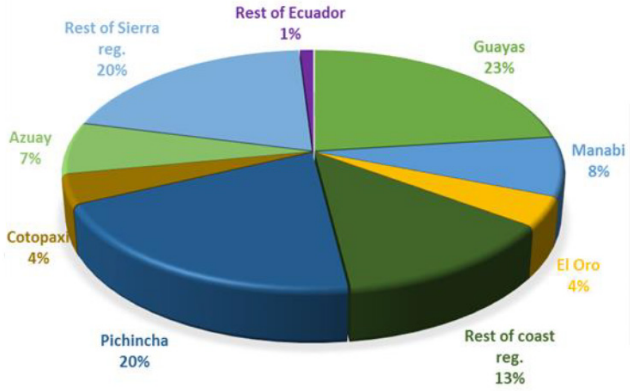

Figure 2. Monthly marketing of gray cement (in tons)[6].

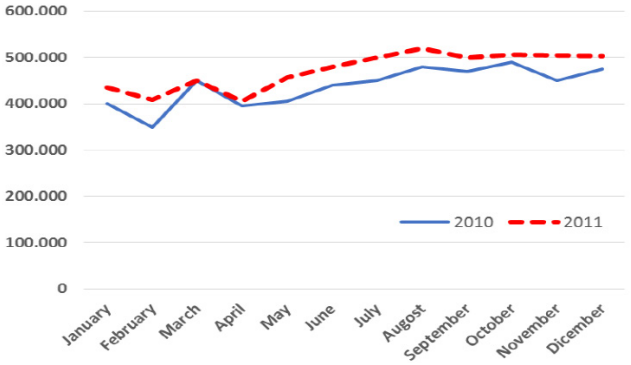

Figure 3. The concentration of Sales by Province[6].

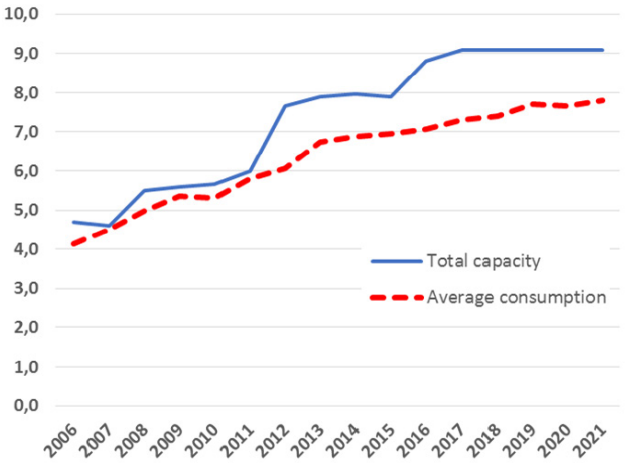

Figure 4. Cement growth projections (in millions of tons per year)[6].

According to figure 4, an increase in cement production has been seen during the beginning of 2018, registering a total of 802.00 metric tons (MT), which meant a rise of 0.2% compared to the level obtained in January 2017[7].

With this productive increment in the cement industry, measures must be taken to contribute positively to the care of the environment. However, the production of some toxic solid particles constitutes a problem in agriculture, and discomfort in surrounding populations.

Granulometric analyses show that 25% of the particles are between 1 and 10 microns that could be mobilized over vast distances. About 3% of these particles are submicronic considered very harmful if they were in high concentrations at ground level.

The contamination also occurs at the air level due to the emission of combustion gases containing carbon monoxide and carbon dioxide in addition to the presence of water pollutants that are produced by the spilling of feed material from the oven, producing pH changes. The presence of suspended and dissolved solids, the presence of leachate in the areas where the contents are stored, is also a potent water pollutant.

Taking into account the process of gradual deterioration of our planet, alternatives that allow balancing this process produced mainly by the hand of man should be considered so, in this work we propose as an alternative, familiarize the scientific community in the application of biotechnological tools in the Cement industry that includes the use of microorganisms and their potential characteristics.

Updating biotechnologies applied to construction materials. progress and challenges

After three centuries of industrialization, human beings see themselves in the overwhelming need to seek potential in natural systems. This potential in the area of construction currently translates into the creation of new bio-inspired materials, which mix biological and engineering processes within a research area called biotechnology.

Martin-Manzanares[8] proposes three possible directions of application of biotechnologies to the construction industry: 1- work with 100% organic material with thermal insulation functionality and structural qualities, 2- the use of micro luminescent micro-organisms for the design of devices with the ability to emit light without electricity consumption, and 3- cementation of granular structures mediating the use of environmentally friendly bacterial populations, without toxicity or corrosion.

Concrete is a material used in construction that dates back to ancient Greece, with approximately 500 B.C., the elements used in its elaboration were modified by the Romans, who implemented in buildings such as the Colosseum and the Pantheon of Agrippa. Its reliability of use in construction, tension, and time generates deterioration in its structure and causes the appearance of cracks, which allow the filtration of rainwater until it reaches the steel reinforcements, which causes them to erode.

Development of materials and construction systems requires the study of microorganisms such as bacteria, fungi or algae, in the improvement of properties of conventional materials such as concrete; in the creation of new construction materials with similar characteristics to existing materials, with the advantage that their production processes are more sustainable; and in the incorporation of some of these microorganisms into new construction systems, which in addition to providing an aesthetic component, develop an energy task in the form of biomass.

Prototypes of use in cementing granular structures have been developed through the use of bacterial populations that deposit calcite, for the manufacture of bricks, soil stabilization and compaction, restoration of monuments and cracks in concrete, and construction of roads and other structures[10].

Henk Jonkers[11,12] developed a method that can be applied to concrete using microorganisms. After several experiments, this researcher chose calcium lactate as a substrate, supplied in biodegradable plastic capsules and added them in the wet mixture of bioproducts.

"We believe that our concrete will revolutionize the way it is currently built because we have been inspired by nature. Plants and animals can heal themselves so, we have achieved that concrete can imitate them," said Jonkers.

The human principle applied in Jonker 's theory is based on the human ability to regenerate bone tissue by mineralization.

In the same line and within this range of construction

products, we have the Bio cement developed by the researchers of Delft Technical University (Holland) considered as a new type of cement with the capacity of self- regeneration in case of cracks or breaks[10]. The mixture of this cement contains bacteria that, on contact with water, can produce limestone that naturally could fill any fissure.

This self-repairable material contains granules with spores of bacteria and calcium lactate, a necessary nutrient that bacteria need to survive. The spores remain inactivated, pending an activation by contact with rainwater introduced by the cracks. Rainwater can activate the mechanism of calcite production that is the product of this reaction to fill the cement gaps.

Currently, researchers have confirmed the sealing of cracks up to 0.5 mm in laboratory phases. It is expected in the future to make it available in real conditions. The self-repairable cement could be on the market in two or three years.

For this process, he used the bacteria *Bacillus pseudofirmus* and *Sporosarcina pasteurii*, which can be found in lakes with high levels of salinity very close to volcanoes. The lifespan is a maximum of 200 years, allowing buildings to maintain their regenerative ability[14]. Bacteria also contribute to oxygen consumption preventing internal corrosion of reinforced concrete. On the other hand, bacteria do not pose a risk to humanity, since they are strict only to the alkaline conditions inside the material consisting of a pH higher than ten or extremely alkaline conditions.

"Self-healing concrete," consists of the same materials that conform conventional concrete (cement, water, fine and coarse aggregates, additives, etc.) added to the bacteria, which give it the quality of self-regenerating. When the concrete is exposed to cracks in the air, moisture, or water penetration, a chemical reaction is generated and as illustrated in figure 5, the self-regeneration products and their chemical composition are observed.

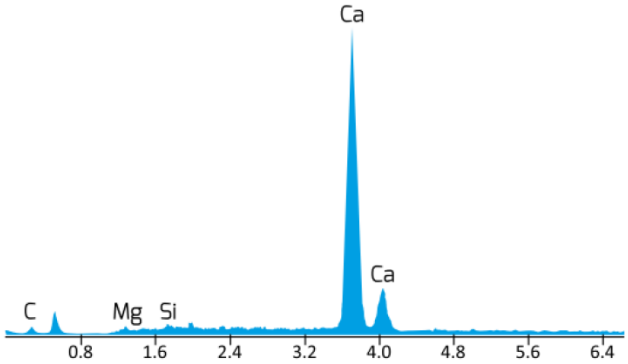

Figure 5. Chemical composition of self-regeneration products. The most substantial balance is Ca, which is linked to the growth of calcites for the production of regenerative concrete.

Microorganisms used in the concrete and bio cement production

The world produced 4.3 billion metric tons of cement in 2014, and this production will continue to increase due to construction demand in the world[15]. Besides, cement production is a process that consumes energy and is harmful to the environment, since it contributes approximately 7% of the world's anthropogenic emissions of CO_2[16].

Despite all the strength and durability, concrete has problems; one of them is the stress of transporting loads that gradually creates microscopic fissures in the material, which allows the entry of water, salts, and sometimes ice[15].

However, natural processes, including earthquakes or weathering or subsidence of the earth and human activities, also perform sufficient functions to degrade or reduce the durability of concrete structures[17].

The formation of ammonia is produced by the hydrolysis of urea when it meets moist air.

$$CO(NH_2)_2 + H_2O \leftrightarrow COOH + 2NH_3$$

The carbonate of the concrete hydrolyzes and originates two products, ammonia, and carbonic acid.

$$NH_2COOH + H_2O \leftrightarrow NH_3 + H_2CO_3$$

From the products, there are two reactions from which bicarbonate, ammonium, and hydroxide ions originate, due to an increase in pH.

$$HCO_3 \leftrightarrow HCO_3^- + OH^-$$
$$2NH_3 + H_2O \leftrightarrow 2NH_{(14)} + 2OH^-$$

By increasing the pH, the bicarbonate balance shifts to form carbonate ions.

$$2OH^- + HCO_3^- + H^+ + 2NH_4^+ \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$$

Through two reactions carried out by the bacteria, the carbonate ion precipitates giving rise to the product known as limestone.

$$Cell + Ca^{2+} \leftrightarrow Cell - Ca^{2+}$$

$$Cell + Ca^{2+} + CO_3^{2-} \leftrightarrow Cell - CaCO_3$$

Bacteria then are the main component that carries out the reactions that allow the material to repair the cracks to be generated, increasing their lifetime.

To help reduce those maintenance costs and make buildings and bridges safer, researchers are now giving concrete the power to heal themselves.

Jonkers and Schlangen[11] have led one of the first concrete jobs, where they take advantage of microorganisms to allow the concrete to heal itself; they mix clay granules containing calcium lactate and spores of limestone producing bacteria in the concrete (they use species of alkali-tolerant bacteria such as *Bacillus pseudofirmus*, which can survive the high pH of the concrete).

These bacterial spores can remain dormant for decades so that when the spores crack with the concrete, the moisture in the air causes the spores to germinate, the bacteria then feed on calcium lactate in the presence of moisture and oxygen to form Limestone, sealing the cracks.

This cure occurs in just three weeks, and bacteria can seal gaps up to 0.8 mm wide[11]. Another example of self-healing of concrete from bacteria is that presented by De Belie, where it packages bacterial spores in a melanin formaldehyde shell, generating a concrete that can cure small cracks up to 1 mm wide in four weeks.

Likewise, the same author mentions that a bacterial limestone producing strain was recently identified that does not require oxygen but uses nitrates, which would be added to the concrete so that microorganisms could potentially do their healing work in the structures of concrete where there is little access to air[18].

There are other methods in which bacteria help in the production of concrete and bio cement. The most studied positive effect is the precipitation of microbe induced $CaCO_3$ (MICP). The MICP in civil engineering has been considered mainly for its application in the fields of protection of natural stone surfaces, concrete crack remediation and soil improvement. Also, the development of the force by mixing bacteria in the concrete has been investigated[18].

The initial application of bacterial calcium carbonate precipitation in civil engineering is for the consolidation of the surface and the protection of construction materials, specifically, historical stones and cement-based materials; the researchers started from the isolation of carbonogenic strains, then the bacteria were isolated mainly from environments that produce carbonates.

The most commonly used metabolic pathway is the bacterial ureolytic hydrolysis of *B. pasteurii* over urea. When the research was carried out on a laboratory scale, the methodology applied was immersion, in which the samples were immersed in bacterial cultures and deposition media during specific periods.

The immersion time varied from 3 to 30 days, depending on the bacterial activity. The bio-$CaCO_3$ layer formed not only decreased water permeability, but both had strong cohesion within the layer and adhesion with the original matrix based on puncture resistance and ultrasonic measurement tests. This is due to the epitaxial growth of new crystals in the preexisting crystals and due to the incorporation of organic bacteria molecules[19].

On the other hand, the cement industry has constantly been looking for procedures that effectively reduce the high energy requirements and environmental costs of cement manufacturing[20].

The answer depends largely on the fact that the bio concrete that is based on the MICP process consists of three materials: alkalophilic microorganisms, substrate solution and calcium ion solution. It has been shown that the MICP-based bio concrete increases the durability of construction materials, the consolidation of sand columns and the repair of monuments and limestone concrete; besides, it can improve the strength and durability of structures, which are considered requirements for concrete or any other construction material[16,21].

Bacteria are not the only microorganisms that can precipitate calcium carbonate; within this group are also algae and microalgae. Seaweeds are nature-friendly organisms and are used in the area of civil engineering to control the chemical reaction of cement, also, avoid voids and decrease the permeability of concrete[22].

Microalgae are a promising means to be used in biocementation, due to their photosynthetic metabolism, algae species such as spirulina, *Arthrospira plantensis* (*Cyanophyta*), *Chlorella vulgaris* (*Chlorophyta*), *Dunaliella salina*, *Haematococcus pluvialis*, *Muriellopsis sp.*, *Porphyridium Cruentum* (*Rhodophyta*) are autotrophic microorganisms that live through the photosynthetic process.

It was shown that through an experiment that was based on nine green algae, one diatom and three cyanobacteria precipitated $CaCO_3$ in batch culture, where they were grown light in a hard water medium containing 68 mg/L of soluble calcium[23].

Several types of microalgae also use the urea hydrolysis mechanism to meet the needs of nitrogen, for example, *Chorella sp* uses urea as a source of nitrogen; Urea is hydrolyzed by urease or by the enzyme urea amidoliase to produce ammonia and bicarbonate, the activity of the urease enzyme can also induce precipitation of calcium carbonate[24]. There are some advantages of using microalgae as a means to produce biocement such as: availability as raw material, easy to grow, can reduce the CO_2 emission that occurs in conventional cement production[22].

On the other hand, its manufacture in the baking oven, requiring 50-85% less energy for its production, which represents 85% less CO_2 released into the atmosphere, which is mainly used as ecological pavement due to its permeable properties[25].

Comparison between bioconcrete and traditional concrete

Mechanical properties and their effects. Concrete based on flexible engineering exhibits more ductile behavior, while ordinary concrete is fragile (Figure 6, 7, 8). Inflexibility measurement tests, the results were 5 times higher than conventional concrete.

Figure 6. Tensile testing of concrete, bending deformation[13].

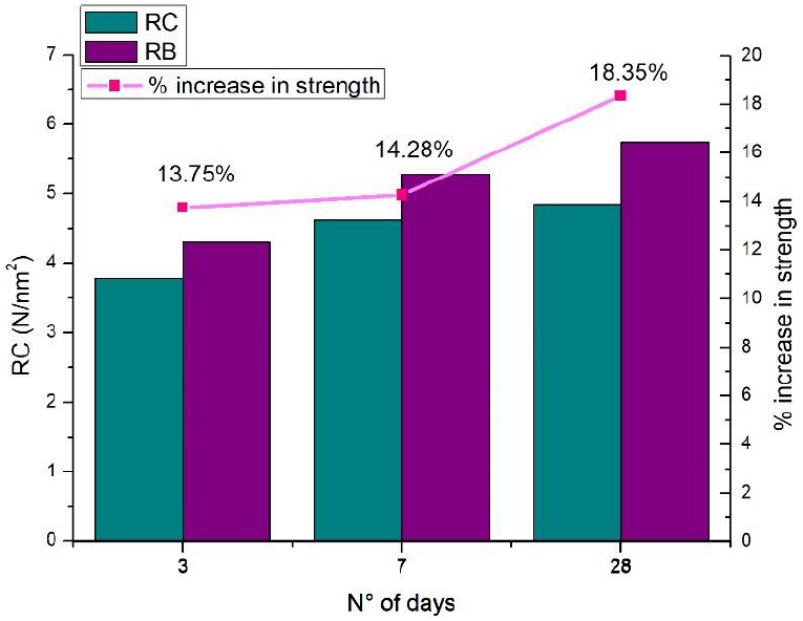

Figure 7. Comparison of the tensile strength values by splitting.
RC: Tensile strength of conventional concrete cylinders. RB: Tensile strength of *B. sphaericus* concrete cubes[13].

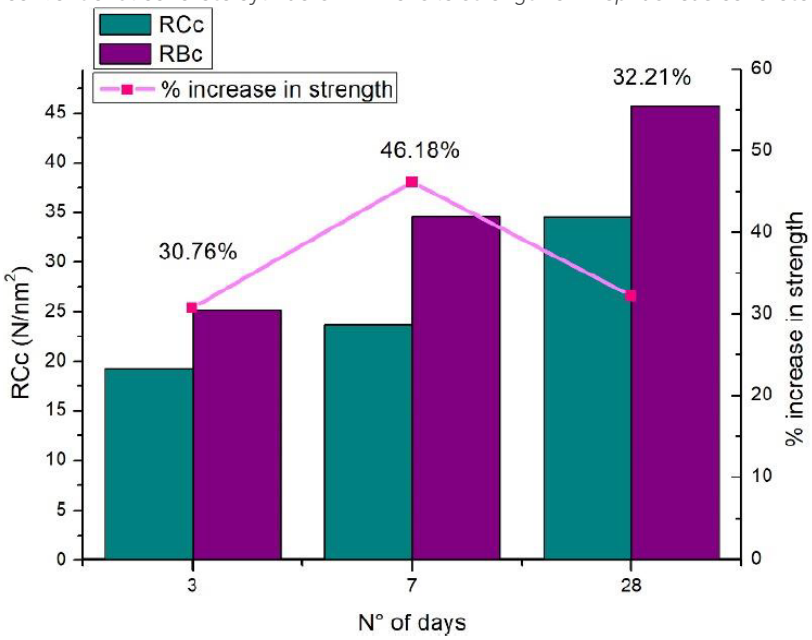

Figure 8. RC: Compressive strength (c) of conventional concrete cylinders. RB: Compressive strength (c) of *B. sphaericus* concrete cubes[13].

The durability of concrete, the effects.

The expense in the repair of the cracks represents a considerable investment reason why to count on this technology avoids losses and inconveniences in the residents of the properties. Although the cost of this bio concrete is 3 times higher than traditional concrete, it is compensated by evading repair costs.

It grants permeability to concrete, has better properties in a hardened state compared to the traditional one that restricts corrosion in marine structures or basements.

Fnvironmental advantages of construction biomaterial production

According to the European Commission[26], the Paris agreement mentions that the cement sector is responsible for around 5% of carbon dioxide (CO_2) emissions, which is the main greenhouse gas and climate change producing gas.

Being concrete, one of the most widely used materials in the world in the construction industry employs 1.6 billion tons of cement, and each ton of cement emits 1 ton of CO_2 into the atmosphere in its manufacture.

Besides that, during the construction process, heavy machinery is used that generates the highest amount of CO_2 emissions. The transport of the materials to the site constitutes 6-8% of the total greenhouse gas emissions for a project.

On the other hand, the commercial and residential construction sector represents 39% of the CO_2 emitted into the atmosphere, generating 30% of solid waste and 20% of water pollution. Based on the previous data, it can be concluded that half of the CO_2 expelled into the atmosphere is related to the construction of buildings throughout all its phases: construction, use, and subsequent demolition. Therefore, the construction sector and its CO_2 emission as a threat of climate change must be considered.

Therefore, to reduce the environmental impact on the construction sector, it is essential to use materials that do not require the use of fossil fuels and cause high carbon emissions.

Among the bioconstruction materials that reduce CO_2 emissions to the environment are:

Ecological calstar paver: this paper is made of at least 40% recycled materials, has the advantage of not needing high energy consumption during CO_2 absorbing flooring, known as GeoSilex, is manufactured with waste from acetylene production, and incorporated into pavements and facades, it makes these surfaces acquire CO_2 absorption capacity, it is marketed as additive paste for concrete, adding to these in a proportion of 3-10% of the weight of the concrete. It should be noted that it is a 100% product made of recycled material, and its carbon footprint during its manufacture is fully amortized, and that once its useful life is finished, it can then be recycled for the production of other materials, which represents a decrease significant in the release of CO_2 to the environment[27].

Green cement based on geopolymers, the main advantage in the production of this type of cement is the reduction of CO_2 emissions during its production process since the processing of common cement uses fuels to heat limestone to a temperature of 1450°C to obtain lime, and by decarbonation of limestone, geopolymer-based cement does not use calcium carbonate in its preparation and can be manufactured at half the temperature (750°C) and by-products of the manufacturing process they can be incorporated back into the production of this cement, which reduces up to 80% the environmental emissions involved in its development and with a production cost 50% lower than that of common cement.

Conclusions

Construction biomaterials are one of these novels, friendly and sustainable alternatives. Recent studies have shown that CO_2 emission used in the construction industry represents approximately half of the CO_2 emitted to the atmosphere so, this industry represents one of the main problems in the greenhouse effect and climate change, and therefore, it is necessary to look for alternatives to conventional methods of manufacturing building materials.

Bioconstruction materials such as ecological pavers, pavements to absorb CO_2 and green cement based on geopolymers represent a desirable alternative to replace conventional materials in the construction induction since these reduce CO_2 emissions to the atmosphere in an 80% in its manufacturing process compared to conventional methods, and in many cases, they represent a 50% decrease in its production costs.

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NEWS AND VIEWS

Phage therapy with mycobacteriophage as an alternative against antibiotic resistance produced by Mycobacterium tuberculosis

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Abstract: Bacteriophages are considered a genetic strategy to combat pathogen bacteria that show resistance to antibiotics. Molecular biology has implemented various control measures to deal with bacteria; the application of bacteriophage directly to tuberculosis viruses is a technological tool currently using. Mycobacteriophage is a type of virus that infects mycobacterium hosts. Because most of them have been genetically modified, they are providing insights into viral diversity. Furthermore, phage therapy is potentially a way to improve the treatment of bacterial infections strictly mediated by bacteriophages of lysogenic and lytic type. Genetic modifications are an essential factor for the development of future phage therapy applications to control the diseases caused by *Mycobacterium tuberculosis*. This review is about the mycobacteriophages to control the antimicrobial resistance caused by *Mycobacterium tuberculosis* thought some applications of phage therapy.

KeyWords: Bacteriophages, phage therapy, resistance, mycobacteriophage, *Mycobacterium tuberculosis*.

Introduction

Bacteriophages are considered as a backbone of the biological universe, forming an enormous, ancient, dynamic, and genetically diverse population, replete with genes that were used to identify the basis of genetic material[1]. Bacteriophages or phages are a type of virus considered the most abundant biological entity on the earth, with an estimated of 10^{31} total particles[2,3,4]. These phages have 20-200 nm in size and they influence gene transfer for the evolution of bacterial species[5]. In the base of this, these viruses are driven by horizontal gene transfer with host and other phages[6]. They can infect and kill bacteria minutes later the contact, replicate, and their progeny are released after bacteriolysis[2].

Moreover, bacteriophages are antibacterial agents because they represent a potential solution to some problems[2]. Although Frederick W. Twort discovered these in Great Britain in 1915 and Félix d´Herelie in France in 1917, they were hindered by the discovery of antibiotics[7]. The bacteriophage has three structures: the head, the tail, and the long tail fibers[8]. The head consists of a core of DNA or RNA genomes and some proteins that are in the capsid coat such as PIII, PIV, PV, PVIII[8]. The long tail has a hollow central core that surrounded by the contractile sheath and ends with a hexagonal base-plate. Furthermore, the tail is specialized for the injection of DNA into the host cell. In the end, the long tail fibers are proteins that are attached to the baseplate´s periphery[9].

Phages have two possible life cycles, which are the lytic cycle and lysogenic, which depend on the interactions with their physical environment of the bacterium. The lytic cycle occurs when the phage inserts its material genetic into the host cell, killing these cells, and releasing mature viruses[10]. On the other hand, the lysogenic life cycle is where phages instead of directly killing their hosts, integrate into their genome in the form of a plasmid. This life cycle can be stable by so much time and even the cells that contain DNA phage can be replicated. Also, the bacteriophage may alter the phenotype of the bacterium/ archaea by expressing genes that are not expressed[10].

Antibiotic resistance is understood as a mechanism that the bacteria develop to continue living in the host by activating the mechanisms to reduce the action of antimicrobial agents to live in the host by a long time[11]. Seven hundred thousand deaths due to antimicrobial resistance are recorded per year if this problem is not controlled. It is estimated that in 2050 there will be 10 million deaths[12]. For this reason, bacterial resistance to antibiotics is a severe problem in contemporary medicine[12,13]. In consequence, since phages can evolve and overcome resistance, they represent an alternative strategy to combat bacterial infections and diseases[12,14].

The difference in language, the protocol of the establishment of clinical trials, and the appearance of antibiotics between the countries of the East and West, has delayed the development of phage therapy[12]. This therapy alternative focuses on virulent bundles, which requires low doses and treatment frequencies to achieve the optimum effect. The most important characteristic of this natural killer, its high specificity of the host reduces the damage to other bacteria[12]. Although the preclinical study of the therapy has 100% success in infections caused by pathogens of resistance to multi drugs, there are no approved antibacterial drugs only ongoing completed clinical trials[2,12]. Due to bacteriophages exhibit specificity in bacterial hosts, mycobacteriophages are taken as a resource to identify clinical isolates of mycobacterium strains[15]. One of them, *Mycobacterium tuberculosis* (*M. tuberculosis*) which is responsible for human tuberculosis and presents a slow growth, the reason why the phage typing accelerates the diagnostc[15]. For this reason, we have focused on the use of specific bacteriophage (mycobacteriophage) to control the antimicrobial resistance that is produced by *M. tuberculosis* through phage therapy, mainly modification of the genome phage.

Phage therapy

The global resistance to antibiotics and the human microbiota was the primary context for using bacteriophages for therapeutic purposes[7]. Viral phage therapy or also called phage therapy is the therapeutic defined by the use of specific bacteriophages to fight pathogenic bacteria that cause infections[16].

The advantages of using phage therapy can be 1. Due to

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the auto dosing phenomenon, which is the capacity of phages to increase their population in the place where their host is, the repeated administration of phages in the therapy site is avoided. 2. The specificity of lytic phages on broad-spectrum antibiotics causes the infection of few bacterial strains. Consequently, there is no alteration of the environmental commensal microbiota. 3. The automatic elimination of phages in the absence of hosts (the result of host lysis). 4. Because the mechanism of action of phages (infection and killing of bacteria) is different from that of antibiotics, it can be used in treatments for diseases caused by bacteria resistant to many drugs. 5. The use of phage proteins to develop therapies simple structure to develop therapies with phage proteins or phage complex for the distribution of vaccines. 6. Efficacy in the prevention and elimination of biofilms 7 Self-modulation of phages concerning bacteria to infect and lyse them[2]. Recent studies of phage therapy use small vertebrate animals and have focused on acute infections, where the bacterium causing the disease can be identified by rapid diagnostic methods[7]. Finally, these studies suggest a significant benefit in the treatment of pulmonary infections resistant to antibiotics, wound, and gastrointestinal infections[7].

Mycobacterium tuberculosis is a bacterium responsible for causes tuberculosis in humans that has been leading cause of death worldwide. According to OMS in 2018, Tuberculosis is responsible for 1.3 million deaths and multidrug-resistant[18] caused some of them. Besides, the bacterium can develop new strategies to live in the host by any time, creating an incurable disease. One of the latest alternative therapies is phage therapy, which is based on the use of mycobacteriophages (phages) to treat and suppress bacterial infections. Mycobacteriophages are a phage that infects both *M. smegmatis* and *M. tuberculosis* bacterium and has that capacity of rapid replication also, can penetrate macrophages by phagocytosis[12] and has complex cell envelope and a double-strand DNA genome and can form a single cluster or many clusters[19]. The antibiotic control resistance of *Mycobacterium* can be by two strategies such as phage as an informative gene of foreign DNA and genetic modification.

Mycobacteriophages therapy in tuberculosis

Mycobacteriophages are essential tools in the development of genetics and to provide clinical tools for the control of tuberculosis due to the high capacity to replicate[4]. Like most phages, mycobacteriophages introduce their genetic material into the host[15], replicate efficiently, express genes at high levels using a variety of regulatory strategies such as three proteins Lysine A, Lysine B, and a holine and some additional proteins capable of lysing the cell membrane. These bacteriophages have a unique characteristic; the rate of growth of the phage is extremely greater than the growth of *M. tuberculosis*; for this reason is more likely to eliminate bacteria[20].

Early gene expression occurs in the third minute, with late transcription initiating about 30 minutes after infection, and continuing until lysis about 180 minutes after infection[15]. Due to this advantage, mycobacterium can be used in different applications. First, phage as informative genes that mark the of foreign DNA for which a derivative of phage L5 was constructed, which carries the luciferase gene (an indicator of the vial mycobacterium), also a phage indicating D29, which is a carrier of the green fluorescent protein. With this strategy, we can supply phages containing the two proteins, and as a result, we can determine the efficiency and specificity of the phages by a luminometer and look at the infected cells by microscopy[20]. The phage with the reporter gene very efficiently infects the bacterium. Therefore the majority of the bacterium population should become fluorescent. According to some investigations, they used Φ2GFP12 compared to that of Φ2GFP10, which are DS6A-based reporter phages[21]. Also, both emit fluorescence at similar intensities; however, fluorophore is more useful to infect and generate more fluorescence in *M. tuberculosis*[22]. In this way, we can see how useful the phage is by infecting bacteria. Finally, this alternative is a good overview of the active phage division potential for bacterial detection and elimination.

Due to the bacteriophages, lithics have a fast lysis[13], which can cause problems by release endotoxins and superantigens[13]. As a consequence, it induces an inflammatory response with dangerous side effects and an uncontrolled replication[23]. For these reasons, several scientists suggest a genetic modification used the lysogenic cycle to enhance phage efficacy to combat antibiotics resistance.

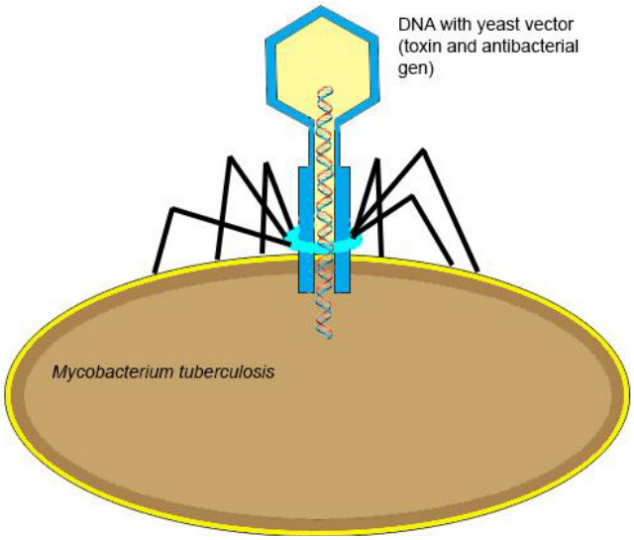

Figure 1. Mycobacteriophage ADN (with YAC inserted) penetrating into *Mycobacterium tuberculosis*.

In the same case, due to *M. tuberculosis* has a reservoir of genes and cassettes, the phage genomes can be manipulated in vivo by molecular cloning, it is another alternative to phage therapy. The vectors can transform *Mycobacterium tuberculosis* into not resistance using phage and yeast artificial chromosome (YAC) as a vector. Fig 1. In some of the integration vectors more used are the following: aphasia, brujita L5, Bxb, Tweety, Giles, omega and Ms6 (52, 68, 72-74, 83, 121, 122)[15]. These can be transformed efficiently and can work in fast and slow strains because the integrases of tyrosine use the attB sites. However, transformation frequencies are extremely low[22]. An example is the use of a lethal agent delivery system (LADS), this technique uses a phage-based in vivo packaging system to create a recombinant phage with the capacity of delivering and naturally expressing the designed antibacterial genes[23]. Besides, with this, the modification of lysis genes phages by a gene encoding which can be a small acid-soluble spore protein (SASP), as well as that, it has the capacity of stopping all cellular activity[23]. However, the effect of natural phage multiplication at the infection site is lost. Moreover, we need to aggregate a toxin, which induces cell death or lyses to eliminate the infected bacterium[24]. Fig 1. According to some investigations, the insertion of the whole page genome inside a yeast artificial chromosome (YAC) vector by homologous recombination to create a recombinant YAC that is then

propagated in the vector host[23]. Then, the phage genome should be modifying inside the vector. As a result, the phage genome would have a toxin and an antibacterial gene.

Finally, the YAC containing the mutant phage can be inserted into a bacterium, followed by replication of engineered phage[20]. Some studies suggest that t7 made up by the insertion of different sequences, give as a consequence the viable phages out these typical genomes by transforming the bacterial host into a new non- resistant bacterium by engineer phage genomes[25]. However, it is very difficult to work with large sequences of DNA in vitro and the need to transform bacterium to obtain viable phages capable of infecting the bacterium, inserting its genome material[26]. Finally, this application is the most efficient because the mycobacterium will have another genetic material, besides it will be transformed into a non-resistant bacterium that can be eliminated easily.

Antibiotic resistance

It is understood as a mechanism that the bacteria develop to continue living in the host by activating the mechanisms to reduce the action of antimicrobial agents to live in the host by a long time[11].

Mycobacteriophages

It is a specific bacteriophage against *M. tuberculosis*. Also, it is an essential clinical tool for the control of tuberculosis due to the high capacity to replicate[4]. Mycobacteriophages introduce their genetic material into the host[5], replicate efficiently, express genes at high levels using a variety of regulatory strategies such as Lysine A, Lysine B and some additional proteins capable of lysing the cell membrane[20].

Modification of the phage DNA.

The mycobacteriophage genetic material will be composed of a vector with toxin and an antibacterial gene. This vector is inserted in yeast artificial chromosome (YAC). Finally, the YAC containing the mutant phage can be inserted into a bacterium, causing it to lose its resistance and be easily eliminated[15,20,24].

Conclusions

In the last few years, the use of bacteriophages as a therapeutic alternative has had a significant impact and interest. Human infections caused by mycobacterium can be understood and combated with phage for mycobacterium hosts. For this reason, phage therapy is a realistic alternative to combat antibiotic resistance, which depends on the strategies used for the limitations that may result from being a therapeutic agent. Furthermore, implementing this therapy option requires the use of a variety of phages to overcome the limited range of hosts and the risk of bacterial mutants resistant to phages. Finally, more research is needed in phage encapsulation to support the development of therapy. We expected that with the identification of more phages, the application of phage therapy will become successful.

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Phage therapy	Phage therapy uses specific bacteriophages to fight pathogenic bacteria that cause infections[2]. We have focused on *Mycobacterium tuberculosis* which causes Tuberculosis.
Antibiotic resistance	It is understood as a mechanism that the bacteria develop to continue living in the host by activating the mechanisms to reduce the action of antimicrobial agents.
Mycobacteriophages	It is a specific bacteriophage against *M. tuberculosis*. Also, it is an essential clinical tool for the control of tuberculosis due to the high capacity to replicate[4]. Mycobacteriophages introduce their genetic material into the host[5], replicate efficiently, express genes at high levels using a variety of regulatory strategies such as Lysine A, Lysine B and some additional proteins capable of lysing the cell membrane[20].
Modification of the phage DNA.	The mycobacteriophage genetic material will be composed of a vector with toxin and an antibacterial gene. This vector is inserted in yeast artificial chromosome (YAC). Finally, the YAC containing the mutant phage can be inserted into a bacterium, causing it to lose its resistance and be easily eliminated[15,20,24].

Table 1. Review paper summary

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NEWS AND VIEWS

Los micro ARNs en patología humana: utilidad clínica y enfoque traslacional

The micro RNAs in human pathology: clinical utility and translational approach

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Resumen: En clínica humana, patologías tan diversas como el cáncer, la sepsis, enfermedades autoinmunes, entre otras; de etiología diferente y un comportamiento fisiopatológico distinto, convergen en un fallo de represión génica que permite la expresión fenotípica de la enfermedad; la posibilidad de tener un marcador biológico que le muestre al clínico estos sucesos es deseable, ya que permitiría tomar estrategias diagnósticas y terapéuticas de forma precoz. Los micro ARNs, son ARNs pequeños y no codificantes que cumplen ese rol "silenciamiento genético", sin embargo, el paso de la investigación básica a la aplicabilidad clínica, es decir, su utilidad traslacional es aún poco difundido en especialidades diferentes a la Oncología. El objetivo de esta revisión, es explicar de la forma más clara, la utilidad actual de los micro ARNs en diversas enfermedades humanas.

Palabras clave: Micro ARNs, enfoque traslacional, clínica humana.

Abstract: In human clinics, pathologies as diverse as cancer, sepsis, autoimmune diseases, among others; of different etiology and a different pathophysiological behavior, converge in a failure of gene repression that allows the phenotypic expression of the disease; The possibility of having a biological marker that shows these events to the clinician is desirable since it would allow early diagnostic and therapeutic strategies. Micro RNAs are small and non-coding RNAs that fulfill that "genetic silencing" role, however, the step from basic research to clinical applicability, that is, their translational utility is still little diffused in specialties other than oncology. The objective of this review is to explain in a more precise way.

Key words: Micro RNAs, translational approach, human clinic.

Introducción

En las últimas dos décadas, de forma inicial a través de un nemátodo *Caenorhabditis elegans* (*C. elegans*) se descubre otro mecanismo poderoso de regulación de la expresión génica a nivel de ARN[1], pequeñas moléculas de ARN denominadas micro ARNs (miARN) los cuales han sido sometidos a intensa investigación y su presencia en la clínica humana parece ser universal, desde el cáncer hasta la patología cardíaca, respiratoria, inmune, infecciosa entre otras[2,3].

Son ARNs pequeños (19 a 24 nucleótidos), no codificantes y no modificados desde el punto de vista evolutivo y cada miARN tiene el potencial de afectar a 500 genes[4]; su papel fisiológico fundamental es la de reprimir genes implicados en la proliferación, apoptosis y diferenciación celular[5], consecuentemente 'silenciamiento de la expresión genética'.

Se forman de la siguiente manera (Figura 1): Empieza en el núcleo celular mediante la acción de la miARN polimerasa II sobre los genes de miARN, de ésta forma mediante transcripción (transeferencia de la información del ADN al ARN), se obtienen pro-miARN[6]. Sobre el pro-miARN actúa la endonucleasa ARNsa III, conocida como DROSHA y se genera el pre-miARN, para ello DROSHA requiere el cofactor DiGeorge Syndrome Critical Region 8 (DGCR8), todo esto a nivel nuclear[6]. Formado ya el miARN, sale del núcleo al citoplasma mediante un transportador, la exportina 5, ahí, mediante una segunda endonucleasa ARNsa III (DICER), el mi-ARN se escinde a miARN maduro (doble cadena) miARN (cadena pasajera), la cadena pasajera se degradará y el miARN maduro se someterá a silenciamiento genético mediante ARNs interferentes[7]. Los miARNs así formados, abandonaran la célula mediante dos formas de liberación: Pasiva; cuando la célula muere de forma programada (cuerpos apoptóticos)y Activa; por secreción celular de exosomas, complejos de ribonucleoproteínas, lipoproteínas de alta densidad y micro vesículas.

Los miARN a su vez interactúan con ARN circulares (ARNcirc), los cuales constituyen moléculas de ARN unidas por un proceso celular denominado splicing o trans-splicing[8] y juegan un rol regulatorio importante. Evidencia reciente ha demostrado que los ARNcirc regulan la función de los miARNs[9], es por esto que la identificación y estudio de ARNcirc es trascendental para el entendimiento de los mecanismos regulatorios de ciertas enfermedades y para su posterior uso como marcadores biológicos en diagnósticos clínicos.

Asociación ARNcirc-miARN y su importancia reguladora

Los ARNcirc fueron descubiertos inicialmente en viroides, patógenos de plantas superiores, conformados por una molécula de ARN cerrada covalentemente[8,10]. Evidencia reciente demuestra que la expresión de ARNcirc en mamíferos es más prevalente y abundante de lo que se pensaba[11,12], siendo altamente conservados evolutivamente[13]; al contrario de los miARNs (moléculas que regulan la expresión de la mayoría de ARN mensajeros[14]), los ARNcirc no han sido muy estudiados.

Los ARNcirc juegan un rol regulatorio importante, interactuado con ARN mensajeros (ARNm), microARN o proteínas de unión con ARN (RBPs)[15]. (Figura 2).

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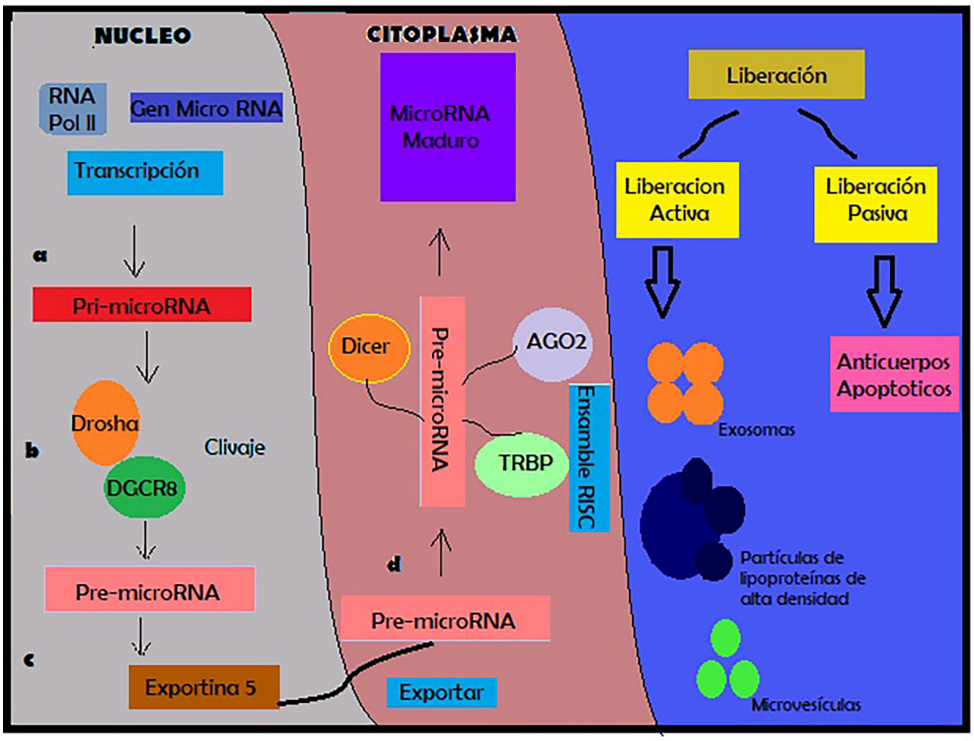

Figura 1. Formación de los mi-ARNs.

Función reguladora: ARNcirc esponja

La principal función reguladora de los ARNcirc descubierta hasta el momento es su mecanismo de acción a manera de esponja, secuestrando miRNAs para regular su función[8,16]. El CDR1 es quizás el ARNcirc mamífero mejor caracterizado, muy abundante en neuronas[15,16], el ARNcirc CDR1 recientemente identificado como regulador negativo del microARN miR-7, demuestra el potencial regulador (ESPOJA) de estos ARN[8]. miR-7 es desestabilizado por un mecanismo que modifica los terminales 5' y 3' del miARN, añadiendo o removiendo nucleótidos[17]. (Figura 3).

Otro ejemplo de estos ARN circulares como esponja es el HRCR (ARNcirc relacionado al corazón)[18]. Estudios sugieren que este juega un rol protectivo en hipertrofia cardiaca secuestrando miR-233[19].

Función de acoplamiento para RBPs

Los ARNcirc también pueden funcionar como sitios de acoplamiento para RBPs para secuestrarlos de su ARN objetivo o para mediar su localización subcelular. Curiosamente, los ARNcirc son particularmente abundantes en el cerebro humano (sinapsis) comparado con otros tejidos analizados[20], sugiriendo su participación en la regulación de ARN locales. Por ejemplo: ARNcirc deben transportar miARNs y RBPs a sitios de sinapsis y liberarlos en respuesta a un especifico estimulo[21].

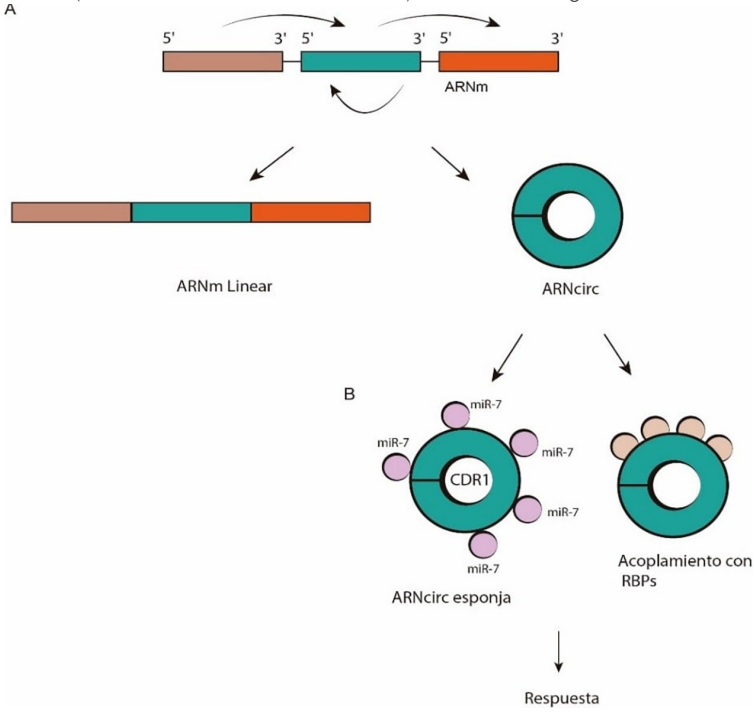

Figura 2. A. Formación de los ARN circulares, B. relación con microARN y acoplamiento con RBPs.

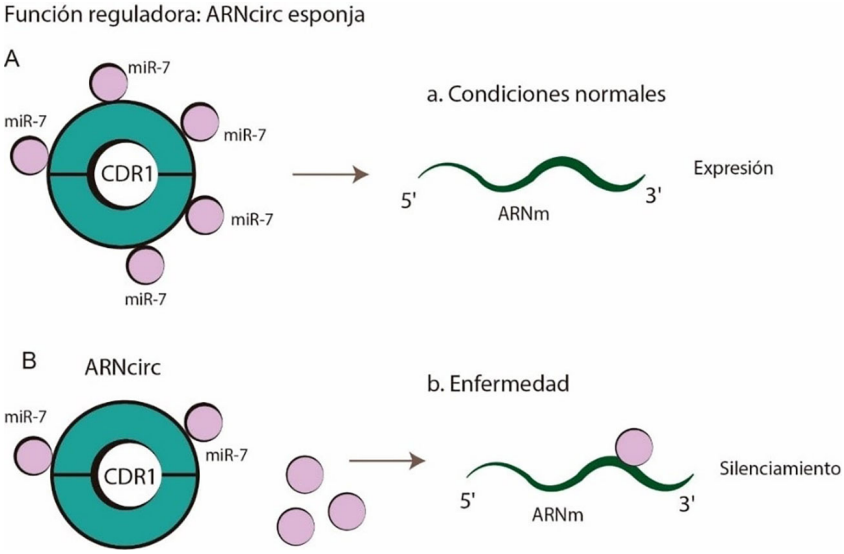

Figura 3. Función de ARNcirc A. ARNcirc CDR1 actúa como esponja, uniéndose a miR-7 en condiciones normales, previniendo su unión con el ARNm lo que conlleva a su transcripción normal. B. En enfermedad ARNcirc no cumple completamente con su función de esponja liberando miR-7 que se unen al ARNm provocando el silenciamiento de ese gen.

Aplicabilidad clínica de los Micro ARNs

El sello distintivo de la función de miARN es su capacidad para suprimir la expresión génica mediante la unión de sus ARN diana, la expresión aberrante de los miARNs afecta la regulación de muchas funciones celulares y redes de genes. Se ha encontrado en tejidos y en sueros de pacientes, como biomarcadores de pronóstico, los miARN también permiten la predicción del curso de una enfermedad[22].

Además del cáncer, existe evidencia suficiente para sugerir que los miARNs están desregulados en infecciones virales, trastornos del sistema nervioso, trastornos cardiovasculares, musculares, diabetes y otras enfermedades lo que implica que la utilización de estos miARNs expresados de forma aberrante como biomarcadores para enfermedades es una valiosa estrategia de diagnóstico.

Sepsis

Los miARNs miR-221 y miR-222 en ratones en quienes se administró lipopolisacárido (un patrón molecular asociado a patógeno PAMP propio de las bacterias gram negativas) mostraron un incremento importante, situación similar se produjo al medirlos en 30 pacientes con sepsis, en ellos, miR-221 y miR-222 se mostraron como reguladores de la reprogramación funcional de macrófagos durante la tolerización (inducción de toleracia) a lipopolisacáridos. La estimulación prolongada con lipopolisacárido condujo a una mayor expresión de miR-221 y mir-222, que regulan el gen 1 relacionado con Brahma (Brg1, también conocido como Smarca4). Este aumento de la expresión provoca el silenciamiento transcripcional de un subconjunto de genes inflamatorios que dependen de la remodelación de la cromatina mediada por SWI / SNF (interruptor / sacarosa no fermentable) y STAT (transductor de señal y activador de la transcripción), que a su vez promueve la tolerancia[7,23].

Niveles elevados de éstos marcadores genéticos se correlacionaron con inmunocompromiso y mayor fallo multiorgánico, de ésta forma se demostró que los miARNs miR-221 y miR-222 podrían servir cómo biomarcadores de inmunoparálisis y mal pronóstico en pacientes sépticos7. Tal vez un limitante de los miRNAs citados, es que también marcan el comportamiento biológico de otras patologías como el cáncer, ya que han sido estudiados como biomarcadores en cáncer de mama, cáncer de pulmón de células no pequeñas, glioblastoma, entre otros, lo que significaría poca especificidad[7,24].

Caserta et al. evalúa miARNs circulantes relacionados con la inflamación en pacientes con estados inflamatorios infecciosos y no infecciosos, identifica seis principales (miR-30d-5p; miR-30a-5p; miR-192-5p; miR-26a-5p; miR-23a-5p; miR-191-5p), determina que en cualquier estado inflamatorio hay descenso de éstos miARNs, pero que es más importante en sepsis (p: <0,0001) y que el comportamiento biológico hacia la baja de los miARNs discrimina adecuadamente estados sépticos severos de estados inflamatorios graves no infecciosos (AUC: 0,742-0,917). Encuentra además correlación inversa de los niveles de miARNs con los niveles de citoquinas proinflamatorias (IL-1, IL-6, etc). Es decir que la disminución de los miARNs en estados sépticos permite la expresión de citoquinas proinflamatorias, por ausencia de silenciamiento. Entonces a menores niveles de miARNs mayor severidad inflamatoria, con mayor capacidad predictiva en estados inflamatorios secundarios a sepsis[23].

Enfermedades cardiovasculares

Un número cada vez mayor de estudios ha demostrado que los miARNs cardiacos son notablemente alterados en la isquemia miocárdica así como en la remodelación ventricular y la reparación cardíaca. Se encontró que la expresión de miARN-320 estaba sistemáticamente desregulada en los corazones isquémicos[4]. La reducción del miARN320 endógeno brindó protección contra la apoptosis de los cardiomiocitos a través de la regulación positiva de la HSP20[12,22].

Uno de los miARN más estudiados en este ámbito es el miARN-132, el cual en modelos farmacológicos, las reacciones de miARN-132 muestran la eficacia terapéutica para bloquear el desfibrilación cardiaca por la normalización del tamaño cardiaco y la inhibición de la fibrosis[25]. Los miembros de la familia de miARN-34 promueven la detención del crecimiento y la apoptosis; la inhibición terapéutica de miARN-34 remodelación inducida por isquemia y mejora de la recuperación cardíaca[26].

La reperfusión del corazón isquémico sigue siendo la intervención más efectiva para mejorar los resultados clínicos, sin embargo, los aumentos anormales en el calcio intracelular durante la reperfusión miocárdica pueden causar la muerte de cardiomiocitos, conocida como lesión por isquemia reperfusión, el miARN-214 está regulado al alza durante la lesión isquémica y la eliminación genética de miARN-214 en ratones causó una pérdida de contractilidad cardíaca, aumento de la

apoptosis y fibrosis excesiva en respuesta a la lesión. Las funciones cardioprotectoras de miARN-214 durante la lesión por isquemia reperfusión se atribuyeron a la represión del ARN mensajero que codifica el intercambiador de sodio / calcio, un regulador clave del flujo de Calcio; y a la represión de varios efectores posteriores de la señalización del calcio que median la muerte celular[27]. Estos resultados sugirieron un papel fundamental para el miARN-214 como regulador de la homeostasis y la supervivencia de los cardiomiocitos.

Cáncer

La expresión de miARNs en el cáncer se debe a que la mayoría está localizado en regiones genómicas relacionadas con su desarrollo. Estudios recientes también han demostrado sus papeles clave en la evolución patogénica, la progresión y la metástasis de los carcinomas[28].

El perfil de expresión de miARN ha sido evaluado recientemente como un biomarcador de diagnóstico confiable para diferenciar entre muestras normales y tumorales. Además de distinguir los tejidos normales de los cancerosos, los marcadores moleculares también se pueden usar para determinar el tejido de origen en tumores de origen primario desconocido[29]. La expresión de miARN mejora (miARN oncogénico) o reduce (supresor tumoral) el crecimiento tumoral a medida que el tumor progresa y se encontró que está asociado con la resistencia a los medicamentos.

Existen datos que demuestran que su expresión anormal es una característica de los procesos neoplásicos (Tabla 1).

miARN	Blancos moleculares	Mecanismo de Acción	Tipos de cáncer
miR-21	PTEN, SPRY2	Promueve invasión y migración (tumorogenesis) al inhibir los reguladores negativos de las rutas de señalización Ras/MEK/ERK	Glioblastoma, mama, colorectal, pulmón, páncreas, piel, hígado, estomago, cervical, tiroides.
miR-34	Proteínas implicadas en el ciclo celular, diferenciación celular y apoptosis.	Regulador clave para supresión de tumores.	Multiples
miR-27	FOXO1 Upregulated	Inhibe el recorrido del ciclo celular e induce la muerte celular.	Cáncer de mama.
miR-31	RhoA Downregulated	Inhibe la invasión local, la supervivencia inicial en un sitio distante y la colonización metastásica.	Cáncer de mama.
let-7	E2F2, c-Myc, KRAS; Downregulated	Reduce los niveles de proteínas c-Myc y E2F2 Inhibe la proliferación celular, la expresión de KRAS y la activación de la proteína quinasa activada por mitógeno	Cáncer de mama.
miR-200	HER2 Downregulated	ZEB1, ZEB2	Cáncer de Próstata.
miR-331	HER2 Downregulated	Bloquea la señalización de PI3K / Akt y las vías de señalización del receptor de andrógenos.	Cáncer de Próstata.
miR-221	PTEN/AKT dowregulation	Expresiones génicas relacionadas con la transición epitelial-mesenquimal a través de la regulación de la señalización PTEN / Akt	Glioma
miR-195	D1, E2F3 dowregulation	Suprime la formación de colonias y bloquea la transición G1-S	Cáncer Hepatocelular.
miR-96	KRAS downregulation	Disminuye la invasión de células cancerosas, migración y crecimiento tumoral lento.	Multiples
miR-29	Proteínas implicadas en el ciclo celular, diferenciación celular y apoptosis.	Regulador clave para supresión de tumores	Pulmón, carcinoma hepatocelular, leucemia, mama

Tabla 1. miARN relacionados con distintos tipos de cáncer.

FOXO1/O3 proteína forkhead; RhoA, Gen homologo familiar A; HER3, receptor de proteína tirosina – quinasa erbB – 3; PTEN, fosfatasa y tensinogeno homologo; AKT, proteína quinasa B, E2F2, transcriptor del factor E2F2.

Diabetes

Los ARN que interactúan con proteínas Piwi (piARN), los pequeños ARN endógenos de interferencia (iARN), los micro-ARN derivados de intrones (miARN) y una serie de ARN no codificantes ejercen una función reguladora, aunque los que mejor se conocen en lo referente a las complicaciones diabéticas son los miARN, que regulan varias vías biológicas clave y funciones celulares implicadas en las complicaciones diabéticas[30]. Entre ellas se cuentan los elementos reguladores de las concentraciones de especies reactivas del oxígeno (ROS), la conexión subyacente entre la hiperglucemia intracelular y las vías que causan complicaciones derivadas de esta patología.

Estudios demuestran que los miARN intervienes en el desarrollo del páncreas, así como para la regulación de la glucosa y contribuyen en el control de las células β, con una participación activa en la regulación de la producción, secreción y acción de la insulina[30,31] (Tabla 2).

CircBase: base de datos que cubre información de ARN-circ, miARN desde el 2013 y frecuentemente se encuentra actualizado con nuevas publicaciones. Se puede acceder a través de http://www.circbase.org/. Esta información se puede descargar y analizar en un contexto genómico, reuniendo datos de varios organismo modelo usados en investigación biomédica.

StarBase v2.0: esta plataforma explora y predice interacciones ARNcirc-miARN. Se puede acceder a través de http://starbase.sysu.edu.cn/, siendo la primera base de datos que identifica la interacción ARN-ARN y proteína-ARN usando análisis CLIP-seq.

Conclusiones

Los miARNs son ARNs pequeños y no codificantes, su papel fisiológico fundamental es el 'silenciamiento de la expresión genética'. Se desregulan en varias enfermedades, de diversa etiología, pero de génesis común "falta de represión génica". Medir estos miARNs expresados de forma aberrante, los hace importantes biomarcadores, que aportan datos diagnósticos y pronósticos valiosos.

miARN	Blancos moleculares	Proceso biológico relacionado
miR-375	MTPN	Expresión del gen de insulina
miR-9	OC2	Inhibición de la secreción de insulina
miR-145	IRS1	Disfunción de la célula β pancreática
miR-192	SIP1	Patogénesis de la nefropatía diabética
miR-133	HERG	Corazón diabético
miR-7	IGF1R	Incremento de la producción de insulina
miR-29a, miR-132 y miR-22	DNMT3A	Expresión de la diabetes mellitus gestacional
miR-103/107	Fasn, C/EBPα	Metabolismo de ácidos grasos
miR-122	AMPK5	Regulación de los lípidos
miR-143	Leptina, ERK5, adiponectina,	Diferenciación de adipocitos
miR-222	Glut 4, PPARγ	Expresión de adipocinas

Tabla 2. miARN relacionados con la diabetes.

Lupus eritematoso sistémico

Se ha publicado la expresión diferencial de los micro-ARN en múltiples tipos de células en pacientes con LES. Debido a que la expresión de miARN está involucrada en la regulación de múltiples aspectos de la respuesta inmunitaria normal, no es sorprendente que los cambios en el contexto del miARN puedan asociarse con la autoinmunidad[20].

La sobreexpresión de varios miARN en los linfocitos T CD4 de los pacientes con LES contribuye al defecto de metilación del ADN, en tal razón la expresión de miARN-126 y miARN-148ª, ambos con efecto al inhibir el DNMT1 favoreciendo a la expresión de la enfermedad[21]. Otro miARN asociado al LES es miR-146ª, que tiene como objetivos directos varios genes relacionados con el IFN, como IRAK1, TRAF6, IRF5 y STAT1[22]. La inhibición de miR-146a provoca una mayor activación del IFN de tipo I, un rasgo de la patogenia característico del LES.

Herramientas bioinformáticas y base de datos disponibles

Actualmente, no existe un enfoque sistemático disponible para identificar ARN circulares en el transcriptoma humano. Debido a que estas moléculas tienen un rol crítico en medicina y en biología, varias bases de datos se han creado para simplificar sus características y funciones.

Exención de responsabilidad

Los autores confirman que el contenido clínico, diagnóstico y terapéutico del reporte de caso son producto del escrutinio médico aplicado al paciente y son responsabilidad estrictamente de los profesionales involucrados en la publicación de este reporte de caso.

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Los autores declaran que no existe conflicto de interés para la publicación de este artículo.

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NEWS AND VIEWS

An overview of synthetic biology

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Abstract: Synthetic Biology is the combination of basic sciences with engineering. The aim of Synthetic Biology is to create, design, and redesign biological systems and devices to understand biological processes and to achieve useful and sophisticated functionalities to improve human welfare. When the engineering community took part in the discussion for the definition of Synthetic Biology, the idea of extraction and reassembly of "biological parts" along with the principles of abstraction, modularity, and standardization was introduced. Genetic Engineering is one of the many essential tools for synthetic biology, and even though they share the DNA manipulation basis and approach to intervene in the complexity of molecular biology, they differ in many aspects, and the two terms should not be used interchangeably. Some of the applications that have already been done by Synthetic Biology include the production of 1,4-butanediol (BDO), the antimalarial drug artemisinin, and the anticancer compound taxol. The potential of Synthetic Biology to design new genomes without immediate biological ancestry has raised ontological, political, economic, and ethical concerns based on the possibility that synthetic biology may be intrinsically unethical.

KeyWords: Synthetic Biology, Genetic Engineering.

Introduction

What is Synthetic Biology?

Synthetic Biology is an arising field of research that integrates basic sciences with engineering. The interdisciplinarity of Synthetic Biology is evident as it has evolved along with the progress made in Biology, Biotechnology, Molecular Biology, and Computer Science. The discovery of DNA as the molecule carrying the organisms' genetic information, the findings regarding the regulation of *E. coli's* lac operon, and the advent of recombinant DNA technology, all paved the way for Synthetic Biology. This field owes its further development to Computer Science, which made possible the construction of models that describe and predict the processes and interactions between and within biological systems. The goal of Synthetic Biology is to create, design, and redesign biological systems and devices to understand biological processes and to achieve useful and sophisticated functionalities to improve human welfare[1–6].

The term "synthetic biology" was not always associated with the design of biological systems. In the 80s, the term was first used in the literature to describe bacteria that were genetically engineered employing recombinant DNA technology. Later, in the early 2000s, synthetic biology was associated with the synthesis of non-natural organic molecules that could function in living systems. The current definition of Synthetic Biology began to crystallize when the engineering community took part in the discussion and introduced the idea of extraction and reassembly of "biological parts" along with the principles of abstraction, modularity, and standardization. Abstraction refers to dissecting the design procedure into several hierarchies as an effective way to handle complexity. The division of the engineering process into several more straightforward abstraction levels (DNA, parts, devices, and systems) allows designers to work at a specific level somewhat independently to build a part, device, or system. Modularity or decoupling is the degree to which a system can be separated into "functional blocks" or orthogonal components. Functional blocks can be combined to construct modules with different functionalities that do not interact with each other. Finally, standardization aims to provide tools and protocols to ensure predictability and reproducibility in biological experimentation. Nowadays, Synthetic Biology is characterized by two main lines of research. The first one is focused on the discovery, characterization, and creation of biological parts, whereas the other seeks to assemble said parts into systems of increasing complexity[1,4–12].

Biological parts are the building blocks in Synthetic Biology. These are segments of DNA that encode for specific and indivisible biological functions such as promoters, ribosome binding sites, protein-coding regions, and transcription terminators (Figure 1). According to the International Genetically Engineered Machine (iGEM) Foundation, biological parts are functional units that cannot be separated into smaller units, and that can be ligated to build sophisticated devices[13]. Two or more parts can be assembled to form construction intermediates that do not comprise a device. Devices are made up of two or more parts that when combined can perform a biological function. The Registry of Standard Biological Parts is a repository of biological parts ran by iGEM that is available for the public. It contains information about the sequence, design, and availability of thousands of parts. The biological parts found in the Registry meet the BioBrick standard. The standardization involves the addition of a BioBrick prefix and a suffix, which are standard cloning sites flanking the part's DNA sequence. The standardization of parts guarantees its compatibility and interchangeability because the restriction enzymes and ligation steps used to combine two BioBricks are independent of its sequences[10,14–18].

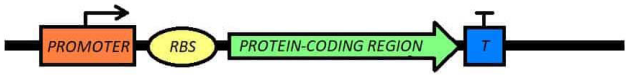

Figure 1. Basic devices with four biological parts: promoter, ribosome-binding site (RBS), protein-coding region, and terminator.

According to the Registry of Standard Biological Parts, there are five assembly standards and three assembly methods for BioBrick-compliant biological parts (Table 1). Assembly standards allow the assembly of parts using the prefix and suffix found on the plasmid backbones containing those parts.

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The BioBrick Standard (RFC 10) was introduced in 2007. This standard is the most used because the vast majority of parts are compatible with it. The RFC 10 uses restriction enzymes that recognize EcoRI, NotI, and XbaI in the prefix and SpeI, NotI, and PstI in the suffix. The main issue with BBF RFC 10 is that it produces an 8bp scar that results in a shift of the reading frame. Thus, BBF RFC 10 impedes the construction of fusion proteins. The standards developed in further years sought to solve this problem by creating scars that could be translated into amino acids. The BioBrick BB-2 Standard (RFC 12) was proposed in 2008. BioBricks compatible with RFC 12 are maintained in plasmid backbones that have EcoRI, NotI, and SpeI as the prefix and NheI, NotI, and PstI as the suffix. The scar that results from assembling parts using RFC 12 translates into the amino acids alanine and serine. The BglBricks Standard (RFC 21) was developed in 2009. BglBricks have restriction sites for EcoRI and BglII in the prefix and BamHI and XhoI in the suffix. The resulting scar corresponds to glycine and serine residues. The Silver Standard (RFC 23) is a modification of RFC 10 as it uses the same enzymes and restriction sites of BBF RFC 10; however, the scar that it produces has 6bp, which encodes for amino acids threonine and arginine. Finally, the Freigbur Standard (RFC 25) uses the same prefix and suffix of RFC 10 but adds NgoMIV and AgeI restriction sites in the prefix and suffix, respectively[13,18,19].

Assembly Standards	Assembly Methods
BioBrick Standard (RFC 10)	3A Assembly
BioBrick BB-2 Standard (RFC 12)	
BglBricks Standard (RFC 21)	Gibson Assembly
Silver Standard (RFC 23)	
Freigbur Standard (RFC 25)	Golden Gate Assembly

Table 1. Assembly standards and assembly methods for the design of biological devices.

Assembly methods are compatible with most of the assembly standards. The Three Antibiotic (3A) Assembly uses the same restriction enzymes of RFC 10. However, the composite part resulting from the ligation of two parts is introduced in a plasmid with an antibiotic resistance that is different from the other two backbone vectors. This technique permits the selection of the vector with the composite part using antibiotic selection instead of using gel electrophoresis to purify the digested parts before ligation. Gibson Assembly is a scarless technique that allows the simultaneous assembly of multiple fragments. It uses a 5' exonuclease, a DNA polymerase, and a DNA ligase. Gibson Assembly does not require specific prefixes or suffixes as it uses PCR primers to produce overlapping BioBricks. Golden Gate Assembly facilitates the assembly of different fragments in one reaction. This technique is based on type IIs restriction endonucleases, usually BsaI, and a T4 DNA ligase. Type IIs endonucleases cut DNA sequences outside their recognition sites, leaving single-stranded overhangs of 4bp. The ligation product of Golden Gate Assembly lacks restriction sites, and the 4bp overlapping fragments can be designed in such a way that multiple parts can be ligated in a single direction[19–23].

The Difference Between Synthetic Biology and Genetic Engineering

There tends to be confusion between Synthetic Biology and Genetic Engineering in which some might even use these terms interchangeably. However, the real problem is whether the difference between these terms is scientific or merely a matter of terminology. Although these two fields of biology share the DNA manipulation basis and approach to intervene in the complexity of molecular biology, they differ in many aspects. According to the Encyclopædia Britannica Synthetic Biology is a field of biology whose main objective is the creation of fully operational biological synthetic systems from the smallest constituents possible[24]. Whereas Encyclopædia Britannica defines Genetic Engineering as "the artificial manipulation, modification, and recombination of DNA or other nucleic acid molecules to modify an organism or population of organisms"[25]. Here, Genetic Engineering becomes one of the many essential tools for synthetic biology because while Synthetic Biology creates synthetic organisms with several biological parts, Genetic Engineering modifies already existing organisms.

One of the major differences between these fields of Biology is the use of engineering. Synthetic Biology relies intensively on the standardized concept of engineering involving the design of optimized genetic circuits with biological parts from many different species as well as industrial analysis and mathematical modeling to achieve this. Genetic Engineering, on the other hand, relies on the alteration of genetic material based on a set of methodologies and is often represented as a hit and miss activity. For this Genetic Engineering is considered a misnomer in which there is hardly any engineering involved. The engineering part in Genetic Engineering is considered a synonym for manipulation of genetics instead of optimization[26,27]. Another important difference between Synthetic Biology and Genetic Engineering is the potential risks. It is general consensus that the risks that Synthetic Biology poses are far more serious than Genetic Engineering due to scientists failing to recognize their limitations and overestimating their ability to control these organisms. Thus, GMOs are closer to patients with organ transplants rather than Frankenstein's monster[28].

Applications of Synthetic Biology

The iGEM competition gathers teams of high schoolers, undergraduates, and graduates every year, from several countries, to present biological systems that have been developed using the biological parts available in the Registry. The goal of the competition is to promote the implementation of Synthetic Biology to design solutions for different problems. The projects presented in the competition are oriented to tackle issues in different areas, including therapeutics, manufacturing, food and nutrition, environment, energy, etcetera. In 2019, several projects were awarded in different categories. For instance, a project (Novosite) in the Therapeutics category had the objective of improving wound healing by creating an antimicrobial, cellulose-based bandage able to deliver peptides and enzymes with antimicrobial activity. The team engineered *Escherichia coli* and *Vibrio natriegens* to produce enzymes and peptides attached to a carbohydrate-binding domain (CBD). In the Manufacturing category, the project Paper Transformer was awarded first place. Paper Transformer was created to produce bacterial cellulose (BC) from short cellulose fibers found in wastepaper. To achieve this, the team engineered *E. coli* to hydrolyze cellulose and synthesize BC employing a dual plasmid system containing three devices: cellulose hydrolysis, BC synthesis, and a regulator. Chlamy Yummy was the award-winning project in the Environment category. The team developed a method for the degradation of polyethylene

terephthalate (PET), one of the most common plastics, to deal with the increasing contamination by plastics. They used *Chlamydomonas reinhardtii* as the chassis to produce PETase and MHETase enzymes, which degrade PET into its monomers.

Outside the context of the iGEM competition, Synthetic Biology has been successfully applied for the manufacture of biofuels and biopharmaceuticals. The most famous examples are the production of 1,4-butanediol (BDO), the antimalarial drug artemisinin, and the anticancer compound taxol. BDO is an important chemical intermediate used to make plastics, elastic fibers, and polyesters. No known organism is capable of synthesizing BDO, so its production relies on petroleum feedstocks. Researchers optimized two heterologous pathways for the synthesis of BDO in *E. coli.* The metabolic routes were divided into upstream and downstream pathways for the biosynthesis of 4-hydroxybutyrate (4HB) and the conversion of 4HB to BDO, respectively. To achieve BDO production in *E. coli* a combination of native enzymes from E.coli and heterologous enzymes from *Porphyromonas gingivalis*, *Mycobacterium bovis*, and *Clostridium acetobutylicum* was used. Additionally, the host metabolism was engineered to channel carbon and energy into the pathways by knocking out several genes involved in the formation of fermentation products and by modifying the host's TCA cycle[29–32].

Artemisinin is a natural compound produced by the plant *Artemisia annua*. The therapeutic properties of artemisinin against multidrug-resistant *Plasmodium spp*. were discovered in the 1970s. Even though artemisinin derivatives are considered as first-line antimalarial drugs, its availability is limited, and its price has fluctuated due to inconsistencies in *A. annua* yields. To ensure steady and higher production of artemisinin, researchers engineered *E. coli* to synthesize the artemisinin precursor, amorpha-4,11-diene, by introducing a heterologous isoprenoid pathway from *Saccharomyces cerevisiae*. The authors expressed the mevalonate pathway of yeast in *E. coli* together with a codon-optimized synthetic variant of the amorphadiene synthase found in *A. annua* (ADS). Two operons, top, and bottom, were assembled for mevalonate pathway expression in bacteria. The top operon transformed acetyl-CoA into mevalonate, whereas the bottom operon converted mevalonate to FPP. Then ADS turned FPP into amorphadiene. Subsequent projects have focused their efforts to produce artemisinic acid, the direct precursor of artemisinin, from the oxidation of amorphadiene[33,34].

Taxol (paclitaxel) is a terpenoid found in the Pacific yew tree (*Taxus spp.*). Taxol is a powerful anticancer drug that has been used to treat several types of cancers, including breast and lung cancer, leukemia, lymphoma, and sarcoma. Similar to artemisinin, the isolation of taxol from its vegetal source is expensive and time consuming due to low yields and the presence of other taxoids with similar chemical structures. To avoid the extraction of taxol from *T. brevifolia*, researchers engineered *S. cerevisiae* to produce paclitaxel by introducing heterologous genes involved in the taxol biosynthetic pathway and the isoprenoid pathway. The authors expressed in yeast heterologous geranylgeranyl diphosphate (GGPP) synthase from *Sulfolobus acidocaldarius* and a codon-optimized variant of taxadiene synthase from *T. chinensis*. GGPP is converted into taxadiene by the taxadiene synthase, which is further transformed into taxol following oxygenation. Also, to favor the production of GGPP, a truncated version of the yeast HMG-CoA reductase was expressed as well as a transcription factor mutant allele[35,36].

The Issues of Synthetic Biology

The fact that synthetic biology aims to fabricate biological interchangeable, standardized sequences of genes and even design new genomes without immediate biological ancestry has raised ontological, political, economic, and ethical concerns based on the possibility that synthetic biology may be intrinsically unethical. Some of the major concerns surrounding synthetic biology rely on questions about whether we have enough knowledge on structures and regulatory mechanisms and the ability to control DNA sequences and synthetic genomes and whether it is safe enough for its use in less-restricted settings. Some of these concerns also include, scientists overestimating their ability to control synthetic organisms and failing to recognize their own limitations, side effects of assuming the techniques work, public safety and social consequences, potential dangers of genetically modified organisms, the resilience of natural ecosystems and ultimate impacts on the habitats and species for which the targets were devised. Other considerations include antibiotic resistance, allergies, carcinogens, toxicity among human health, and horizontal gene transfer[27-32].

Also, as in many emerging technologies, there is a preoccupation for dual-use applications and the deliberate misuse of the technology for nefarious purposes. In this case, synthetic biology has given rise to the potential bioterrorism and biowarfare with the synthesis of lethal biological weapons if fallen into the wrong hands. For example, in 2013 the National Science Advisory Board for Biosecurity (NSABB) advised against the publication of papers including H5N1 influenza ¨gain of function¨ with the concern that this information could allow H5N1 influenza to become transmissible from mammal to mammal and act as a shortcut for the development of the deadly biological weapon. Another example of the potential misuse of synthetic biology could be the creation of pathogens more toxic than the preexisting, considering that this has happened before with traditional genetic engineering techniques with a vaccine-resistant strain of the mousepox virus. Some even believe that synthetic biology can pose a threat higher than nuclear technology. This is mainly because the information for synthetic biology and life sciences, in general, is mostly of public domain contrary to that of nuclear technology and because in the future synthetic biology may be cheap and portable contrary to nuclear technology, which is bulky and expensive. A potential way to reduce the risk of harmful misuse of synthetic biology is by applying regulations and policies that ensure enforcement of chemical and biological weapon conventions and rules for DNA sales benchtop DNA synthesizers[37-39,43-45].

Some emerging issues question how synthetic organisms will interact with already existing species and whether these will disrupt communities or be invasive and how will issues like these be regulated to avoid ¨garage biology¨. Also, synthetic biology has given rise to doubts on the impact that engineered organisms intended to generate services to benefit people will have on natural ecosystems that already deliver these services. Besides, there are also uncertainties in whether there will be interactions between synthetic and natural organisms, and if the public notion of what is natural will change and challenge the basis for conservation action. There are also concerns about humans ¨playing god¨ which could have a religious interpretation of humans taking the role of a higher being by avoiding the constraints of timescales and evolution. Therefore, ignoring the need for a natural template to create life from non-living inorganic matter ignoring human

limitations. Another ethical concern lies on whether synthetic biology may fall in between machines and living things because usually in synthetic biology organisms are referred to as ¨genetically engineered machines¨ or intracellular processes as ¨genetic circuits¨ thus allowing these metaphors to interpret synthetic biological organisms as machines. Also, this metaphor assumes that the behavior of a complex object or organism could be explained by reference to its parts[39,45,46].

In the political and economic side, synthetic biology raises concerns in Latin American countries such as Ecuador, Peru, Venezuela, Brazil, Colombia, and Mexico being these, countries with massive biodiversity of fauna, flora, bacteria, and microorganisms arguing that synthetic biology could strengthen the gap between developing and developed countries. This is because biotechnology companies can obtain patents for synthetic organisms, DNA synthesizer machines, and their digitalized genome maps on the argument that they did not exist in nature previously, for industrial purposes. The benefits of synthetic biology will reflect the economic interests of those able to invest, develop and patent them. Latin American countries have economies based on agriculture, with crops of potatoes, banana, corn, beans, and thousands of medicinal and culinary plants, which could jeopardize the raw material of new biotechnological productions. Therefore, there could be out-and-out biopiracy or bioprospecting to produce modifications in commonly used living organisms, to privatize them, leading developing countries to pay royalties for these. This could raise questions such as how will a balance be achieved between private risk and gain and public benefit and safety[37,39].

Bioethics currently have a higher priority for other ethical controversies that are nearer in the future, such as abortion, artificial intelligence, stem cells, human-non-human chimeras, and animal treatment; thus, synthetic bioethics haven't been evaluated in depth. The Presidential Commission for the Study of Bioethical Issues issued a report in December of 2010 stating the *New Directions: The Ethics of Synthetic Biology and Emerging Technologies* in which several issues are considered for precautionary and risk analysis. However, it is argued that insufficient work has been done to address the risks of this discipline, which requires attention, so strategies for mitigating the potential dangers be discussed accordingly. Also, it is considered that the preexisting traditional regulations related to laboratory management and pathogens are not enough for the emerging field of synthetic biology[42,45,47].

Conclusions

To summarize, Synthetic Biology is the combination of basic sciences with engineering with the goal of creating, designing, and redesigning biological systems and devices to understand biological processes and to achieve useful and sophisticated functionalities to improve human welfare. Synthetic Biology is based on the idea of extraction and reassembly of "biological parts" along with the principles of abstraction, modularity, and standardization. It is important to remember that while Genetic Engineering is one of the many essential tools for synthetic biology and they share the DNA manipulation basis and approach to intervene in the complexity of molecular biology, they differ in many aspects, and the two terms should not be used interchangeably. The iGEM competition for the implementation of Synthetic Biology has attracted projects such as the creation of an antimicrobial, cellulose-based bandage able to deliver peptides and enzymes with antimicrobial activity, production of bacterial cellulose (BC) from short cellulose fibers found in wastepaper, and the degradation of polyethylene terephthalate (PET). Some of the applications that have already been done by Synthetic Biology include the production of 1,4-butanediol (BDO), the antimalarial drug artemisinin, and the anticancer compound taxol. The fact that Synthetic Biology has the potential to design new genomes without immediate biological ancestry has raised ontological, political, economic, and ethical concerns based on the possibility that synthetic biology may be intrinsically unethical.

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ESCUELA DE CIENCIAS BIOLÓGICAS E INGENIERÍA

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YACHAY TECH
UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL

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Docencia, investigación, extensión y proyección social al servicio del territorio

UCO
Universidad Católica de Oriente
Vigilada Mineducación

Fortalezas **institucionales**

- Biotecnología
- Limnología
- Derechos Humanos – Posconflicto
- Internacionalización
- Inclusión Social
  - SER Servicio Educativo Rural
  - Educación de Alfabetización
- MIS – Instituto de formación para el trabajo y el desarrollo humano
- Formación humanística "Ruta Humanística en el currículo - Cátedra abierta Madre de la Sabiduría"
- Investigación y desarrollo tecnológico
- Comprometida con la calidad
- Centro de Estudios Territoriales
- Biodiversidad
  - Herbario
  - Ictiología
  - Litoteca

Áreas del **conocimiento**

- Ciencias Agropecuarias
- Ciencias de la Educación
- Ciencias de la Salud
- Ciencias Económicas y Administrativas
- Ciencias Sociales
- Derecho
- Ingenierías
- Teología y Humanidades

- 26 programas de pregrado
- 16 programas de posgrado
  - 1 doctorado
  - 8 maestrías
  - 7 especializaciones

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