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The works must be unpublished and refer to topics of Biological and Health Sciences, Medical Mycology, Dermatology, Immunology, Human Ecology, Parasitology, Pediatric Infectious Diseasesand other topics related to Medicine and Health Sciences.

Presentation of Content

In the first article we present, *Auxiliary Expert System in Nutrition*, by SÁNCHEZ-VILLLASEÑOR, Carlos Alberto, ANGUIANO-BELLO, Ernestina, CARRILLO-QUIROZ, Anastacio and BARCENAS-NAVA, Areli, with affiliation in the Instituto Tecnológico de Iguala, as the following article we present, *Preliminary evaluation of the bacteriological quality of air in lagoon of Cajititlán, Jalisco, Mexico*, by CASAS-SOLÍS, Josefina, ROSAS-RAMÍREZ, Aurora and GARCÍA-VELASCO, Javier, with ascription in the Universidad de Guadalajara, as following article we present, *Molecular identification of mycobacteria species present in patients with cutaneous tuberculosis in Yucatán, Mexico*, by CAAMAL-LAW, Ángel D., PUC-FRANCO, Miguel A., ANDUEZA-PECH, María G., CERÓN-ESPOINOSA, José D. and VARGAS -GONZÁLEZ, Alberto, with secondment at the Universidad Autónoma de Yucatán, as the last article we present, *Histological study of platelet-rich plasma on lesions induced in the lab rabbit femur*, by MAR TÍNEZ-AGUILAR, Pablo Isaac, GARCÍA-SUASTEGUI, Wendy Algeria, HANDAL-SILVA, Anabella and MORÁN PERALES, José Luis, with secondment at the Benemérita Universidad Autónoma de Puebla.

Content

Article **ECORFAN Journal Republic of Guatemala**
June 2018 Vol.4 No.6 1-4

Auxiliary Expert System in Nutrition

Sistema auxiliar de expertos en nutrición

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Abstract

The present work designs and implements an auxiliary expert system in nutrition for mobile platforms, with which, access to nutritional information will be facilitated to any user who has the need to obtain a personalized food plan and has difficulties to assist with a human expert. Using the Android programming language, using the SCRUM development methodology, which involves in stages the activities defined during the development of the project, in this way, the development of this becomes more agile and allows to deliver higher quality to the user. The expert system guides users, taking into account some of their physical characteristics and activities, to take a food plan appropriate to their profile to bring better nutrition and prevent diseases related to poor diet. Through the data provided by the user, the expert system identifies, through the knowledge acquired from a human expert, the calorie consumption allowed for each user, thus obtaining a personalized food plan.

Expert system, Food Plan, Nutrition, Android

Resumen

El presente trabajo diseña e implementa un sistema experto auxiliar en nutrición para plataformas móviles, con el cual, se facilitará el acceso a información nutricional a todo usuario que tenga la necesidad de obtener un plan alimenticio personalizado y tenga dificultades para asistir con un experto humano. Utilizando el lenguaje de programación Android, empleando la metodología de desarrollo SCRUM, la cual conlleva por etapas las actividades definidas durante el desarrollo del proyecto, de esta manera, el desarrollo de este se vuelve más ágil y permite entregar mayor calidad al usuario. El sistema experto orienta a los usuarios, tomando en cuenta algunas de sus características físicas y actividades que realiza, a tomar un plan alimenticio apropiado a su perfil para llevar una mejor alimentación y prevenir las enfermedades relacionadas a una mala alimentación. A través de los datos que proporciona el usuario, el sistema experto va identificando, por medio del conocimiento adquirido de un experto humano, el consumo permitido de calorías para cada usuario, obteniendo así un plan alimenticio personalizado.

Sistema Experto, Plan Alimenticio, Nutrición, Android

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Introduction

Currently nutrition is a matter of national interest, since good nutrition means preventing diseases and reducing health costs. However, food offers are many and cause confusion due to ignorance, which is why poor diet is common. This requires the intervention of a human expert with knowledge on the subject, but like any expert, it will always be more difficult to access it than to access a system where your knowledge is stored. Due to the above, the interest in creating a nutritional expert system for mobile platforms was aroused.

Justification

Currently there is no nutritional expert system on mobile platforms focused on being used by the population in general, which is why this project is born trying to satisfy the need to opt for a personalized food plan without having to resort to a human expert.

Problem

Currently, Mexico occupies the first place in a world level in childhood obesity and the second place in adult obesity, which end up leading to a prevalence of overweight of 70% in adulthood. In the long term, obesity favors the appearance of diseases such as diabetes, heart attacks, high cholesterol levels or kidney failure, among others. The main cause to which it points is the habits of consumption of unhealthy foods, not to mention with access to experts in nutritional health.

Hypothesis

Through the use of the nutritional expert system it is expected that the whole person does not have access to a nutritional health professional, can carry out a personalized nutritional plan that will help them not to pay for illness due to poor diet, and in this way contribute to the decrease in the percentage of diseases related to poor diet.

Objective

General Objective

Design, develop and implement an auxiliary expert system in the elaboration of food plans..

Specific Objectives

- Design and develop a database to store information of users and users.
- Investigate and implement new formulas for the design of a personalized food plan.
- Design and develop the system using the Android programming language.

Theoretical Frame

The Expert System branch of the Artificial Intelligence are informatics system simulate the learning, memorazing, reasoning, communication and action process consequence of an expert human in every branch of the science.

The technology of an expert system has a database of knowledge with accumulate experience of expert human and a whole rule for apply this database in especially.

This characteristic allow keep data and knowledge get logic conclusions, take decisions, learn of The experience and facts communicate with expert human, explain because the decisions taked by consequence of all this.

The expert system facilitates access to nutritional information to any user who has the need to obtain a personalized food plan and has difficulties in attending with a human expert.

A personalized food plan promotes good nutrition which means prevention of diseases and reduction of health costs.

The expert system guides users, taking into account some of their physical characteristics and activities, to take a food plan appropriate to their profile to bring better nutrition and prevent diseases related to poor diet.

Android is an object-oriented programming language, designed especially for people who depend on presentation.

The system is developed in this language because its features allow it to be installed on most mobile devices.

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Research Method

The method used in the development of the expert system is the SCRUM development model.

Research Sort

Use technology for research because to solve a direct need of society, use innovation tools such as the expert system to make the knowledge of an expert more accessible to those who need it.

Theoretical Method

Use the method of analysis-synthesis because it is mainly made for the methods and stages used for software development, we can isolate the process of identifying food plans and put it in a user-friendly interface, because it uses an abstract programming guide to the objects.

Software Development

The first screen of The Expert System shows a simple menu with images and text making this more interactive with the user. The window has 5 buttons: Personal information, menu, food list, table of equivalences and tips.

Figure 1 Main menu screen.

click on Menu, it shows a window where you will have to fill each field with your personal data so that the system can analyze them and make the food plan

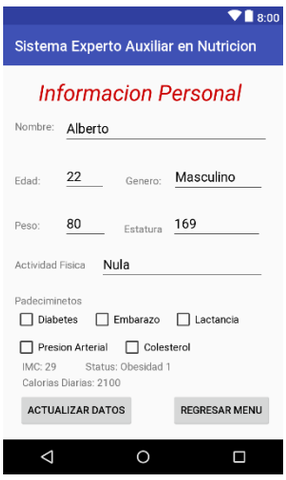

Figure 2 Personal Information Window

Once you have finished filling in the data, you can consult your personalized food plan by clicking on the Menu button on the main screen. In the menu window, the recommended consumption portions of each food group are shown, for each of the 5 meals

Figure 3 Confirmation window to exit of the system

Click on the Food List button, it shows a window where you can consult, by groups, information of the food stored in the database.

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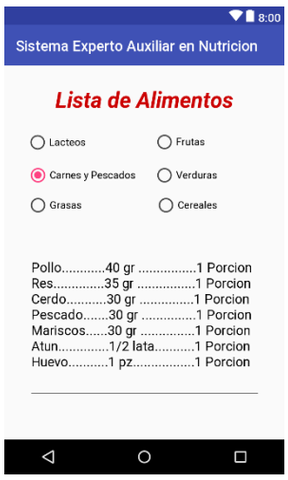

Figure 4 food list window

Click on the Equivalence Table button, a window with information is displayed so that the user can calculate the portion sizes of some foods using the size of their hands.

Figure 5 Equivalence table window

Click on the Tips button, open a window where some tips are shown, which are usually given by the human expert.

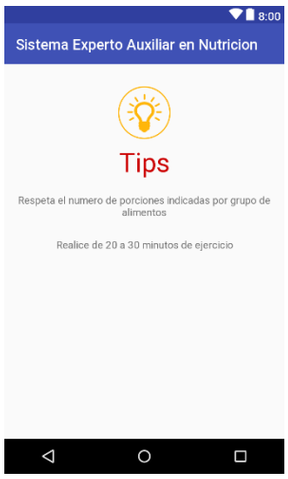

Figure 5 Tips window

Results

With the implementation of the expert system, the user will obtain a personalized food plan, decreasing the use of financial resources and optimizing their time by not having to attend with a human expert. In terms of long term, the user will have a tool to help prevent diseases related to poor nutrition, such as obesity, overweight and malnutrition.

Conclusions

Access to the knowledge of an expert in nutritional health will be easier with the implementation of this tool.

Users can obtain a food plan, without worrying about attending with a human expert. Also with this option you can consult a diet when they have the time available.

Referencias

Instituto Nacional de Salud Pública. Encuesta Nacional de Salud y Nutrición de Medio Camino 2016 Informe Final de Resultados. Disponible desde:https://www.gob.mx/cms/uploads/attachment/file/209093/ENSANUT.pdf

Rich, E., Knight, K. (1991) Artificial Intelligence. McGrall-Hill.
Winston, P. (1992) Artificial Intelligence. Addison-Wesley.
Nilsson, N. (1986). Principes of Artificial Intelligence.

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Preliminary evaluation of the bacteriological quality of air in laguna de Cajititlán, Jalisco, Mexico

Evaluación preliminar de la calidad bacteriológica del aire en laguna de Cajititlán, Jalisco, México

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Abstract

The Laguna de Cajititlán, is located in the central portion of the state of Jalisco, approximately 25 km from the city of Guadalajara. This area presents fragmentation of habitat, caused by population growth, the development of new subdivisions around the lagoon, overexploitation of the soil and water and insertion of economic activities that have generated the loss and affectation of natural resources. The purpose of this work was to know the bacteriological quality of the air in the Laguna de Cajititlán, Jalisco and the health risks of the population. The sampling was done in 5 points in summer. 100 liters of air were taken using different culture media for the determination of aerobic mesophiles, Escherichia coli, Salmonella and Staphylococcus aureus by the M Air T Millipore system. The samples were incubated at 37oC for 48 hours, the CFU were quantified and biochemical tests were applied for their identification. The total percentage for Gram positive and Gram negative bacteria shows 62% and 38% respectively. The microbiological diversity found as a function of the frequency observed is as follows: Escherichia coli 30.8%, Staphylococcus aureus 51% and Salmonella 4.8%, all considered pathogenic bacteria.

Wastewater, Bioaerosols, Pathogens, Health, Threats, Risks

Resumen

La Laguna de Cajititlán, se localiza en la porción centro del estado de Jalisco, a 25 km aproximadamente de la ciudad de Guadalajara Esta área presenta fragmentación del hábitat, ocasionada por el crecimiento demográfico, el desarrollo de nuevos fraccionamientos alrededor de la laguna, sobreexplotación del suelo y agua e inserción de actividades económicas que han generado la pérdida y afectación de los recursos naturales. El propósito de este trabajo fue conocer la calidad bacteriológica del aire en la Laguna de Cajititlán, Jalisco y los riesgos a la salud de la población. El muestreo se realizó en 5 puntos en verano. Se tomaron 100 litros de aire utilizando diferentes medios de cultivo para la determinación de mesófilos aerobios, *Escherichia coli*, *Salmonella* y S*taphylococcus aureus* mediante el sistema M Air T Millipore. Las muestras se incubaron a 37ºC durante 48 horas, se cuantificaron las UFC y se aplicaron pruebas bioquímicas para su identificación. El porcentaje total para bacterias Gram positivas y Gram negativas muestra un 62% y un 38% respectivamente. La diversidad microbiológica encontrada en función de la frecuencia observada es como sigue: *Escherichia coli* 30.8%, S*taphylococcus aureus 51% y Salmonella 4.8* %, todas ellas consideradas bacterias patógenas.

Aguas Residuales, Bioaerosoles, Patógenos, Salud, Amenazas, Riesgos

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Introduction

The lagoon of Cajititlán is located in the central portion of the state of Jalisco, approximately 25 km from the city of Guadalajara under the jurisdiction of the municipality of Tlajomulco de Zúñiga. It is located between the coordinates 20 ° 23 '48 "and 20 ° 28' 11" north latitude and the 103 or 14 '290 "and 103 ° 27' 38" west longitude. It has an approximate area of 14.3 km2 with a maximum northwest-southeast length of 7.5 km, it has a northeast-southwest distance of 2 km, with an average depth of 1.69 m.

This body of water is the most important in the area and is supplied by a series of streams that fall both from the Sierra El Madroño and the drains of other hills located in its surroundings. The climate of the zone is classified as temperate subhumid, with rainfall in summer (Cortés, 2000). This area presents fragmentation of the habitat, caused by the demographic growth, the development of new subdivisions around the lagoon, overexploitation of soil and water and insertion of economic activities that have generated the loss and affectation of natural resources. An important factor that makes this body of water more vulnerable to the pollutants poured into it, this is an endorheic basin, so these pollutants accumulate in this.

The streams that flow into the lagoon incorporate large volumes of domestic and industrial wastewater, while the runoff that crosses the cultivation areas or livestock activities contribute agrochemicals dangerously contaminated (Velázquez-López et al., 2012).
Recently there have been episodes of massive deaths of both species of flora and fauna, and the real causes of these phenomena have not been established, which makes its study imperative because of the social and environmental reach of this body of water.

Theoretical framework

There is a close relationship between development, environment and health. The current development models generate impacts on the environment, as a consequence of the use of available resources (Arbeláez et al., 2010). Contaminants can be incorporated into the environment in the form of gases, dissolved substances, particles or in solid form.

These are incorporated into the aqueous medium through routes such as the atmosphere and the soil (runoff and leaching phenomena). This is generated through point or diffuse sources, the difference between the two is that a pollutant originating in a point source can be collected, measured and treated; In addition, the point source can be identified or georeferenced. A diffuse source consists of various point sources that could be controlled. One of these sources is water that originates in the collection and discharge of urban, industrial wastewater and certain agricultural activities (POFA, 2012).

Several studies on pollution focus only on gases and inert particles, when most of them are biological particles, mainly bacteria, fungi and viruses that cause diseases. These airborne or bioaerosal particles are those that are in suspension in the air alive or dead constituted by products of their metabolism, bacterial endotoxins, mycotoxins, peptidoglycans, as well as pollen, and even small insects and their residues, algae, protozoa and dust mites, which are sometimes pathogenic (Vélez-Pereira and Camargo-Caicedo, 2014).

The atmosphere does not have a native microbiota, but it is a means of dispersing many types of microorganisms from other environments. Certain bacteria and their many metabolites affect atmospheric chemistry through physical processes such as the nucleation of ice and the formation of droplets. clouds, and can also affect the global climate and the hydrological cycle (Hurtado, et al., 2014).

A biological particle to be considered as a bioaerosol must contain a diameter of 3 nm for viruses, from 0.25 to 20 μm for bacteria, from 17 to 58 μm for pollen from plants and from 1 to 30 μm for fungi, the most important are those with a diameter less than 3μm because of their size can penetrate the lower airways such as the pulmonary alveoli where they can cause damage to the bronchial epithelium, induce inflammation or neoplasia, degeneration, or can be transported to the pulmonary blood to spread throughout the body and cause aggressive infections for humans (Ghosh et al., 2015; Quarato et al., 2017). Bio-aerosols can contribute up to 25% to atmospheric aerosols and their exposure to them can cause adverse health effects, including infectious diseases, acute toxic effects, allergies and cancers (Li et al., 2017).

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In Latin America, studies on air quality and social perception are very limited, although in the region there are large cities with serious air pollution problems such as Mexico City, Sao Paulo and Rio de Janeiro, where the population is exposed to levels that exceed the established limits, according to estimates of the World Health Organization. (Catalán-Vázquez, 2006). Anthropogenic activities, such as vehicular traffic, wastewater treatment plants (Rosas, 2003), solid waste management centers, the movement of animals in exposed soils, agricultural practices and the manipulation of compost among others, they release a large amount of bacteria into the atmosphere, causing pollution of the surrounding areas.

In several samples of urban and home dust in Mexico City has been isolated E. coli, bacteria indicator of fecal contamination, and that constitutes 40% of total coliform bacteria isolated in the dust, indicating a potential risk of contamination by this and other pathogenic bacteria, as well as by viruses or parasites. It has been reported that bacteria are present in the atmosphere of extramural environments and that their inhalation represents a risk to health, either in its vegetative form or part of its structural compounds called "biogenic compounds", such as membrane lipopolysaccharides. external of Gram-negative bacteria and teicoic acids of Gram-positive. (Rosas et al., 2004).

In studies on the characterization of bioaerosols both in air of open and closed spaces, the genera of Cladosporium, Rhizopus, Fusarium and C. sphaerospermum have been identified as the most frequent microorganisms. Penicillium, Aspergillus and Cladosporium as the possible causes of respiratory allergies (Mortazavi and Ariya., 2015). Factors such as solar radiation, dehydration and rehydration, thermal effects, meteorological physics, the formation of radicals and ions and air turbulence are potentially harmful and lethal for microbial cells in the air (Karra and Katsivela, 2007).

Several studies agree on the need to carry out continuous monitoring of the concentration of bioaerosols in the air in order to assess the potential threats to health to which the population is exposed due to the presence of pathogens present in them, and to establish measures mitigation of this risk, thus improving their quality of life.

In addition to generating data for the establishment of environmental quality indicators that provide the basis for formulating a proposal for an environmental standard that considers the bacteriological quality of air in open and working spaces (Ki et al., 2014, Sánchez-Monedero et al., 2006).

Justification

In the surroundings of the Laguna de Cajititlán, according to INEGI data, in 2010 there were 59,625 people, states the State Water Commission. The Laguna de Cajititlán has a natural storage capacity of 54 million cubic meters. The main pollutants are nutrients mainly nitrogen and phosphorus, which cause the appearance of algae or aquatic weeds and fecal coliforms, caused by the discharges of wastewater, due to poor or no treatment. It is of vital importance to know if this body of water and the activities carried out in its surroundings contribute to the air pathogenic bacteria that put at risk the health of its inhabitants.

Problem Statement

Therefore, the information obtained from the air quality bacteriological studies allow us to assess the potential health threats to which the population is exposed due to the presence of these pathogens, as well as to establish mitigation measures to that risk, thus improving its quality of life, in addition to generating data for the establishment of indicators and the proposal of an environmental standard that considers the bacteriological quality of air in open and working spaces. The purpose of this work was to know the bacteriological quality of the air in the Laguna de Cajititlán, Jalisco and the threats to the health of the population.

Objective

To know the bacteriological quality of the air in the Laguna de Cajititlán, Jalisco.

Methodology to be developed

The Laguna de Cajititlán is located in the central portion of the state of Jalisco, approximately 25 km from the city of Guadalajara under the jurisdiction of the municipality of Tlajomulco de Zúñiga.

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It is located between the coordinates 20 ° 23 '48 "and 20 ° 28' 11" north latitude and the 103 or 14 '290 "and 103 ° 27' 38" west longitude (Figure 1). For the selection of the sampling points, those places where the greatest water contamination is perceived, such as bad odors, presence of noxious fauna, garbage and wastewater discharges, were taken into account.

Figure 1 Location map of the air sampling points in the Laguna de Cajititlán, Jalisco, Mexico. (modified from INEGI, 2012).

For sampling, the mechanical method was used with an M Air T Millipore air analyzer system (Figure 2). Petri dishes with trypticasein soy agar (AST) were placed for the determination of aerobic mesophiles, MacConkey agar for the isolation of Salmonella sp. and Escherichia coli and mannitol salted agar (MAS) for Staphylococcus aureus. Each of the media were independently placed in the head of the air analyzer, 100 liters were selected, and the equipment indications were followed.

Subsequently, the samples were transferred to the laboratory in coolers and incubated at a temperature of 37 ° C for 48 hours. At the end of the incubation the number of colony forming units (CFU) in a Quebec colony counter of each of the exposed plates was quantified and reserved for later isolation and identification of bacteria present (Rosas, 2003).

Figure 2 Air analyzer (Millipore M Air T®)

For the isolation and identification, they were selected by colonial morphology of each of the selective media and transferred to enrichment tubes, incubating them for 24 hours at 35 +/- 2º C. The samples were analyzed and bacteria identification was performed. according to what is described in Official Mexican Standard 113, 114 and 115 (SSA, 1995 and 2011).

Each of the colonies were grouped according to their staining characteristics in: Gram positive or Gram negative. For each group, the conventional tests for Gram-positive cocci were carried out. Fermentation tests for mannitol and hemolysis activity, oxidase, coagulase, catalase were determined. And the group of Gram negative bacilli TSI, LIA, MIO, FAD, citrate, malonate, urea, RM-VP, among others.

The results of the biochemical tests were interpreted by the metabolic changes of each species and compared with the Bergey manual for their identification. Subsequently the bacteriological quality and the frequency of the bacterial diversity at each point were determined.

Results

The results obtained indicate a variation in the concentration of bacteria between 750 and 2150 CFU / m3 in the different points sampled. (Table 1); these bacterial levels of bioaerosols found vary widely by region and site, which indicates a high level of microbial contamination in the air of this area. In a similar work they reported that the area of the Tijuana River, Mexico has a concentration 200 times higher than its reference area (beach) (Hurtado, et al., 2014).

Sampling Site	Coordinates	Aerobic mesophiles UFC/ m^3
Cajititlan	20º 25' 58"N 103º 18' 490"	2135
Fractioning the Reservation	20º 25' 50"N 103º 20' 400"	920
Cuexcomatitlan	20º 25' 48"N 103º 21' 370"	795
San Juan Evangelista	20º 24' 25"N 103º 19' 200"	1605
Los Cedros	20º 40' 96"N 103º 28' 361"	2120

Table 1 Cantidad de mesófilos aerobios en el aire en la Laguna de Cajititlán, Jalisco, México

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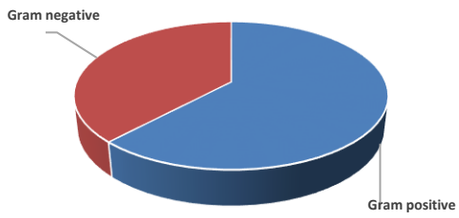
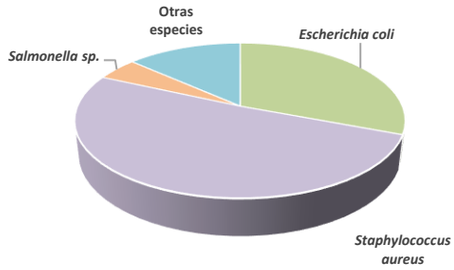
It is important to highlight that these results represent the concentrations of bacteria at the moment in which they are sampled, which is why meteorological factors such as relative humidity, temperature, speed and wind direction determine the concentration, the viability of the same and their transport. (Vélez-Pereira and Camargo-Caicedo, 2008, Li et al., 2017). In this study, a higher percentage of Gram positive bacteria (62%) was determined in the total of the sampled sites (Graph 1). The relative abundance of Gram-positive bacterial cells (cocci) could be explained by their structural composition of the cell wall, which is more resistant to hostile environments such as desiccation and solar radiation. This allows these bacteria to remain viable for longer in the air and to be pathogenic, causing infectious diseases in humans when they are inhaled or ingested in food and water (Hurtado, et al., 2014).



Graphic 1 Total percentage of Gram positive and Gram negative bacteria isolated in the air in the Laguna de Cajititlán, Jalisco, Mexico

The bacterial frequency of the five sampling points was determined, in all the points the following bacterial groups were identified: Escherichia coli with 30.8%, Staphylococcus aureus with 51%, in Salmonella 4.8%, and other bacteria 13.4% (Graphic 2), their concentrations depend on the sites sampled. Several pathogenic bacteria such as E. coli is an opportunistic pathogen and is found in high concentrations in fecal matter and wastewater, so it is an indicator of pathogenicity; Staphylococcus aureus can cause diseases of the skin, upper respiratory tract and oral cavity (Hurtado, et al., 2014).



Graphic 2 Bacteriological diversity isolated in the air in the Laguna de Cajititlán, Jalisco, Mexico

The atmosphere is considered as a means of transporting organic, inorganic and microbiological contaminants that disperse and affect the environment globally (POFA, 2012). This is a problem whose importance is evident in most of the world, which affects human health, plants and animals (Yassi, 2002).

It also registers a higher bacterial frequency in places densely populated by settlements, discharges of wastewater that are close to lakes or highly polluted rivers, such as the Laguna de Cajititlán, a body of water that with the various anthropogenic activities that are carried out in their environment they contribute to the air the presence of pathogenic bacteria that put the health of the population at risk. These findings coincide with those studied in similar works in open spaces (Catalán-Vázquez, 2006).

Conclusions

The presence of Escherichia coli, Staphylococcus aureus and Salmonella, in all the sites studied represents a threat to the health of the population of Cajititlan, Jalisco; the predominance of E. coli indicates contamination of fecal origin.

It is likely that the high incidence of gastrointestinal and respiratory diseases presented by the population located in the study area are caused by the bacteria found. It is important to carry out more studies on the emission sources of bioaerosols, the exposure of the population, and the risks for health.

References

Arbeláez M., Gasselin P., Hacon S. y Ruiz A. (2010). Indicadores de salud ambiental para la toma de decisiones. En L. A. C. Galvão, J. Finkelman, y S. Henao (Ed. OPS Mc Gram Hill Interamericana). *Determinantes ambientales y sociales de la salud (pp.*155-167).

Catalán-Vázquez, M. (2006). Estudio de la percepción pública de la contaminación del aire y sus riesgos para la salud: perspectivas teóricas y metodológicas. *Revista del Instituto Nacional de Enfermedades Respiratorias. México*, 19(1), 28-37.

ISSN-On line: 2414-8849
ECORFAN® All rights reserved.

Cortés R. C. (2000). *Florística de la Región de Cajititlán, Municipio de Trajomulco de Zúñiga, Jalisco, México.* (Tesis de Licenciatura). Licenciatura en Biología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara.

Ghosh B., Lal H. y Srivastava A., (2015). Review of bioaerosols in indoor environment with special reference to sampling, analysis and control mechanisms. *Environment International*, 85, 254–272.

Hurtado L, Rodríguez G., Lopez J., Castillo J. E., Molina L., Zavala M. y Quintana P. J. E. (2014). Characterization of atmospheric bioaerosols at 9 sites in Tijuana, Mexico. *Atmospheric Environment journal*, 96, 430-436.

INEGI Instituto Nacional de Estadistica y Geografia (2012). Recuperado de http://www.inegi.org.mx/default.aspx

Karra S. y Katsivela E. (2007). Microorganisms in bioaerosol emissions from wastewater treatment plants during summer at a Mediterranean site. *Water research,*41, 1355–1365.

Ki J. H., Hannah B. K.y Byung U. L. (2014). Concentration of environmental fungal and bacterial bioaerosols during the monsoon season. *Journal of Aerosol Science*, 77, 31–37.

Li, Y., Lu, R., Li, W., Xie, Z.y Song Y. (2017). Concentrations and size distributions of viable bioaerosols under various weather conditions in a typical semi-arid city of Northwest China. *Journal of Aerosol Science,* 106: 83-92.

Mortazavi R. y Ariya P. A. (2015). The impact of renovation on indoor airborne bacterial and fungal populations. *Indoor and BuiltEnvironment*, 26(10), 1351-1361.

POFA (2012) Diagnóstico Integral del Polígono de Fragilidad Ambiental (POFA) y su entorno 2012 CIATEJ. CIATEJ.

Quarato, M., De Maria, L., Gatti, M.F., Caputi, A., Mansi,F., Lorusso, P. Birtolo F. y Vimercati, L. (2017). Air pollution and public health: A PRISMA-Compliant systematic Review. *Atmosphere,* 8: 183. doi:10.3390/atmos8100183.

Rosas, A. (2003). *Evaluación ambiental del proceso de tratamiento de aguas residuales y los riesgos a la salud en la comunidad universitaria del CUCBA* (Tesis de maestría), Maestría en Salud Ambiental, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara.

Rosas, I., Cravioto, A. y Ezcurra, E. (2004). *Microbiología ambiental*. *SEMARNAT*. México.

Sánchez-Monedero, M., Roig, A., Cayuela M. y Stentiford, E. (2006). Emisión de Bioaerosoles Asociada a la Gestión de residuos orgánicos. *Ingeniería,*10(1), 39-47.

SSA Secretaria de Salud (2011) *NOM-113-SSA1-1994*, *Norma Oficial Mexicana* bienes y servicios. Método para la cuenta de microorganismos coliformes totales en placa, Diario Oficial de la Federación, 10 de junio de 2011. Recuperado de http://www.salud.gob.mx/unidades/cdi/nom/113ssa14.html.

SSA Secretaria de Salud (1995) *NOM-114-SSA-1994*, *Norma Oficial Mexicana* Bienes y servicios. Método para la determinación de salmonella en alimentos, Diario Oficial de la Federación, 22 de septiembre de 1995. Recuperado de http://www.salud.gob.mx/unidades/cdi/nom/114ssa14.html.

SSA Secretaria de Salud (2011) *NOM-115-SSA1-1994*, *Norma Oficial Mexicana* Bienes y servicios. Método para la determinación de staphylococcusaureus en alimentos, Diario Oficial de la Federación, 10 de junio de 2011. Recuperado de http://www.salud.gob.mx/unidades/cdi/nom/115ssa14.html

Velázquez –López, L., Ochoa-García, H. y Morales-Hernández J. (2012). Agua y conflictos ambientales de la ribera de Cajititlán, Jalisco. En Tetreault, D.; Ochoa-García, H. y Hernández-González E. (Coords.) Conflictos socioambientales y alternativas de la sociedad civil. Guadalajara: ITESO.Repositario Institucional del ITESO (pp. 181-213).

Vélez-Pereira, A. y Camargo-Caicedo, Y. (2008). Comportamiento Aerodinámico y viabilidad de las partículas Biológicas. *Revista RE'TAKVN*, 1(1), 44-56.

ISSN-On line: 2414-8849
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Vélez-Pereira A. M. y Camargo, C. Y. (2014). Análisis de los factores ambientales y ocupacionales en la concentración de Aerobacterias en unidades de cuidado intensivo del hospital universitario Fernando Troconis, 2009 Santa Marta-Colombia. *Revista Cuidarte*, 5(1):595-605.

Yassi, A., Kjellström, T., de Kok, T., y Guidotti, T. (2002). Salud Ambiental Básica. México: PNUMA

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Molecular identification of mycobacteria species present in patients with cutaneous tuberculosis in Yucatán, Mexico

Identificación molecular de especies de micobacterias presentes en pacientes con tuberculosis cutánea en Yucatán, México

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Abstract

The control of tuberculosis is a priority in the policies of the Ministry of Health in Mexico. In the present work, the detection of Mycobacterium sp. Was performed through the Polymerase Chain Reaction (PCR), as well as smear and culture tests. The study was conducted with 8 skin biopsies from different patients with suspected cutaneous tuberculosis, from the Dermatological Center of the State of Yucatan. Bacteriological studies were performed using the Ziehl-Neelsen method and the culture was carried out by incubation of the samples in Lowenstein-Jensen medium at 37 ° C for eight weeks. For PCR, the DNA was amplified with a specific pair of primers for the Hsp65 gene, retaining in all Mycobacterium spp. a size of 439 bp of expected amplification. The growth of Mycobacterium spp. Was observed in three samples after incubation in Lowenstein-Jensen media. The PCR-RFLP indicated other species of Mycobacterium (M. abcessus, M. fortuitum and M. leprae). The sensitivity of the PCR in relation to culture and sputum smear was 87.5%

Tuberculosis, Mycobacterium, Sputum Smear, Culture, PCR

Resumen

El control de la tuberculosis es una prioridad en las políticas de la Secretaría de Salud en México. En el presente trabajo, se realizó la detección de *Mycobacterium sp*., mediante la Reacción en Cadena de la Polimerasa (PCR), así como pruebas de baciloscopía y cultivo. El estudio se realizó con 8 biopsias de la piel de diferentes pacientes con sospecha de tuberculosis cutánea, del Centro Dermatológico del estado de Yucatán. Los estudios bacteriológicos, se realizaron mediante el método de Ziehl-Neelsen y el cultivo se llevó a cabo mediante la incubación de las muestras en medio Lowenstein-Jensen a 37 ° C durante ocho semanas. Para la PCR, el ADN se amplificó con un par específico de cebadores para el gen Hsp65, conservando en todos *Mycobacterium spp*. un tamaño de 439 pb de amplificación esperado. El crecimiento de *Mycobacterium spp*., se observó en tres muestras después de la incubación en medios de Lowenstein-Jensen. El PCR-RFLP indicó otras especies de *Mycobacterium* (*M. abcessus, M. fortuitum y M. leprae*). La sensibilidad de la PCR en relación con el cultivo y la baciloscopia fue del 87,5%.

Tuberculosis, Mycobacterium, Baciloscopia, Cultivo, PCR

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Introduction

Mycobacterioses are infections caused by mycobacteria other than those belonging to the Mycobacterium Tuberculosis Complex (MTC). The current situation of these diseases is unknown, because neither infections nor isolates are reported. Even so, it is believed that the prevalence of non-tuberculous mycobacteria (NTM) is increasing every year, possibly due to the fact that human beings are more in contact with certain types of environments, as well as the demographic changes of the population and the population. increase in people with some immunosuppression (Cassidy PM., y cols., 2009; Lee AS., y cols., 2009; Glapinski J., y cols., 2004; Yew WW., y col., 2011)

Most of the pathogenic species of MNT can cause skin and soft tissue infections, many of them are distributed worldwide and are fast growing, among the main ones, we find M. fortuitum, M. chelonae, M. abscessus, M. marinum and M. ulcerans (Amresh Kumar Singh y cols. 2015). In the state of Yucatán, the diagnosis is made through skin biopsies of patients with symptoms suggestive of this disease. The techniques used are smear microscopy and culture, but these have the disadvantage of low sensitivity and specificity in skin samples. However, through molecular techniques it is intended to overcome the limitations of conventional methods.

The GenoType ® Mycobacterium Common mycobacteria/additional species (CM/AS) assay (Hain Lifescience; Nehren Germany), is a commercial kit that uses the amplification of the gene RRNA 23S, together with the reverse hybridization with probes of specific oligonucleotides immobilized on membrane strips. Altogether the GenoType ® Mycobacterium CM/AS, identifies 31 species of Mycobacterium, including the complex M. tuberculosis, and a wide spectrum of MNT, being a reliable test for the identification of species of Mycobacteria (Lee AS., et al., 2009).

Material and methods

A descriptive, prospective, open, observational and transversal study was carried out. Samples of patients from the Yucatan Dermatological Center with clinical symptoms suggestive of cutaneous mycobacteriosis were included.

The samples were processed in the microbiology laboratory of the "Dr. Hideyo Noguchi" Regional Research Center, all patients signed informed consent letter to participate in the study. Samples included skin aspirates or biopsies of approximately 10 x 4 mm.

Smear and cultivation

All samples were cultured in Löwenstein-Jensen (L-J), for which the aspirates were sown directly, while the biopsies were macerated in a mortar with 2 ml of saline solution. For the sowing in L-J were taken 100 µ L of the supernatant and incubated at 37 °c for 4 weeks, supervising weekly to corroborate growth. The presence of resistant acid-alcohol bacilli was determined by the staining of Ziehl-Neelsen.

DNA extraction

For DNA extraction, a colony of bacterial culture or 400 M L was taken from the sample maceration. The InstanGeneTM Matrix commercial case was used (Bio-Rad Laboratories; Hercules, CA) following manufacturer's instructions.

Polymerase chain reaction

For each of the amplified genes, the following mixture was performed for the reaction: 12.5 µ L of Go Taq ® Green Master Mix 2x (promega Corporation 2800 Woods Hollow Road), 0.5 µ L of each initiator (1 M m), 10 M L of extracted DNA and 1.5 µ L of water free of nucleases to obtain a final volume of 25 M L. For the determination of gender was conducted a PCR aimed at amplifying a region of the gene that encodes for HSP65, which is retained in all Mycobacterium spp., with an expected enlargement size of 439PB, 50 using the first: HSP65 Fw 5 ´-ACC AAC GAT GGT GTG TCC AT-3 ´ and HSP65 Rv 5 ´-CTT GTC GAA gcc CAT ACC CT-3 ´

The conditions for amplification were as follows: 1 cycle at 94 °c for 5 minutes, then 35 cycles of 94 °c for 1 minute, 60 °c for 1 minute, 72 °c for one minute and a final extension cycle of 72 °c for 7 minutes.

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For the determination of the M. Tuberculosis complex is amplified a region of the gene RpoB that encodes the beta subunit of the RNA polymerase, with an expected magnification size of 230 Pb, using the first: MTC Fw 5 '-TAC GGT CGG CGA GCT GAT CCA AA-3 ' and MTC Rv 5 '-ACA GTC G GC GCT TGT GGG TCA AC-3 '. The conditions of cycling for the detection of the species are: 1 cycle at 94 °c for 5 minutes, then 35 continuous cycles will be repeated at 94 °c for 1 minute, 55 °c for 90 seconds and 72 °c for 1 minute, at the end 1 cycle at 72 °c for 5 minutes.

To determine the species M. leprae, sequences directed to amplify a conserved region of the folp1 gene found in the species M. leprae with an expected size of 281 bp were used. using the primers: Fw 5'-GCTTCTCGTGCCGAAGCG-3 'and Rv 5'-CCATCGCGGGATCTGCTCGCCC-3'.
Cycling conditions were: 1 cycle at 94 ° C for 5 min, then 35 continuous cycles of 94 ° C for 1 min, 62 ° C for 1 min, 72 ° C for one min, at the end of one cycle at 72 ° C for 7 min and left at 4 ° C.

GenoType® Mycobacterium CM / AS.

For the identification of mycobacterial species, the GenoType® Mycobacterium CM / AS kit was used, following the manufacturer's instructions and using the reagents provided by it. The complete protocol consists in the amplification of a sequence of the 23S rRNA gene (specific for the genus Mycobacterium), for which a BIO-RAD iCyclerTM thermocycler was used, the reaction was carried out as follows: 12.5 µl of Go Taq ® Green Master Mix 2X; 0.5 µl of each primer; 5 µl of extracted DNA and 6.5 µl of nuclease-free water, the amplification conditions were those suggested by the manufacturer. Once the DNA was amplified, the hybridization of the amplification products to specific oligonucleotides immobilized in the membrane strips continued, the development of the strips and the interpretation of the results was carried out according to the manufacturer's instructions.

Results

We included 40 samples from patients with clinical symptoms suggestive of mycobacteriosis, of which only 15 were positive for mycobacteria (37.5%).

Of all the samples, only 8 grew in the L-J culture and had a positive sputum smear (20%) (Figures 1 and 2).

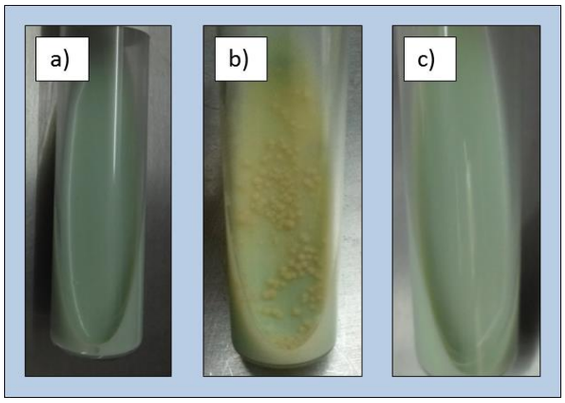

Figure 1 Culture results in Löwenstein-Jensen. a) negative control. b) positive culture. c) negative culture

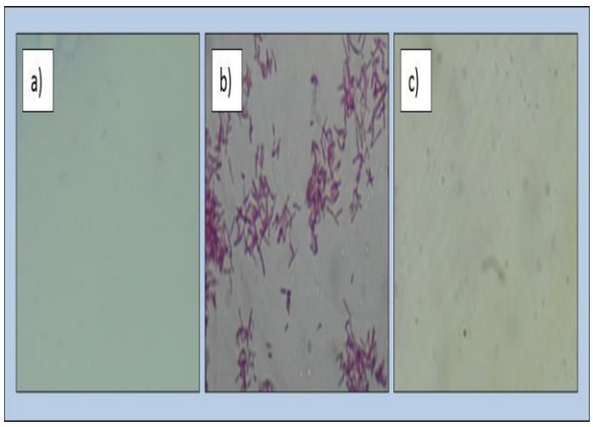

Figure 2 Results of smear microscopy. a) negative control. b) positive Bk c) negative Bk

Of the 40 samples, 15 were positive to the Mycobacterium genus when the hsp65 gene was amplified, however, all were negative to the rpoB gene, which corresponds to the Mycobacterium tuberculosis complex, indicating that they were non-tuberculous mycobacteria.

To the 15 positive samples to Mycobacterium genus, the identification of species was made, using the GenoType® Mycobacterium CM / AS, obtaining as a result that 7 of them only corresponded with the Mycobacterium genus, while 5 of them corresponded with M. fortuitum and 3 with M. abcessus (Figure 3).

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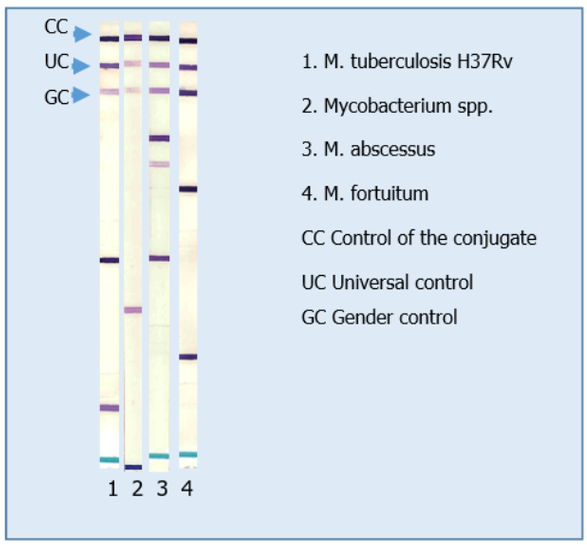

Figure 3 Results of GenoType Mycobacterium CM / AS. 1. In all the strips, the internal controls corresponding to lines 1, 2 and 3 appear; 1. Control of M. tuberculosis strain H37Rv (lines 10 and 16); 2. Mycobacterium spp. (line 12); 3. M. abscessus (lines 5, 6 and 10); M. fortuitum (lines 7 and 14)

The 8 samples that were not identified with the GenoType® Mycobacterium CM / AS, were amplified for the folp1 gene, which corresponds to the species M. leprae, and coincided with the symptoms of the patients.

At the end of the study, of the 40 samples, 15 were positive for mycobacteria, finding the following species: 5 M. fortuitum, 3 M. abcessus and 7 M. leprae.

Discussion and conclusion

PCR resulted in a higher positivity rate for Mycobacterium spp. than culture and smear microscopy. This may be due to the low number of bacteria present at the time of staining; in this regard, other studies indicate that $\geq 10^4$ bacteria per ml are required to obtain a positive result in Ziehl-Neelsen (Almaguer, J., et al 2009). In relation to the higher sensitivity of the PCR, it could be explained by the identification of mycobacterial DNA in samples with a negative result by smear microscopy (Suárez, M. J., et al., 2010). On the other hand, it is known that the culture requires 10 bacilli for isolation, considerably smaller than those suggested for smear microscopy (Parimango, D., et al., 2007). The sensitivity of the PCR in relation to the culture, which is considered the reference method for the diagnosis of tuberculosis, was 87.5%, culture 25% and baciloscopy 12.5%.

The positive results that were obtained by PCR in this study can be due to several factors, including the number of bacilli present in the sample that can give negative results for the culture and smear microscopy. In other cases, patients may have received treatment that would result in the death of the bacilli and would cause the absence of viable bacilli that limit the growth and visualization of colonies in the cultures (Frankel, A., et al., 2009; Tincopa , OW; Sánchez, LS 2003)

The results of this study confirm the importance of molecular techniques in the diagnosis of cutaneous tuberculosis (TB), especially for the correct identification of MNT and M. leprae, since conventional methods have significant disadvantages, which directly affect the treatment inadequate (Frankel, A., et al., 2009; Kandola, P., Meena, L. 2014; Silva, C., et al., 2007).

In this work, it was possible to identify M. fortuitum, M. abcessus with the help of GenoType® Mycobacterium CM / AS, which are opportunistic mycobacteria associated with skin infections, although their pathogenicity is lower than M. tuberculosis, demonstrating that it is a Useful tool for the diagnosis of MNT (Maroñas, L.; Postigo, M. 2013; Yang M., et al., 2016).

M. leprae was also found in patient samples and because this disease is still valid in the state of Yucatan, an accurate and timely diagnosis is important (Cenaprece 2012).

In conclusion, the use of molecular biology techniques for the identification of MNT in patients with TB, is of vital importance, since it guarantees an accurate diagnosis and an adequate treatment.

References

Almaguer, J.; Ocampo, J.; Rendón, A. 2009. Panorama actual en el diagnóstico de la tuberculosis cutánea. Revista española de cardiología; 100, 7, 562-570.

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ECORFAN® All rights reserved.

Amresh Kumar Singh, Rungmei S. K. Marak, Anand Kumar Maurya, Manaswini Das, Vijaya Lakshmi Nag, and Tapan N. Dhole, 2015. "Mixed Cutaneous Infection Caused by Mycobacterium szulgai and Mycobacterium intermedium in a Healthy Adult Female: A Rare Case Report," Case Reports in Dermatological Medicine; Article ID 607519, 4 pages,. doi:10.1155/2015/607519

Cassidy PM, Hedberg K, Saulson A, McNelly E, Winthrop, KL. 2009. Nontuberculous mycobacterial disease prevalence and risk factors: a changing epidemiology. Clinical Infectious Diseases; 49(12):124-129.

Frankel, A.; Penrose, C.; Emer, J. 2009. Cutaneous Tuberculosis A Practical Case Report and Review for the Dermatologist. JCAD; 2, 10, 19-27.

Glapinski J; Walkiewicz R.; Safianowska A.; Grubek-Jaworska H. 2004. Computerized Mycobacterium Type Recognition Using Numerical Data Base of Mycolic Acids Elution Profiles. Biocybernetics and Biomedical Engineering;24(1):25-38.

Kandola, P.; Meena, L. 2014. Extrapulmonary tuberculosis: Overview, manifestations, diagnostic and treatment techniques. Adv. Mater. Rev. 1, 1, 13-19.

Lee AS, Jelfs P, Sintchenko V, & Gilbert GL. 2009. Identification of non-tuberculous mycobacteria: utility of the GenoType Mycobacterium CM/AS assay compared with HPLC and 16S rRNA gene sequencing. Journal Of Medical Microbiology;58(7):900-904.

Maroñas, L.; Postigo, M. 2013. Micobacterias cutáneas: un reto diagnóstico. Más Dermatol; 19, 5-13.

Parimango, D.; Chávez, M.; Lujan, M.; Otiniano, M.; Muñoz, E. 2007. Comparación de los métodos Ogawa y Löwenstein-Jensen en el aislamiento de Mycobacterium tuberculosis pulmonar. Hospital Regional Docente de Trujillo, Perú. Rev. Med. Vallejiana; 4, 1. 24-31.

Silva, C.; Bermúdez, V.; Arraiz, N.; Bermúdez, F. 2007. Fármacos de primera línea utilizados en el tratamiento de la tuberculosis. Archivos Venezolanos de Farmacia y Terapéutica; 26,1.

Situación actual y desafíos de la lepra en México. 2012. Disponible en: http://www.cenaprece.salud.gob.mx/programas/interior/micobacteriosis/descargas/pdf/situacionactual_manualprocedimientos.pdf

Suárez, M. J.; Quiroz, J.; Jiménez, E.; Salazar, L. 2010. Diagnóstico molecular de Mycobacterium tuberculosis en cortes histológicos embebidos en parafina: investigación exploratoria. REVISTA MÉDICA DE LA UNIVERSIDAD DE COSTA RICA; 4, 1, 68-78.

Tincopa, O. W.; Sánchez, L. S. 2003. Tuberculosis cutánea. Dermatología Peruana; 13, 3, 195-214.

Yang M, Huh HJ, Kwon HJ, Kim JY, Song DJ, Koh WJ, Ki CS, Lee NY. 2016. Comparative evaluation of the AdvanSure Mycobacteria GenoBlot assay and the GenoType Mycobacterium CM/AS assay for the identification of non-tuberculous mycobacteria. J Med Microbiol. Dec;65(12):1422-1428. doi: 10.1099/jmm.0.000376.

Yew WW, Sotgiu G, Migliori GB. 2010. Update in tuberculosis and nontuberculous mycobacterial disease. American Journal Of Respiratory And Critical Care Medicine;184(2):180-185.

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Histological study of platelet-rich plasma on lesions induced in the lab rabbit femur

Estudio histológico del plasma rico en plaquetas sobre lesiones inducidas en el fémur del conejo de laboratorio

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Abstract

We analized the effect of platelet rich-plasma (PRP) on a induced lesion (IL) over femoral diafsis in rabbits to evalued the bone recuperation time. We used 10 male New Zeland rabbits with 1.8 at 2.0 kg of body weight. The rabbits were assigned in two experimental groups: induced lesion (IL) treated with PRP group and IL control group without PRP. IL consisted in a trephine on the femoral diafisis that consisted in a 5mm diameter hole. The PRP was obtanined by blood extraction of 3 ml that was centrifuged at 1400 rpm in 7 minutes and recovered plasma and newly centrifuged at 200 rpm in 15 minutes; each PRP fraction was actived with 10% $CaCl_2$ and then was collocated over the trephine. We obtained histological samples of IL region from second to sixth week. The PRPactivated treatment on IL makes to recovered the bone structure and the speeds process of bone healt from third week in the laboratory rabbit.

New Zeland Rabbit, Platelet Rich-Plasma, Induced Bone Lesion, Femur Histology

Resumen

Se analizó el efecto del plasma rico en plaquetas (PRP) sobre una lesión inducida (LI) en la diáfisis femoral del conejo, para evaluar el tiempo de recuperación ósea. Se utilizaron 10 conejos machos de la raza Nueva Zelanda Blanco con un peso de 1.8 a 2.0 kg. Los conejos fueron divididos en 2 grupos: grupo con lesión inducida (LI) tratado con PRP y grupo testigo con lesión inducida sin PRP. La LI consistió en un trépano en la diáfisis del fémur por medio de un orificio de 5 mm de diámetro. El PRP se obtuvo por extracción de 3 ml de sangre que fue centrifugada a 1400 rpm por 7 minutos tomando el sobrenadante, que nuevamente se centrifugó a 2000 rpm por 15 minutos; cada fracción obtenida de PRP fue activada con cristales de $CaCl_2$ al 10% y se colocó directamente sobre el trepano. Se obtuvieron muestras histológicas de la región con LI a partir de la segunada semana de evolución. El tratamiento con PRP activado en la LI mejora notablemente el tiempo de consolidación del hueso y acelera el proceso de regeneración ósea a partir de la tercera semana de evolución en el conejo de laboratorio.

Conejo Nueva Zelada, Plasma Rico en Plaquetas, Lesión Ósea Inducida, Histología del Fémur

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Introduction

In the Veterinary Practice, attention to injuries of the muscular skeletal system is frequent. In order to prevent serious and even lethal consequences in domestic animals that come to the hospital or clinic, the care of fractures must be done carefully to avoid causing situations that could aggravate the picture, for this routine maneuvers are applied as they are: Diagnose the fracture, immobilize and, if necessary, perform a surgical intervention to coact the separated segments, applying implants, placing brackets or fixing the affected parts (Santoscoy, 2010).

Bone fractures in most cases can heal correctly with minimal but careful handling, for example: an immobilizer can give excellent results, as long as the animal can reduce its movement and move away from conditions that lead to relapse, aggravating the injury or injury. Therefore, the rapid restoration plays an important role for the animal to recover in the broad sense.

The rejection of implants and the lack of consolidation of fractured parts has led to the search for biomaterials to promote bone regeneration, finding a response in platelet-rich plasma (PRP) as a useful adjuvant.

This compound is the supernatant that is obtained once the anticoagulated blood is subjected to centrifugation, generating a plasma that contains more platelets than the peripheral blood. The use of PRP was proposed by Marx and Carlson in 1986, for the placement of bone grafts in oral and maxillo-facial surgery, but aroused interest in other areas of biomedicine, such as orthopedics, otorhinolaryngology and reconstructive surgery. (Carrillo et al, 2013).

The use of PRP as a surgical complement in Veterinary Orthopedics has been recommended for its modulating and stimulating action on the proliferation of stem cells derived from mesenchymal stem cells, in addition to causing the release of some growth factors such as: TGF-beta, PDGF, FGP, IGF, among others (Faudez et al, 2010). In the literature, multiple techniques on the use of PRP are described, mainly in methods of maxillofacial surgeries and spinal surgery. However, there are few control studies that determine and quantify the magnitude of the effects of PRP.

The purpose of the present study was to analyze the effect of PRP placed on an injury induced in cortical bone, assuming that the process is similar to what occurs in a fracture, using the laboratory rabbit (New Zealand White) as a study model. The process of consolidation of the bone lesion was analyzed by means of conventional histological techniques with the general purpose of promoting the use of PRP as a reducer of fracture consolidation times.

Justification

Several studies have shown that bone fractures heal successfully by using various regenerative techniques and materials that stimulate bone tissue and surrounding organs. However, according to Butterfield et al. (2004) it has not been possible to demonstrate the efficacy of PRP treatment in osteoregeneration processes in rabbits. Other studies have demonstrated their effectiveness using the model of the miniature pig and recommend it as an adequate alternative to successfully heal bone and maxillofacial lesions (Schlegel et al, 2004; Schlegel et al, 2007).

Aghaloo et al. (2004) conducted a study on rabbit parietal bone grafting a surgical defect with autogenous bone and treatment with PRP. A radiographic, histological and histomorphometric analysis showed a slight tendency to increase bone density when PRP was used. In another similar study, demineralized bovine bone was used and a higher bone density could be shown in the cases with autogenous grafted bone, with respect to the groups grafted only with demineralized bovine bone and PRP (Laguna et al, 2006).

Roldan et al. (2004) using the Wistar rat model, observed that the application of PRP did not enhance bone formation in bovine inorganic bone or in grafts with autogenous bone. In another study on the frontal bone of pigs, the preparation of the graft bed with PRP does not seem to have an influence on osseointegration. Zechner (2003), however, created mandibular defects in twelve dwarf pigs and when applying PRP and installing the implants observed a better peri-implant bone regeneration in the initial phases (6 weeks), equaling the stimulation of osteogenic cell proliferation at 12 o'clock. weeks.

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For all the above, we can infer the importance and differentiation in the methods of obtaining the PRP in addition to the defect to be treated. There are reports on its application in muscle, tendon injuries or in its use in maxillofacial surgery, but these are preliminary clinical studies without long-term follow-up (Laguna et al, 2006).

Problem

The use of PRP in Veterinary Orthopedic Medicine is a little used method, since it implies the use of optimal infrastructure to be able to perform the correct extraction, in addition the diffusion of its use as a surgical adjunct is poor. Although it is known that the fracture process involves the release of multiple biochemical factors that lead to platelet activation, in this work we studied the effect of PRP administration on experimentally damaged bone tissue and bone recovery will be evaluated histologically in a cortical bone like the lab rabbit's femur.

The present work aims to show that the PRP is a useful resource for the acceleration in the regeneration of fractures or injuries in long bones, which require a surgical intervention to ensure the immobilization of the affected limb. The data obtained may be used in cases of surgical intervention in veterinary clinical management in the orthopedic area, in order to assess the time and effectiveness of bone recovery.

Hypothesis

The use of PRP in an induced fracture in the femur of the rabbit will reduce the time of bone repair with respect to the use of the conventional surgical technique used for the management of fractures in cortical bones.

General objective

To analyze the use of PRP on a fracture of the femur induced in the experimental model of the rabbit and bone reconstruction by means of conventional histology.

Theoretical framework

In the red bone marrow originates different phases of progenitor cells, which differ in cells of the erythrocytic, granulocytic, megakaryocytic and agranulocytic series.

The final result of this production is the emission of erythrocytes, leukocytes and platelets Megakaryocytopoiesis begins with the development and formation of megakaryoblasts, these are a large cell with a single nucleus, this cell is progenitor of promegakaryocytes, which are cells Larger than megakaryoblasts and with a multilobed nucleus, the precursor cells of the promegacariocytes are megacaricitos, which are cells with a single nucleus and abundant cytoplasm (Geneser et al, 2000).

Platelet formation takes place in the cytoplasm of the megakaryocytes through the formation of a structure known as proplaqueta, which suffers a fragmentation process resulting in small cells of discoidal and anucleated form, these are marked as platelets. Platelets are cells that fulfill different functions and have a specific structure (Reagan et al, 1999).

Platelets form a major role during the hemostasis process, after a vascular lesion initially seals the vascular defects and generates contact with the exposed extracellular matrix elements such as collagen and Von Willebrand Factor, in addition they provide a surface on which Active coagulation factors are recruited and grouped. The participation of platelets in the processes of hemostasis and thrombosis depends on the action of 3 events: platelet adhesion, change in shape and platelet aggregation (Milagros et al, 2000).

The bone is a specialized form of dense connective tissue, the extra cellular components suffer a calcification, providing the main function to the bones, being a supporting device, since they insert the muscles; In addition to providing protection to the thoracic viscera and the central nervous system, the bone system is of great importance for the regulation of the metabolism of calcium and phosphorus in the blood, which enables its homeostasis, bone growth and repair of the bones. fractures (Geneser et al, 2000).

Bone histology is diverse and functionally complex. The main cell types of the bone are: Osteoprogenitor cells: These are stem cells of pluripotent mesenchymal origin, present in the vicinity of the entire bone surface. In the bones of adults it retains its osteoprogenitor capacity that can be manifested through stimuli (such as a fracture). Osteoblasts:

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These are bone-forming cells derived from osteoprogenitor cells. It is disposed on the osteoid border, these cells synthesize, transport and organize the numerous proteins of the bone matrix and are in charge of initiating the mineralization process due to the accumulation of phosphate and calcium granules in their mitochondria, this content will pass to the calcification vesicles that will intervene directly in calcification and osteoregeneration.

Osteocytes: These are osteoblasts incorporated into the calcified bone matrix. These cells communicate with each other and with the cells of the bone surface through a wireless network of cytoplasmic processes that pass through tunnels in the bone matrix, called canaliculi, allowing the transcellular transport of substances captured by cells of the bone covering, in addition to the existing diffusion by means of the extracellular fluid that surrounds the extensions in the canaliculi. What is extremely important to start the remodeling of the bone tissue.

Osteoclasts: These are the cells responsible for bone resorption, these are the cause of bone destruction in bone remodeling. Bone is formed where mesenchymal connective tissue or cartilage previously exists. If the ossification takes place from mesenchymal connective tissue it is called intramembranous ossification, because it develops between conjunctive membranes that will give rise to the membranous bones such as the cranial vault. If the ossification takes place from the cartilage it will be called endochondral ossification and it is the one that originates the axial skeleton, the appendicular and the chondrocranium.

In both types of ossification the bone tissue that is deposited will be of the immature type, later this tissue will be organized and will become laminar, forming first all the spongy type bone and subsequently the one that will be compact. In any type of ossification, calcification of the extracellular matrix is essential. This calcification is granted by the secretion of the ossification vesicles (Geneser, 2000). Tissue regeneration occurs through a series of events at the cellular and molecular level, which are regulated by signal proteins. These substances with paracrine actions are released by the surrounding tissues damaged and emit signals to the cells of the hematopoietic system.

Platelets assume control of vascular regeneration when the subendothelium comes into contact with the plasma and induces a series of chain reactions that lead to the formation of a platelet plug and a blood clot, in addition to the secretion of biologically active proteins which they are secreted by the platelet structure (Barret et al, 2010). In the platelets there are alpha granules, which contain numerous proteins that influence healing or known as growth factors derived from platelets. The alpha granules also secrete proteins that will function as defense against external pathogens, through signal proteins that will attract macrophages, in addition to the leukocyte cells of the plasma. The platelets activate these proteins around 10 minutes after clot formation, reaching 95% activation in about 1 hour. After this the platelets synthesize and secrete additional proteins while they remain alive between 5 and 10 days (Morales, 2014).

When the platelets begin to diminish their action, the macrophages that arrived through the bloodstream stimulated by the platelets assume the responsibility of the regulation of the healing establishing the place of the regeneration by hand of the platelets (Maczy et al, 2012; Schlegel et al. al, 2004).

There are chemical signals that tissue cells produce and release to have paracrine effects on surrounding organs, which allow us to establish all the physiological conditions for the maintenance and regeneration of organs. Several studies have shown that bone fractures heal successfully by using various regenerative techniques and materials that stimulate bone tissue and surrounding organs. However, according to Butterfield (2004) it has not been possible to demonstrate the efficacy of PRP treatment in osteoregeneration processes in rabbits. Other studies have demonstrated their effectiveness using the model of the miniature pig and recommend it as an adequate alternative to successfully heal bone and maxillofacial lesions (Schlegel et al, 2004; Schlegel et al, 2007).

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For all the above, we can infer the importance and differentiation in the methods of obtaining the PRP in addition to the defect to be treated. There are reports on its application in muscle, tendon injuries or in its use in maxillofacial surgery, but these are preliminary clinical studies without long-term follow-up (Laguna et al, 2006).

Research Methodology

Biological material

Ten male rabbits of the New Zealand White breed with a weight of 1.8 to 2.0 kg were used. The rabbits were divided into 2 groups: group with induced fracture treated with PRP (Group FI + PRP; N = 5) and control group with fracture induced without PRP (FI; N = 5).

Anesthesia Procedure and Surgical Technique

The surgical technique and post-surgical accommodation were carried out in accordance with the guidelines set by the CICUAL-BUAP and NOM-062-ZOO-1999. All animals were subjected to the anesthesia protocol recommended by the Institutional Committee on Animal Use and Care of Cornell University (Gourdon, 2010, Martin & Kirsipuu, 2017).
For the process of sedation and anesthesia, the marginal vein was canalized using a No. 4 catheter, in order to avoid rupturing the vein or obstruction by coagulum. During the entire surgery 10 ml / kg / hour 0.9% NaCl was infused in each patient.

The anesthesia of the animals was performed with a Ketamine-Xylazine mixture (20 mg / kg / ketamine + 5 mg / kg / xylazine; iv) (Martin & Kirsipuu, 2017). Once the animal was anesthetized, the area to be incised with lidocaine was marked and blocked to reduce the pain, where the dermis and the epidermis were incised with a # 24 scalpel, to be able to approach the thick fascia lata muscle, which was incised to find the aponeurosis of the biceps femoral and vastus lateralis muscles; this tissue was debrided to expose the femoral diaphysis.

To avoid affecting the biomechanics of the bone, a trepano of small dimensions was performed in the femoral diaphysis with a manual drill of Jacob, until forming a hole of 5 mm in diameter and entering the medullary canal and touching the bone in its contralateral side.

Obtaining Plasma Rich in Platelets

On the day of surgery, five animals were assigned to the group with FI + PRP to obtain the PRP fraction. For this, the following steps were carried out, according to the Maczy and collaborators technique (2012):

Venous puncture: puncture was performed in the area of the neck in the jugular vein since this area is the least traumatic for the patient, blood must be obtained 10 minutes before surgery, the amount of blood required will depend on the weight, size of the patient and the defect to be treated. In the present work 3 ml of blood was extracted in each patient.

Extraction of blood: blood was collected in a tube with sodium citrate, as an anticoagulant to have a better activation process, since sodium citrate promotes platelet aggregation because it acts as a calcium scavenger and prevents the premature activation of platelets.

Platelet separation: this phase of the separation must be carried out with the appropriate equipment and ensure obtaining the highest concentration of platelets per unit volume, avoid rupture thereof and avoid premature activation of platelets. When the anticoagulated blood was centrifuged, 3 fractions were obtained according to their density: 1) Lower layer: density of 1.09, composed of red blood cells. 2) Medium layer: density of 1.06, composed of white blood cells and platelets (PRP). 3) Top layer: density 1.03, composed of plasma.

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This protocol is based on the separation of the formed elements from the blood and is carried out as a function of the density from highest to lowest. In this study the double centrifugation protocol was used, which consists in carrying out the first centrifugation process at 1300 RPM for 7 minutes, achieving the separation of the whole blood in a lower strip of red blood cells and a yellowish upper plasma, which will be platelet poor plasma (PPP). In order to obtain the plasma fraction with greater purity, the upper plasma fraction was extracted with a micropipette and centrifuged again at 2000 RPM for 15 minutes to obtain the fraction with the highest content or concentration of platelets (Beca et al, 2007; Martínez et al, 2002).

Activation of Plasma Rich in Platelets

Each sample of PRP was extracted with another micropipette and placed in another sterile tube, until the time of activation with 10% calcium chloride crystals to reverse the coagulation, add a proportion of 0.05ml of calcium chloride per 1ml of PRP, this mixture was later deposited directly in the trephine of each exposed femur of the experimental group (Figure 1) (Danche, 2006).

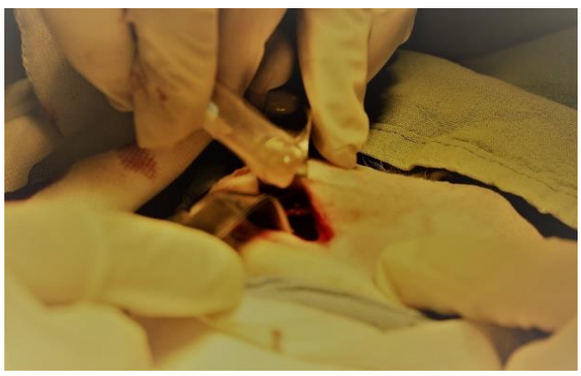

Figure 1 Form in which the PRP was applied; platelet activation with calcium chloride 10% produces a reaction transforms the PRP into a gel of easy application

Postoperative care

The 10 animals submitted to the surgery received isolation in the Claude Bernard Bioterio for their recovery. Hosted in individual cages, they received water and balanced feed ad libitum; the cleaning of the cages was done every day. All the animals were monitored daily until their sacrifice. During their lodging, all the animals received antibiotic therapy and postoperative analgesia with Enrofloxacin (5 mg / kg; s.c.) and meloxicam (3.5 mg / kg; s.c.), for five days.

The wound was subjected to daily cleansing with 1% chlorhexidine gluconate solution, for ten days until the healing was completed.

Autopsy and Obtaining Samples

To obtain samples of the femur, one animal from each group was transferred to the Neuroendocrinology Laboratory of the Department of Biology and Toxicology of Reproduction, of the Institute of Sciences of the BUAP, where euthanasia was applied to the 2nd, 3rd, 4th, 5th and 6 weeks with sodium pentobarbital (60 mg / kg, ip), to obtain samples of the left femur in each interval of postoperative evolution. The bone samples were obtained by cutting the femoral shaft with a saw. In all cases, the right femur was dissected as a control. The samples were fixed in 10% formaldehyde for 24 hours, before being processed for demineralization (Kemmerman et al, 1995), paraffin block assembly and histological section (Luna, 1975).

Histology of the Bone Tissue Samples

The demineralization process was carried out in the space of four weeks per sample, removing the acid on a daily basis and checking with the chemical methods every third day. From the fourth week, the bone tissue samples were ready to be included in paraffin. Strips were formed with 6 consecutive cuts and were left to float in the tub until the strip was extended avoiding creases, and were mounted on a slide with 3% inclusion gelatin to adhere the tissue cut to the glass.

Once assembled the tissue sections were allowed to dry at room temperature and placed in a closed chamber with formaldehyde vapors for 24 hours, before being dyed. All the dry sections were subjected to a stain with the hematoxylin-eosin technique (Luna, 1975).

Results

The results of the present study confirm the efficacy of PRP treatment for healing fractures or bone lesions. In general, after three weeks of evolution, a high proliferation of differentiated osteoblasts of progenitor cells was found in the histological sections of the injured bone. Similarly, large amounts of osteoprogenitor cells related to the endosteum and osteoblasts were found in areas with tissue formation.

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In addition, it was very common to find more collagen fibers in the group treated with PRP than what was observed in the animals of the control group. There were also signs of greater formation of canaliculi in the animals treated with PRP with respect to the control.

Figure 2 shows the appearance of an intact bone compared to the appearance of a lesion (induced fracture) in the second week of evolution. The animal with lesion without treatment with PRP still shows clear signs of tissue alteration characterized by the presence of leukocytes; however, no signs of necrosis were observed.

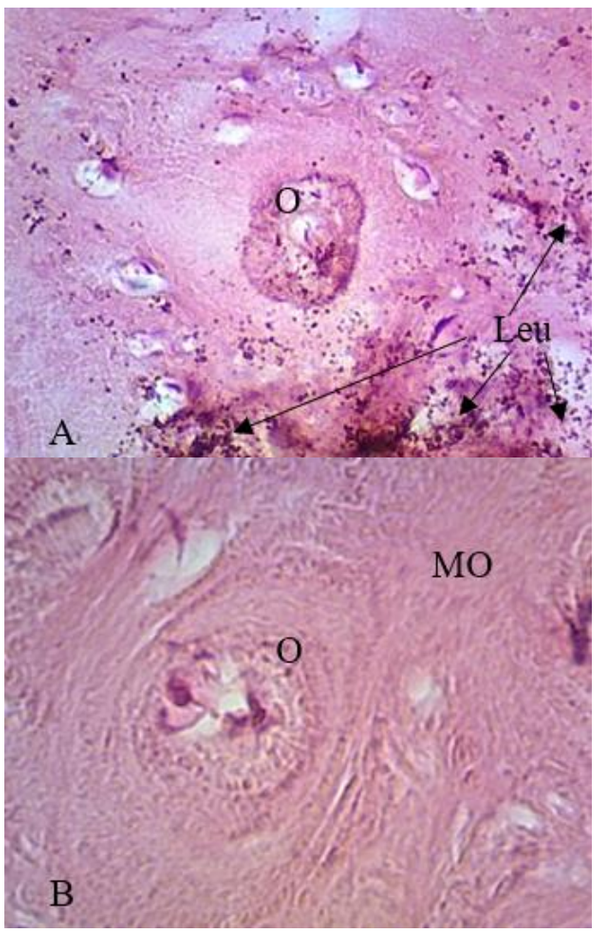

Figure 2 Images at 100X that show the appearance of the femoral shaft with the induced lesion (A) with respect to the diaphysis of a bone without fracture (B) at the second week of evolution. In A, the dense infiltration of leukocytes visible in the third postoperative week can be observed, even in areas of bone consolidation. The intact bone shown in photograph B allows us to observe the region around an osteone in healthy bone. O: Osteona; Leu: Leucócitos; MO: Bone matrix

From the third week of evolution, there is a visible regeneration of the mineralized bone matrix in the group of animals treated with PRP with apparent signs of regeneration clearer than in a control animal without treatment.

In both groups, the process of bone matrix formation is evident; however, in the animals treated with PRP, a greater degree of consolidation could be observed in the areas of bone formation, which was not observed in the control group (Figures 3 and 4).

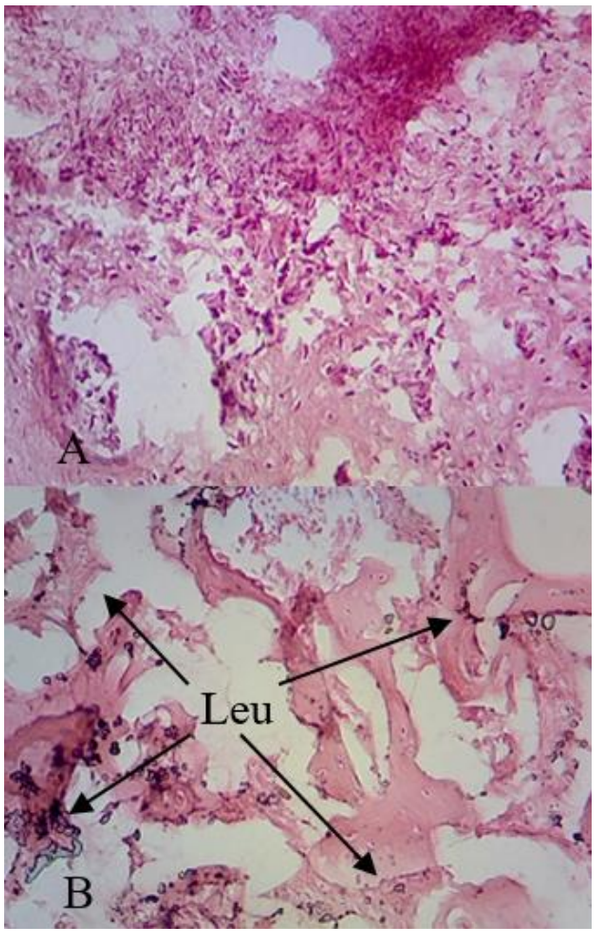

Figure 3 Images at 40 X that show the injured areas at the second week of evolution. A: treatment with PRP; B: bone of the control animal. Compared to the control, a better consolidated bone matrix is observed in the animal treated with PRP and virtual absence of leukocytes in the field

From the fourth week on, no great differences in bone regeneration between the experimental groups are observed; however, it was possible to notice the difference in the bone matrix since in the group treated with PRP, the process of mineralization of the matrix is more advanced and almost reaches its totality in comparison to the control group where the matrix remains largely cartilaginous (Figure 4).

The process of regeneration, restructuring and bone remodeling is favorable in the reduction of healing time in animals treated with PRP. It was possible to compare the presence of a zone of high cell proliferation in the group treated with PRP, which would reduce the bone healing time.

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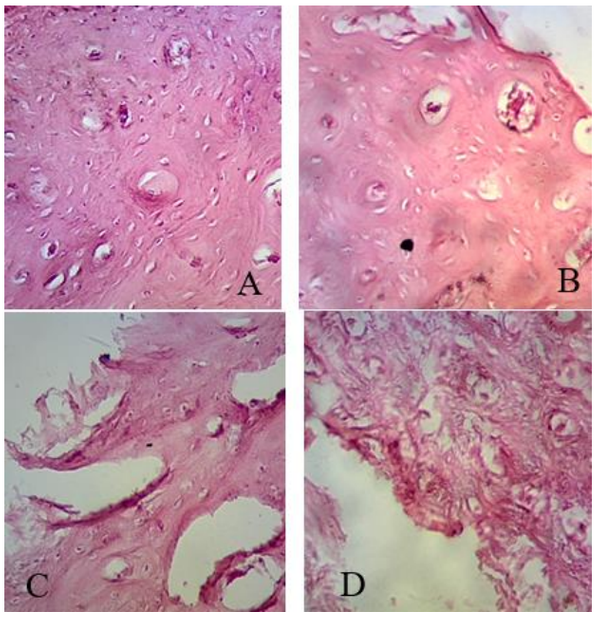

Figure 4 100X images showing the appearance of the bone matrix (MO) in animals treated with platelet-rich plasma (PRP) (Right)) and their controls (Left) by the third week. In the photographs of the control animals (B and D), a process of consolidation of the MO is apparently slower than in the animals treated with PRP, where the cartilaginous matrix (MC) in the regeneration zone is less dense than in the animals. the animals treated with PRP. In the animals that received the treatment with PRP (A and C), a greater quantity of osteocytes (OC) is noted and differentiated cells converted into osteoblasts (OB) can be found; however, in the control group, areas with a large population of osteoclasts (OT) predominate; CA: capillaries; ZM: Spinal Zone

Discussion of results

After assessing the effectiveness of bone regeneration after an induced lesion treated with PRP, the histological sections analyzed showed effective changes, particularly in areas of high bone regeneration that were more evident than in the animals that did not receive the treatment. The apparent reduction of time to heal the induced fracture could be a sign of the stimulus induced after the application of PRP.

Experimental data report that the use of PRP induces acceleration in bone regeneration in the first weeks after an injury or surgery in maxillofacial surgery (Danche, 2002, Fontana et al, 2004, Roldán et al, 2004). These works coincide in that effective results can be observed up to the fourth week with respect to the controls. However, as of the fourth week, a decrease in the speed of bone regeneration is noticeable, to the point of being minimally exceeded by the control group at 8 weeks.

Zechner (2003) suggests a PRP action dependent on time. In a study conducted in pigs, he affirms that PRP favors osteorregeneration around the sixth week; in his work he describes that, from that moment the effect of the PRP ceases in such a way that there are no structural differences in the regenerated bone with respect to what was observed at 12 weeks.

Several studies suggest that platelets significantly increase the proliferation of bone cells in adult individuals, stating that platelets act as local regulators in posttraumatic bone regeneration, possibly because of the growth factors released by platelets and attracted by means of chemotaxis (Gruber et al, 2002; Soffer 2004).

It has also been observed that the effect of PRP at the site of application lasts 5 days, but its effects on the acceleration of bone regeneration can be observed up to 4 weeks after its administration (Weibrich, 2004, Marx and Carlson, 1998). We chose to follow the technique proposed by Maczy and collaborators (2012), since it is an efficient, low cost method and preserves the autologous procedure since the activation is carried out with calcium chloride. However, it should be mentioned that there is no method that can be called "standardized", although there are comparative studies between the different methods of obtaining the PRP, none seems to be better than another, because apparently everything depends on the experimental conditions in the moment of its application. It has already been described that there are determinant variables such as the type of bone, its approach and even, the properties of the surrounding tissues where it is applied and the activation process (Beca et al, 2007).

Recent studies have estimated the concentrations of growth factors and cytokines contained in the PRP with various cellular concentrations and it has been observed that the amount of these elements is lower compared to the amount of plasma. Similarly, these studies indicate that low centrifugation speeds produce PRP with low purity and suggest that red blood cells release free radicals in large quantities, which could generate damage to the tissue that is desired to stimulate (Montesinos et al, 2017). The protocols analyzed and compared between different researchers allow us to suggest the use of the PRP obtaining technique that was used in this work, as long as the fracture model is similar.

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The rabbit as an experimental animal seems to be adequate because the process of bone formation and remodeling is three times faster than in other species (Roberts et al, 1988). In humans the remodeling process lasts between 6 and 9 months; This period of time is known as sigma. Thus, the sigma for the dog lasts 3 months and for the rabbit 6 weeks (Danche et al, 2006). The choice of trepan size (5mm diameter) was to wait for the prompt regeneration of the organism without PRP treatment, in addition to avoiding possible affections and unnecessary suffering for rabbits. On the other hand, the choice of femoral diaphysis as a test tissue for induced fracture was based on the fact that ossification is endochondral, where cartilage is formed first and then bone, which is important for the interpretation of the regeneration process bone with orientation to the reduction of fractures surgically.

It is important to note that the manipulation of blood is accompanied by possible changes in the quality of blood cells; These imponderable changes can be significant alterations that can affect the functioning and the interaction of the cells. The objective of the activation of the PRP before its application is to inhibit these alterations and obtain an optimal functioning of the PRP (Carrillo et al, 2013; Saez et al, 2007).

Conclusion

The treatment with activated PRP in bone lesions significantly improves the time of bone consolidation and accelerates the process of bone regeneration after a lesion from the third week of evolution in the laboratory rabbit.

References

Aghaloo T, Moy P & Freymiller E. Investigation of platelet rich plasma in rabbit cranial defects: A pilot study. Oral Maxillofac Surg 60:1176-1181. 2002.

Barret KE, Barman SM, Boitano S & Broocks HL. Control hormonal del metabolismo del calcio y fosfatos y fisiología del hueso. En: "Ganong: Tratado de Fisiología Médica". Capítulo 23; McGraw Hill. Mexico. 363-374. 2010.

Beca T. Hernández G, Morantes S & Bascones A. Plasma rico en plaquetas. Una revisión bibliográfica. Av Peridon Impanton 13:39-52. 2007.

Butterfield K, Bennet S & Grohowitz G. Effect of platelet rich plasma with autogenous bone grafts for sinus augmentation in a rabbit model. Oral Maxillofac Surg 33: 56-9. 2004.

Carrillo P, González A & Macías I. Plasmas rico en plaquetas, herramienta versátil de la medicina regenerativa? Cir Cir 74-82. 2013.

Danche TJ. Evaluación de la regeneración ósea mediante aplicación de PRP en un modelo experimental animal. Tesis maestría. DEA. U.C.M. 2002.

Danche TJ. Influencia del plasma rico en plaquetas en la regeneración ósea. Estudio densitométrico y morfométrico en calota de conejas osteoporoticas. Tesis doctoral. Universidad Rey Juan Carlos. 2006.

Faudez R. Plasma rico en plaquetas (prp) y su uso en cirugía veterinaria. Hospitales Veterinarios 2: 2010.

Fontana S, Olmedo DG & Linares JA. Effect of platelet-rich plasma on the peri-implant bone response. experimental study. Implant Dent 13(1):73-8. 2004.

Geneser F. Histologia sobre bases biomoleculares: Tejido Esquelético. Capitulo 12: Editorial Médica Panamericana. México. pp. 2000.

Gourdon J. Rodent Anesthesia. CARE 101.01. Cornell Center for Animal Resources an Education. Cornell University. (2010)

Gruber R, Varga F & Fisher MB. Platelets stimulate proliferation on bone cells: involvement of platelet-derived growth factor, microparticles and membranes. Clin Oral Impl Res 13:529-35. 2002.

Kammerman JR, Prophet EB & Barnes CF. Histotecnología Ortopédica. En: Métodos Histotecnológicos. Capítulo 13. Eds. E.B. Prophet, B. Mills, J.B. Arrington & L.H. Sobin. Registro de Patología de los Estados Unidos de América (ARP) / Instituto de Patología de las Fuerzas Armadas de los Estadios Unidos de América (AFIP). 73-81. 1995.

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Laguna G. J. Plasma rico en plaquetas. Revista española de Cirugía oral y Maxilofacial. 28. 2006.

Luna, L.G. Manual of histology staining methods of the armed forces Institute of Phatology. Mc Graw Hill. New York. 52. 1975.

Maczy G, Artega M, Benito M & Benito M. Aplicación del plasma rico en plaquetas (PRP) y sus derivados en implantología dental y cirugía plástica. Invest clin. 53: 408-418. 2012.

Martin M & Kirsipuu V. Rabbit anesthesia. CARE 103.01. Cornell Center for Animal Resources an Education. Cornell University. (2017)

Marx R & Carlson E. Platelet-rich plasma. Growth factors enhancement for bone grafts. Oral Surg Oral Med Oral Pathol 85:638-46. 1998.

Milagros G & Cristina C. Características Estructurales y funcionales de las plaquetas. Rev Cubana Agiol Cir Vas 2: 32-41. 2000.

Montesinos GP, Bojórquez MA, Ramírez HH, Sánchez MA, Pedrero PL, Santa cruz JF. Plasma rico en plaquetas: Estudio comparativo de cuatro protocolos para su obtención. Rev Cent Dermatol Pascua. 26: 2. 2017.

Morales MD. Estudios de los factores que pueden modificar la calidad del plasma rico en plaquetas (PRP). Universidad CEU Cardenal Herrera. 2014.

Reagan, WJ, Sanders TG & DeNicofa DB. Hematopoyesis. En: Hematología Veterinaria: Atlas de especies domésticas comunes. Capítulo 1. Harcourt Brace. España. 47-49. 1999.

Roberts E. Bone Tissue Interface. J Dental Education 52:804-809. 1988.

Roldan JC, Jepsen S & Miller J. Bone formation in the presence of platelet-rich plasma vs bone morphogenetic protein-7. Bone 34(1):80-90. 2004.

Santoscoy E. Ortopedia, neurología y rehabilitación en pequeñas especies. Manual moderno. México. 530pp. 2010.

Schlegel K, Donath K, Rupprecht S, Falks S, Zimmermann R, Felszeghy E & Wiltfang J. De novo bone formation using bovine collagen and platelet-rich plasma. Biomaterials 25. 5387–5393: 2004.

Schlegel K, Zimmermann R, thorwart M, Wilhelm F, Kronghoi B, Nkenky E & Felszeghy E. Sinus floor elevation using autogenous bone or bone substitute combined with platelet-rich plasma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 104: 15-25. 2007.

Saez TB, Calvo BJ, Puig GA. Calidad del plasma rico en plaquetas: estudio de la activación plaquetaria. Ergon Rev Esp Cir Oral y Maxilofac 29, 240-248. 2007

Soffer E, Ouhayoun JP & Dosquet C. Effects of platelet lysates on select bone cell functions. Clin Oral Impl Res 15: 581-588. 2004.

Weibrich G, Hansen W & Kleis G. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 34(1):665-671. 2004.

Zechner W, Tangl S, Tepper G, Furst G, Berhhart T, Haas R, Mailath G & Watzek G. Influence of plateler rich plasma on osseus heling on dental implants: Histologic and histomorfometric study in mini pig. Oral Maxilo Fac Implants 18:15-22. 2003.

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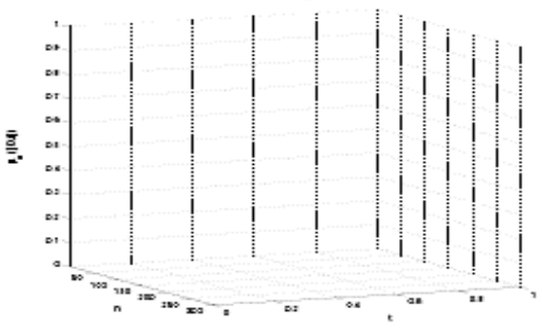

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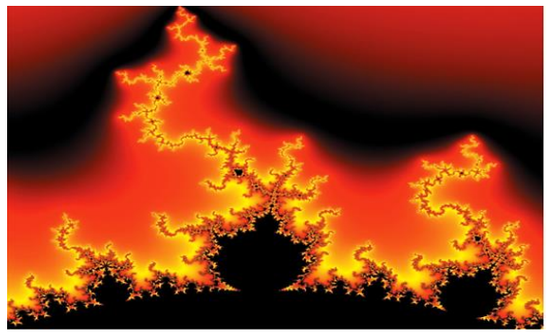

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