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RESEARCH ARTICLE

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Comparison of the phenotypic profile of antimicrobial resistance in the oral microbiota of non-smokers, tobacco smokers, and electronic cigarette vapers -a pilot study

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ABSTRACT

Introduction: The microbiota is a community of microorganisms that live in a specific environment. Their type and number depend on multiple internal and external factors. Oral is the second most diverse and populated microbiota of the body. Smoking and vaping induce changes in its composition, and it has been demonstrated that they can lead to an increase in antimicrobial resistance. **Objective:** To compare the phenotypic profile of antimicrobial resistance in the oral microbiota of non-smokers, tobacco users, and electronic cigarette vapers. **Methods:** An observational, descriptive, cross-sectional, and comparative study was carried out. Three groups of non-smokers, smokers of conventional tobacco, and electronic cigarette (EC) vapers of tobacco flavored e-juice were formed. Oral cavity samples were obtained, incubated, and seeded in agar plates. Bacteria were isolated and identified performing Gram staining, oxidase, indole, and biochemical test panels. Susceptibility tests were performed using a MicroScan autoSCAN-4 system and the Kirby-Bauer test. **Results:** Variation was observed in the populations of bacteria that were isolated in each of the groups, but the non-smokers showed the most pathogens. In the non-smoking group, *Staphylococcus sciuri* was the most common bacteria, *Staphylococcus sciuri* and *Enterobacter cloacae* were the most abundant in the smoking group, and in the EC vapers group, the most common bacteria were *Staphylococcus epidermidis* and *Staphylococcus sciuri*. **Conclusion:** Multidrug resistance was observed in all the groups. However, EC vapers showed the highest proportions of antimicrobial resistance, raising a major concern.

Key words: cigarette; e-cigarettes; vaping; microbiota; drug resistance; antimicrobial drug resistance.

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RESUMEN

Introducción: La microbiota es una comunidad de microorganismos que vive en un ambiente específico. El tipo y el número depende de múltiples factores internos y externos. La microbiota oral es la segunda más diversa y poblada del cuerpo. Fumar y vapear inducen cambios en su composición y esto puede conducir a un incremento en la resistencia antimicrobiana. **Objetivo:** Comparar el perfil fenotípico de resistencia antimicrobiana en la microbiota oral de no fumadores, consumidores de tabaco y vapeadores. **Metodología:** Un estudio observacional, descriptivo, transversal y comparativo se llevó a cabo. Se formaron tres grupos de no fumadores, fumadores de tabaco convencional y vapeadores de tabaco saborizado con e-juice (EC). Se obtuvieron muestras de la cavidad oral, incubadas y cultivadas en placas de agar. Las bacterias fueron separadas e identificadas realizando tinción de Gram, oxidasa, indol y páneces de pruebas bioquímicas. Las pruebas de susceptibilidad fueron realizadas usando el sistema MicroScan autoSCAN-4 y mediante el método Kirby-Bauer. **Resultados:** Se observó variación en las colonias de bacterias aisladas en cada uno de los grupos. En el grupo de no fumadores, la bacteria más común fue *Staphylococcus sciuri*; en el grupo de fumadores, lo fueron *Staphylococcus sciuri* y *Enterobacter cloacae* y, en el grupo de los vapeadores EC, las más comunes fueron *Staphylococcus epidermidis* y *Staphylococcus sciuri*. **Conclusión:** Se observó resistencia a fármacos en todos los grupos. Sin embargo, el grupo de vapeadores EC tuvo las mayores proporciones de resistencia antimicrobiana, planteando una gran preocupación.

Palabras clave: cigarrillo; cigarrillos electrónicos; vapear; microbiota; resistencia a fármacos antimicrobianos.

1. INTRODUCTION

Microbiota, also known as microflora, is the term given to a community of microorganisms including archaea, bacteria, fungi, protozoa, and viruses that live in a specific environment.¹ It comprises 10–100 trillion symbiotic microbial cells found in the skin and mucosal epithelium: oral cavity, respiratory tract, and gastrointestinal and urogenital tracts.² They are fast-evolving entities that respond to external perturbations rapidly in ways that affect the phenotypic responses, being able to reduce or increase the risk of developing certain diseases.³ Their type and number depend on the genetic background, type of birth, age, dietary habits, personal hygiene, use of antibiotics, and environmental exposure of each person, among others. In addition, it is constantly modified during lifetime.⁴

The oral microbiota is the second most diverse and populated microbiota of the body. It plays a key role in homeostasis by protecting the mouth from pathogenic colonization, reducing nitrate species, and facilitating food digestion.⁵ It comprises adherent microorganisms that can stick to the surfaces of gums and teeth; however, non-adherent microorganisms can also be found until they are removed by mechanical flushing.⁶ Up to date, its composition remains controversial as several species have not been identified. Still, since the beginning of the Human Microbiome Project, the most commonly found bacteria in healthy individuals include Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria. Non-bacterial microorganisms, such as fungi, protozoa, and viruses have been also reported.⁷

Smoking tobacco cigarettes is considered the leading cause of preventable diseases worldwide as it increases the risk of developing cardiovascular disease and certain types of cancer, among others.⁸ Brook I. and Gober A.E. have demonstrated that active or passive exposure to cigarette smoke eliminates indigenous bacteria, such as *Peptostreptococcus* and *Prevotella*, and allows the presence of pathogenic bacteria as *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumonia* in the upper airways.^{9,10} Furthermore, Lacombe A. et al. have reported that it also increases the profile of antimicrobial resistance in *Staphylococcus aureus*.¹¹

Electronic cigarettes (ECs), also known as vape pens, emerged as a possible solution to fight the tobacco epidemic and their use has rapidly increased, especially among adolescents. They are battery-powered devices that generally contain fewer toxic substances than conventional tobacco cigarettes; however, several health issues, including death, have been addressed.¹² Recently, some researchers have analyzed the effects of ECs in the oral microbiota. For example, Campos M.A. et al. compared if there were any differences in the oral microbiota when tobacco smokers replaced his habit with EC vaping.¹³ Stewart C.J. et al. compared the oral and gut microbiota of tobacco smokers and EC users,¹⁴ but, to our knowledge, none have studied their antimicrobial resistance profile.

The objective of our study was to compare the phenotypic profile of antimicrobial resistance in the oral microbiota of non-smokers, tobacco users, and EC vapers. Due to the size of the sample studied, this pilot study is part of a larger one,



which will be carried out when conditions allow it. Depending on the results obtained then, we will determine the statistical treatment to be carried out.

2. MATERIALS AND METHODS

An observational, descriptive, cross-sectional, and comparative study was carried out.

2.1 Study population

The study population consisted of three groups: non-smokers ($n = 11$), conventional tobacco smokers ($n = 8$), and EC vapers of tobacco flavored E-juice ($n = 11$). Each group was made up of female individuals whose ages ranged from 18 to 21 years. It was sought that the volunteers had a similar consumption rate of tobacco or tobacco-flavored E-juice among them. In order to participate in this study, the volunteers had to answer a few questions to make sure they suffered no diabetes, hypertension or obesity; they were not under any pharmacological treatment and showed no clinical manifestations of respiratory infection, such as fever, headache, conjunctivitis, rhinorrhea, sore throat or cough at the time of the study. Then, they signed an informed consent.

2.2 Sample collection

Sterile plastic bottles (Delta-Lab, Barcelona, Spain) and a bottle with 250 ml of drinking water were given to each person. The samples were taken in the morning, before subjects ate and brushed their teeth. They rinsed their oral cavity and gargled with drinking water that was spat in the sterile plastic bottle. Samples were sent to campus immediately in less than 60 minutes.

2.3 Bacterial isolation

Blood and heart infusion (BHI) broth mediums (Becton Dickinson; Franklin Lakes, NJ, USA) were inoculated with 10 ml samples. Subsequently, they were incubated at 37 °C for 24 h. After incubation, 10 μ L BHI broth medium was taken and seeded in blood agar (Becton Dickinson; Franklin Lakes, NJ, USA) using the cross-streak method and incubated again at 37 °C for 24 h. Pure colonies were obtained from the blood agars where the samples were seeded. The identification consisted of Gram staining, oxidase, indole, and biochemical test panels: The oxidase test was done using a BBL DrySlide Oxidase kit (Becton Dickinson; Franklin Lakes, NJ, USA), following the manufacturer's specifications. The indole test was carried out using a BBL DrySlide Indole kit

(Becton Dickinson; Franklin Lakes, NJ, USA), according to the manufacturer's specifications.

2.4 Biochemical tests

The biochemical tests were performed using a MicroScan autoSCAN-4 (System Beckman Coulter®; Kraemer Blvd. Brea, CA, USA). The MicroScan Pos Combo Panel Type 33 (PC33) and MicroScan Neg Combo Panel Type 68 (System Beckman Coulter®; Kraemer Blvd. Brea, CA, USA) systems were used. In both cases, the manufacturer's specifications were followed using Prompt® Inoculation System-D (inoculation system wands) and Pluronic® suspension (30 ml of stabilized aqueous surfactants), Inoculator-d grids, and the MicroScan RENOK system. The plates of the MicroScan Pos Combo Panel systems were incubated at 37 °C for 18 h, without CO₂ and with 40–60% humidity. The plates were revealed with peptidase reagents, potassium hydroxide, Kovac's reagent, 0.5 N, N-Dimethyl-1-Naphtylamine, 10% ferric chloride, sulfanilic acid, and 1-Naphthol (System Beckman Coulter®; Kraemer Blvd. Brea, CA, USA). Finally, they were read using a MicroScan autoSCAN-4 reader (System Beckman Coulter®; Kraemer Blvd. Brea, CA, USA) and the data were analyzed with LabPro Command Center System Ink version 4.42 (System Beckman Coulter®; Kraemer Blvd. Brea, CA, USA).

2.5 Susceptibility tests

Susceptibility tests were performed using two methods: MicroScan autoSCAN-4 system (System Beckman Coulter®; Kraemer Blvd. Brea, CA, USA) and the traditional disk diffusion technique (Kirby–Bauer method) as shown below.

Positive Combo Panel Type 33 (μ g/mL): amoxicillin/clavulanic acid 4 μ g/2 μ L; ampicillin 2–8 μ g; ampicillin/sulbactam 8 μ g/4 μ g–16 μ g/8 μ g; ceftriaxone 8 μ g, 32 μ g; ciprofloxacin 1–2 μ g; clindamycin 0.5–4 μ g; erythromycin 0.5–4 μ g; gentamicin 4–8 μ g; levofloxacin 1–4 μ g; moxifloxacin 0.5–4 μ g; oxacillin 0.25–2 μ g; penicillin 0.3 μ g, 0.12 μ g–0.25 μ g, 2 μ g, 8 μ g; rifampicin 1–2 μ g; Synercid 0.5–2 μ g; tetracycline 4–8 μ g; trimethoprim/sulfamethoxazole 0.5/9.5–2/38 μ g; and vancomycin 0.25–16 μ g.

Negative combo panel type 68 (μ g/mL): amikacin 16–32 μ g; amoxicillin/clavulanic acid 8 μ g/4 μ g–16 μ g/8 μ g; ampicillin 8–16 μ g; ampicillin/sulbactam 8 μ g/4 μ g–16 μ g/8 μ g; cefazolin 2–4 μ g; cefepime 4–16 μ g; ceftriaxone 1–2 μ g, 8 μ g, 32 μ g; cefuroxime 4–16 μ g; ciprofloxacin 1–2 μ g; ertapenem 0.5–2 μ g; gentamicin 2–8 μ g; imipenem 1–8 μ g; levofloxacin 2–4 μ g; meropenem 1–8 μ g; piperacillin/tazobactam 16–64 μ g; tetracycline 4–8 μ g; tigecycline 2–4 μ g; tobramycin 4–8 μ g; trimethoprim/sulfamethoxazole 2 μ g/38 μ g.

The Kirby–Bauer method was based on the Clinical and Laboratory Standards Institute protocol. The pure colonies obtained from the blood agar were re-suspended in bacteria suspension, depositing two or three medium-sized colonies (2–3 mm) in a broth inoculum of BBL Crystal (Becton Dickinson; Franklin Lakes, NJ, USA). The inoculum obtained was adjusted using a Mac Farland 0.5 reader (expected CFU/mL 1.5×10^8) and seeded on Müeller Hinton 150 × 15 mm² BD medium agar (Becton Dickinson; Franklin Lakes, NJ, USA). The antibiotic discs were placed using a Sensi-Disk dispensing system. The antibiotic panel was composed of amoxicillin/clavulanic acid (20 µg/10 µg), cefazolin (30 µg), cefepime (30 µg), cefoperazone (75 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), meropenem (10 µg), penicillin (10 µg), tetracycline (30 µg), and vancomycin (5 µg) (Franklin Lakes, NJ, USA).

3. RESULTS

3.1 Bacterial population isolated

A variation was observed in the populations of bacteria that were isolated in each of the groups. The largest number of bacterial species (15), each with a different frequency, was isolated from non-smokers. Within this group, the following results were found: *Staphylococcus sciuri* was the most frequent microorganism with a total of 5 (20.8%), followed by *Micrococcus* and related species with a frequency of 3 (12.5%) (Figure 1). In the smoking group, eight bacteria

species were isolated, each with a different frequency. The *Staphylococcus sciuri* and *Enterobacter cloacae* bacteria had higher prevalence with 4 (20%) isolates (Figure 2). In the EC vaper group, a total of ten species of bacteria were isolated, each with a different frequency. It was found that the species with the highest prevalence were *Staphylococcus epidermidis* and *Staphylococcus sciuri*, with a frequency of 3 (21.4%) (Figure 3).

3.2 Phenotypic profile of antimicrobial resistance

To obtain a complete picture of the phenotypic profile of antimicrobial resistance of each isolate, the results obtained by the Kirby–Bauer method and those obtained from the MicroScan system should be complementary. In the non-smoking group, the antibiotics which presented 50% or more percentage of resistance were Penicillin (83.33%), Erythromycin (70.83%), Ceftriaxone (64.28%), Vancomycin (62.50%), Ampicillin (60%), Amoxicillin/Clavulanic Acid (54.54%), and Ampicillin/Sulbactam (50%). In the smoking group, the antibiotics which presented 50% resistance or higher were Penicillin (90%), Erythromycin (80%), Ampicillin (71%), Vancomycin (70%), and Amoxicillin/Clavulanic Acid (50%). In the EC vaper group, the antibiotics which presented resistance of 50% or more were Penicillin (92%), Ampicillin (80%), Erythromycin and Amoxicillin/Clavulanic Acid (79%), Cefazolin (77%), Vancomycin (69%), Ceftriaxone (60%), and Cefepime (54%) (Figure 4).

Bacteria isolated from the oral microbiota of non-smokers

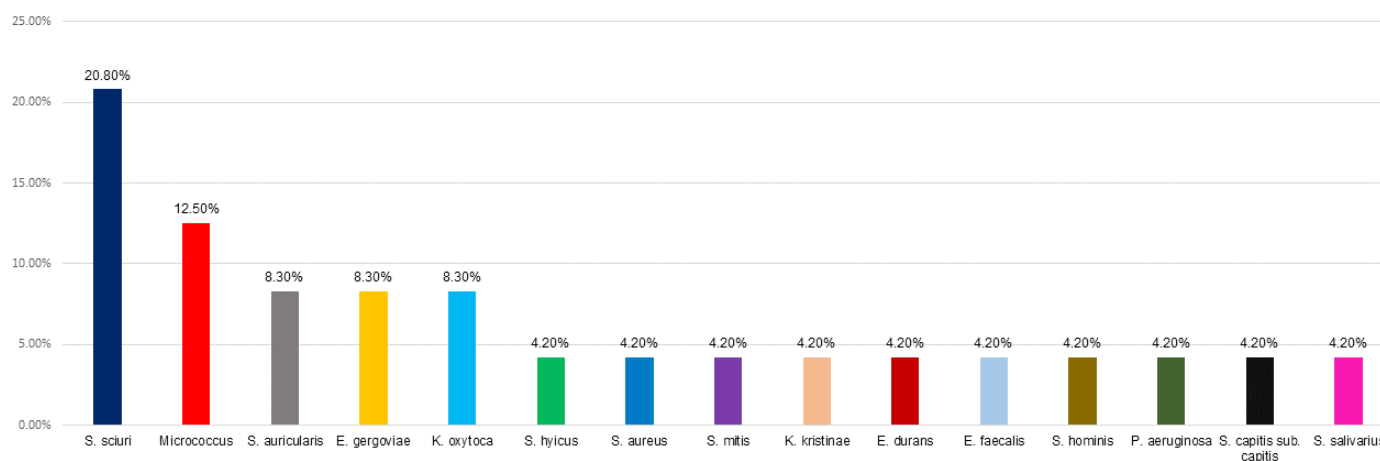


FIGURE 1. Bacteria isolated from the oral microbiota of non-smokers: *Staphylococcus sciuri* was the most frequent microorganism, with a total of 5 (20.8%), followed by *Micrococcus* and related species with a frequency of 3 (12.5%), while *Staphylococcus auricularis*, *Enterobacter gergoviae*, and *Klebsiella oxytoca* species presented a frequency of 2 (8.3%). The remaining ten species of the isolation presented the same frequency of 1 (4.2%): *Staphylococcus hyicus*, *Staphylococcus aureus*, *Staphylococcus mitis*, *Kocuria kristinae*, *Enterococcus durans*, *Enterococcus faecalis*, *Staphylococcus hominis*, *Pseudomonas aeruginosa*, *Staphylococcus capitis*, and *Streptococcus salivarius*.

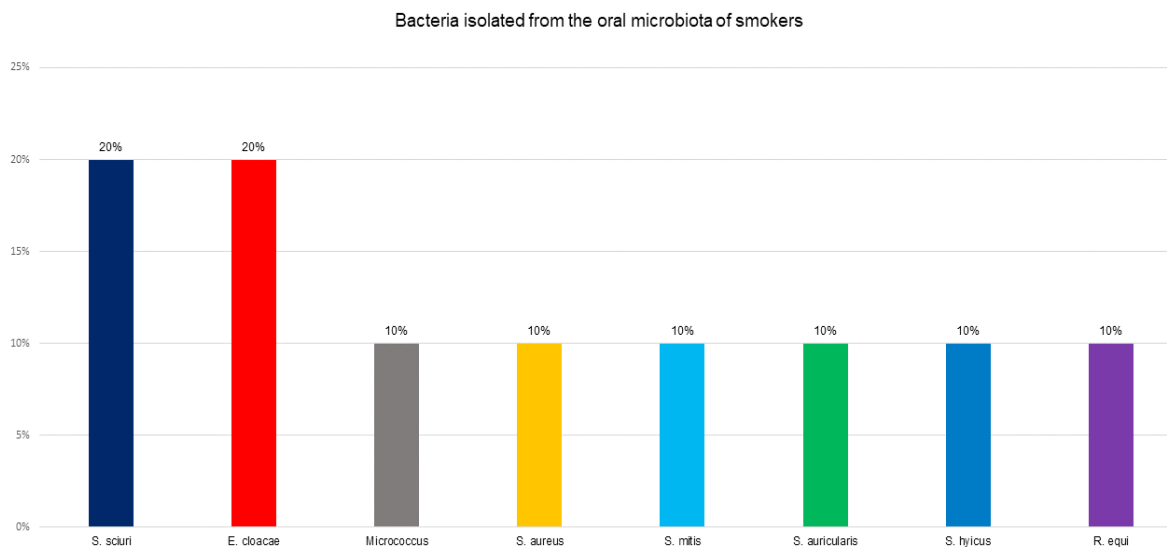


FIGURE 2. Bacteria isolated from the oral microbiota of smokers: *Staphylococcus sciuri* and *Enterobacter cloacae* bacteria had a higher prevalence of 4 (20%). The remaining isolated microorganisms had a frequency of 1 (10%): *Micrococcus* and related species, *Staphylococcus aureus*, *Staphylococcus mitis*, *Staphylococcus auricularis*, *Staphylococcus hyicus*, and *Rhodococcus equi*.

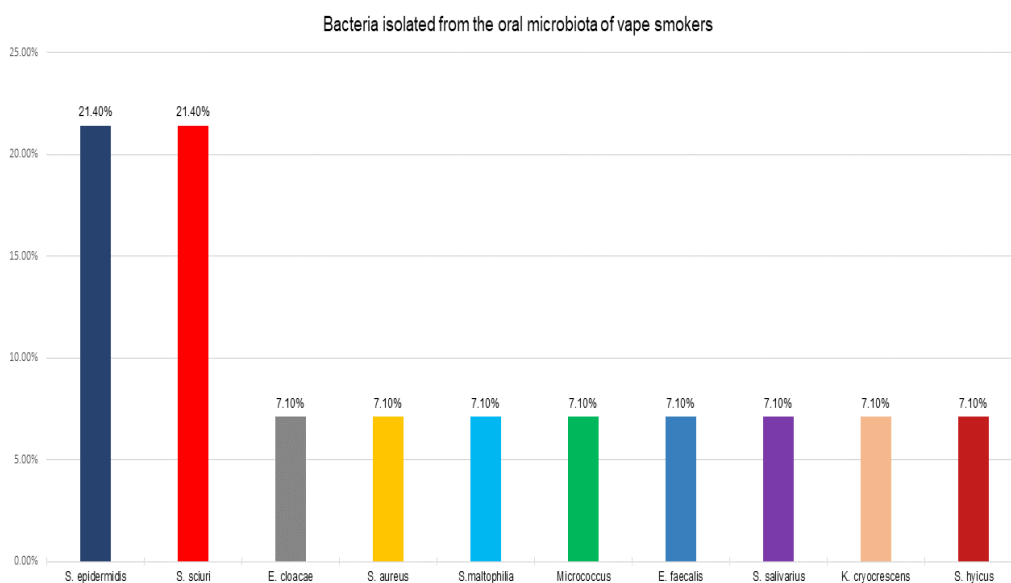


FIGURE 3. Bacteria isolated from the oral microbiota of vape smokers: *Staphylococcus epidermidis* and *Staphylococcus sciuri* with a frequency of 3 (21.4%). The remaining eight species found presented the same frequency of 1 (7.1%): *Enterobacter cloacae*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Micrococcus* and related species, *Enterococcus faecalis*, *Streptococcus salivarius*, and *Kluyvera cryocrescens*.

4. DISCUSSION

The microbiota comprises structurally, and functionally organized communities of microorganisms attached to the skin and mucosal surfaces that can communicate and collaborate to maintain ecological stability.¹⁵ Its composition is entirely different between body sites and it depends

on several internal and external factors, such as genetic background, gestational date (preterm or term birth), type of birth (vaginal delivery or cesarean), milk feeding methods (breast milk or artificial milk), age (neonate or elder), dietary habits (predominance of carbohydrates or fatty acid intake), personal hygiene (periodicity, methods, and products),

Antimicrobial resistance profile of bacteria from microbiota

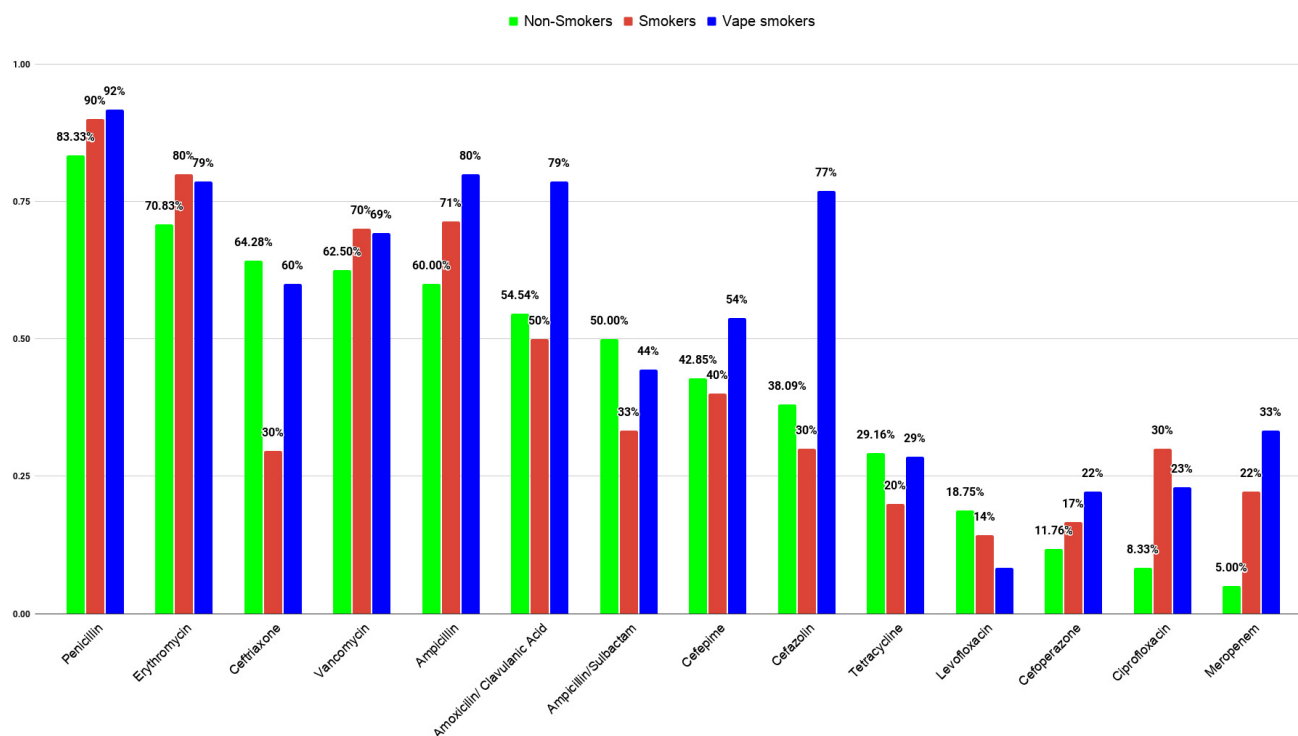


FIGURE 4. Antimicrobial resistance profile of bacteria from the oral microbiota of non-smokers, The resistance obtained in non-smokers was Penicillin 83.33%, Erythromycin 70.83%, Ceftriaxone 64.28%, Vancomycin 62.50%, Ampicillin 60%, Amoxicillin/Clavulanic Acid 54.54%, Ampicillin/Sulbactam 50%, Cefepime 42.85%, Cefazolin 38.09%, Tetracycline 29.16%, Levofloxacin 18.75%, Cefoperazone 11.76%, Ciprofloxacin 8.33%, and Meropenem 5.0%. The resistance in the smokers group was Penicillin 90%, Erythromycin 80%, Ampicillin 71%, Vancomycin 70%, Amoxicillin/Clavulanic Acid 50%, Cefepime 40%, Ampicillin/Sulbactam 33%, Ciprofloxacin 30%, Cefazolin 30%, Ceftriaxone 30%, Meropenem 22%, Tetracycline 20%, Cefoperazone 17%, and Levofloxacin 14%. The resistance obtained in the Vape Smokers group was Penicillin 92%, Ampicillin 80%, Erythromycin and Amoxicillin/Clavulanic Acid 79%, Cefazolin 77%, Vancomycin 69%, Ceftriaxone 60%, Cefepime 54%, Ampicillin/Sulbactam 44%, Meropenem 33%, Tetracycline 29%, Ciprofloxacin 23%, Cefoperazone 22%, and Levofloxacin 8%.

smoking (active or passive), antibiotic administration (glycopeptides, lincosamides, macrolides, and quinolones), and environmental exposure.^{4,16} A person’s microbiota is shaped throughout their entire life.

The most diverse and studied microbiota are those in the gastrointestinal tract and the oral cavity.¹⁷ The latter is a complex anatomic region containing several structures that can become microbial habitats, such as gingiva, gingival sulcus, teeth, tongue, lips, cheeks, and hard and soft palates. This complexity promotes the existence of very varied ecological niches that include aerobic and anaerobic environments.¹⁸ The taxa which are expected to be present in the oral cavity of healthy people include Gram-positive bacteria, such as *Abiotrophia* spp., *Actinomyces* spp., *Bifidobacterium* spp., *Corynebacterium* spp., *Eubacterium* spp., *Lactobacillus* spp., *Peptostreptococcus* spp., *Propionibacterium* spp., *Pseudoramibacter* spp., *Rothia* spp., *Stomatococcus* spp.,

and *Streptococcus* spp.; Gram-negative bacteria, such as *Campylobacter* spp., *Capnocytophaga* spp., *Desulfobacter* spp., *Desulfovibrio* spp., *Eikenella* spp., *Fusobacterium* spp., *Haemophilus* spp., *Leptotrichia* spp., *Moraxella* spp., *Neisseria* spp., *Prevotella* spp., *Selenomonas* spp., *Simonsiella* spp., *Treponema* spp., *Veillonella* spp., and *Wolinella* spp.;¹⁹ fungi like *Aspergillus* spp., *Aureobasidium* spp., *Candida* spp., *Cladosporium* spp., *Cryptococcus* spp., *Fusarium* spp., and *Saccharomycetales* spp.; and protozoa such as *Entamoeba gingivalis* and *Trichomonas tenax*.²⁰ The presence of archaea and viruses has been also described.

A deviation from symbiosis leads to dysbiosis, and it has been demonstrated that the smoke of the cigarettes produces changes in the bacterial population of the oral cavity. Lee S.H. et al. observed that the composition of the oral microbiota between non-smokers and former smokers is very similar; however, it differs from that of active



smokers.²¹ Yu G. et al. showed that the alpha biodiversity (understood as the heterogeneity of a bacterial community based on the number of species present and their relative abundance) was higher in the oral cavity of non-smokers than in that of smokers, as the latter lose richness of the oral mucosa.²² Consequently, Shen P. et al. discussed the notion that cigarette smoke predisposes the nasal colonization by *Streptococcus pneumoniae*.²³ In our study, variation was observed in the populations of bacteria that were isolated in each of the groups, but the non-smokers exhibited the most. This correlates with the evidence available in the literature, being the alpha biodiversity of the non-smokers higher than that of smokers and EC vapers.

In a comparative pilot study of the oral microbiota among non-smokers, tobacco smokers and EC vapers, significant alterations were seen in the tobacco smokers but no alterations were detected in EC vapers compared to the non-smokers.²⁴ Furthermore, it has been reported that the EC vapor of flavorless E-juice, unlike cigarette smoke, does not cause inhibition of the oral microbiota bacterial growth, especially in commensal bacteria as *Streptococcus* spp.²⁴ On the other hand, the sweetening or flavoring components of cigarettes and ECs can contribute to oral dysbiosis. This has been demonstrated in mentholated cigarettes.²⁵ and it has been suggested that this may also happen with flavored E-juices.²⁶ In our study, we observed that all the groups presented pathogenic bacteria: The non-smoking group had *Enterobacter gergoviae*, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa*; the smoking group had *Enterobacter cloacae* and *Rhodococcus equi*; and the EC vaper group had *Enterobacter cloacae*, *Enterococcus faecalis*, *Kluyvera cryocrescens*, and *Stenotrophomonas maltophilia*. However, EC vapers had the highest percentage in samples, followed by the smoking group and the non-smokers. Although the proportion of pathogens is low in the last group, the presence of bacteria should be studied, especially that of *Pseudomonas aeruginosa*, a commensal pathogen that causes necrotizing pneumonia.

Furthermore, Lacoma A. et al. reported that cigarette smoke increases the profile of antimicrobial resistance in *Staphylococcus aureus*,¹¹ and as the use and acceptance of ECs is continuously rising, it is important to know whether it also increases the profile of antimicrobial resistance. In our study, multidrug resistance was seen in each of the groups, but the highest levels were present in smokers and EC vapers, especially in the latter. Penicillin was the only antibiotic with pharmacological resistance (over 80%) in all the groups. The antibiotics with the lowest pharmacological resistance level were Meropenem (5%) among non-smokers, Levofloxacin (14%) in smokers, and Levofloxacin (8%) in the vaper smokers' group. In contrast, Amoxicillin/Clavulanic Acid

and Cefazolin showed an important increase in resistance due EC smoking habit. It is important to highlight that drugs with a high percentage of resistance are not an option in case of infection.

The presence of multidrug-resistant bacteria in all the groups evidences the need for urgent measures to fight a global health problem, as multidrug-resistant infections are expected to be the first cause of death by 2050.^{27,28} Specifically, smoking has not been considered a factor driving antimicrobial resistance; however, current evidence supports it, mainly due to the changes to the oral microbiota.²⁴ To our knowledge, this report is the first phenotypic profile of antimicrobial resistance by EC vapers in our country. This pilot study has multiple limitations, such as the size of the sample and the homogeneity of the sex; still, it is a basis to conduct more comprehensive studies that clarify the proposed variables.

As originally stated, it should be noted that the microbiota within the different populations studied presented differences. In the case of EC vapers, there was a difference in part due to the microorganisms found in the microbiota of this population. The greatest resistance to antibiotics was found in the three populations studied within this group. Furthermore, EC vapers had the highest amount of pathogenic bacteria within the oral cavity. This is undoubtedly a relevant finding since ECs originally emerged as an alternative to replace conventional cigarettes. In their beginnings, ECs were considered an alternative with less harmful effects than those caused by the conventional cigarette. However, this and other studies have shown that ECs also have harmful effects in those who use them.²⁶ This is relevant to our study given that we found pathogenic bacteria along with these effects, which affect the body. For example, in the study by Kassinen and Krogus-Kurikka cited in the National Center for Biotechnology Information Search database,²⁹ the irritable bowel syndrome, one of the most common gastrointestinal disorders, was found to be largely bacteria-mediated. We found a large number of Gram-negative and Gram-positive bacteria from the phyla Proteobacteria and Firmicutes, respectively. Within the first phylum are *Enterobacter cloacae*, *Enterococcus faecalis*, *Kluyvera cryocrescens*, and *Stenotrophomonas maltophilia*. These pathogenic bacteria found in higher concentrations among EC vapers promote gastrointestinal disorders, as irritable bowel syndrome, eventually involved in amino acid synthesis, cell junctions, and inflammatory response. They are also related to a weakening of the epithelial barrier, which would finally explain diseases such as irritable bowel syndrome.²⁹ This is where the importance of our work lies since these changes were found within the group of EC vapers.



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Canine oral microbiota: A source of potentially pathogenic polyresistant bacteria

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ABSTRACT

Introduction: The current rise in polyresistant bacterial strains is due to self-medication, failure to comply the treatment, incorrect prescription of antibiotics by the physician, and even the abuse of these in agriculture industries. In this research, we suggest another possible source. Humans, being in coexistence with dogs and in contact with their saliva, could have been exposed to polyresistant microorganisms that are potential pathogens to. Identify, isolate and analyze these possible microorganisms were the main objectives of this study. **Materials and methods:** Oral samples (n=28) from domestic dogs were taken and cultured. Bacterial colonies (n = 160) were obtained and subjected to identification and antimicrobial sensitivity tests. **Results:** From 160 isolated colonies, the most prevalent species was *Staphylococcus haemolyticus*. Other bacteria such as *Enterococcus faecium*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* were also found in a lesser proportion. There was an increased resistance of the bacteria against cell wall synthesis antibiotics. The resistance towards vancomycin was the highest, followed by cefalotin and cefixime. In contrast, all bacteria were sensible to imipenem. **Conclusion:** The resistance observed against protein synthesis inhibitors showed a high resistance towards erythromycin and clarithromycin but a high sensibility to amikacin and gentamicin. In this study several human pathogens that are the cause of infectious diseases were identified in the oral microbiota of dogs. Furthermore, another risk of polyresistant bacteria transmission is proposed with the determination of each bacterial resistance.

Key words: antimicrobial resistance; antibiotics; oral microbiota; canines.

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RESUMEN

Introducción: La reciente aparición de cepas de bacterias polirresistentes puede deberse a la automedicación, falla en el seguimiento del tratamiento, prescripción incorrecta de antibióticos por el médico, así como su sobreuso en la industria agrícola. En este artículo se sugiere otra posible fuente. Los humanos, al estar en estrecho contacto con los perros y con su saliva, pueden estar expuestos a microorganismos polirresistentes que son potencialmente patogénicos en los humanos. **Objetivo:** La identificación, aislamiento y análisis de dichos posibles microorganismos. **Materiales y métodos:** Se tomaron muestras de saliva de 28 perros domésticos para posteriormente ser cultivadas. Se obtuvieron colonias bacterianas (n = 160) para su identificación y sensibilidad antimicrobiana. **Resultados:** De las 160 colonias aisladas, la especie con mayor prevalencia fue *Staphylococcus haemolyticus*. Otras bacterias como *Enterococcus faecium*, *Escherichia coli*, *Proteus mirabilis* y *Pseudomonas aeruginosa* se encontraron en una menor proporción. Se observó un incremento en la resistencia hacia los antibióticos que inhiben la síntesis de pared celular, siendo la vancomicina el antibiótico con mayor resistencia bacteriana, seguido de cefalotina y cefixima. En contraste, todos los cultivos bacterianos fueron sensibles a imipenem. **Conclusión:** La resistencia observada a macrólidos y aminoglucósidos fue muy variable, con una alta resistencia hacia claritromicina, y una alta sensibilidad a amikacina y gentamicina. Varios patógenos que causan enfermedades infecciosas en humanos fueron encontrados en la microbiota oral de los perros. Esto, junto con la determinación de sus resistencias, representa una posible forma de transmisión de bacterias polirresistentes hacia los humanos.

Palabras clave: resistencia; antibióticos; microbiota oral; caninos.

INTRODUCTION

Bacterial resistance has increased in the last years, making treatments and therapeutic decisions more complex and difficult. Second- and third-line treatments are more commonly used to treat infections by exogenous pathogens. The need for understanding routes of antimicrobial resistance transmission must be studied. One important route is present between humans and animals by direct contact or through the food chain.¹ It has been identified that gram-negative bacteria can develop transmission mechanisms of antimicrobial resistance between commensal bacteria and pathogenic bacteria, even between different species.² As an example, nonpathogenic commensal species, like *E. coli* strains, can be resistant to antimicrobials. These species may transfer antimicrobial resistance genes to actual pathogenic species by horizontal gene transfer using plasmids, transposons, and integrons that carry these genes.¹

The highest isolation of multiresistant bacteria in animals is found in pets, followed by farm animals and the lowest in wild animals.³ This could be due to the daily close contact between the owner and the pet. The oral microbiota of dogs is one of the microsystems that is in direct contact with humans. It is known that the transmission of normal canine microbiota and periodontal pathogenic bacteria to humans is possible by daily contact as dogs tend to lick their owners.⁴ The identification of the same species in both dog and owner has been described. Bacteria of the genus *Porphyromonas*, *Prevotella*, and *Tannerella* have been the most frequently reported in such cases.^{4,5}

The canine oral microbiota mainly consists of bacteria from the Firmicutes and Bacteroidetes phyla.^{6,7} Current studies identify the oral pathogens present in dogs, establishing that the bacteria is a mixture of aerobic and anaerobic microorganisms that usually include *Pasteurella canis*, *Streptococcus* spp., *Staphylococcus* spp., *Fusobacterium* spp., *Bacteroides* spp., and *Capnocytophaga canimorsus*.⁸ Other studies have shown different species such as *Porphyromonas cangingivalis*, *Porphyromonas gulae*, *Actinomyces canis*, and *Neisseria weaveri* including the genus *Fusobacterium* spp.⁹ These species normally differ from those present in human oral microbiota.¹⁰

Periodontal diseases as gingivitis and periodontitis are extremely frequent in dogs. In these diseases, an alteration occurs in the composition of the normal microbiota by the colonization of several pathogenic microorganisms. Some of them are strict anaerobic and facultative anaerobic bacteria, mainly of the genera *Streptococcus* spp., *Staphylococcus* spp., *Enterobacteriaceae* spp., *Corynebacterium* spp., *Clostridium* spp., and the species *Eikenella corrodens* and *Pasteurella multocida*.¹¹

In recent years, there has been an increase in the number of infections caused by different polyresistant bacteria affecting humans.² Nevertheless, there are scarce studies on the profile of antimicrobial resistance in the canine oral microbiota as it has been somewhat ignored as a possible source. The present study analyzed the antimicrobial resistance of aerobic bacteria in the oral microbiota of domestic dogs whose owners live in middle- and upper-class areas in Mexico City.

MATERIAL AND METHODS

Sample collection

The group consisted of 28 male dogs (age 1–2 years, weight 30–40 kg, and height 60–65 cm). All dogs presented a good state of health when examined and auscultated. This reduced variables that could interfere with the study. Using sterile swabs, saliva samples were collected from the dogs in middle- and upper-class households in Mexico City (n=160). The swab was taken from the internal part of the cheeks of the oral cavity, gums, and teeth. Samples were immediately incubated in BHI (Brain heart infusion) (Becton Dickinson Dr, Franklin Lakes, NJ, USA) at 37 °C/24 h. All the samples were processed in the microbiology laboratory of the Health Science School at Anahuac University.

Bacterial Isolation

After incubation, blood agar was used to culture 10 µl BHI medium (Becton Dickinson Dr, Franklin Lakes, NJ, USA) and the mixture was incubated at 37 °C for 24 h. The plates were examined and each morphotype was counted and subculture to obtain pure cultures. The pure colonies obtained were re-cultured in blood agar (n = 140) (Becton Dickinson Dr, Franklin Lakes, NJ, USA) at 37 °C/24 h.

Biochemical tests for bacterial identification

Gram staining, oxidase and indole test, and the biochemical tests described in the identification kit (Carbohydrate fermentation, citric acid, malonate utilization, and esculin hydrolysis, among others) were performed on the colonies obtained from blood agar (n = 140).

The micro and macro morphology of the bacteria were assessed by gram staining. BD BBL™ Gram Stain Kit (Becton Dickinson Dr, Franklin Lakes, NJ, USA). BBL™ Dryslide™ Oxidase (Becton Dickinson Dr, Franklin Lakes, NJ, USA) and BBL™ DrySlide™ Indole (Becton Dickinson Dr, Franklin Lakes, NJ, USA) were used for oxidase and indole testing.

The biochemical identification was performed using the BBL™ Crystal™ ID Kit (Becton Dickinson Dr, Franklin Lakes, NJ, USA), BBL™ Crystal™ MIND software, and the BBL™ Crystal™ Autoreader (Becton Dickinson Dr, Franklin Lakes, NJ, USA). The biochemical tests were carried out on the colonies obtained from the blood agar. Inoculum broth was adjusted to 0.5 McFarland standard (expected 3.0×10^8 CFU/mL) and incubated without CO₂, 40–60% humidity, at 37 °C

for 18 h. The 0.5 McFarland standard reading was made by nephelometry using the CrystalSpec™ Nephelometer (Becton Dickinson Dr, Franklin Lakes, NJ, USA).

Antimicrobial susceptibility test

The antimicrobial susceptibility was tested by the Kirby–Bauer test under the protocol of the Clinical and Laboratory Standards Institute. Pure colonies (2–4) were taken from the blood agar and re-suspended in BBL™ Crystal™ Inoculum Broth (IB). Then, they were adjusted to 0.5 McFarland standard (Expected 1.5×10^8 CFU/mL). A bacterial suspension was cultured in BD® BBL Müller Hinton medium (150 x 15 mm). The antibiotics used were cell wall synthesis inhibitors, aminoglycosides, macrolides, and DNA inhibitors: amoxicillin/clavulanic acid (AmC-30), piperacillin (PIP-100), piperacillin/tazobactam (TZP-110), ampicillin 10 (AM-10), cephalotin (CF-30), cefazolin (CZ-30), cefaclor (CEC-30), cefuroxime (CXM-30), cefixime (CFM-5), ceftizoxime (ZOX-30), meropenem (MEM-10), imipenem (IPM-10), vancomycin (Va-30), amikacin (AN-30), gentamicin (GM-10), kanamycin (K-30), spectinomycin (SPT-100), tetracycline (Te-30), erythromycin (E-15), azithromycin (AZM-15), clarithromycin (CLR-15), clindamycin (CC-2), chloramphenicol (C-30), nalidixic acid (NA-30), ciprofloxacin (CIP-5), and gatifloxacin (GAT-5). The medium and antibiotics employed were purchased from Becton Dickinson (Becton Dickinson Dr, Franklin Lakes, NJ, USA).

RESULTS

Bacterial identification

By using biochemical assays, we performed the isolation and identification of each bacterial culture obtained from the oral swab samples (n = 160). 20 Swab samples were cultured but there was no bacterial growth. The remaining pure samples were then evaluated (n = 140). In this study, *Staphylococcus haemolyticus* was the most frequently isolated species (20.7%) (Figure 1), followed by *Enterococcus faecium* and *Escherichia coli* (15.7% both). *Proteus vulgaris* and *Pseudomonas aeruginosa* were found in 10.7% of the samples studied. Other bacteria identified and isolated were *Providencia rettgeri*, *Enterococcus durans*, *Bacillus cereus*, *Staphylococcus simulans*, and *Gemella morbillorum*, each with 5% frequency (Figure 1). The remaining 1.5% corresponds to unidentified bacteria.

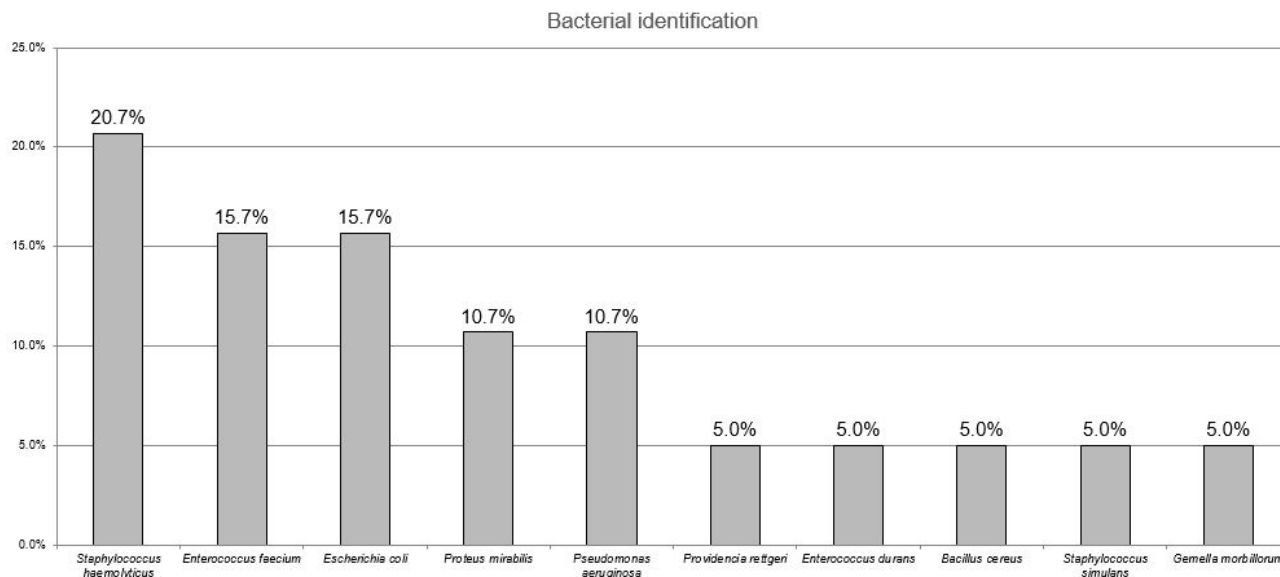


FIGURE 1. Identification of bacterial species from pure colonies (n = 140).

Antimicrobial susceptibility to inhibitors of bacterial cell wall synthesis

In this study, we tested each group of the most commonly prescribed antibiotics worldwide such as semi-synthetic penicillins with and without β -lactamase inhibitors, protein synthesis inhibitors antimicrobials, and DNA replication targeting antibiotics. First, we tested two semisynthetic penicillins not combined with β -lactamase inhibitors for bacterial resistance. We found a marked resistance for ampicillin (47.1%) and piperacillin (26.4%) (Figure 2). The susceptibility to antibiotics was also tested in combination with β -lactamase inhibitors. Resistance to amoxicillin/clavulanic acid was 42.1% and 10.7% for piperacillin/tazobactam (Figure 2). In this study, 62.9% of all the bacterial isolates were resistant to cephalothin and 42.1% were resistant to cefazolin, which are first-generation cephalosporins (Figure 2). Analyzing resistance to second-generation cephalosporins, 26.4% of the bacteria were resistant to cefaclor and 42.1% to cefuroxime. Resistance against third-generation cephalosporin cefixime was 63.6% and vs ceftizoxime, 47.1% (Figure 2).

In this study, resistance to carbapenems was low, 15.7% of isolated cultures were resistant to meropenem, unlike imipenem no resistance was observed. From the bacteria identified, 84.3% showed resistance to vancomycin (Figure 2).

Antimicrobial susceptibility to protein synthesis inhibitors

The antibiotic resistance against macrolides was 62.9% for erythromycin, 47.1% for azithromycin, and 68.6% for

clarithromycin (Figure 3). Resistance to aminoglycosides like amikacin and gentamicin was 15.7%. Resistance to kanamycin and spectinomycin was 31.4 and 68.6%, respectively (Figure 3). Resistance to chloramphenicol, clindamycin, and tetracycline was 0.7, 73.6, and 37.1%, respectively (Figure 3).

Antimicrobial susceptibility to DNA replication inhibitors

Finally, the Kirby-Bauer test showed bacterial culture resistance against the following quinolones: nalidixic acid (57.9%), ciprofloxacin (21.4%), and gatifloxacin (5.0%) (Figure 4).

DISCUSSION

In this study, we aimed to identify and describe polyresistant and potentially pathogenic bacteria from saliva samples belonging to healthy canine oral microbiota. The most prevalent species found in this study was *Staphylococcus haemolyticus*. This bacterium is a member of coagulase-negative *Staphylococcus* (CNS). This group consists of several species other than *Staphylococcus aureus* that have become increasingly important as opportunistic pathogens in health centers around the world. Among the species in CNS, *Staphylococcus haemolyticus* is recognized as an emerging and important human pathogen. It has been associated with severe infections, such as endocarditis, urinary tract infections, septicemia, peritonitis, wounds, bones and joint.¹²

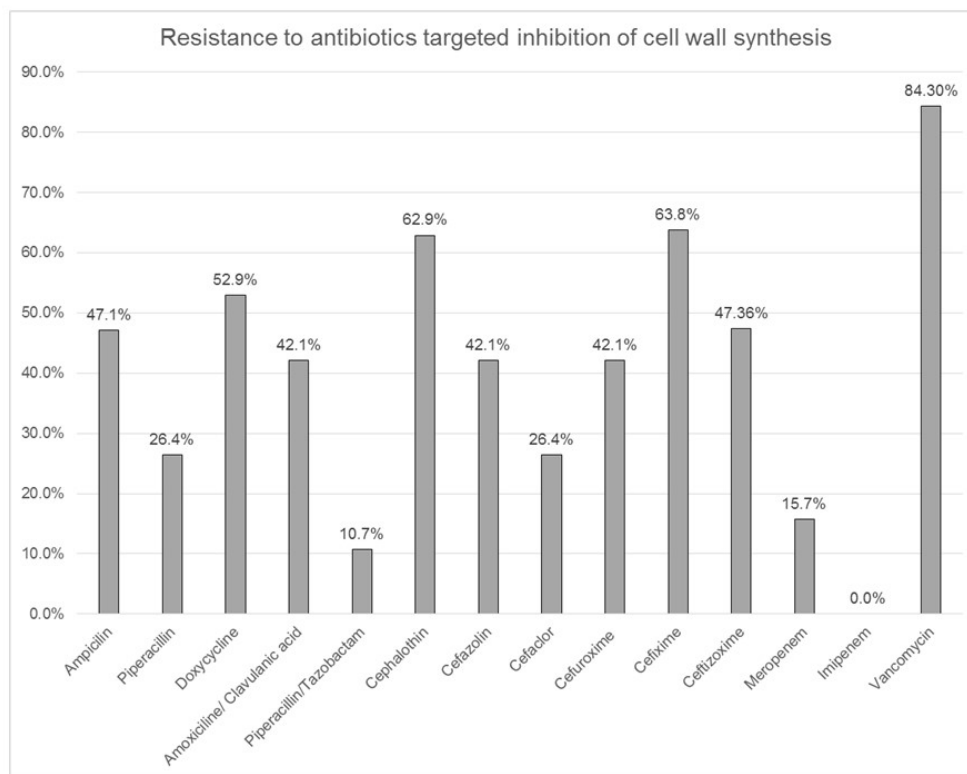


FIGURE 2. Antimicrobial susceptibility to inhibitors of bacterial cell wall synthesis. Ampicillin (AM-10), piperacillin (PIP-100), amoxicillin/ clavulanic acid (AmC-30), piperacillin/tazobactam (TZP-110), cephalothin (CF-30), cefazolin (CZ-30), cefaclor (CX-30), cefuroxime (CXM-30), cefixime (E-15), ceftizoxime (ZOX-30), meropenem (MEM-10), imipenem (IPM-10), vancomycin (Va-30). The highest resistance observed was against Vancomycin, first class cephalosporin, cephalothin, and the third class cephalosporin; cefixime.

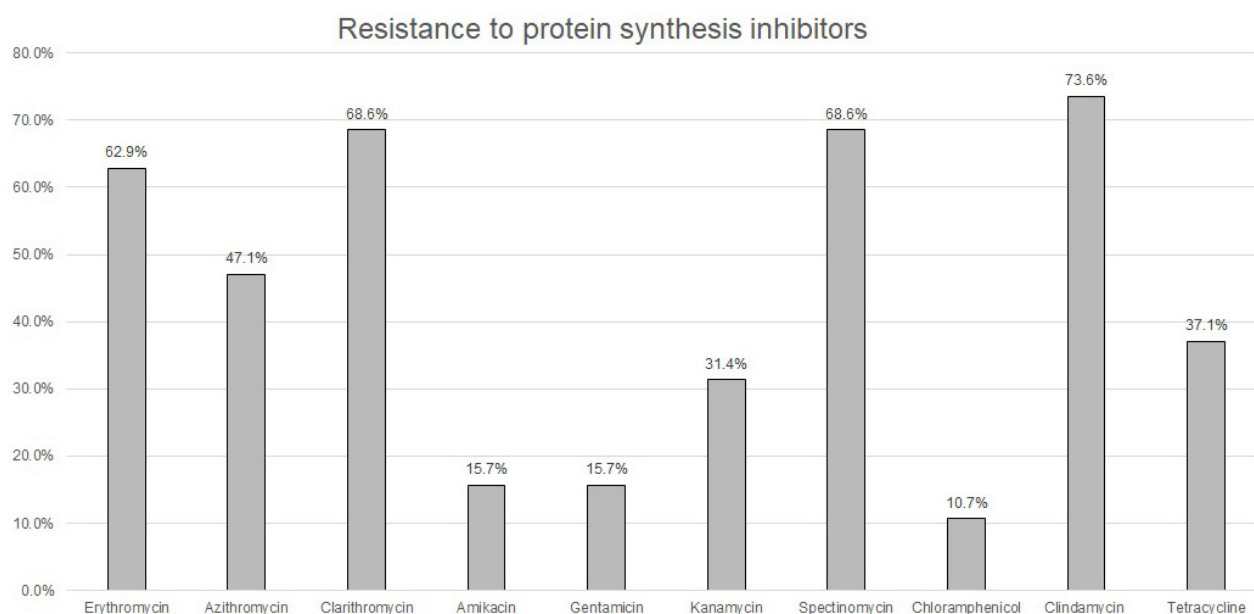


FIGURE 3. Antimicrobial susceptibility to protein synthesis inhibitors. Erythromycin (E-15), azithromycin (AZM-15), clarithromycin (CLR-15), amikacin (AN-30), gentamicin (GM-10), kanamycin (K-30), spectinomycin (SPT-100), chloramphenicol (C-30), clindamycin (CC-2), tetracycline (Te-30). High resistance against macrolides is shown while broad-spectrum antibiotics (gentamicin) targeting gram-negative bacteria have higher rates of susceptibility.

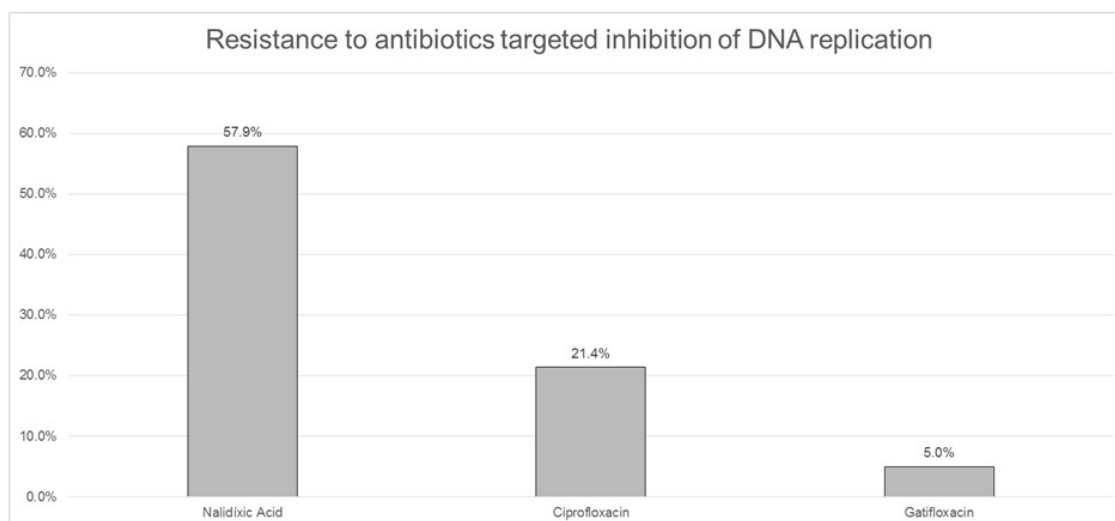


FIGURE 4. Antimicrobial susceptibility to DNA replication inhibitors. Nalidixic acid (NA-30), ciprofloxacin (CIP-5), gatifloxacin (GAT-5). Nalidixic acid showed the highest resistance.

This bacterial isolation contrast with the results showed by Yamasaki et al. where *Tannerella forsythia* and *Porphyromonas gulae* were the most commonly isolated bacteria in the oral microbiota of dogs.¹³

We also identified *E. faecium* in a great proportion. The genus *Enterococcus* belongs to the *Enterococcaceae*, a family of gram-positive, catalase-negative cocci. *Enterococcus* has been recognized as the third or fourth most important cause of nosocomial infections worldwide. It is also responsible for urinary tract infections and bacteremia, being the second and third most important cause, respectively.¹⁴ The third most isolated bacteria were *Escherichia coli*; this is one of the most widely distributed and known bacteria in the world. It is associated with multiple nosocomial and community-acquired infections such as urinary tract infections, diarrhea, and even bacteremia and sepsis.

We also identified *Proteus Mirabilis* in a great quantity. The genus *Proteus* consists of gram-negative bacilli that belong to the *Enterobacteriaceae* family. Strains of *Proteus* have been isolated from the intestinal tract of various mammals including human beings, as well as houses and hospitals. This bacterium is considered one of the main causes of urinary tract infections acquired both in the community and hospitals.¹⁵

An important pathogen isolated from the oral microbiota of dogs was *Pseudomonas aeruginosa*. It is considered the leading cause of nosocomial infections and is associated with several diseases including endocarditis, pneumonia, otitis, and bacteremia, as well as gastrointestinal, osteoarticular, ocular, skin and soft tissue infections, and bacteremia.¹⁶ Due to its presence in hospital settings, this bacterium has also

been associated with multiresistance, which makes it one of the hardest pathogens to treat.

Following the identification, *Providencia rettgeri* is a gram-negative bacillus belonging to the *Enterobacteriaceae* family. It is associated with soft tissue infections, infections caused by contaminated urinary catheters, and bacteremia.¹⁷ We also isolated *Bacillus cereus*, a gram-positive bacillus found in different foods, soil, and water. It produces food poisoning associated to rice intake and produces diarrhea accompanied by severe cramps and profuse vomiting.

Other bacteria found was *Enterococcus durans*. It has been isolated from animal and human intestines, which, unlike other *Enterococci*, is rarely associated with infections in humans due to its low pathogenicity. However, it has been linked to a fatal case of aortic valve endocarditis.¹⁶ *Staphylococcus simulans*, another CNS, is rarely identified as a possible cause of infections in humans. Out of the few reported cases, there is evidence that it produces soft tissue infection¹⁸ or septicemia.¹⁹ *Gemella morbillorum* has been isolated as a normal inhabitant of the respiratory, gastrointestinal, and genitourinary tract in humans. *Gemella morbillorum* has been reported to cause infections in the central nervous system, pneumonia, pleural empyema, mediastinitis, osteomyelitis, hepatic abscess, and peritonitis as well as cardiovascular infections.²⁰

We found that most of the bacteria present in the oral cavity of the dogs were gram-negative, mostly aerobes and possible high pathogens also compatible with species long implicated in human infectious diseases. There are four main causes that have been associated with antimicrobial resistance in the clinical environment: self-medication, error

in the medical prescription of antibiotics, lack of adherence to antimicrobial treatment, and the excessive use of antibiotics as growth promoters in the agriculture industry, in consequence generating food products with antibiotics and resistant bacteria.

In this investigation, we propose a possible fifth mechanism in which humans come into contact with potentially pathogenic polyresistant bacteria. This mechanism is domestic dogs and close contact with canine oral microbiota and saliva through licking and kissing. Infants and children would potentially have a greater contact with these pets and in result with the dog microbiota. There is also evidence that supports the connection between the bacteria in the oral cavity of dogs and their contact with the owners. Yamasaki et al. described how families co-share the oral microbiota with their pets.¹³ The high resistance noted in this study against β -lactams (ampicillin, amoxiclav) could be because most of the isolated bacteria were gram-negative and are naturally more resistant to these antibiotics. Additionally, the high resistance against vancomycin is due to the same factor; unlike gram-negative bacteria, gram-positive bacteria are highly susceptible to β -lactams because of its high penetrance into bacteria.²¹ Vancomycin is used principally against methicillin-resistant *Staphylococcus aureus*. There was also a marked resistance against cephalothin, cefixime, ceftizoxime, and cefazolin. These antibiotics are usually used to prevent wound infections in surgery or act as broad-spectrum drugs to inhibit bacterial growth. Resistance against these antibiotics was found in more than 40% of the bacteria. These results should be considered as a potential evidence of polyresistant bacteria in addition to the naturally resistant opportunistic microorganisms that infect the human.²² While there were many bacterial cultures susceptible to aminoglycosides, there was a high prevalence of bacteria resistant to azithromycin, clarithromycin and erythromycin. The resistance against macrolides could be an important factor to catalog these bacteria as polyresistant. The presence of polyresistant and potentially pathogenic bacteria in the canine oral microbiota reported in this study should be considered a potential hazard and a serious public health problem. We believe that information on the measures to be taken while in close and frequent contact with canines should be provided to pet owners and health professionals.

Future studies should focus on the correlation between clinical cases of nosocomial infections and those acquired in the community with possible exposure to these microorganisms. Furthermore, the limitation in this study is the lack of correlation between antibiotic resistance and isolated bacteria. This could encourage other studies to focus their research on this aspect. In addition, we must point out that pets are also susceptible to the colonization of polyresistant bacteria from humans, making this a vicious cycle. This study

represents a new approach to describe the resistance profile of the oral canine microbiota.

CONCLUSION

The recent outbreak of infections caused by polyresistant bacteria is a major national and international health problem. There are several reasons for antibiotic resistance. This study proposes a potential, yet sometimes ignored, source of resistant bacteria. Based on the results obtained, contact with oral canine microbiota and saliva can be a risk factor to acquire infections from polyresistant and pathogenic bacteria.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Identifying glial scar tissue using infrared thermography: a spinal cord injury pilot study

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ABSTRACT

Introduction: Glial scarring after a spinal cord injury (SCI) can represent both a physical and a molecular barrier for axonal regeneration and thus its removal has been found to be helpful in the recovery process. For this removal to be feasible in humans, an efficient method is needed to clearly identify glial tissue without inflicting more damage. **Objective:** To evaluate infrared thermography as a tool for identifying glial scar tissue in chronic SCI. **Material and methods:** An exploratory experimental pilot study was performed on Sprague-Dawley rats divided into sham and SCI (T9). All animals were subjected to a baseline thermography performed after a laminectomy that was either followed by closure of the surgical planes (sham group) or injury infliction (SCI group). Five weeks later, a second thermography was performed. Afterward, the spinal cord (T8-T10) was removed and processed for glial fibrillary acidic protein (GFAP) immunohistochemistry, which was used as a gold standard for identifying reactive astrocytes and glial scar. All animals received the same care throughout the study. **Results:** The thermography did not reveal a statistical difference for the baseline values ($p = 0.24$); however, a significant difference in thermography values was found 5 weeks later ($p = 0.01$). This difference significantly correlated with astrocyte counts at the site of injury ($r = -0.57$; $p = 0.03$, Spearman's correlation). **Conclusions:** Infrared thermography could be useful to evaluate the extent of glial scar after SCI.

Key words: spinal cord injury; infrared thermography; glial scar.

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RESUMEN

Introducción: La cicatriz glial de una lesión de médula espinal (LME) es una barrera física y molecular para la regeneración axonal, por lo que su resección es útil en la recuperación en modelos animales. Para que esta resección sea factible en humanos, es necesario un método claro de identificación de tejido cicatricial sin provocar mayor daño. **Objetivo:** Este estudio busca evaluar la utilidad de la termografía infrarroja en la identificación de la cicatriz glial en LME crónica. **Materiales y métodos:** Se realizó un estudio piloto exploratorio experimental con ratas Sprague-Dawley divididas en un grupo sham y un grupo de LME (T9). Se realizó una termografía basal poslaminectomía en todos los animales, seguida de cierre de la herida o LME y se obtuvo una segunda termografía 5 semanas después. Se retiró la médula (T8-T10) y se realizó inmunohistoquímica anti-GFAP (proteína ácida fibrilar glial) como estándar para el marcaje de células de la glia. Todos los animales recibieron los mismos cuidados. **Resultados:** No hubo diferencia estadística entre los valores basales de la termografía ($p = 0.24$); sin embargo, 5 semanas después se observó una diferencia significativa en los valores presentados en la zona de lesión de los animales con LME ($p = 0.01$). Esta diferencia presentó una correlación significativa con la cantidad de astrocitos ($r = -0.57$; $p = 0.03$, prueba de correlación de Spearman). **Conclusiones:** La termografía puede ser de gran utilidad para conocer la extensión de la cicatriz glial observada después de una LME.

Palabras clave: lesión de médula espinal; termografía infrarroja; cicatriz glial.

1. INTRODUCTION

Depending on the level and severity of the injury, spinal cord injury (SCI) patients are subject to dealing with life-long medical and economic consequences that have a detrimental effect on their quality of life.^{1,2} With this in mind, researchers have studied possible treatment options that can either improve or even permanently re-establish the functions of the spinal cord. These strategies approach the problem through the pathophysiology of the injury, and this has led to therapies such as cyclooxygenase inhibition, antioxidants, apoptosis inhibitors, and immunophilin ligands, among others.³ Nevertheless, despite the success of some of these therapies, a review of several studies suggested that removing the glial scar before administering any of these therapies is the best strategy to promote neurological recovery.⁴

After SCI, a glial scar begins to form in the acute stage and remains active throughout the chronic phase. The scar is mainly constituted by astrocytes that undergo reactive astrogliosis (hypertrophy, increased proliferation, and secretion of GFAP, vimentin, nestin, cytokines, and proteoglycans), constituting a physical barrier to prevent damage expansion.⁵ Nevertheless, this glial scar also acts as a physical and chemical barrier for neural growth and axonal regeneration.^{4,6} Different studies have shown that the glial scar has a very dynamic function and is equally important during the early stages of injury as it is restrictive in the chronic one. Initially, these cells release endothelial growth factors and vasoconstrictors to prevent more bleeding, antioxidants to counteract the glutamate-induced excitotoxicity, and regenerating factors such as FGF-2 and S100 β for tissue restoration.⁷ In the long run, however, these effects diminish or disappear and what remains is a physical and molecular barrier for restoration, largely due to the increase in chondroitin sulfate proteoglycans (CSPGs)

secreted from the scar, which in turn inhibit axonal growth.⁸ In line with this, several studies have proven in experimental models that the surgical removal of this scar tissue, alone or in combination with other therapeutic strategies, can be successful in improving motor capacity below the injury site.⁹ That is why the design of a method to precisely identify and successfully remove the glial scar without creating more damage remains an important area of research. This problem can be addressed by using an infrared thermography camera. This device processes thermal data through electromagnetic radiation and converts it into an electronic image.^{10,11} The camera detects heat from the studied tissue. Since the scar has a low metabolic activity, and thus lowers the temperature, infrared thermography might help recognize the extent of the glial scar. This would provide an opportunity to carefully remove the scar without inflicting further damage to the neural tissue.¹² The aim of this study was to determine whether infrared thermography could effectively identify glial scar in an injured spinal cord.

2. MATERIALS AND METHODS

An experimental pilot study with laboratory animals was carried out. The research protocol was approved by the IRB and the Internal Committee for Laboratory Animal Use and Care (CICUAL) at Universidad Anáhuac México.

2.1 Experimental design

Since this was an exploratory study, the research team used a criteria sample size of 11 Sprague-Dawley female rats ranging in weight from 210 to 250 grams, with no prior diseases or

interventions. All animals were subject to a laminectomy (T9), followed by a baseline thermography of about 1.5 cm proximal and caudal to T9. Then, randomization determined which animals were immediately closed (sham, $n = 4$) and which were injured (experimental, $n = 7$). Both groups received the same post-surgical care for 5 weeks, after which the same area was exposed again for a second thermography. This timeline allowed for the glial scar to gradually evolve from the subacute stage into a more mature and less active tissue within 2 weeks post-injury.¹³

2.2 Tissue management

The animals were euthanized by perfusion with prior heparin and anesthesia (sodium pentobarbital). Then, the spinal cord was removed and fixed with paraformaldehyde for 24 h and 30% sucrose for 72 h; it was then frozen with Tissue-Tek. Longitudinal sections of the spinal cord were used in GFAP immunohistochemistry, with GFAP as the gold standard in reactive astrocyte identification to have a comparative parameter for the thermography. In every case, 40- μm sections were made until visualizing the central canal, and three consecutive sections were used in immunohistochemistry. Finally, sections with the most gliosis were chosen and the area with the most GFAP marking was photographed (20x).

2.3 Laminectomy and experimental SCI

After applying anesthesia using intramuscular xylazine/ketamine (10 mg/kg/50 mg/kg), careful skin and muscle tissue incisions allowed for visualization of the 9th thoracic vertebra and its removal to expose the spinal cord. The animals in the experimental group were then subjected to a moderate contusion injury using an IH Spinal Cord impactor (Precision System & Instrumentations) with a force of 200 kdyn at a 2-mm distance. Polyglycolic acid and nylon sutures were used to close muscle tissue and skin, respectively. After surgery, 1 mL saline solution and 0.1 mL ketoprofen (intraperitoneal) were administered.

2.4 Postoperative care

The animals were housed in pairs with food and water *ad libitum*. They received manual bladder voiding for two weeks and a prophylactic dose of 0.1 mL enrofloxacin (subcutaneous) every other day during the first week post-op. Oral acetaminophen (0.2 mL) was administered every 12 h for the first two weeks. The animals received veterinary care for the whole duration of the experiment as established in the National Institutes of Health guidelines¹⁴ and the Official Mexican Standard NOM-062-ZOO-1999.¹⁵

2.5 Infrared thermography

A FLIR E6-XT infrared camera was used and always maneuvered by the same person at a 10-cm distance (verified with a measuring ruler) straight above the exposed tissue to get a clear image for analysis. The data from these images was analyzed using the accompanying software (FLIR Thermal Analysis and Reporting) by pinpointing the exact T8-T10 zone in each thermogram and obtaining the temperature.

2.6 Immunohistochemistry

The removed spinal cords (T8-T10) were embedded with Tissue-Tek and placed in a cryostat at $-22\text{ }^{\circ}\text{C}$ to make 40- μm longitudinal sections until visualizing the central canal; 3 consecutive sections were taken for immunohistochemistry. The sections were incubated in PBS with 3% hydrogen peroxide, rinsed with PBS, and then incubated in PBS and 0.3% Triton. Subsequently, the primary antibodies were added (rabbit polyclonal GFAP antibody, 1:1000; Merck) at $4\text{ }^{\circ}\text{C}$ for 2 nights. Once rinsed with PBS, samples were incubated with donkey anti-rabbit IgG antibody (1:500, Millipore) at room temperature for 1.5 h. Samples were rinsed with PBS and incubated in A+B conjugate (Vector PK 6100) for 1.5 h. They were rinsed again with PBS, and finally, the DAB kit (Vector SK-4100) was applied. The sections were placed on gelatinized slides and covered with Cytoseal 60 and a coverslip.

2.7 Astrocyte counts

In every case, the section with the most gliosis was analyzed with 20x magnification, always focusing on the most marked region within the analyzed section. This selected region was photographed and analyzed using ImageJ to count the marked astrocytes for further analysis. This software recognized GFAP-marked astrocytes and allowed the researcher to manually count them.

3. STATISTICAL ANALYSIS

The Shapiro-Wilk test showed that the thermography data did not have a normal distribution ($p = <0.001$), but the astrocyte count data did show a normal distribution ($p = 0.15$). Therefore, a Mann-Whitney U test was used to compare intergroup thermography, a Student's t-test compared astrocyte counts, and a Spearman's correlation determined the correlation between thermography and gliosis (assessed by Image J program for astrocyte count). SPSS v.22 and GraphPad Prism 5 were used for analysis.



4. RESULTS

Table 1 shows the general results obtained from this study: baseline and 5-week thermography as well as astrocyte count for the same spinal cord segment. A preliminary difference can be observed in GFAP-marked astrocytes in the sham vs. the experimental group. In addition, lower temperatures are shown by the injured animals (in the second thermography) compared to the temperatures in the *sham* group; further analyses detailed in the figures below were performed to draw valid conclusions.

Figure 1 shows a randomly selected infrared thermogram taken from one of the animals in the study (immediately after the laminectomy). The images were used for further analysis given that they also save the temperature range of the exact moment they were taken and allow for the exact determination of the temperature of any area within the image.

Figure 2A shows the thermography analysis that proved no difference existed before the intervention. Both groups demonstrated very similar baseline temperatures (*sham* 30.45 mean \pm 0.50 SD, experimental 29.91 \pm 0.46, $p = 0.15$). Figure 2B, however, illustrates how the temperature in the injury zone varies significantly between the groups 5 weeks later (*sham* 32.68 \pm 0.32, experimental 30.39 \pm 1.35, $p=0.01$).

Figure 3 is also a randomly selected microscopic image to portray how astrocytes were marked with anti-GFAP immunohistochemistry for the astrocyte count. In Figure 4, the difference in astrocyte counts between groups was evaluated, finding a significant difference between the *sham* (13.75 \pm 2.98 mean \pm SD) and the experimental group (54.71 \pm 7.63) ($p = 0.0001$). This difference reveals

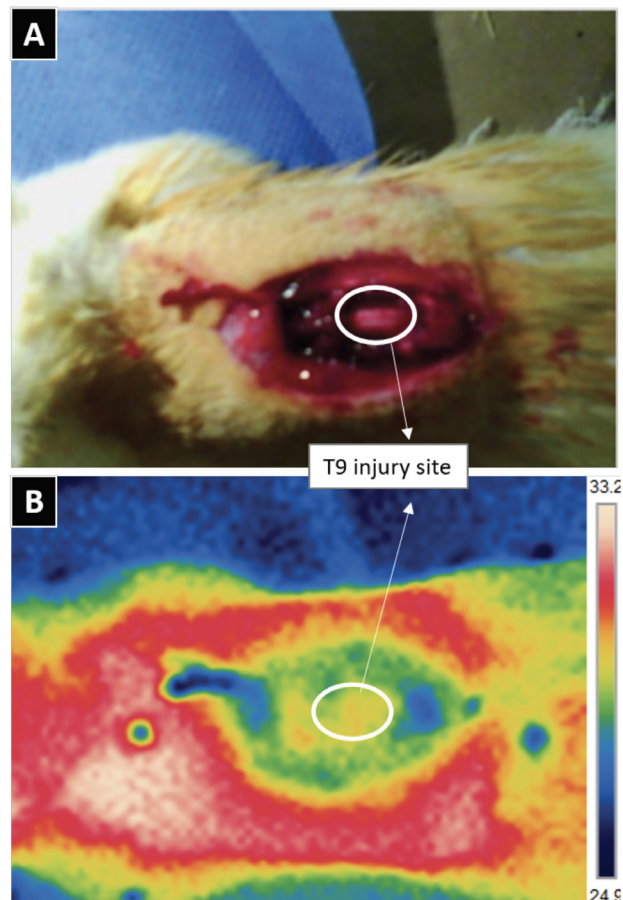


FIGURE 1. Infrared thermogram taken immediately after the laminectomy, before group assignment. A. Simultaneous picture capture with thermogram; the circled area was later analyzed. B. Thermogram of the picture shown above (A) allows for precise temperature evaluation. Temperature range (24.5–33.2°C) automatically calibrated is shown to the right. Images were randomly selected for graphic representation of a thermography.

TABLE 1. Thermography and gliosis results per animal.

Animals	Baseline thermography	5-week thermography	Number of GFAP-marked astrocytes
<i>Sham</i> (1)	31.2 °C	32.4 °C	10
<i>Sham</i> (2)	30.2 °C	32.9 °C	15
<i>Sham</i> (3)	30.1 °C	32.4 °C	13
<i>Sham</i> (4)	30.3 °C	33 °C	17
<i>Experimental</i> (1)	30.1 °C	30.2 °C	70
<i>Experimental</i> (2)	30.3 °C	28.9 °C	52
<i>Experimental</i> (3)	29.7 °C	31.7 °C	54
<i>Experimental</i> (4)	30 °C	30.3 °C	51
<i>Experimental</i> (5)	30.5 °C	31.3 °C	58
<i>Experimental</i> (6)	29.7 °C	28.4 °C	46
<i>Experimental</i> (7)	29.1 °C	31.9 °C	52

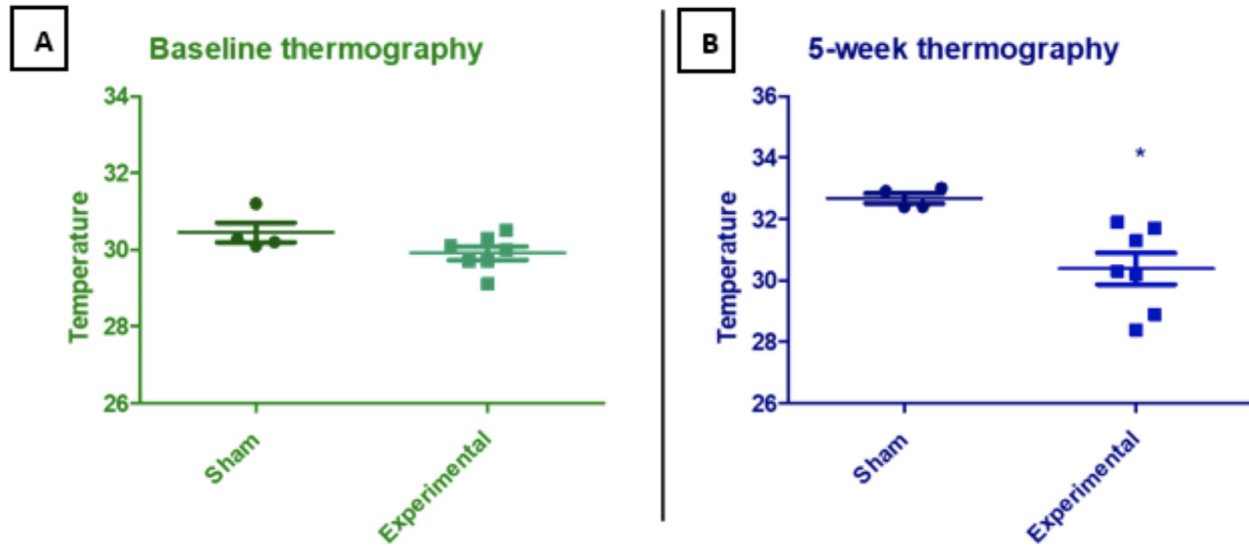


FIGURE 2. N = 4, 7 (*sham*, experimental). **A.** Mann-Whitney U test found no statistical difference between temperatures of the groups; $p = 0.15$. **B.** Mann-Whitney U test found a statistical difference in temperatures between groups; $p = 0.01$. Data shown as mean \pm SEM.

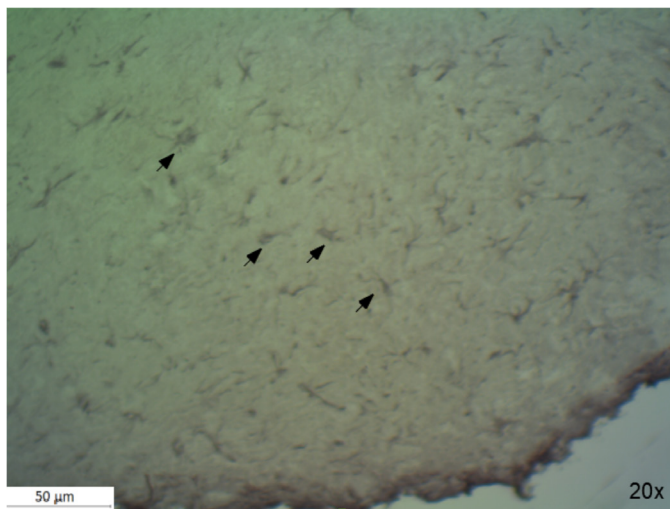


FIGURE 3. Anti-GFAP immunohistochemistry. Microscopic 20x image of a histologic section of injured spinal cord (after 5 weeks) with anti-GFAP immunohistochemistry. The arrows point at some of the marked astrocytes.

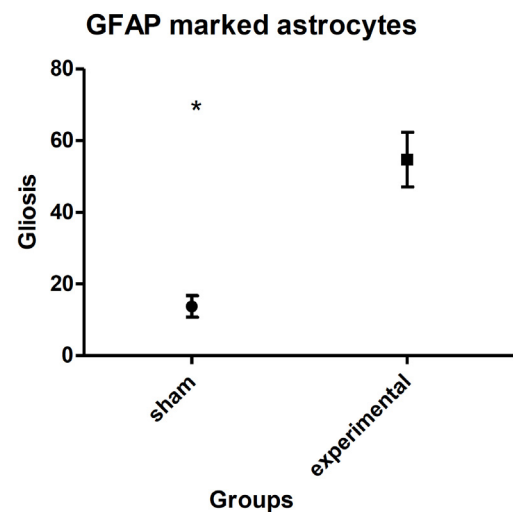


FIGURE 4. GFAP-marked astrocytes. N = 4, 7 (*sham*, experimental). Student's t-test found a statistical difference between the number of marked astrocytes by immunohistochemistry in the two groups; $p = 0.0001$.

the number of activated astrocytes at the site of SCI vs. an unharmed spinal cord. In addition, it provides an important evidence of GFAP as a precise identifier of such cells in the glial scar.

Finally, Figure 5 shows the correlation between the two variables, thermography, and glial scar (represented by GFAP-marked astrocytes). A negative r of 0.57 with a statistical significance ($p = 0.03$) establishes that the lower the temperature is, the higher the gliosis in that same area is (a pattern seen in the injured animals). Moreover, the graph

showed two different distributions among the animals in the *sham* vs. the experimental group since the animals without SCI consistently had higher temperatures and lower gliosis, reinforcing our hypothesis.

5. DISCUSSION

The results show that there are temperature changes in injured vs. healthy tissue in the spinal cord and, in the case of SCI by contusion, these changes correlate with the amount



Thermography and anti-GFAP immunohistochemistry correlation

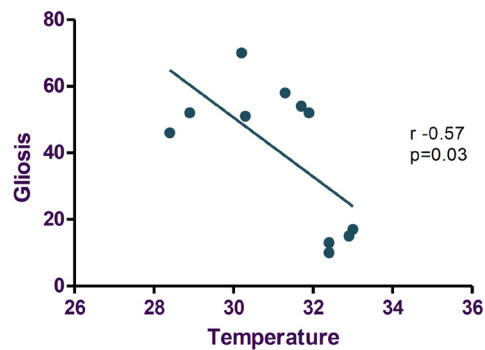


FIGURE 5. Thermography and anti-GFAP immunohistochemistry correlation. $N = 4, 7$ (*sham*, experimental). Spearman's correlation found a negative correlation ($r = -0.57$) between temperature and gliosis ($p = 0.03$). Each dot represents an animal; the animals in the *sham* group show higher temperatures and less gliosis than those in the experimental group.

of gliosis. This leads us to understand that temperature is a useful criterion for identifying healthy vs. scar tissue in the spinal cord. Considering the current research efforts surrounding glial scars and their role in SCI recovery and axonal regeneration, these findings are significant in putting forth novel strategies. Exploring noninvasive ways to identify this tissue is pivotal in terms of developing viable treatment options.¹⁶

This scar tissue is currently being thoroughly investigated since, even though the scarring process is very important for recovery in the early stages after a SCI, this same scar becomes a recovery barrier during the chronic stages of SCI.^{17,18} Accordingly, a study by Rodríguez-Barrera R, *et al.* proved that chronic scar removal combined with immunization with neural-derived peptides resulted in changes that led to improved motor function.⁹ Tom EJ, *et al.* showed the success in using the bacterial enzyme chondroitinase ABC for CSPG digestion in combination with a graft, resulting in functional axonal regeneration in an SCI model.¹⁹ Zhang S, *et al.* used rose Bengal scar ablation with promising results, including a significant increase in myelination.²⁰ Other research groups have tried to remove this scar without favorable results, either because the removal was performed in acute stages after SCI or because their method for identifying the scar tissue was invasive and had effects of its own.²¹⁻²³

Some reports, however, do not recommend glial scar removal based on findings regarding a continuous role the glial scar plays in guiding axonal regeneration throughout chronic stages of SCI. This should be considered and studied further, even though most of the evidence supports scar removal at these chronic stages of injury.²⁴

The present study proved equal temperature in the studied spinal cord area before the intervention, and lower temperature in that same area of the injured animals vs. those in the *sham* group after 5 weeks. Since all the animals underwent laminectomy, this elicited an inflammatory response expected after any intervention, which leads to a rise in local temperature. Still, the animals subjected to a SCI after the laminectomy exhibited lower local temperatures at the site of injury 5 weeks later compared to those in the control group. This was to be expected since the *sham* animals were also subjected to a laminectomy and, therefore, there was an inflammatory response. However, the animals with SCI had tissue necrosis and vascular changes besides inflammation.²⁵ The variations in temperature were subtle but statistically significant when compared between groups.

It is important to consider that this technique is innovative and there is no universal standardized use. The research team took the necessary precautions, including always taking the same distance, always with the same operator, and in random order between all animals, as to decrease possible bias. Nevertheless, even taking these precautions, there is some unavoidable human error as with every operator-dependent machine, as well as temperature variations during the interventions that could be further monitored and controlled in later studies. Future projects could blind the analysis from the operation for better results.

The immunohistochemistry results were also fairly expected since there was a significant rise in astrocyte count in the spinal cords of those animals in the experimental group,²⁶ and the correlation results further strengthen the hypothesis of the study.

This is a pilot study with a small animal sample and thus additional data are needed to proceed towards clinical research. Still, the concept is solid and should encourage this line of research that ultimately seeks to improve the lives of SCI patients.

6. CONCLUSIONS

Our results show that infrared thermography is effective in detecting minimal temperature changes in live tissue. The lower temperatures detected in the spinal cord tissue were associated with higher gliosis among animals in the experimental group (SCI). These findings can be explored in new studies on chronic scar removal in SCI patients without inflicting more damage to the surrounding healthy tissue.





7. ACKNOWLEDGMENTS

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8. CONFLICT OF INTERESTS

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Anxiety disorder and psychological dependence on smoking in adults from two communities in the State of Mexico

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ABSTRACT

Introduction: Smoking is a public health problem associated with chronic degenerative diseases. The coexistence of tobacco consumption and anxiety disorders was detected, which represent the most frequent psychiatric illnesses around the world. **Objective:** Analyze the prevalence of symptoms of anxiety and their severity as a consequence of tobacco dependence in young adults from two communities in the State of Mexico. **Material and methods:** This is a descriptive, observational, and cross-sectional study. The measurement instrument was a survey consisting of the Fagerström test, the Glover-Nilsson test, and Beck anxiety inventory. Prior informed consent applied to adults aged 18–35 years from the municipalities of Huixquilucan and Atlautla, State of Mexico with a calculated sample of 300 subjects. The analysis was carried out using SPSS Statistics, with a significance level of $\alpha = 0.05$. **Results:** 50.2% of the population were active smokers and 15.8% showed high suspicion of anxiety disorders independent of smoking. In smokers, 15.1% had scores for an anxiety disorder while more than 60% showed mild anxiety symptoms and 18.9%, moderate anxiety. A high prevalence of moderate dependence on nicotine was found in those smokers whose age of initiation of consumption was 16–19 years and who also had previous failure to abandon consumption. **Conclusion:** Anxious symptoms were found in 66% of those surveyed. This is relevant since mental illnesses have shown a significant increase, generating long-term problems in the overall health of adults.

Key words: anxiety; tobacco; addiction; dependence; disorder.

RESUMEN

Introducción: El tabaquismo es un problema de salud pública asociado a enfermedades crónico- degenerativas. Se ha detectado una elevada coexistencia de tabaquismo con trastornos de ansiedad, que representan el conjunto de enfermedades psiquiátricas más frecuentes en el mundo. **Objetivo:** Analizar la prevalencia de síntomas de acuerdo a severidad y sospecha de ansiedad como consecuencia de la dependencia de tabaco en adultos de dos comunidades del Estado de México. **Material y métodos:** Este es un estudio descriptivo, observacional y transversal. El instrumento de medición fue una encuesta conformada por las pruebas de Fagerström y Glover Nilsson y el inventario de ansiedad de Beck. Se obtuvo el previo consentimiento informado aplicados en adultos jóvenes de 18–35 años de los municipios de Huixquilucan y Atlautla, Estado de México, con una muestra calculada de 300 sujetos (menos 3 sujetos que no contestaron a cabalidad las escalas, quedando con un total de 297 sujetos). El análisis se realizó con el software SPSS, con nivel de significación $\alpha = 0.05$. **Resultados:** El 50.2% de la población era fumadora activa y 15.8% mostraron alta sospecha de trastornos de ansiedad independiente del tabaquismo. En fumadores, 15.1% mostraron puntajes para trastorno de ansiedad, más del 60% tuvo síntomas ansiosos leves y 18.9% tuvo ansiedad moderada. Se encontró alta prevalencia de dependencia moderada de nicotina en aquellos fumadores cuya edad de inicio de consumo fue de 16–19 años, quienes además presentaron fracaso previo en abandonar consumo. **Conclusión:** Se encontró sintomatología ansiosa en el 66% de los encuestados. Es importante profundizar puesto que las enfermedades mentales muestran un incremento significativo y generan problemas a largo plazo en la salud global del individuo.

Palabras clave: ansiedad; tabaco; adicciones; dependencia; trastorno.

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INTRODUCTION

Smoking is a public health problem of great importance. It is important to make a study of its incidence in the young Mexican population. The World Health Organization (WHO) has reported an estimated projection of 8 million tobacco-related deaths by 2030. Anxiety disorders are one of the most prevalent groups of mental illnesses in the world, incapacitating an economically productive population group. They lead to complications such as depressive disorders, substance abuse or even suicide.^{1,2} There are studies indicate that the presence of symptoms such as anxiety and depression are present in smokers, as well as low tolerance to frustration and hyperactivity, anhedonia, autolytic behaviors and a poor ability to cope, as well as to adapt to it. Patients who present or have anxiety traits are predisposed to substance abuse, known as “addictive personality”, described by the Cloninger’s psychobiological model for addictions. The importance of establishing both anxiety disorders and harmful tobacco as comorbidities, in order to reduce the incidence of both, since substance abuse is usually related to personality or other psychiatric symptoms.³

Smoking is defined by WHO as a drug addiction, and the American Psychiatric Society classifies nicotine as a psychoactive substance that produces dependence without abuse. Although quitting smoking is an almost instantaneous act, a large number of people do not succeed on their own despite having good reasons for quitting and knowing the severe long-term consequences of such substance abuse.⁴ Several studies have been conducted in active smokers with or without a diagnosis of psychiatric illness, in which depression is associated with smoking. In addition, tobacco consumption is strongly correlated not as a method of reassurance on the part of the consumer but as a continuous cycle that consists of craving (desire or desire to consume the substance). This cycle is related to symptoms of dependence and substance abstinence, which can further complicate future attempts to quit smoking.^{3,4} According to the results of Centro de Integración Juvenil and the National Survey on Addictions, tobacco is the main starting drug. WHO indicates anxiety is an anticipation of future harm or misery, accompanied by a feeling of dysphoria and/or somatic symptoms of tension. The target of anticipated damage can be internal or external. It is an alert signal that warns of imminent danger and allows the person to take the necessary measures to face a threat.^{4,5}

Anxiety disorders are one of the most common mental disorders, seconded by depressive disorders, with a worldwide prevalence of 12%. They appear from an early age with an average of 15 years and a higher prevalence between 25 and 45 years. They are more frequent in women

than in men in a 2:1 ratio. Below are the criteria to diagnose generalized anxiety disorder according to the Diagnostic and Statistical Manual of Mental Disorders IV or DSM-IV.⁶

- A) Excessive anxiety and worry about a wide range of events or activities.
- B) Difficulty to control a state of constant worry.
- C) Anxiety and worry associated with three or more of the following symptoms:
 1. Restlessness or impatience
 2. Easy fatigue
 3. Difficulty concentrating or having a blank mind
 4. Irritability
 5. Muscle tension
 6. Sleep disturbances
- D) Center of anxiety and worry not limited to the symptoms of a disorder.
- E) Anxiety, worry or physical symptoms causing clinically significant distress or impairment in social, occupational, or other areas.
- F) Alterations not due to the direct physiological effects of a substance or a disease and not exclusive in the course of a mood disorder, a psychotic disorder or a pervasive developmental disorder.^{7,8}

According to the WHO, anxiety is defined as the anticipation of possible harm or suffering, accompanied by a sensation of dysphoria and somatic symptoms of tension. The stimulus that causes the expected damage can be internal or external. It is an alarm signal that warns of imminent danger and allows the person to take the necessary measures to face possible threats.⁹ It has been postulated that there could be a type of personality associated with addictive behaviors, which would serve as a marker of risk for its development. There is limited evidence of this predictive personality, which serves to mark people at high risk of developing a toxic dependence. Growing evidence shows that the genetic influence on the initiation of smoking behavior is mediated by personality: There is a positive relationship between sensation seeking and dopamine D4 receptor polymorphism.¹⁰

Patients who smoked without trying to give up the habit were found to have three characteristics: anhedonia, or lack of

pleasure when doing activities, low tolerance for frustration, and personality traits with a tendency to present anxiety symptoms or attacks. In a study published in the American Journal of Psychology, a model proposed by Leventhal and Zvloezyk showed the existence of emotional smokers; that is, people with exclusively psychological dependence on smoking. Physiological dependence on nicotine was excluded as part of their addiction, which does not constitute a related withdrawal symptom. Here we are talking about physiological dependence, proposing an alternative reason for smokers who are dependent on smoking for emotional characteristics, and the complex psychiatric processes independently related to dependence generated by nicotine.^{10,11} This model explains the existence of crucial factors for emotional dependence on tobacco: anhedonia, low tolerance to frustration and hypersensitivity to anxiety, as well as risk factors for presenting anxiety and depressive disorders. In addition, smoking behavior patterns are manifested without a pleasant sensation when smoking; that is, they are related to anhedonia. Another study published by the same authors proposes an explanatory model of smoking and dependence, which implies a constant feeling of reward, and in turn this will increase over time.^{11,12} There is evidence of a relationship between cigarette smoking and various psychopathological disorders, especially depression and various anxiety disorders.¹³ In a study carried out in Galicia, the relationship between cigarette consumption and anxiety traits was analyzed in two representative samples of primary and secondary school students.¹⁴

The objective of this study was to analyze the prevalence of symptoms according to severity and suspicion of anxiety as a consequence of tobacco dependence in adults from two communities in the state of Mexico. The results indicate similarities in both samples: Those who never smoked had a significantly lower score in trait anxiety than those who smoked. In addition, men have a lower score in anxiety than women in all the comparisons made. These results indicate the consistent relationship between anxiety and smoking from early ages to adulthood. The implications of these results in the treatment and prevention of smoking and anxiety problems are discussed.

MATERIALS AND METHODS

A descriptive, observational and cross-sectional study. The participants were adults from 18 to 65 years of age, men and women, who were residents of the municipality of Huixquilucan, and Atlautla in the State of Mexico. From 1596 subjects, we obtained a calculated sample for a finite population of 300 subjects. Three subjects were discarded during the data analysis for not completing the instrument,

and 297 subjects remained. The measurement instruments were self-applied scales (prior informed consent) validated in the Mexican population: Fagerström test for nicotine dependence to identify subjects with tobacco addiction, the Glover-Nilsson scale for psychological dependence on tobacco, and Beck anxiety Inventory.

We researched whether or not the subjects were active smokers and had previous attempts on quitting tobacco consumption; the three scales were subsequently applied. The application was carried out from April to June 2019 in both municipalities. Subsequently, each survey was evaluated, scoring the severity of each symptom and variable introduced.

For nicotine dependence evaluated through the Fagerström test, the results are interpreted as: 0–3 mild dependence, 3–6 moderate dependence, and > 6 high dependence on nicotine. For psychological dependence on smoking according to the Glover-Nilsson scale, the scores are: < 12 mild psychological dependence, 12–23 moderate psychological dependence, 24–32 severe psychological dependence, and 33 or more, very severe psychological dependence.

For quantification of anxiety symptoms and suspicion of anxiety disorders by Beck inventory, the interpretation is as follows: 0 without anxiety symptoms, < 21 mild anxiety, 21–34 moderate anxiety, and 35 anxiety disorder. This information was collected in a database analyzed with SPSS Statistics, setting the level of significance at $\alpha = 0.05$.

Statistical analysis

A descriptive analysis of relative frequencies for qualitative variables in percentages and quantitative variables with central and deviation trends.

RESULTS

The results show that 50.2% of the studied population were active smokers (see Table 1). Adolescence is the period when most adults started smoking. The highest age at which smoking starts is 16–18 years, gradually rising from early adolescence (15 years). After 18 years, beginning to smoke becomes rarer (Figure 1).

Anxiety symptoms in adults regardless of smoking habit

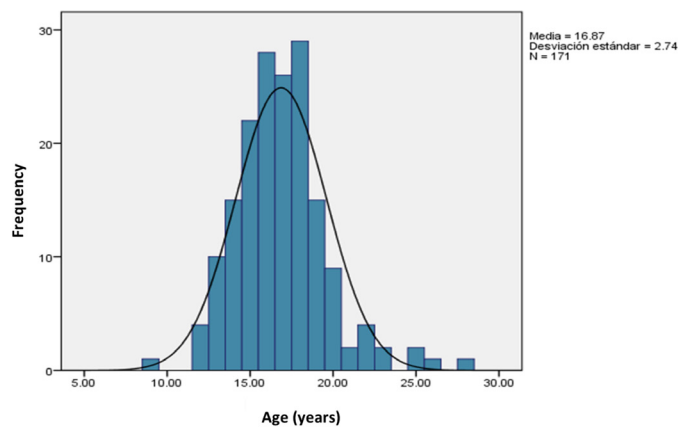
Regardless of the presence or absence of smoking, it was found that 66.2% of adults presented symptoms and signs

**TABLE 1.** Smoking and non-smoking adults in both communities.

		Frequency	Percentage	Valid percentage	Accumulated percentage
Valid	Yes	149	50.2	50.2	50.2
	No	147	49.5	49.5	99.7
	No answer	1	.3	.3	100.0
	Total	297	100.0	100.0	

TABLE 2. Anxiety symptoms in adults from both communities regardless of smoking measured using Beck anxiety inventory.

		Frequency	Percentage	Valid percentage	Accumulated percentage
Valid	No anxiety	17	5.7	5.7	5.7
	Mild anxiety	196	66.2	66.2	72.0
	Moderate anxiety	45	15.2	15.2	87.2
	Severe anxiety	34	11.5	11.5	98.6
	None	5	1.4	1.4	100.0
	Total	297	100.0	100.0	

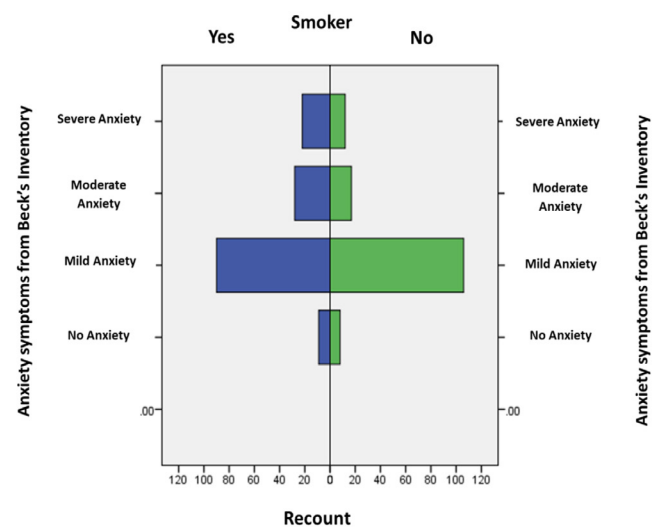
**FIGURE 1.** Age of beginning of cigarette consumption in adults by frequency.

of mild anxiety while 15.2% scored for moderate anxiety. 11.5% presented symptoms of severe anxiety and 5.7% of the population did not present symptoms of anxiety measurable by the Beck scale ($\alpha = 0.005$ with a 95% confidence interval, CI) (Table 2).

Symptoms of anxiety in smokers and non-smokers

Anxiety levels were evaluated both in smokers and non-smokers. The results obtained from the Beck anxiety inventory ($\alpha = 0.005$, 95% CI) showed that 66.2% of the

subjects presented mild anxiety, 15.2% moderate anxiety, and 11.5% severe anxiety and 5.7% showed no symptoms of anxiety (Table 2). Figure 2 presents a similar frequency of mild anxiety in both smokers and non-smokers. However, there is a significant increase in moderate to severe anxiety symptoms measured by Beck inventory in smokers, compatible with the literature indicating anxiety disorders are often found along with substance abuse (See Figure 2).

**FIGURE 2.** Smokers with anxiety symptoms by severity measured by Beck anxiety inventory.

Anxiety disorders in smokers

Only 15.7% of the smokers had a high score (> 36) for suspecting the presence of a severe anxiety disorder ($\alpha = 0.005$, 95% CI); 84.3% did not present any symptoms of anxiety (Table 3). Subjects with anxiety symptoms were given recommendations to seek further assessment from mental health professionals (psychologists, psychiatrists, and local physicians) to make a deeper, more individualized interrogatory and manage the disorder. We can infer that adults who started smoking from an early age, especially at 16 years, have more difficulties and failed attempts at quitting smoking. They are followed by those who started tobacco consumption at ages 18 and 19. In contrast, subjects who started smoking at age 17 had no previous attempts at quitting smoking (Figure 3).

Psychological dependence on tobacco measured by Glover-Nilsson scale

After the application of the Glover-Nilsson scale for psychological dependence on smoking, 55.3% of the

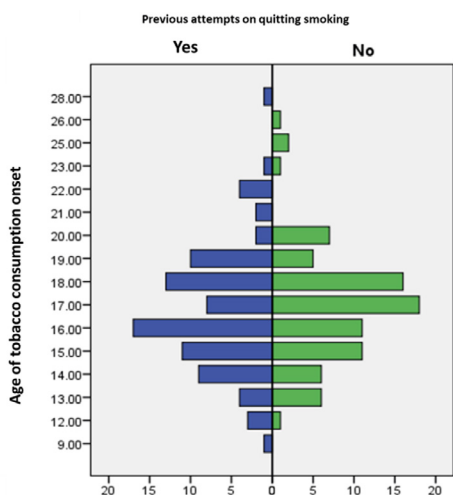


FIGURE 3. Failure to abandon tobacco consumption and age of tobacco consumption onset.

smokers presented mild psychological dependence, 28.3% had moderate psychological dependence, 9.4% showed severe psychological dependence, and 5.7% had a very severe psychological dependence (Table 4). Figure 4 shows similar results in subjects with high nicotine dependence and severe psychological dependence. A prevalence of 15.7% was found for probable anxiety disorders, independent of the smoking habit. In smokers, there was a prevalence of 15.1% for anxiety disorder, more than 60% showed scores equivalent to mild anxiety symptoms, and 18.9% scored for moderate anxiety. A high prevalence of moderate dependence on nicotine was found in those smokers whose starting age was 16–19 years and who also had previously failed to quit smoking.

DISCUSSION

The results were obtained by measurement scales validated in the Mexican population to simplify the self-application

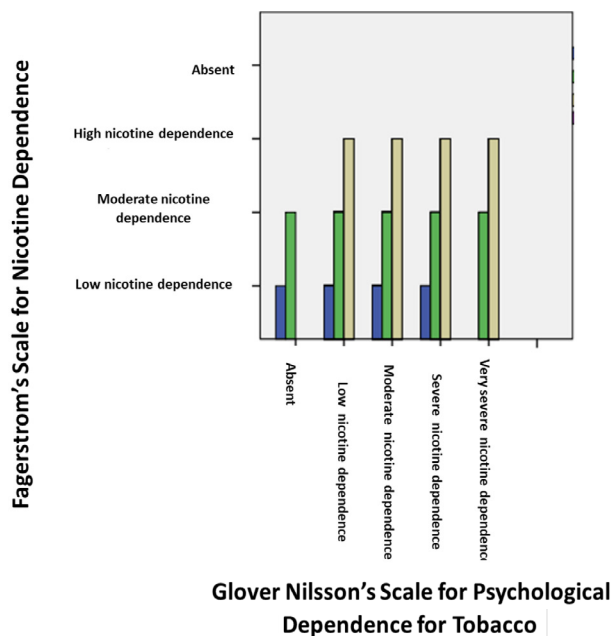


FIGURE 4. Nicotine and psychological dependence measured by Fagerström and Glover-Nilsson scales.

TABLE 3. Probable anxiety disorders in adult smokers from Huixquilucan and Atlautla measured according to Beck anxiety inventory scale.

		Frequency	Percentage	Valid percentage	Accumulated percentage
Valid	No anxiety disorder	134	84.3	84.3	84.3
	Probable anxiety disorder	25	15.7	15.7	100.0
	Total	159	100.0	100.0	

**TABLE 4.** Psychological dependence on tobacco use measured using the Glover-Nilsson scale.

		Frequency	Percentage	Valid percentage	Accumulated percentage
Valid	No signs of dependence	2	1.3	1.3	1.3
	Mild dependence	88	55.3	55.3	56.6
	Moderate dependence	45	28.3	28.3	84.9
	Severe dependence	15	9.4	9.4	94.3
	Very severe dependence	9	5.7	5.7	100.0
	Total	159	100.0	100.0	

and interpretation as much as possible. This study could be potentially used and improved in subsequent studies as a screening device for young adults. This population is vulnerable to substance abuse and co-existing anxiety disorders, both of which can go underdiagnosed, severely affecting life quality. The shortened version of the Fagerström questionnaire for nicotine dependence was used (6-questions instead of 8) since it omits questions on the amount (mg) of tobacco contained that the vast majority of the people surveyed are unaware of. Beck inventory for anxiety symptoms was applied. This study can be extended by comparing populations of a cohort from this original study or other populations from other communities.

In addition, anxiety disorders are prevalent: 34 subjects in the population showed high suspicions of anxiety disorder with a prevalence of 15.7%. This percentage is worth observing since it is similar to that previously reported in literature. Then, we can infer that mental anxiety disorders are an insidious and growing problem in mental and public health that have long been disregarded. Similarly, its association with substance consumption and abuse, in this case tobacco, has been well studied. There is a high prevalence in subjects who showed mild anxiety symptoms, closely followed by moderate and severe anxiety. These percentages are even higher in smokers, with an increase in symptoms of moderate and severe anxiety. The dependence on nicotine was found to be greater than psychological dependence on smoking. This also related to peaks in the age of initiation of tobacco use at 16, 17 and 18 years, who also presented no attempts to quit their smoking. Then, there is evidently a high prevalence of anxiety disorders coexisting with smoking (one of many forms of comorbid substance abuse). Therefore, both entities should be equally studied in each case.

With the original data from the group suspected of having an anxiety disorder, we can follow the subjects' evolution if they seek out mental health professionals. Furthermore, the

changes and impact derived from early screening of anxiety symptoms and tobacco abuse can also be tracked. The aim is to perfect such procedures in the long run as prevention tools for public mental health and substance abuse control in the adult population. Indeed, the study has limitations regarding the number of participants and the lack of further intervention; however, it can be expanded and replicated.

Therefore, this study can be extended to relate to the use of other substances since anxiety disorders are multifactorial diseases of great importance. Having important complications associated like depression and substance abuse, which can cause severe dysfunction in multiple areas of an individual's life, it can be used as a quick, inexpensive screening tool in working population on the long run that can impact positively in the detection of common mental health disorders, such as anxiety disorders and tobacco abuse.

CONCLUSIONS

Symptoms of anxiety were present in 66% of those surveyed, so we can conclude that it is important for public health to develop more of these investigations in our country since mental illnesses have shown a significant increase worldwide. In particular, it is important to talk about anxiety disorders, diseases that affect the productive working adult population. They are clearly related to consumption and abuse of substances, in this case tobacco, which is estimated to cause high morbidity and mortality. These disorders are also linked to dependency and the inability to abandon consumption. Therefore, additional studies are required to demonstrate and expand the correlations in larger populations for the comprehensive management of these two highly relevant entities for public health in Mexico.

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CONFLICT OF INTERESTS

The authors declare no conflict since individualized authorization was received.

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Dysphagia as a predictor of malnutrition risk in older adults: a brief review of literature

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ABSTRACT

Dysphagia is known as any alteration of the swallowing process that affects the ability to eat a The use of mild and aromatic nd/or drink and presents in 30–40% of hospitalized patients. In the elderly, it is highly relevant due to its association with the development of frailty, malnutrition, and the high risk of morbidity and mortality. A set of factors has been proposed as a cause of dysphagia, such as anatomical and physiological alterations, neurological pathologies, drugs, and sarcopenia. Thickening liquids and texture-modified foods may be beneficial for patients with dysphagia, but they can also promote malnutrition if not prescribed properly. That is why nutritional support is key to avoid the risk of aspiration and malnutrition, which in turn, contribute to the development of dysphagia.

Key words: dysphagia; elderly; malnutrition; frailty; nutritional support.

RESUMEN

Se conoce como disfagia a cualquier alteración en el proceso de la deglución que afecta la capacidad para ingerir alimentos y bebidas, presentándose con una frecuencia del 30 al 40% en pacientes hospitalizados. En el adulto mayor es de gran relevancia por su asociación con el desarrollo de fragilidad, desnutrición y el alto riesgo de morbi-mortalidad. Se ha propuesto un conjunto de factores que contribuyen al desarrollo de la misma como las alteraciones anatómicas y fisiológicas, patologías neurológicas, fármacos y sarcopenia. Es por ello que el soporte nutricional resulta de vital importancia con el fin de evitar el riesgo de broncoaspiración y desnutrición que, a su vez, contribuyen al desarrollo de disfagia.

Palabras clave: disfagia; adulto mayor; desnutrición; fragilidad; soporte nutricional.

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INTRODUCTION

Dysphagia is known as any alteration in the swallowing process that makes it difficult for food and/or liquids to pass from the oral cavity to the stomach and can be caused by different reasons.^{1,2} Oropharyngeal dysphagia is prevalent under different conditions among older adults and has been recognized as a geriatric syndrome by the Dysphagia Working Group.³

Dysphagia is one of the most frequent alterations in the elderly due to anatomical and physiological changes during aging, decrease in muscle mass and sarcopenia, and the development of diseases. In addition, the use of some drugs is associated with its presence.

Its diagnosis is of great importance since this condition reduces the quality of life and increases the risk of aspiration pneumonia, dehydration, social isolation, alterations in nutritional status, and morbi-mortality in patients who suffer from it.

The prevalence of dysphagia in the general population has been estimated to be between 2 and 16% and it can increase up to 30–40% in hospitalized patients.^{1,2} It has been observed in up to 64% of patients who have suffered a stroke and over 80% of those with dementia. However, dysphagia is poorly diagnosed due to variations between diagnostic methods.⁴

It is also related to malnutrition due to diminished intake, inadequate nutritional composition of texture-modified diets, and muscle weakness, leading to the persistence and worsening of malnutrition, sarcopenia, and dysphagia.

METHODS

A brief review of the literature was performed using PubMed as database. Key words identified as search terms were dysphagia, elderly, malnutrition, frailty, and nutritional support. These were combined using the AND Boolean operator.

Our inclusion criteria required articles written in English and Spanish that address dysphagia, dysphagia in elderly or malnutrition. The exclusion criteria comprised studies focused only on pediatric population, abstracts, and articles without full text.

RESULTS

Brief physiology of swallowing

To carry out an adequate swallowing, coordination, expansion, and displacement of the pharynx are required so

that the food bolus passes through the structures and the upper esophageal sphincter, while the airway is protected.⁵

This complex process includes three phases and requires the coordination of mouth, pharyngeal and laryngeal muscles, and esophagus, which are innervated by the cranial nerves V, VII, IX, X, and XII. The oral phase, considered voluntary, is responsible for chewing, bolus formation, propulsion, and passage to the pharynx. In this phase, various muscles are required, such as the tongue, cheek, orbicularis oris, and buccinator muscles.⁶ The pharyngeal phase allows the nasopharynx to be closed by the soft palate, while the hyoid bone and larynx are raised and the vocal cords are brought together to protect the airway. The pharynx contracts and the cricopharyngeal sphincter is relaxed, so that the food passes into the esophagus. After this step, the esophageal phase includes the peristaltic contraction of the esophagus and relaxation of the lower esophageal sphincter so that the bolus reaches the stomach. This is slower than the pharyngeal phase and may exceed 10 seconds.⁶ The pharyngeal and esophageal phases are considered involuntary, which implies that there is no control over them.^{7,8}

Swallowing physiology and disorders in the elderly

Physiologically, during aging, alterations occur in the organism that predispose older adults to dysphagia, such as sensory and motor variations, decreased salivation, and changes in taste and smell that may contribute to a reduction in the swallowing process.² Sensory changes include decreased smell and loss of taste appreciation, caused by medicines, among others.^{9,10}

The use of drugs, such as tricyclic antidepressants (due to their anticholinergic effect and decreased saliva), benzodiazepines, risperidone, and haloperidol is common among these patients. Still, it must be remembered that they have effects on the state of consciousness, may cause dry mouth, and have extrapyramidal effects that make swallowing even more difficult.^{11,12} Likewise, sedative drugs, such as antipsychotics, opioid analgesics, hypnotics, and anxiolytics directly impact the central nervous system, mainly due to their antidopaminergic action. They can alter swallowing and the cough reflex, thereby increasing the risk of suffocation.^{9,13}

Dysphagia is also associated with adulthood because adults present diseases that are related to this alteration more frequently. Among these conditions are neurological diseases, Parkinson's disease (due to the degeneration of dopaminergic pathways in the substantia nigra, striatum, and enteric nervous system), Alzheimer's disease, and cerebrovascular disease (CVD). It is also linked to head and neck cancer, dementia, and amyotrophic lateral sclerosis



in which a progressive function loss of the cortical motor neurons can cause spasticity of the mandibular muscles.^{1,8,14} It is also caused by traumatic brain injury, myasthenia gravis, cerebral palsy, myopathy, and Guillain-Barré syndrome due to involvement of the oropharyngeal muscles.²

Even in the absence of such diseases, cerebral atrophy may be the result of ageing and the consequent decline in nerve function, while a regional-dependent muscle atrophy may affect swallowing.¹⁵

There are other causes of dysphagia, like anatomical and physiological deficiencies in the mouth, pharynx, larynx, and esophagus, including decreased muscle mass and connective tissue elasticity. They lead to loss of strength and movement as well as weakness of the lip muscles, causing inability to completely seal them, and that of facial muscles. This increases the risk of retaining food in the mouth, difficulty forming the bolus, and passage through the oral cavity.⁸ In addition, other factors that contribute to this process are the loss of muscle strength and tone, decreased speed, precision, and coordination of movements, reduced esophageal propulsion and peristalsis, and tooth loss.^{1,2}

Among the changes that negatively impact the swallowing process are the ossification of the hyoid bone and the thyroid and cricoid cartilages, atrophy of the intrinsic muscles of the larynx, dehydration of the laryngeal mucosa, loss of elasticity of the laryngeal ligaments, flabbiness of the vocal cords, and loss of the geniohyoid muscle, all of which may occur approximately in 15% of men and 31% of women.^{9,16}

Dysphagia can be caused by sarcopenia, as seen in tongue atrophy in older populations, geniohyoid muscle atrophy, pharyngeal wall thinning, and diminished muscle mass of the anterior belly of the digastric and temporalis muscles.¹⁷

A study in community-dwelling older adults found that there is a moderate correlation between decreased grip strength and decreased posterior tongue strength.^{9,17} The muscles that participate in swallowing are mostly made up of type II fibers, which are the first affected in the processes of malnutrition and sarcopenia when compared to type I fibers.^{18,19}

Fat infiltration into the skeletal muscle causes weakness since it compromises the integrity of the muscle and increases the risk of frailty. Fat infiltration has been detected in the geniohyoid muscle, which participates in the movement of the hyoid bone for swallowing.⁹

The decline in muscle strength and the loss of teeth affect the choice of food, efficiency of chewing, and the swallowing of oral residue, impacting the choice of food texture and, therefore, its nutritional composition.^{9,10}

Frailty

Oropharyngeal dysphagia can be considered a geriatric syndrome, and at the same time it is closely related to frailty. Although there are several definitions, frailty can be described as the state of vulnerability associated with adverse events that increases the risk of morbidity and mortality. The prevalence of frailty in the Mexican elderly is estimated to be 39%.^{20,21}

Geriatric frailty syndrome is assessed when there are three or more of the following five criteria^{9,22,23}:

1. Weakness measured by dynamometry
2. Decrease in walking speed, evaluating the time in which 4.57 meters are traveled
3. Low level of physical activity
4. Low energy or exhaustion reported according to the geriatric depression scale
5. Unintentional weight loss of more than 4.5 kg or 5% in the previous year

Dysphagia has been identified to participate both as a factor contributing to frailty and as a consequence thereof.⁹ This is a consequence of physical, psychological and nutritional factors, being malnutrition a marker of this syndrome.⁵

Among the factors that increase the risk of developing frailty are diabetes mellitus, high blood pressure, chronic obstructive pulmonary disease, decreased upper limb strength, recent hospitalizations, low levels of physical activity, and economic and social factors, among others.^{20,21}

Frailty represents a vicious circle influenced by various factors, such as aging and low food intake due to anorexia of ageing, the underlying pathology that leads to loss of muscle mass and thus sarcopenia. The latter causes reduced strength and maximum oxygen volume, which in turn lead to a decrease in walking speed, and is also associated with a lower resting metabolic rate. These effects restrict activity and lower energy expenditure, which ultimately contribute to malnutrition and greater sarcopenia.^{22,23}

Dysphagia diagnosis

The assessment of dysphagia includes medical history, a physical exam, and instrumental examinations.²⁴ There are screening tools to measure the risk of dysphagia, as the Eating Assessment Tool-10 (EAT-10), developed in the United States in 2008. According to a study published in 2018, EAT-10 was



observed to be a predictor of aspiration. This tool consists of 10 questions that provide information on functionality, emotional impact, and physical symptoms that dysphagia could cause. A score of 3 or higher could be interpreted as a potential risk of dysphagia.^{1,3}

The clinical examination also includes a water swallowing test, where the patient is asked to swallow 50 ml of water in 5-ml aliquots. If the patient chokes, coughs or presents any alteration in the voice quality, dysphagia is diagnosed; still, silent aspiration may not be detected.⁶

A videofluoroscopic swallowing study (VFSS) or a modified barium swallow (MBSS) can be used to make an objective assessment, which is currently considered the gold standard in diagnosis.^{25,26} VFSS allows to identify alterations in the efficacy or safety of swallowing. Efficacy is more frequently associated with malnutrition and/or dehydration, while safety is linked to tracheobronchial aspiration.²⁷

Dysphagia and its impact on nutritional status

Regarding nutrition, dysphagia decreases or alters food and/or liquid intake, contributing to an impoverishment of the nutritional state. This subsequently hampers the functional capacity and, therefore, could promote frailty.²

Dysphagia is highly associated with malnutrition and must be treated since malnutrition increases the risk of complications, length of hospital stays, hospital costs, mortality, and infections by reducing immune function.¹

Although data in the literature are scarce, 3–28% of patients suffer dysphagia and malnutrition since several definitions and diagnostic methods have been used to assess both entities. However, the coexistence of malnutrition and dysphagia is frequent.²⁸

Malnutrition is defined as the imbalance of energy, protein, and other nutrients that cause negative effects on body composition, physical functionality, and clinical results. The risk of malnutrition can be detected using various screening tools such as the MNA-SF (Mini Nutritional Assessment-Short Form) proposed by the European Society for Clinical Nutrition and Metabolism (ESPEN). This society suggests that malnutrition is indicated by a body mass index below 18.5 kg/m² and unintentional weight loss. Whether it is greater than 10% and indefinite or greater than 5% in the last 3 months, it must be accompanied by BMI <20 kg/m² in adults over 70 years of age, <22 kg/m² in adults under 70 years of age or a fat-free mass index <15 kg/m² in women and <17 kg/m² in men.³

It remains unclear whether malnutrition occurs before or after hospital admission. Then, nutritional screening is of utmost importance due to the large number of risk factors related to malnutrition, such as dysphagia, low BMI, decreased muscle strength, cognitive decline, various comorbidities, and polypharmacy.²⁹

During hospitalization, there is physical inactivity that compromises the functional capacity. It is known that prolonged immobility is related to a slower recovery, so a longer hospital stay increases hospital costs. In addition, older adults have lower biological reserves that promote recovery (Figure 1).⁵

Comprehensive management of dysphagia

There are different ways of managing dysphagia, as compensatory measures that involve the implementation of techniques that allow food and drink intake or provide alternative sources of nutrition. However, these measures only provide immediate benefits and refer to posture adjustments, swallowing maneuvers, and diet modifications; still, they do not solve the underlying problem.²

Oral hygiene is a factor of great importance and scarcely mentioned. It should be performed routinely even when the person lacks teeth to avoid bacterial colonization and decrease the risk of aspiration pneumonia.^{11,19}

Among other interventions for the management of dysphagia, modifications in diets (thickening liquids) have been frequently used to prevent aspiration and its consequences. Dietary modifications are usually guided by the VFSS swallowing study, which examines swallowing liquids and solids of different sizes and consistencies.^{30,31}

Currently, the classification proposed by the IDDSI (International Dysphagia Diet Standardization Initiative) can be used to identify the consistency and viscosity of foods and beverages according to the severity of dysphagia.³²

The diets modified in consistency are based on the fact that thick liquids have a slower flow, providing more time for the closure of the airway and thus reducing the risk of aspiration. Although they are easier and safer to chew and swallow, their prescription must be carefully monitored.³⁰

There is still insufficient evidence to suggest that modifying the texture of food and thickening liquids benefit adults with dysphagia to prevent pneumonia and its consequences. Instead, an increased risk of dehydration and malnutrition has been observed together with this type of modifications.^{16,30}



The absorption of the drugs these patients take is also modified and the increase in viscosity can delay the dissolution and disintegration of these drugs. Furthermore, thickened liquids may increase the risk of post-swallow oral and pharyngeal residues and there is a high risk of insufficient oral intake.^{30,33,24}

Thickeners are commonly used to modify the viscosity of liquids and food since they reduce tracheobronchial aspirations. However, the adherence to treatment only reaches 48–56% due to the organoleptic characteristics of these foods. In addition, they may require more effort for their preparation. Some studies have reported a greater risk of dehydration at a higher viscosity while a greater tolerance and attachment are found at a lower viscosity, as in the consistency of nectar.¹¹

With the aim of reducing the risk of suffocation and work in the oral cavity, textures have been used to modify foods. These modifications can range from softening food enough to break it with a fork to creating a puree consistency that does not require chewing. Generally, when there is a high risk of suffocation, it is suggested to consume small bites of food whose size (1.5 x 1.5 cm) does not occlude the airway if the food is not chewed.⁹ This risk is also present in the intake of fibrous, firm, sticky, dry, and crisp foods or those that can cause airway occlusion.⁹

It has been suggested that adults over the age of 65, and men in particular, have a 7-fold risk of choking compared to children aged 1–4.⁹ The modification of purees contributes to a high risk of malnutrition since there is usually a lower intake and purees are low in calories, protein, and micronutrients as compared to foods in regular diets.³⁰ This type of management

faces problems such as lack of compliance and lower quality of life given that patients tend to dislike modified diets in consistency and find them less satisfactory.^{30,33}

During aging and in order to adapt to changes, adults often modify their choice of food textures to make chewing easier, which can contribute to weight loss. These modifications may also cause a decrease in energy density and changes in taste, making food unappetizing to the eye and contributing to a decrease in intake and a greater risk of malnutrition.^{9,24}

The use of mild and aromatic spices as well as natural flavorings including garlic, ginger, pepper, oregano, and capsaicin is recommended to promote the sensory appreciation of these foods.^{9,10} There is evidence that this type of diet could have some benefits, but there is also contradicting evidence pointing out the negative impact on quality of life. Then, it should be adopted with caution and under strict surveillance, allowing the patient to choose whenever it is possible.³⁰

The difficulty in indicating the type of diet to use is the lack of standardization and the presence of variable consistency of thickened liquids prepared by staff within and between hospitals. The same problems are likely present when texture-modified food is produced in-house.³⁰

Modified textures and thickened liquids should be used in persons with chronic dysphagia to enhance nutritional status (grade B recommendation). Texture-modified diets and thickened liquids should be prescribed after carrying out a clinical swallowing exam and/or instrumental assessment (Good practice point).²⁴ The oral diet can be adapted and even use oral nutritional supplements to cover the macro and micronutrient requirements of these patients, improve their

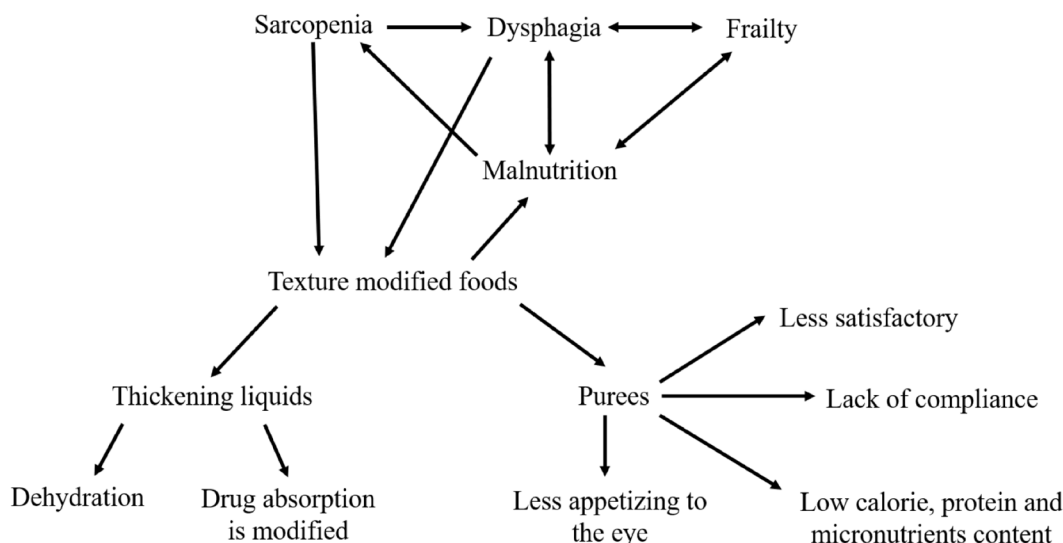


FIGURE 1. Mechanisms associating dysphagia and malnutrition.



nutritional status, reduce morbidity and mortality, and allow them the pleasure of eating.^{11,34}

The European Society for Swallowing Disorders (ESSD) suggests some strategies described by Ney et al. to promote food intake. Among them are eating slowly to control the food bolus, preventing food or drink consumption when tired or in a hurry, putting small portions of food or drink in the mouth (e.g. using a teaspoon), avoiding distractions at mealtimes to focus on swallowing, stopping from mixing solids and liquids in the same bite and alternating them to avoid residue, and using sauces and condiments that favor bolus formation. It is extremely important that caregivers or family members who share mealtimes with older adults have the necessary training.^{11,34}

ESPEN recommends the use of oral nutritional supplements in those patients at risk of malnutrition, with multimorbidity and frailty, after orthopedic surgery, and with anorexia. It is also indicated when there are some dietary restrictions for chronic diseases and <75% of unintentional weight loss or requirements, as long as oral appetite and nutrition are not compromised.^{11,34}

On the other hand, enteral nutrition by nasogastric tube or percutaneous endoscopic gastrostomy (PEG, grade A recommendation) could be considered in patients with severe dysphagia to improve nutritional status. However, the use of this type of nutrition is still controversial. The main indication for enteral nutrition is severe neurological dysphagia in which support should start as soon as possible together with swallowing therapy with the aim of reducing the risk of nutritional complications.^{11,34}

The nasogastric tube is usually suggested for short periods of time; that is, less than 4–6 weeks when there is no risk of aspiration. For example, it is recommended in patients with acute dysphagia, stroke or head injury. It should be considered that nasogastric tubes can cause side effects like mucosal erosion, kinking, and shifting.^{6,11,34} Meanwhile, PEG is recommended for periods longer than 4–6 weeks, as in chronic or progressive diseases, generally after CVD or dementia; it does not exclude aspiration risk.^{35,36}

In patients with mild to moderate dementia, it is suggested to use oral nutritional supplements or even nasogastric tube or PEG to cover the requirements and prevent malnutrition. Nevertheless, in patients with terminal dementia, the ESPEN guidelines and the American Geriatrics Society recommend avoiding tube feeding, as studies show artificial enteral nutrition in patients with advanced dementia is associated with morbidity and mortality.^{36,37,38}

Nutrition is a basic right that cannot be denied, but before making a decision in clinical practice, four principles must

be taken into consideration: beneficence, non-maleficence, respect for autonomy, and justice. Observing these will provide patients with an effective treatment for dysphagia and respect for their decisions, preferences, and goals.^{39,40}

In order to decide whether to apply a treatment, two aspects can be taken into account: knowing the patient's values and expectations and clarifying the cost-benefit. Furthermore, it is suggested to choose the treatment of the frail elderly based on the severity of the condition and efficacy and safety alterations identified during VFSS.^{11,27}

Oral feeding is a basic need, yet enteral nutrition could be considered a medical treatment and thus can be prescribed, started, and stopped. Before starting, it is important to clarify the duration time and the expected results.^{39,40}

The current treatment of oropharyngeal dysphagia consists of compensatory measures, rehabilitation or both. It includes modification in viscosity and lower chin, which are effective but not standardized. In consequence, it is not possible to determine the particular intervention required by each person.¹¹

Swallowing rehabilitation

Swallowing rehabilitation involves performing exercises that target specific muscles and improves lips and tongue functionality as well as bolus formation in the oral cavity. Rehabilitation has high degrees of evidence, A or B, depending on the technique used.^{11,34}

Position

Among the postural changes that could be easily implemented is sitting at 90° during the feeding and at least 30 minutes after finishing meals. Lowering the chin allows for airway closure and is easy to perform, besides having an A grade of evidence.¹¹

When tongue movements are impaired but the pharyngeal phase is intact, titling the head backwards helps guide the bolus into the pharynx.⁶

Other maneuvers and postures have an evidence grade of B, such as double swallowing to decrease waste before the next inspiration.¹¹

CONCLUSIONS

Social coexistence revolves around food and drinks, so swallowing difficulties have both social and emotional



consequences for older adults. In addition, they entail a high risk of dehydration, malnutrition, and aspiration pneumonia.

It is of utmost importance to evaluate the clinical conditions that lead patients to develop dysphagia. Only then will they be offered the best nutritional alternatives, such as nutritional supplementation, food enrichment, and artificial enteral nutrition. These alternatives improve the quality of life and do not represent more risks than benefits to the patients' health.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest in the preparation of this work.

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Can exercise be used as a protective agent against disease severity in COVID-19 and as treatment during subsequent rehabilitation?

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ABSTRACT

In a matter of months, COVID-19 has spread worldwide, and it has affected not only human lives but also the socioeconomic structure. Disease severity increases with the presence of other factors such as age, diabetes, and hypertension. Exercise has been shown to control blood pressure and blood sugar level; it enhances the immune system and age-related physiological changes. Given its ability to control all of these factors, exercise can be used as a protective agent against disease severity in COVID-19 and as treatment during subsequent rehabilitation.

Key words: COVID-19; severity; exercise; hypertension; diabetes; age; rehabilitation; physical therapy.

RESUMEN

En cuestión de meses la COVID-19 se ha expandido a todo el mundo y su impacto no solo ha sido en vidas humanas, sino también en la estructura socioeconómica. La severidad de la enfermedad aumenta en presencia de factores tales como edad, diabetes e hipertensión. Se ha demostrado que el ejercicio puede controlar la presión sanguínea y los niveles de azúcar en sangre, además de aumentar la función inmune y cambios fisiológicos relacionados con la edad. Dada a la habilidad de controlar todos estos factores, el ejercicio tiene el potencial de ser usado como factor protector para reducir la severidad de la COVID-19 y como tratamiento durante la rehabilitación subsecuente.

Palabras clave: COVID-19; severidad; ejercicio; hipertensión; diabetes; edad; rehabilitación; terapia física.

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1. INTRODUCTION

Since its first appearance in December 2019, COVID-19 has spread from its place of origin in China to the whole world in months. It has an incubation period of 5 days and has symptoms like fever, dry cough, and fatigue.¹ The main form of transmission is via droplets, and it can affect several tissues besides the lungs, such as the central nervous system, heart, liver, gastrointestinal tract, and kidneys.² The most common risk factors for disease severity for COVID-19 are diabetes, age, and hypertension.³⁻⁶ Until now, there is no known cure, and the best strategy for controlling its spread has been the implementation of preventive measures, such as social distancing, use of face masks, quarantine, the proper cleaning of surfaces, and hand washing.⁷ COVID-19 has had an impact on every aspect of the world socioeconomic landscape and is estimated to have long-term repercussions.⁸

Benefits of physical activity have been well documented, from a better quality of sleep, anxiety and weight management, and lower cardiovascular disease risk to reduction of all-cause mortality, among others.⁹⁻¹² The terms physical activity and exercise are sometimes used interchangeably, but one should not be confused with the other. Exercise must be a planned, structured, repetitive, and purposeful physical activity with measurement methods.¹³ Zbinden-Foncea, Deldicque, and Howley reviewed the possible role of exercise as a preventive strategy to decrease the severity of COVID-19, focusing on its role in regulating the inflammatory status.¹⁴ This review will focus on the role of exercise during the COVID-19 pandemic, from reducing risk factors for disease severity to its role during rehabilitation in disease recovery.

2. SUBTOPICS

Blood pressure control

Although hypertension is considered a risk factor for COVID-19 severity^{15,16}, the changes in the pro-inflammatory status and immune suppression that accompany hypertension are the reason why it is considered a risk factor.¹⁷ Nevertheless, data suggests that patients with poor blood pressure control have higher rates of adverse outcomes like mortality, ICU admission, heart failure, respiratory failure, and mechanical ventilation.¹⁸⁻¹⁹ One possible treatment line for preventing adverse outcomes in these patients is to keep blood pressure under control. After an acute bout of exercise, there is a decrease in blood pressure known as post-exercise hypotension (PEH). There are several mechanisms for this change in pressure, and it is likely a combination of neural and hormonal mechanisms.²⁰⁻²² One strong candidate for PEH

is post-exercise baroreflex. After exercise cessation, there is an increased sympathetic nervous activity that causes a reset in the baroreflex, thus decreasing blood pressure.^{23,24} As little as 30–60 min aerobic exercise per week is enough to lower blood pressure.²⁵ Even in resistant hypertension, interval walking exercise decreases blood pressure.²⁶ Thirty minutes of moderate treadmill walking lowers systolic and diastolic blood pressure over 8 hours of prolonged sitting.²⁷ Aerobic exercise in the evening results in a better hypotensive effect when compared to exercise done in the morning in hypertensive individuals, although both options are effective in lowering blood pressure.²⁷ High-Intensity Interval Training (HIIT) can provide a longer²⁸ or the same²⁹ hypotensive effect as continuous aerobic exercise. Isometric handgrip has been proposed as a cost-effective, low-equity alternative to decrease blood pressure.³⁰⁻³² However, aerobic exercise yields a better blood pressure control response when compared with isometric exercise.^{33,34}

Glycemic control

It has been hypothesized that people with diabetes appear to have worse outcomes because of high levels of blood glucose and changes in their physiology that accompany diabetes. Among these changes are increased ACE-2 expression and furin levels, impaired T cell function, increased interleukin-6 (IL-6) levels, and impaired neutrophil chemotaxis and phagocytosis.^{15,35,36} In a cohort of 7,337 patients with confirmed COVID-19 and type-2 diabetes, individuals who had well-controlled glucose levels (6.4 mmol/L) had a higher lymphocyte count and lower neutrophil and serum IL-6 levels. Furthermore, they had reduced organ injury and lower death rates compared to patients with poorly controlled glucose levels.³⁷ It could be argued that a possible prevention strategy for the diabetic patient is to keep their blood glucose under control. Glycemic control (GC) refers to the regulation of glucose that a human has in their system. The mechanisms of GC as a response to exercise are a combination of increased blood flow, permeability, and glucose requirements during exercise.³⁸ Exercise is a recommended lifestyle change for GC in individuals with type-1 and type-2 diabetes.^{39,40} Resistance and aerobic exercise decrease glucose in type-1 diabetes; resistance exercise decrease mean glucose for 24 hours post-exercise.⁴¹ Both continuous and interval aerobic exercises result in decreased glucose in type 2 diabetes.⁴² Even walking exercise programs can be useful in decreasing glucose levels. An interval-style walking program resulted in decreased mean and maximum glucose concentration compared to continuous walking.⁴³ Exercise can also be used as a prevention strategy for the development of type-2 diabetes. HIIT has been shown to prevent the progression of type-2 diabetes in prediabetic individuals.⁴⁴



Exercise as a countermeasure for age-related physiological changes

Aging is related to a series of changes that affect all systems within the human body. These changes comprise an increase in arterial wall stiffness⁴⁵, a decrease in muscle strength, size, bone mineral density (BMD)⁴⁶, and lung structure, which affects gas exchange⁴⁷, and a decrease in immune cell count and function⁴⁸, among others. All these changes lead the individual to a state known as frailty⁴⁹⁻⁵² and it appears that these changes in which the organism is unable to adapt and maintain homeostasis is the reason why the elderly are amongst the at-risk population^{53,54}. One possible alternative to manage this population is to prevent these changes from happening altogether. The direct connection between aerobic exercise and longevity has been shown in animal studies. The survival chances increased ~25% when the animal had higher VO₂max while doing aerobic exercise, lowering cholesterol levels and arterial pressure and increasing glucose tolerance, alveolar ventilation, and pulmonary diffusion.^{55,56} Exercise can improve cardiorespiratory function through various mechanisms such as an increase in muscle mass and heart function.⁵⁷ High and low-intensity resistance exercise leads to improved oxygen consumption and cardiorespiratory endurance.⁵⁸ Aerobic and endurance exercise, coupled with interval training, produced an increase in VO₂max and skeletal muscle area and strength.⁵⁹ A combination of aerobic and resistance exercise leads to increased muscle strength and body composition changes in sarcopenic obese individuals.⁶⁰ A cross-sectional study showed that men who performed moderate-to-vigorous exercise showed a lower risk of osteoporosis.⁶¹ A 5-month resistance and aerobic exercise intervention effectively reduced BMD loss during caloric restriction in older adults, although resistance exercise showed better results.⁶² Resistance exercise can

improve muscle size, quality, and power production as well as physical function in older adults.⁶³ Both supervised and home-based exercise improved lower limb strength and walking speed in frail and pre-frail individuals.⁶⁴ Changes in immune parameters can still be obtained despite age. Master athletes exhibited lower senescent lymphocytes compared to healthy age-matched controls.⁶⁵ The aerobic exercise yielded a better immune response than resistance exercise in the elderly.⁶⁶ Both acute and chronic exercise has a positive effect on immune parameters in the elderly.⁶⁷ Altogether, evidence supports the notion that the stimulus by exercise leads to changes in the physiology, specifically when it comes to blood pressure control, glycemic control, and the delay and even reversal of age related changes. This may confer some protection against COVID-19 severity.

Role of exercise in cardiopulmonary rehabilitation

The role of exercise in pulmonary rehabilitation has been well established, and rehabilitation can start as early as in the intensive care unit (ICU). Elastic bands have been used effectively and safely in the ICU to prevent a decline in upper body strength and trunk control.⁶⁸ Cycling combined with functional electric stimulation increased cardiac output in patients during bed stay at the ICU.⁶⁹ British Thoracic Society Guidelines places exercise as an integral part of pulmonary rehabilitation given that it enhances muscle size and function, pulmonary capacity, and residual capacity.⁷⁰ Exercise can also help regulate symptom control and quality of life. Moderate aerobic exercise resulted in better symptom control and quality of life in patients with chronic obstructive pulmonary disease (COPD).⁷¹ Considering the COVID-19 lockdown, rehabilitation must be done in a home-based setting. Home-based telerehabilitation in individuals with COPD leads to

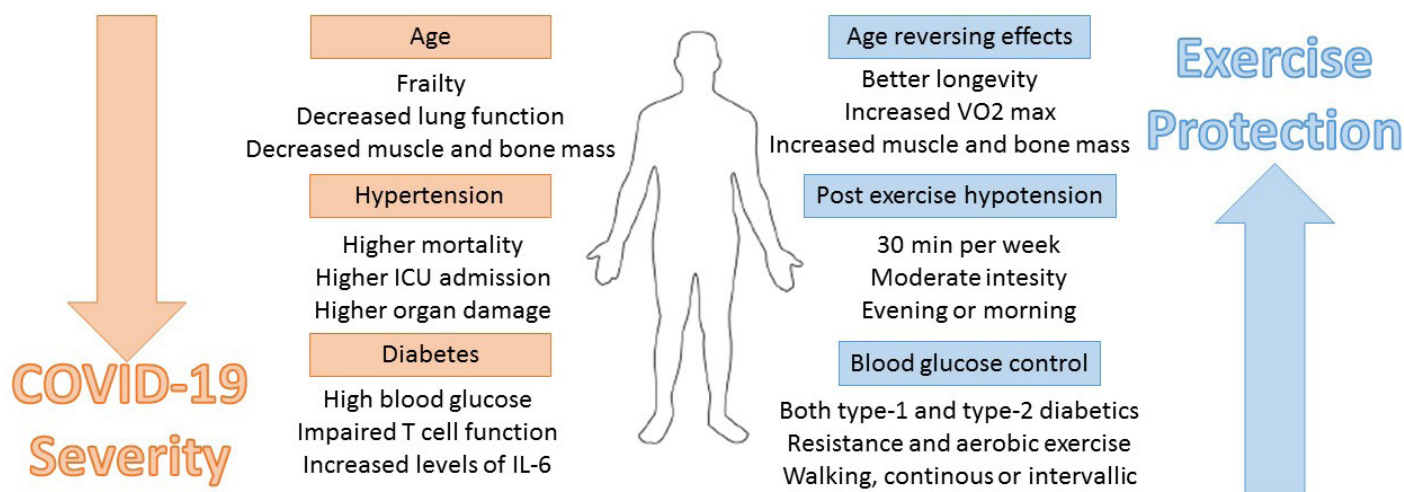


FIGURE 1. Relationship between exercise and COVID-19 risk factors.

increased exercise capacity and quality of life.⁷² Six months of telerehabilitation with COPD and heart failure effectively reduced hospitalization rate, exercise tolerance measured by 6-minute walk test, increased quality of life, and reduced dyspnea.⁷³ Individuals undergoing home-based cardiac rehabilitation showed improvement in VO₂max and quality of life.⁷⁴ Exercise can also provide a treatment option for non-disease side effects of the COVID-19 pandemic, such as mental exhaustion, depression, sedentarism, mood changes, self-esteem, and even social isolation.⁷⁵⁻⁷⁷ Exercise can provide a viable treatment option for the rehabilitation of COVID-19 patients in every step of the way, from ICU stay to outpatient rehabilitation and even at-home exercise programs (Figure 1).

3. CONCLUSION

Exercise could be used as a low-cost treatment for reducing risk factors and mortality from COVID-19. HIIT appears to be a time-effective option to reduce COVID-19 disease severity factors. Several challenges need to be considered, such as a correct exercise prescription by a certified physical therapist. Therapy must be based on specific needs, abilities, and monitoring of the individual and equipment needed to ensure a correct and safe exercise execution, all while maintaining preventive measures against COVID-19.

4. CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Social intelligence, an elementary competence in the development of the doctor-patient relationship

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ABSTRACT

Introduction: The doctor-patient relationship is one of the great foundations of the vocation of medicine in the service of humanity. There can be no professional care without a solid foundation centered on trust and assertive communication. It is only then that competences such as Social Intelligence (SI) can play a defining role since they provide the physician with the practical skills, theoretical knowledge, and relevant attitudes to establish a professional relationship with the patient. Daniel Goleman presents the concept of SI as a continuation of his work on EI, where the whole cognitive process arises from self-knowledge, the ability to delve into the different spheres of the emotional-affective spectrum and self-regulation. Later, the social relationship is reached when the emotional recognition of others is based on the individual capacity for empathy which, together with a harmonized regulation, leads to the development of healthy social skills. **Objective:** To present the relevance of SI competence in the proper development of the doctor-patient relationship. **Methods:** A systematic review was carried out based on the PRISMATM statement (2009), using PubMedTM, MedigraphicTM, and ResearchgateTM as search engines. A total of 115 articles were evaluated. Results: Based on its diverse definitions, accepted since 1920, social intelligence is an essential component. However, it is clear that there is a huge contradiction because it is not really considered in formal education. Over the years, the concept of SI has evolved, while the doctor-patient relationship has become increasingly important. **Conclusion:** The general consensus is that the need to humanize the medical sciences leads us to reflect on the scarcely studied humanistic competences. Then, we can promote a comprehensive medical education to promote a holistic conception of health as those who suggest medicine is a vocation do.

Key words: social intelligence; patient-doctor; anthropocentrism; human dignity; emotional intelligence; communication; humanism.

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RESUMEN

Introducción: La relación médico-paciente es uno de los fundamentos de la vocación médica al servicio de la humanidad. No puede haber atención profesional sin una base sólida centrada en la confianza y la comunicación asertiva. Las competencias blandas como la Inteligencia Social (IS) juegan un papel determinante ya que dotan al médico de las habilidades prácticas, conocimientos y actitudes relevantes para establecer una relación profesional con el paciente. Goleman presenta el concepto de IS como continuación de su trabajo sobre la IE, donde todo el proceso cognitivo surge del autoconocimiento, la capacidad de ahondar en las diferentes esferas del espectro emocional-afectivo y del autoconocimiento. Posteriormente se llega a la relación social, donde el reconocimiento emocional de los demás se fundamenta en la capacidad de empatizar que, junto con una regulación armonizada, conduce al desarrollo de habilidades sociales. **Objetivos:** Presentar la relevancia de la IS en el desarrollo adecuado de la relación médico-paciente. **Metodología:** Se llevó a cabo una revisión sistemática basada en la Declaración PRISMA™ 2009, utilizando PubMed™, Medigraphic™ y Researchgate™ como motores de búsqueda. Un total de 115 artículos fueron evaluados. **Resultados:** Con base en la diversidad de definiciones aceptadas desde 1920, la Inteligencia Social es un componente esencial en la educación y práctica médica; sin embargo, evidentemente existe una enorme contradicción porque no se tiene realmente en cuenta en la educación formal. Con el paso de los años, el concepto de inteligencia social ha evolucionado y, en el caso de la relación médico-paciente, es cada vez más importante. **Conclusión:** El consenso generalizado de la necesidad de humanizar las ciencias médicas nos lleva a reflexionar sobre las competencias humanísticas poco estudiadas. Así, se promoverá la educación médica integral en beneficio de una concepción holística de la salud de manera similar a la propuesta por los defensores de la medicina como una vocación.

Palabras clave: inteligencia social; médico-paciente; antropocentrismo; dignidad humana; inteligencia emocional; comunicación; humanismo.

1. INTRODUCTION

1.1 Social intelligence

1.1.1 Social intelligence and its evolution

To understand social intelligence (SI) it is important firstly to set a comprehensive background of the definition and structure of intelligence, a concept which is constantly changing and progressing. In the 1920s, Thorndike proposed to divide intelligence into three dimensions: abstract, mechanical, and social; this set of dimensions would help the human being to understand and handle ideas, objects, and people.¹⁻⁴ Seven years later, Spearman Thurstone established a monolithic theory of human intelligence, contemplating seven factors, which he considered mental capacities, without addressing the concept of social intelligence specifically.⁵⁻¹⁴ In 1933, Vernon defined social intelligence as the human ability to interact with other people and thus get along with them. In 1938, Welchester approved Spearman's ideologies, which stated that SI is considered to be general intelligence, but he went further and established that it is a form of intelligence applied directly to social situations¹⁵⁻²¹ and discarded the notion of it only being a component of intelligence.²²⁻²⁶ Thirty years later, Guilford brought up the Structure of Intellect, where intelligence is composed of operations, content, and products. which rise to 120 specific intellectual abilities, in possible combinations of 5 operations, 4 contents and 6 products.²⁷ In turn, Guilford considered SI was directly linked

to behavioral contents as well as the interactions between individuals. The latter fundamentally take into account the attitudes, needs, desires, moods, perceptions, and thoughts of the human being to help generate empathy towards others.²⁸ Finally, in 1983, Dr. Gardner proposed the theory of multiple intelligences in which interpersonal intelligence, the basis and fundament of SI was found, defining it as that which is related in understanding and acting. In it, different moods, temperaments, motivations, and intentions, expressed both through verbal and non-verbal means, can be observed.²⁹

1.2 Social intelligence in the theory of multiple intelligences

At the end of the past century, Howard Gardner presented the theory of multiple intelligences, offering a broad, individualized, and contradictory vision to the socio-educational paradigm of the time.³⁰ He emphasized that, in terms of its nature, the human being shows intellectual qualities in different areas of knowledge, music, linguistics, logic-mathematics, vision-space, kinesthesia, nature, and even those that are intrapersonal and interpersonal. He focused on a pedagogical approach and a comprehensive humanistic vision.³¹⁻³³ This theory proposed a novel educational standpoint, where human development goes far beyond mere academic-school training.³⁴ It gave rise to a perspective centered on the person as a determining pillar in the process of social construction.³⁵⁻⁴⁰



Even though the development of sympathetic social skills is sequenced and to a certain extent natural, it should not be forgotten that based on the approach made by Gardner⁴¹, intrapersonal and interpersonal intelligence can, and should be, fostered in students to achieve a person's comprehensive development.⁴²⁻⁴⁸

1.2.1 Intrapersonal intelligence

The individual's ability to recognize in themselves the distinct emotional, affective, and intellectual spheres is what Dr. Gardner⁴⁸⁻⁵⁰ identifies as intrapersonal intelligence. It arises from the cognitive process of introspection and self-regulation of what is commonly known as emotional intelligence (EI).

1.2.2 Interpersonal intelligence

To acknowledge the importance of SI founded through interpersonal intelligence, it is important to analyze the very nature of human being. Humans are gregarious, which derives in the importance of social skills emerging from inner interaction. Interpersonal intelligence allows us to socialize harmoniously.⁵¹⁻⁵²

From the development of these two kinds of intelligence, we acquire humanistic competences, such as EI and SI. We also get the ability to acknowledge one's emotions, regulate them, and finally act in consequence, developing a harmonious social construction.

1.3 Emotional intelligence

EI is defined as the ability to perceive and identify the emotions, both in others and ourselves to discriminate between them and use the information to guide thought and act accordingly.⁵³ Nowadays, emotional intelligence is one of the most promoted competences; however, it has not really been developed. It is essential to delve into the composition of the emotional core itself to understand and study the construction and structure of EI in a timely manner.

1.4 Social Intelligence

Anthropologically, the human being is social by nature, a quality that consequently arises from the individual recognition of the different stimuli that allow us to live harmoniously in society.⁵⁴ This ability arises from the sequenced development of intrapersonal intelligence that later triggers interpersonal skills called SI.⁵⁵ Studies carried out by Daniel Goleman⁵⁵ show that SI originates in the structural foundation of mirror neurons, in charge of the empathic and sympathetic

responses generated in the process of natural-social coexistence. Therefore, it is a developable competence based on a biological argument.⁵⁶ The sequenced understanding between EI and SI reflects on the different spheres that make up the emotional-affective spectrum.⁵⁷

1.5 Doctor-patient relationship

1.5.1 Importance of doctor-patient relationship

The doctor-patient relationship is the foundation of the medical profession. In his speech "Medical Ethics" Laín Entralgo, a Spanish anthropologist and physician⁵⁸, stresses the importance of focusing professional attention on personalistic criteria (Elio Sgreccia). Then, the person's dignity is promoted as a fundamental criterion of the medical vocation.⁵⁹ So, in the twenty-first century, it is basic to promote concrete ways that will allow the health professional to acquire the theoretical-practical skills (competences) to promote an assertive communication with their patients.⁶⁰ Old models, such as paternalism, must be put aside so that medical education can generate a paradigmatic shift.

1.5.2 Social intelligence and doctor-patient relationship

In recent years, medical education has solely focused on technical-scientific aspects.⁶⁰ It seems that "The Person", who in reality is this teleological foundation and central aspect of the medical vocation, has been left aside.⁶⁰ Achieving the education of clinical competencies in line with humanistic competences centered on personalism is perhaps one of the greatest challenges of the twenty-first century.⁶¹ This constant depersonalization detracts from the doctor-patient communication⁶² and consequently generates a greater need to live attached to the most innovative techniques for diagnosis and treatment. Not understanding oneself individually, and consequently not understanding others, is a major obstacle in achieving a medical vocation.⁶³ It would be of great interest to evaluate the actual state of SI in medical practice since there is a great lack of research in this sense. However, the generalized opinion agrees on an urgent need to humanize medicine.⁶³

The situation and the globalized context in the face of the COVID-19 pandemic have made us reflect on the importance of focusing our attention on the humanistic and scientific competences that can strengthen this anthropological need.⁶⁴ The COVID-19 pandemic has revealed a crucial aspect of the doctor-patient relationship. Without a doubt, it is one of the practical aspects that have been most affected by the current epidemiological situation. Patients have had to fight for their life away from their relatives, accompanied only by



their doctors. That is the fundamental importance, to retake humanistic aspects that promote comprehensive training for the benefit of patients and health care professionals.⁶⁵

1.6 Education in health sciences

The professional training of a doctor must aim to pursue not only theoretical-scientific knowledge but also service, the natural essence of the vocation.⁶⁶ Having fundamentals that allow all health professionals to recognize, manage, and interpret emotions—their own and those of others—is key to guarantee a high-quality human care.⁶⁶ There is a general awareness of the urgent need to include curricular programs that favor human development oriented to soft skills. However, it seems that medical training still favors a constant dehumanization over the training years.⁶⁶ That is why it is essential to promote research in this sense so that medical schools and universities actively contribute to the harmonious construction of society.

2. METHODS

A systematic review was carried out based on the PRISMA™ statement using PubMed™, Medigraphic™, and Researchgate™ as a search engine as well as the keywords Social Intelligence, Emotional Intelligence, Doctor-Patient Relationship, and Communication Skills. The inclusion criteria were relevance and consonance with medical profession (physician and nursing exercise), in accordance with the development of doctor-patient relationship (medical education, philosophical aspects, and clinical practice). The

exclusion criteria were EI and SI applied to non-medical professions. A total of 115 articles were evaluated (Figure 1).

Risks of bias: It is necessary to promote quantitative studies that allow an objective approach to the practical and concrete benefits that soft skills grant in the exercise of the medical profession. This review is based on qualitative studies. Due to the lack of research on the development of SI as a competence to acquire a humanistic doctor-patient relationship, this systematic review does not include any meta-analysis.

3. RESULTS

The number of articles identified in the databases was 115, 10 of which were removed due to duplication. Twenty were excluded because they did not contain the variable relevance and consonance with medical profession (physician and nursing exercise), in accordance with the development of doctor-patient relationship (medical education, philosophical aspects, and clinical practice). Eighty-five articles were considered eligible and, finally, 75 articles were included in the systematic review.

Based on the diversity of definitions and SI structures (Table 1), we can analyze the importance given to this soft skill since 1920 social abilities have considered essential component of intelligence development, however there has been a huge contradiction in the educational paradigm implemented which truly does not include formal education in this sense, we must mention that it is essential that every medical student must be instructed or guided in order that the doctor-patient relationship is optimal for both the doctor and

TABLE 1. Definitions of intelligence and their evolution in time.

Definition of intelligence	Taking social intelligence into account	Author	Year
Three dimensions help the human being to understand and handle ideas, objects, and people.	Yes	Thorndike	1920
It is the sum of 7 skills, which are mental capacities.	No	Spearman Thurstone	1927
It is a set of capabilities integrated into a 4-level hierarchical structure.	Yes	Vernon	1933
It is the ability to act with a specific purpose, think rationally, and interact effectively with the environment.	Yes	Welchster	1944
It has a 3-dimensional composition, considering operations, content, and products, creating over 120 skills.	Yes	Giulford	1968
It is the ability to solve problems or produce products important in a cultural context or a given community.	Yes	Gardner	1983
NA	Yes	Goleman	1995

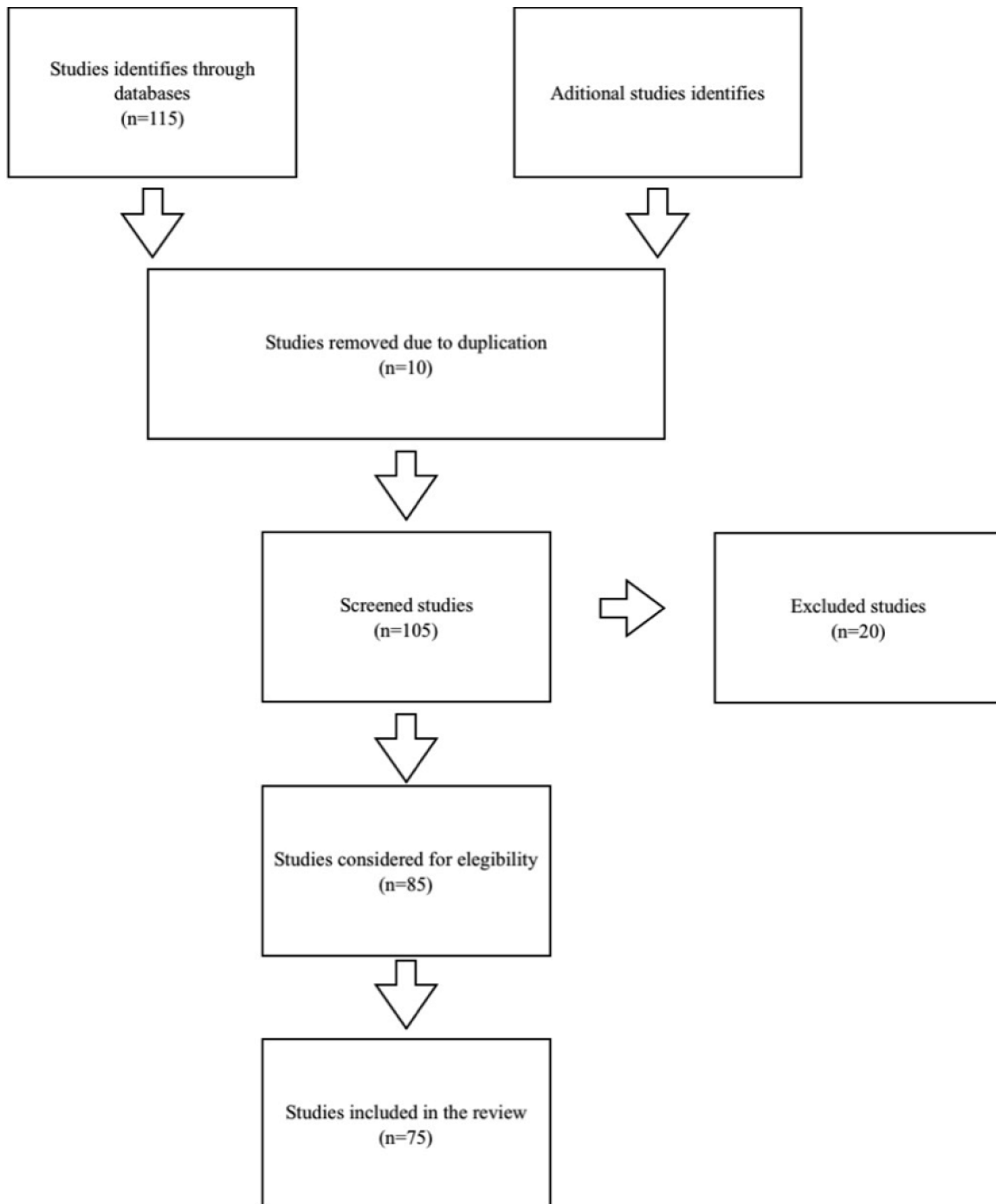


FIGURE 1. Review methodology.

the patient.⁶⁴ The general consensus that modern medicine is much more technical than humane should be reason enough to study and complement the phenomena. Over the years, the concept of SI has evolved and has become increasingly important in the case of the doctor-patient relationship. This is because medicine is a profession that emphasizes human contact, so it is essential that physicians have the skills and abilities that SI covers (Table 2).⁶⁵⁻⁶⁶

Social skills:

1. Assertive communications/active listening: expressing oneself clearly and directly, respecting others.
2. Emotional validation: a process of learning, understanding, and expressing acceptance of another person's emotional experience.



3. Nonverbal language: the transference of any information through the use of any nonverbal means (> 80% of communication).
4. Conflict resolution and negotiation: a formal or informal competence that allows two different parties to find a peaceful resolution to any kind of conflict.
5. Respect: the defense and promotion of human dignity, treating or thinking of someone else based on these concepts.
6. Credibility: quality of being trusted or believed in.

The effective practice of medicine requires the ability of the physician to understand and identify the individual's temperament, motivations, humor, and intentions. So, they are able to interpret the social context of the patient.⁶⁷⁻⁶⁹

In the doctor-patient relationship, where SI represents the basis of an assertive communication between both parts in some situations (Table 3). For instance, there are cases where the doctor must communicate serious situations (diseases, deaths, among others) that involve pain, sadness, and deep emotions. In such cases, communicating adequately and assertively is vital and radiates in the skills and social capacity that both parties have. Communication must, however, be guided by the doctor.⁷⁰⁻⁷²

4. DISCUSSION

Communication skills are decisive in the harmonious construction of society and critical to the doctor-patient relationship.⁷³ Based on a deliberative model, SI is truly essential to an assertive communication that can be ensured in defense and promotion of human dignity. The social skills that SI guarantees are crucial to improve the doctor-patient relationship (Table 4).

TABLE 2. Social intelligence competences.

	Emotional aspects	Skills
Oneself	Emotional intelligence	
Towards others	Empathy and sympathy	Social skills: <ul style="list-style-type: none"> • Assertive communications/active listening • Emotional validation • Nonverbal language • Conflict resolution and negotiation • Respect • Credibility

TABLE 3. Doctor-patient relationship models.

Paternalism: There is no respect for the patient's autonomy, so there is no basis to argue for the need for communication skills.	Informative: The therapeutic decision is made entirely by the patient; humanistic skills as empathy or sympathy are not exercised.
Interpretative: The physician "interprets" the autonomic values of the patient subjectively, violating the person's dignity and reducing their autonomy.	Deliberative: This model promotes the person's dignity, requiring the presence of humanistic competences that favor adequate communication focused on the person's preponderant value.

TABLE 4. Benefits of promoting social intelligence in doctor-patient relationship.

Physician	Patient
Human dignity-based practice	
Assertive communication: true communication based on the patient's needs respecting corresponding values	
Professional practice	Experience of an ethical practice
Accompaniment	
Medical vocation centered on the person	





The general consensus regarding the need to humanize the medical sciences⁷⁴ leads us to reflect on the scarcely studied humanistic competences. Then, we will be able to promote a comprehensive medical education and a holistic conception of health as those who suggest medicine to be a vocation. EI and SI are tools that should not be underestimated⁷⁵ since they are the basis of a professional practice through the humanistic development of a doctor-patient relationship. The vision surrounding the importance of these competences draws attention to the actual medical education curricula. Today, the professional and human practice of medicine needs a humanistic commitment more than ever to complement the great scientific-technical development. Then, it is important to take into consideration both the direct and indirect curricular structure in medical schools. By doing so, humanistic attitudes can promote the acquisition of theoretical and practical knowledge and ensure the imprint character in health sciences students.

Individual and personal knowledge unquestionably leads to the development of social skills based on empathy. So, the doctor-patient communication is promoted from a more personalistic route, focusing on the dignity of the human person (Elio Sgreccia). Universities and health sciences professors must make a significant effort to lead medical education away from the prevailing over-technification. They should train medical students to benefit from the integral conception of the person and thus make them aware of the honor of being part of the health care system.

As Edmund Pellegrino stated: "medicine is the most humane of sciences, the most empiric of arts, and the most scientific of humanities." One of the great challenges for medical sciences in the twentieth century is to combat the threat that dehumanization poses to professional healthcare practitioners and education. Medical education has a critical role to play in this situation.

5. CONCLUSIONS

The notion of a technical-scientific approach with a predominance over humanistic aspects must be challenged through the constant and direct promotion of the essential and determining vision of the medical vocation. There is no greater honor than to accompany a patient in a moment of great fragility and vulnerability since the doctor is clothed with a "generous soul and a spirit eager for science" (Aesculapius). This spirit fervently seeks not to belong to itself but to dedicate its life, honor, and assets to all those who put their health into the hands of the one who seeks to accompany them.

Medicine must be seen as a reason to become a better person. This inspiring and aspirational vision will only be achieved through constant education directed through the teaching of comprehensive clinical-humanistic competences. Those abilities will allow the doctor to connect with himself and others (Social Intelligence). In this way, the doctor can work actively towards the harmonious construction of a better and fairer society.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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Comparison between insulin delivery methods: subcutaneous, inhaled, oral, and buccal

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ABSTRACT

Diabetes mellitus is a complex and chronic metabolic disorder characterized by hyperglycemia. Part of the treatment for this condition is the administration of insulin, a protein that directly influences blood glucose levels. It is traditionally administered parenterally; however, new alternative ways for its application seek greater efficiency and less invasiveness. The objective of this article is to compare the different methods of insulin administration, taking into account new technologies. This review article provides an overview of diabetes and insulin, highlights the advantages and disadvantages of insulin delivery methods (subcutaneous, inhaled, oral, and buccal), and shows the new technologies that include biotechnological applications.

Key words: diabetes; insulin; subcutaneous; inhaled; oral; buccal.

RESUMEN

La *diabetes mellitus* es un desorden metabólico crónico y complejo, caracterizado por hiperglicemia. Parte del tratamiento para este padecimiento es la administración de insulina, proteína que influye directamente en los niveles de glucosa en sangre. Tradicionalmente esta se administra de forma parenteral; sin embargo, en la actualidad existen formas alternativas para su aplicación que buscan mayor eficacia y menos invasividad. El objetivo del presente artículo es comparar los diferentes métodos de aplicación de la insulina, tomando en cuenta las nuevas tecnologías. Este artículo de revisión ofrece una descripción general de la diabetes y la insulina, destaca las ventajas y desventajas de los métodos de administración (subcutánea, inhalada, oral y bucal) y muestra las nuevas tecnologías que incluyen aplicaciones biotecnológicas.

Palabras clave: diabetes; insulina; subcutánea; inhalada; oral; bucal.

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INTRODUCTION

Diabetes mellitus (DM) is a complex and chronic metabolic disorder characterized by hyperglycemia.^{1,2} It is classified into two main types, type 1 (DM1) and type 2 diabetes (DM2).³ DM1 is an autoimmune disease in which the pancreatic beta-cells are destroyed while DM2 is a combination of insulin resistance and a deficiency in pancreatic insulin production.¹ Between 5 and 10% of the patients suffer DM1 and 90–95% have DM2.⁴

Some of the complications of DM are retinopathy, nephropathy, and neuropathy as well as an elevated risk of suffering other illnesses like coronary, peripheral artery and cerebrovascular diseases, obesity, cataracts, erectile dysfunction, non-alcoholic fatty liver disease, and infections.³

Both types of DM require lifestyle changes, for instance, healthy eating and physical exercise.⁵ The treatment of DM1 comprises insulin and/or insulin analogues while the management of DM2 includes oral antidiabetics, such as metformin, thiazolidinediones, sulfonylureas, dipeptidyl peptidase-4 inhibitors, and sodium-glucose cotransporter-2 inhibitors. Insulin is necessary in DM2 in the following cases: when patients with oral antidiabetic treatment at maximum doses do not reach their glycemic control goals ($HbA_{1c} < 6.5\%$) in 3 months, when oral antidiabetic drugs are contraindicated, or during pregnancy.⁶

Insulin is commonly self-administered subcutaneously by patients through injections.⁷ The Worldwide Injection Technique Questionnaire Study was conducted in 2014–2015 among 13,000 insulin-injecting patients from 42 countries, including Mexico. It was found that the incorrect provision of insulin through injections can cause a number

of complications. Among those are unexpected hypoglycemia or glucose variability due to factors such as lipohypertrophy (30.8% of diabetic patients), poor rotation at injection sites (29.4% of diabetic patients), insulin leakage (36.9% of diabetic patients), and the reuse of needles.^{8,9} To improve the optimization of insulin administration it is recommended to seek alternative insulin delivery methods.⁹ Therefore, this review aims to compare subcutaneous insulin administration with inhaled, oral, and buccal delivery methods to exhibit the advantages and disadvantages of each.

INSULIN

It is a hormone that increases glucose assimilation, stimulating glycolysis and favoring glycogen synthesis in the liver and muscles.¹⁰ It is used in patients with DM1 whose production of this hormone is null and DM2 patients with resistance to or limited production of it. Having different insulin preparations and administration methods allows patients to have greater application comfort, contributing to their well-being.

Types of insulin

Types of insulin are characterized based on their pharmacokinetic profiles, concentration, and the onset, peak and duration of action¹¹ (Table 1).

Rapid-acting and insulin bolus

The application of insulin bolus (rapid-acting insulin together with basal insulin and food) provides flexibility in the dosage.¹¹ Its onset action is at 0.5–1 h, with a peak action at 2–4 h

TABLE 1. Onset of action, maximum effect, and action duration of insulin preparations after subcutaneous injection.

TYPE OF INSULIN	START	MAXIMUM PEAK	DURATION
RAPID ACTING INSULIN			
Aspart insulin	10–20 min	40–50 min	3–5 h
Lispro insulin	15–30 min	30–90 min	3–5 h
Glulisine insulin	20–30 min	30–90 min	1–2.5 h
SHORT-ACTING INSULIN			
Regular insulin	30–60 min	2–5 h	5–8 h
INTERMEDIATE ACTION INSULIN			
Isophane insulin (NPH)	1–2 h	4–12 h	18–24 h
LONG-ACTING INSULIN			
Insulin glargine	1–1.5 h	None	20–24 h
Insulin detemir	1–2 h	6–8 h	Up to 24 h
Insulin degludec	1–2 h	None	> 24 h

and an activity duration of 6–8 h. It can be accompanied by different methods to accelerate its absorption; however, research is trying to improve its mechanism of action.^{12,13} It can be administered before, during or after meals, and the dose can be adjusted based on carbohydrate consumption.¹¹

Short-acting or concentrated insulin

It is two to five times more concentrated than its commonly used counterpart. It is used in patients who have severe insulin resistance or consume steroid-type medications. Its peak of action corresponds to the type of insulin concentrated and can range from 30 min to over 1 h. It is useful in patients who require more than 200 units a day.^{11,14}

Intermediate-acting insulin

Intermediate-acting insulin analogues have an onset action between 1 and 2 h, with a maximum action of 6–10 h and an activity duration of 10–16 h. The rate of absorption is reduced by adding protamine to the preparation.¹³

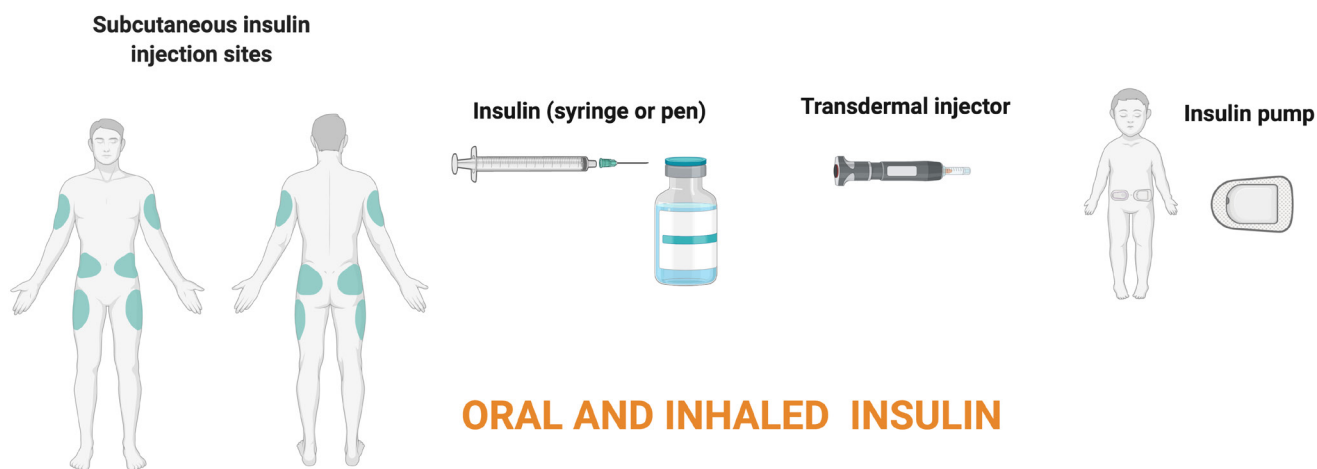
Long-acting or basal insulin

It is an ideal option for patients with DM2 just starting their treatment. It is usually injected at night, every 24 h and suppresses hepatic glucose production. It generates a better glycemic control with minimal side effects, resulting in optimal plasma insulin concentrations.¹⁵ It is administered independently of food intake, and patients should continue to receive the established dose upon reaching normal glucose levels.¹²

METHODS OF INSULIN ADMINISTRATION

To date, insulin methods of administration have been limited mainly to subcutaneous injections and parenteral pumps.¹² Then, research is looking for alternative administration methods that allow the gradual improvement of diabetes management to increase patient compliance and reduce invasiveness.¹⁶ These methods include inhalable forms that use microspheres¹⁷, closed-loop delivery systems with insulin pumps¹⁸, and oral forms associated with microencapsulation techniques (Figure 1).

SUBCUTANEOUS INSULIN



ORAL AND INHALED INSULIN

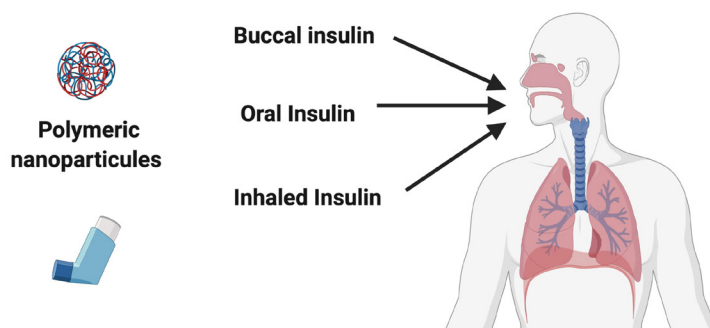


FIGURE 1. Most common routes for insulin administration.



Subcutaneous insulin

It is the conventional administration route. Preferred injection sites include the abdomen (except a 2-inch radius around the navel), arms, thighs, and buttocks because of their high rates of absorption. The injection site should be rotated to prevent lipohypertrophy or lipoatrophy.¹⁹ This delivery method uses syringes, pens, jet injectors, and pumps.

Syringes

Syringes used for insulin injections are marked in insulin units and have 0.3, 0.5, 1, or 2 ml of capacity while needles vary in length. The patient's injection technique needs to be reviewed periodically by a physician. Recommendations that diminish painful injections are not injecting cold insulin, not changing the direction of the needle, avoiding needle reuse, keeping the muscles of the area relaxed, and puncturing the skin quickly.^{19,20}

Insulin pens

Available in disposable forms²¹ and in durable reusable forms, these devices use cartridges instead of vials. There are low-dose pens that deliver insulin in half-unit increments.¹⁹ The dose is set by rotating the knob of the pen.²¹ It is recommended to leave the needle applied for at least 5 s after the knob of the pen is all the way down to make sure the delivery is complete.¹⁹ They are used by 80–90% of European patients. Nowadays, “smart” pens provide dose calculation and have tracking features.²¹

Insulin jet injectors

They inject insulin as a fine stream into the skin, without a needle.¹⁹ They deliver insulin at a high velocity (usually >100m/s) across the skin in the subcutaneous tissue and, unlike injections, they dispense the insulin over a larger area.²²

Insulin pump

It is a small, computerized device that delivers insulin through a thin tube that passes under the skin. It releases insulin the way the body would naturally, with a constant flow throughout the day and night, called basal insulin, and an additional dose at mealtime, called a bolus. More than a million patients currently use this treatment modality.²³ It improves administration control, reducing the risk of long-term complications. It is part of closed-loop treatment systems, in which subcutaneous insulin infusion and devices

that continuously monitor glucose levels are connected to generate an automatic response.²⁴

Inhaled insulin

It proposes the absorption of insulin through the pulmonary system. The distant lung presents a large, highly perfused surface area that allows for a rapid absorption of small particles into systemic circulation.²⁵ Inhaled insulin evades hepatic metabolism and enzymatic degradation in the gastrointestinal (GI) tract, and it prevents painful and invasive procedures. It might be ineffective in patients with respiratory problems.²⁶ Inhaled insulin was the first reported alternate route of administration that was successful. However, the cost and contraindications made it commercially unsuccessful.²⁷ In 2014, the FDA approved an inhaled form of insulin in technospheres administered via a compact inhaler device. This method consists of a dry powder insulin formulation absorbed into a carrier, namely technospheres, that are propelled into the deep lung.²⁸

Oral insulin

It has been since the discovery of insulin, a very desired aim.²⁹ It is the friendliest way of administering insulin, the one that most closely mimics physiological insulin delivery, increasing portal insulin concentration via intestinal absorption.³⁰ However, apart from the innate physical instability, oral insulin faces various physiological challenges, such as chemical and enzymatic degradation in the GI tract, intrinsic poor oral absorption, and rapid systemic clearance, resulting in low bioavailability and insufficient therapeutic effect.¹⁶ The development of nanotechnology has offered hope to increase its efficacy. Several nanoparticles composed of biodegradable polymers, such as chitosan, alginate, mucin, yeast, liposomes, polymeric nanovesicles, and polymeric hydrogels have been developed for the microencapsulation of insulin.^{12,30}

Chitosan is an optimal oral administration vehicle³¹ derived from the alkaline deacetylation of chitin.³² It is used as a drug delivery system due to its degree of acetylation and its molecular weight, which affect its aqueous solubility and hydrophobicity. Chitosan microparticles increase their solubility under acidic conditions, benefiting drug delivery in the GI tract. Its natural mucoadhesive properties and its ability to transiently open epithelial tight junctions and make it a great candidate for intestinally absorbed, orally administered drugs.^{31,32}

Another recently developed vehicle are yeast-alginate microcapsules (YMC).³³ This method commonly employs



Saccharomyces cerevisiae to incorporate differentially charged insulin peptides³⁴, in a way that the YMC are able to open tight junctions and enter the systemic circulation through M cell-mediated endocytosis.³⁵ Additionally, in order to inhibit the action of the acidic environment in the stomach, alginate is used as a coating agent to efficiently deliver YMC to their absorption site.³⁶

Other organic and inorganic polymeric compounds have also been tested for the same purpose. These include dextran-based and PLGA-based (poly(lactic-co-glycolic acid)) microparticles. Dextran has been used to encapsulate insulin-polyanion complexes, improving drug entrapment and loading efficiency, facilitating mucoadhesiveness, and improving GI permeability. However, the layer-by-layer method proposed is complex, costly, and time-consuming.^{16,37} On the other hand, PLGA has been proposed as a potential microcarrier of insulin, due to its biocompatibility and biodegradability. Nonetheless, up to date hydrophobic PLGA has been proven unsuccessful to encapsulate hydrophilic insulin, generating loss of stability and loading efficiency.¹⁶

Buccal insulin

It presents some of the advantages of oral insulin, besides, it increases drug bioavailability because it bypasses enzymatic degradation in the GI tract and hepatic metabolism.³⁸ It is generally presented in the form of mucoadhesive buccal films.²⁷ The buccal mucosa has ready accessibility, high tissue permeability, high vascularization, and increased rate of cell growth, which reduces cytotoxic effects related to high drug concentration.³⁹ However, with hydrophilic peptides like insulin, the speed of diffusion through buccal tissue is not considered to be fast enough when compared to subcutaneous injection. Thus, buccal delivery films must usually be integrated with permeation enhancers that effectively loosen junctions between epithelial cells to increase transbuccal drug delivery.³⁸ The design, mechanism, and disintegration times of different mucoadhesive films varies widely in the literature. They are normally composed of film forming polymers, plasticizers, stabilizers, colorant, sweeteners, and flavors.⁴⁰ The main component, after the insulin peptides, is the polymers, which must exhibit good adhesive properties and enhance permeability of the active ingredient.³⁸ Several polymers have been suggested, with chitosan standing out for exhibiting similar properties as those found in oral insulin.³⁹ Glycan-coated gold nanoparticles (GNP) have shown promising results in clinical trials.²⁷ Alternatively to films, aerosolized short-acting buccal insulin products have been proposed, and are currently being clinically tested.³⁰

ADVANTAGES AND DISADVANTAGES OF INSULIN DELIVERY SYSTEMS

Syringes. The incorrect use of injections, including lack of injection site rotation and reuse of needles^{9,20}, can cause skin hyperpigmentation, induration⁴¹, and lipohypertrophy, the latter characterized by thickened fatty lumps in the subcutaneous tissue of the area where multiple insulin injections were applied⁴². In turn, this can increase the levels of unexplained hypoglycemia, originate uncontrolled glycated hemoglobin, and increase insulin costs.^{20,21} The erroneous application of the injections in a few cases can cause lipoatrophy, a rare complication that consists of scarring lesions due to an immunological reaction to insulin or the excipients added to its formulation⁴³. The errors mentioned above apply for the use of pens as well.²⁰

Insulin pens. Compared with syringes, insulin pens have long term advantages for glycemic control, cause less hypoglycemia events, increase dose accuracy, and are the preferred insulin administration method. They are easier to use⁴⁴, induce less pain⁴⁵, reduce the injection force required, have a shorter needle length, are more discreet⁴⁶, and improve adherence.¹⁹ Some pens incorporate mechanisms to store the dose, time, and date of previous insulin injections.⁴⁶

Insulin jet injectors. They do not use needles, so they are ideal for patients who cannot use syringes or with needle phobias⁴⁷; however, they form a hole in the skin.⁴⁸ One possible advantage is the faster absorption of rapid-acting insulin because it decreases postprandial glycemia in diabetic patients.⁴⁷ Its disadvantages are a relatively high cost^{19,48} and a probable traumatization of the skin. Because of this, they should not be a routine option for patients.¹⁹ Compared with insulin pens, insulin jet injectors have a faster flow rate and a larger area of local subcutaneous absorption. A drawback of jet injectors is the post administration "wetness" of the skin because it can be contaminated with dust or other impurities if it is not taken care of.⁴⁹

Insulin pump. It reduces the number of injections while patients require a puncture every 2 or 3 days compared to daily injections. It is more precise as the sensing of glycemic indexes allows a more adequate and effective administration. It is flexible and allows the patient to adjust the doses depending on their caloric intake. It automatically calculates insulin bolus doses for corrections using glucose measurements from the patient's finger and allows data to be stored, which can be used for patient analysis.^{50,51} The main disadvantage of this method is its high cost as well as the fact that the pump only delivers rapid-acting insulin and cannot deliver insulin bolus; however, it does allow dose



adjustments according to patient needs. It can lead to skin damage in case of trauma or mishandling and the risk of developing ketoacidosis in case of poor function.^{50,52}

Inhaled insulin. It has a high absorption rate that mimics the bioavailability observed in subcutaneous insulin. Licensed options on the market have not yet achieved success over traditional administration methods.²⁷ Earlier versions of these devices tended to be bulky, difficult to use, and costly since nebulizer replacements tended to have a short lifespan.²⁶ Even in newer products, their implementation with diabetic patients has been low given the limitations found in patients with diminished respiratory capacity.^{11,28}

Oral insulin. It is probably one of the most effective, less invasive and patient-friendly routes on the market. However, the inherent instability of insulin has proven to be a great obstacle in its development. It is perhaps the best route to mimic the physiological excretion of insulin, due to the fact it is absorbed into the portal system and distributed in the same route. However, the acidic conditions in the GI tract in conjunction with hepatic metabolism contribute to a less than desired bioavailability in the systemic circulation. Oral delivery systems technology vary widely in nature and mechanisms, creating diverse factors that might interfere with the desired absorption rate.⁵³

Buccal insulin. Buccal insulin poses similar advantages to those of oral insulin, additionally increasing absorption through the highly vascularized buccal mucosa, and avoiding acidic, enzymatic, and hepatic degradation through direct absorption into the systemic circulation. One of its obstacles is the development of highly permeable polymers that allow a high rate of absorption through the oral mucosa. Also, many factors may alter drug function, including food consumption, variable saliva secretion, and hot or cold beverage intake. Finally, adverse effects must be considered, such as the risk of swallowing or low compliance due to below average palatability.⁵⁴ Table 2 shows a summary of the advantages and disadvantages of buccal insulin.

DISCUSSION

Many attempts have been made to introduce less invasive, patient-friendly, and more efficient modes of insulin administration. Still, the scientific evidence and the variety of socioeconomic factors presented in this review have promoted the use and overall acceptance of subcutaneous administration as the golden standard for diabetes insulin therapy. The side effects and disadvantages of this method tend to be sidelined just by the overall effectiveness of this route. No other route has been able to recreate this effectiveness or bioavailability nor has it been able to cope

with the economic factors that interfere with its development, production, and marketing. It is inferred that this apparent standstill in insulin administration innovation is closer to an end than it has ever been. In this context, this review has centered on analyzing and understanding the different innovations that have transcended from basic science to potential clinical applications in the treatment of diabetes, especially where insulin therapy is concerned.

In recent years, biotechnological innovation has helped the introduction of new technologies that allow the development of oral and buccal routes. Even though still far away from being as accepted as traditional subcutaneous methods, these routes hold the potential for being more patient friendly, reliable, and able to fully recreate the physiological excretion of insulin. Nevertheless, the advancement of these methods still has a lot of distance to cover, going from prototypes to actual licensed administration methods. Buccal and oral forms are not the only proposed alternative methods of administration. Inhalable forms, and alternate subcutaneous forms, such as insulin pumps, have also been developed, and some of them are currently available on the market. These methods, even though less preferable than the ideal non-invasive oral or buccal methods, have paved the way to wider development and interest from the pharmaceutical companies to introduce alternative attractive methods of insulin therapy in the diabetes market. Overall, it is our opinion that even though traditional subcutaneous methods are still the golden standard in diabetes treatment, the current and constant scientific innovation will eventually provide the effectiveness, efficiency, reliability, and patient friendliness needed for the future success and overall acceptance of the alternative methods discussed in this review, and many other more that are still unthought of.

CONCLUSION

Subcutaneous insulin is the only option for the treatment of type 1 diabetes and the use of insulin improves glycemic control in patients with type 2 diabetes. Therefore, the deep knowledge of the different types of insulin as well as the advantages and disadvantages of the routes of administration is the basis for the physician to determine the most appropriate treatment. In this context, it is imperative that research continues focusing on new biotechnological options to obtain more effective, safe, and less invasive treatments to help improve the patient's quality of life.

CONFLICT OF INTEREST

The authors received no funding for this work and have no conflicts of interest to disclose.



TABLE 2. Advantages and disadvantages of insulin delivery methods.

METHODS	ADVANTAGES	DISADVANTAGES
SUBCUTANEOUS		
Syringes	<ul style="list-style-type: none"> • The most used • Less expensive than pens, jet injectors, and pumps • Can mix two insulins 	<ul style="list-style-type: none"> • Frequently erroneous self-injections • Hyperpigmentation • Indurations • Lipohypertrophy • More painful than pens, jet injectors, and pumps • Needle phobia • Less accurate than pens • Decreased adherence
Insulin pen	<ul style="list-style-type: none"> • Compared to syringes: • Less painful • Easier to use • Improved adherence • Increased dose accuracy • Fewer hypoglycemia events • Long term advantages in glycemic control 	<ul style="list-style-type: none"> • Frequently erroneous self-injections • Hyperpigmentation • Indurations • Lipohypertrophy • Needle phobia • More expensive than syringes • Cannot mix two insulins
Insulin jet injector	<ul style="list-style-type: none"> • Less painful than syringes and pens • No needles • Faster absorption of rapid-acting insulin • Reduced risk of transmitting diseases 	<ul style="list-style-type: none"> • Relatively high cost • Probable traumatization of the skin • Keeping skin dry after the application is necessary
Insulin pump	<ul style="list-style-type: none"> • Less injections than syringes • More accurate • Lower risk of complications • More flexible release • Adaptable to the patient's needs 	<ul style="list-style-type: none"> • High cost • Needle phobia • Risk of developing diabetic ketoacidosis in case of malfunction • Possible skin damage • Time-consuming pump replacement
INHALED		
Insulin inhaler device	<ul style="list-style-type: none"> • Large surface area for absorption • Perfused epithelial cells assure high bioavailability • Highly permeable and thin barrier • Most enzymatic and hepatic metabolism bypassed • Non-invasive efficient route 	<ul style="list-style-type: none"> • Not recommended for patients with respiratory problems • Cough, allergic reactions and long-term damage to the lungs • Lack of long-term adverse effects studies • Bioavailability compromised by variable respiratory factors
ORAL		
Oral insulin	<ul style="list-style-type: none"> • The least invasive • The physiological release of insulin mimicked • Increased compliance • Easy to use • Reduced cases of hyperinsulinemia 	<ul style="list-style-type: none"> • Low pH, enzymatic activity, and hepatic metabolism and decreased final bioavailability • Varied technology mechanisms creating variability in the absorption and intestinal permeability
Buccal insulin	<ul style="list-style-type: none"> • High patient acceptability • Low degree of acidic, enzymatic or hepatic degradation • Highly vascularized buccal mucosa allowing direct access to systemic circulation • Easy to use 	<ul style="list-style-type: none"> • Low permeability of mucosa requiring specialized polymers and agents • Drug effectivity altered by saliva secretions, food consumption, and beverage temperature • Compliance affected by low palatability



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