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NOVA en OJS

La revista *NOVA* entra a ser parte de las publicaciones científicas de *Acceso Abierto* por medio del sistema de administración y publicación de revistas y documentos periódicos en internet *Open Journal System* -OJS-. Este sistema está diseñado para reducir el tiempo y energías dedicadas al manejo exhaustivo de las tareas que involucra la edición de una publicación seriada, permitiendo un manejo eficiente y unificado del proceso editorial. Con esto se busca acelerar el acceso en la difusión de contenidos e investigaciones científicas producidas dentro y fuera de la universidad en los temas relacionados con las ciencias biomédicas.

OJS, es un software desarrollado por Public Knowledge Project – PKP- de la Facultad de Educación de la University of British Columbia, utilizado ahora por la Universidad Colegio Mayor de Cundinamarca, dentro de un proceso liderado, con el apoyo de la oficina de investigaciones, por la editora de la revista *NOVA* Olga Lucia Ostos y el ingeniero Camilo Andrés Angulo Muñoz -diseñador, y gestor de la plataforma y editor de textos en formato HTML y EPUB-. En el siguiente enlace podrán tener acceso a la plataforma *Open Journal System* de *NOVA* y a los documentos en PDF, HTML y EPUB allí disponibles, así mismo, encontrarán las normas para los autores, la sección de registro, donde los autores pueden inscribirse para hacer envío de sus artículos, y las bases e índices bibliográficos a las que pertenece la revista.
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International guest editor

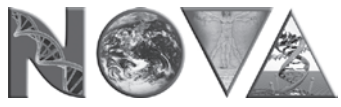
María Rodríguez Cortés
Ph.D. (c). Biomedical Sciences
Master in chemical sciences
Biological Pharmaceutical Chemist

Many people always talk about what characteristics a scientist should have, in my opinion, a scientist is like a child, a person whose creativity is always active and lives rediscovering the world, paying attention to small details that seem invisible to others but that become so important for ending changing the reality we know. Scientists are critical and persevering, and although many think that we are only locked up in the laboratory, the scientist is the friendliest being that many can meet because he understands from his humility that he must form giant teams and networks so that science fulfills its objective. Scientists in this special edition of the journal have those characteristics.

The importance of networks and interaction in science is vital to continue in the scientific advance, that was something that I confirmed making this edition. Being in the preparation of this journal as an invited editor lets me have contact with talented scientists. I feel so proud to see in this edition quality research from different parts of Latin America, giving us an idea of the potential of the region. All authors in this number are admirable scientists.

In my doctoral training, I learned to face failure, value small achievements, and not stop dreaming. I learned in the classes to see the neurosciences in another way, I understood that beyond knowing a lot about a subject, you must love it to break down the aspects concerning it thoroughly that you are investigating so as not to lose love for what you do. Then I remembered the importance of working for science in the dissemination of knowledge. It's an honor to be part of this journal and continue with the legacy of my mentors.

Enjoy this special number with amazing research from scientists in Mexico, Colombia, and Perú who work every day on solutions in microbiology, public health, critical medicine, and cancer research. Latin America is full of talent and has the potential to grow in science. I appreciate so much the opportunity to work with my research network and give you excellent articles that the editorial board chose after an exhaustive process looking forward to the quality and innovation.



NOVA Publicación Científica
en Ciencias Biomédicas

Review article

Chemodrug resistance: Cancer's fight for survival

Jafet Alcántara Colin[1], Antonio Sandoval Cabrera[2,3], Daniel Alejandro Martínez Quintero [2], Jonnathan Guadalupe Santillán Benítez[4]

Abstract

Introduction. Chemoresistance is a multifactorial phenomenon implicated in all failed therapies and accounts for 90% of all cancer deaths and 30% of relapses. **Objective**. To understand the genetic mechanisms by which cancer cells acquire resistance to chemo drugs. **Methodology**. A non-systematic review study was carried out, in which genes and proteins involved in chemoresistance were searched using the terms "Cancer Drug resistance [Title/Abstract]". From the articles obtained, highly involved genes, emerging genes, and proteins related to resistance were recognized. To obtain more specific information about genes, their interactions, and proteins associated with metabolism, the tools "The Human Protein Atlas", "STRING CONSORTIUM 2022," and The Small Molecule Pathway Database were used for their review. **Results**. From this review it was found that there are genes highly related to resistance such as: *ABCA3, ABCB1, ABCB2, ABCC1, ABCC2, ABCG2, CYP2D6, CYP3A4, GSTA1*. Recently recognised genes such as*: FOXO3, FOXM1, Skp2, Snail, Twist1, ZEB1* and *SLCO1B3*. **Conclusions**. It is necessary to taking account new approaches related to cancer treatments considering chemoresistence and the genes related to the resistence.

Keywords: cancer, resistance, chemoresistance, cytochrome P450.

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Introduction

In 2020, cancer was the leading cause of death worldwide, with close to 10 million deaths, with lung cancer being cancer with the highest incidence in men, with 31.5 cases per 100,000 men worldwide. breast cancer in women with 47.8 cases per 100,000 women, and leukemia in children under 19 years of age had 3.2 new cases per 100,000 individuals (1). According to a 2020 study by Bukowski k. *et al* ., estimate that at least 90% of cancer deaths are due to patients' resistance to treatment (2). It is estimated that by 2040, there will be 26 million new cancer cases worldwide, and approximately 15 million patients will require chemotherapy (3). Therefore, it is essential to have more effective treatments against cancer, in which new approaches are considered that adopt the influence that chemoresistance can have and how it affects the success of therapy against this disease.

Chemoresistance occurs when cancer cells are more tolerant to treatment; with the outcome of therapeutic losses effect. The bases of chemoresistance lies in different molecular mechanisms, one of the well described is the efflux pumps corresponding to the family of ATP-Binding Cassette (ABC) genes. These genes codify for ABC proteins wich transports small molecules, including chemo drugs, outside the membrane. Other of the main mechanisms is drug metabolizm through to the cytochrome p450 (CYP450) superfamily. (4,5).

Recently, cancer treatments have been based on the administration of regimes of more than two drugs at long-term and high doses. The drawback of the long and intensive treatments is the possibility of acquire treatment resistance, resulting in a higher mortality rate and cancer relapses. The focus of this review is to understand how cancer cells acquire resistance to chemopharmaceuticals.

Methodology

In order to retrieve the most relevant information related to the subject, the National Center for Biotechnology Information (NCBI) database, The Human Protein Atlas (https://www.proteinatlas.org/) and STRING CONSORTIUM 2022 were used. (https://string-db.org/).

To obtain information related to the history of chemoresistance, we searched for the PubMed.gov search engine (https://pubmed.ncbi.nlm.nih.gov/), using the words "Drug resistance", we further analyzed detailed the oldest articles that the search yielded, also in this section the Google Scholar search engine was used to search information related to Dr. Paul Ehrlich, the information was acquired according to the interest of continuing to search for relevant ideas at the beginning of the resistance to drugs.

For the rest of the sections, the PubMed.gov search engine was used by introducing the word "Cancer Drug resistance [Title/Abstract]", these were limited to their appearance in the title and abstract, thus obtaining 1,042 results. , then they were filtered as follows: in text availability, free and complete texts were selected; In type of article, review, books, and documents were selected; On the date of publication, the years 2017 to 2022 were selected, obtaining 198 results. We applied additional filters to these results: only in humans and English as a language, getting 69 results. The information of the articles related only to cancer in general and not to other diseases or any type of article was acquired, thus obtaining a total of 36 articles.

To search for information related to chemopharmaceuticals, the name of the chemopharmaceuticals was introduced in the search engine, and the articles related to it were selected.

Cell location

Protein visualization at the cellular level was performed using The Human Protein Atlas database (https://www.proteinatlas.org/).

Drug metabolic pathways

Enzymes retaled to metabolizm of each drug were obtained from The Small Molecule Pathway Database (SMPDB; https://smpdb.ca/). A total of 11 meatabolic pathway related to anticancer drugs and paracetamol were obtained.

History of resistance to chemo pharmaceuticals

The meaning of "drug resistance" could be analyzed in two concepts. In one way the term "drug", refers to a set of molecules that help treat a disease or condition; the drug is made up of the active molecule or ingredient and a presumably inert excipient. In the other way "resistance" is associated with the property of tolerating conditions outside the optimal operating range. Collectively, drug resistance is when cancer cells or microorganisms, do not respond to a drug that usually weakens or destroys them. Drug resistance occurs before, during, or after administering the treatment (6)

The origin of drug resistance is not completely clear, however, we can dated its beginnings with the contributions of the German beginning with the contributions of the German doctor and bacteriologist, Paul Ehrlich, who was awarded the Nobel Prize in medicine in 1908. In addition to his contributions in the discovery of arsphenamine, the first effective medicinal treatment against syphilis, in 1878 he published the theory of selective toxicity, in which Paul Ehrlich mentions that staining is selective of biological tissues, due to specific chemical characteristics of cells and

in 1906 he coined the term chemotherapy (7–9). Later in 1916, the scientist Seinai Akatsu of the Rockefeller Institute published an article entitled "The drug-fastness of spirochetes to arsenic, mercurial, and iodide compounds *in vitro* ", in which he described the following: "it has been known for some time While in trypanosomiasis, trypanosomes that have survived the first effect of an arsenic germicide, such as atoxyl or arsacetin, offer greater resistance to a subsequent dose of the same drug" , with the latter we can say that knowledge of the effect (10)of drug resistance is more than 100 years old, remembering that the first treatments against different diseases date from the use of medicinal compounds after the contributions of Paul Ehrlich. An important event of the last century was the establishment of the HeLa cell line derived from a squamous cell carcinoma of the cervix, being the first cancer cells that have contributed to the pharmacological study of chemical compounds, despite this, today the chemoresistance continues to affect the efficacy of drug treatments (7,11–14).

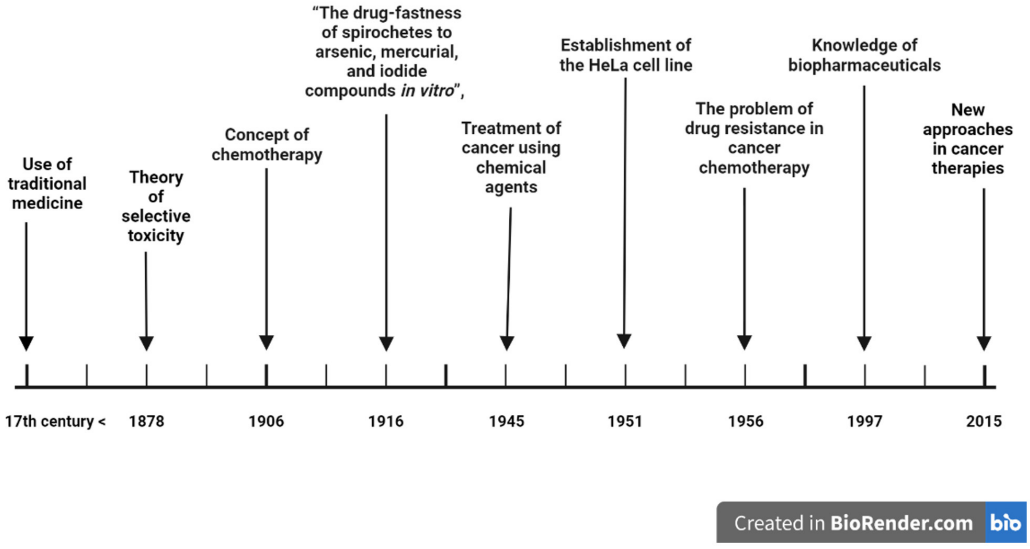


Figure 1. Chemoresistance time line.

Chemopharmaceuticals and their resistance mechanism

Cancer treatments are based on the administration of multidrugs (4 to 6) at different concentrations, which are selectively directed for different molecular targets; the drugs are employed depending on the severity and the stage of the disease, for example, treatments for acute lymphoblastic leukemia consist of a combination of genotoxic drugs, antimetabolites, mitotic spindle inhibitors, and glucocorticoids (GCs) (15). Treatments usually consist of three phases: Remission induction; Consolidation/intensification, maintenance (16).

There are different chemo pharmaceuticals, which can be classified depending on the molecular target; among these, we have: alkylating agents such as oxazsafosforinas, nitrogen mustards, hydrazine, platinum-based agents, there are also antimetabolite agents such as pyrimidine antagonists, antagonists of purines, purine analogs, antifolates, and ribonucleotide reductase inhibitors. There are some spindle inhibitors like taxane and some vinca alkaloids, there are also topoisomerase I and II inhibitors (2).

One of the most used chemopharmaceuticals is doxorubicin (Dox), it is an antibiotic belonging to the anthracycline family, extracted from the bacterium *Streptomyces peucetius* , the use of Dox as a drug against cancer was started in the fifthys years. Dox is a DNA intercalator, by being located between the DNA chain, it prevents the topoisomerase II enzyme from carrying out replication, making in the first instance that the cancer cell cannot replicate and in turn, prevents the transcription of genes necessary for the cell, for this reason, the cancer cell dies. Despite its efficiency in treating solid and blood tumors, it has been seen that patients acquire resistance against the drug, and it has also been associated with hepatic, renal, and cardiac cytotoxicity (5,17). Another drug widely used in cancer is tamoxifen; this chemo pharmaceutical is a selective modulator of the non-steroidal antineoplastic estrogen receptor. Tamoxifen competitively inhibits estradiol by binding to estrogen receptors, thus, preventing, thus preventing the receptor from binding to the estrogen response element in DNA. The result is a reduction in DNA synthesis and the cellular response to estrogen (18). However, drug resistance to tamoxifen is also known to exist (17,19). Cisplatin is an antitumor drug widely used to treat various types of cancer. Despite its remarkable efficacy, most tumors show intrinsic or acquired resistance to this drug. The main biological target of cisplatin is genomic DNA, causing many DNA lesions that block transcription and replication (17,20).

Resistance to chemotherapeutics is on the rise, with more and more individuals with resistance profiles to different drugs, making cancer therapies inefficient (21).

Before understanding the mechanisms by which cancer cells develop resistance to chemopharmaceuticals, it is necessary to understand that tolerance refers to cells' ability to survive transient exposure to a chemical agent. On the other hand, we have that persistence is the capacity of a clonal subpopulation to survive to the treatment(22); for example, suppose there are 100 cells in a culture to which a therapeutic agent x is administered, after some time (t=1), it is performed a cellular count obtaining ten cells, so it could be said that each cell had tolerance to the therapeutic agent, then another count is performed at t=2 with the same culture conditions, coun-

ting 20 cells, the ten cells presented before and their descendence; therefore, this new clonal subpopulation was persistent to the treatment, since the concentration of drug x will no longer have a therapeutic *effect*. The above approach is mainly used with microorganisms, but it helps us understand the effect of cell resistance.

It is currently considered that a cancer disease can contain cancer cells that are sensitive or resistant to drugs, so the two states are not mutually exclusive, both occurring at the same time. Resistance, whether intrinsic or acquired, is irreversible and arises from the accumulation of alterations in cancer cells or their tissue microenvironment, with the sole objective of promoting cancer cell survival. Intrinsic resistance is associated with pre-existing (inherited) genetic mutations which are heterogeneous among cancer cells from the same individual. Pre-existing unresponsive cell subpopulations will be selected after drug treatment, resulting in the activation of intrinsic pathways used as a defense against environmental toxins. After drug treatment, acquired resistance may appear (22,23). We can see chemoresistance from the point of view of evolution, cancer cells, like any other organism, constantly evolve for survival, developing mechanisms to adapt to the environment's adverse conditions. Cancer is a chronic degenerative disease, where cells divide uncontrollably, despite this, cancer cells are highly adaptable, evolving to survive, it is possible that we can take advantage of some mechanisms of cancer cells and use them rationally in favor of our survival. Some of the most common cellular and molecular resistance mechanisms in cancer are listed below.

Molecular target modification

This mechanism is mediated by molecular target changes, these changes involve sites of drug binding, and thus the new molecular sites cannot be recognized by the drug. In the case of proteins as molecular targets, a genetic mutation causes the protein to change its conformation, or the site for which the drug was designed, causing loss of affinity for the target site (2,4,24,25).

Production of metabolizing or biotransforming enzymes

Biotransformation is a method that cells use so that drugs can be more easily excreted and this process is generally carried out in two phases. Phase I biotransformation is primarily mediated by cytochromes P450 (CYPs) and, to a lesser extent, flavin-containing monooxygenases. This phase is associated with reduction reactions. Phase II biotransformation involves a larger number of families of enzymes, including UDP- glucuronosyltransferases (UGT), sulfotransferases, N-acetyltransferases, and glutathione S-transferases (GST); this process is based on conjugation reactions (26). The resistance mechanism is based on the

overexpression of these metabolizing enzymes, which have been found to increase their expression, especially the enzymes of the CYP540 family, the GST superfamily, the UGT superfamily, gamma-glutamyl transferases and thiopurine methyltransferases (2,4).Recently, the importance of amino acid metabolism in chemoresistance has been discussed, because amino acids help cancer cells counteract therapies by maintaining redox homeostasis, supporting biosynthetic processes, regulating epigenetic modification, and providing metabolic intermediaries for cell metabolism. (27).

Tumor microenvironment

It is well known that the environment in which cancer cells develop is highly heterogeneous, enriched by extracellular signals that stimulate signaling pathways involved in metabolism, cell division, cell remodeling, and the production of transcription factors. These signals are made up of different molecules, among which we have hormones, cytokines, and interleukins (IL) such as IL-1, IL-4, IL-6, and IL-8; there are also different growth factors such as FG2, FGF9 and FGF10, all these molecules are secreted into the environment by secretory cells, lymphocytes, and fibroblasts generally. Additionally, the extracellular matrix is a three-dimensional network that provides structural and biochemical support to cancer cells, being an important component in the tumor microenvironment. (2,4,23,28–31). Also, cancer cells can produce vesicles containing molecules such as microRNA (miRNA) or messenger RNA (mRNA) inside, which can promote chemoresistance. It has been observed that extracellular vesicles are involved in processes such as stromal activation, angiogenesis, evasion of the immune system, reprogramming of energy metabolism, transfer of mutations and metastasis, thus, extracellular vesicles can modulate the tumor microenvironment in favor of the resistance (32–37).

Efflux pumps

Membrane cell have proteins that function as channels, which allow the passage of small molecules and ions. Efflux pumps are protein complexes that push drugs out of the cell, preventing the drug from carrying out its therapeutic effect. The efflux pumps belong mainly to the ABC transporter superfamily, of which there are 7 families (ABCA-ABCG) with 48 members in total. But only the ABCB1, ABCC1 and ABCG2 members are the ones that have seen to be involved with resistance to chemopharmaceuticals (2,4,38).

Epigenetics

Epigenetics is the study of changes that activate or inactivate genes without changing the DNA sequence, due to age and exposure to environmental factors (diet, exercise, medications and chemicals). Epigenetics refers to a non-genetic cellular memory, which records environmental and

developmental cues (and alternative cellular states in unicellular organisms), is the basis of epi-(above)-genetics (39,40). Resistance is related to DNA methylation, histone modification, chromatin remodeling, and non-coding RNA related alterations (2,4,41,42). Histone deacetylase 3 (HDAC3) belongs to the group of genes associated with cancer and has been implicated as a regulator of responses to anticancer drugs, angiogenic potential, and tumorigenic potential (43).

Coding and non-coding RNA

Messenger RNA (mRNA) is the coding sequence of each gene, it is the sequence that is translated into protein, and resistance is associated with modifications that this sequence can undergo, this sequence changes could modify some basic cellular proceceses, such as, alternative splicing, the modification of adenosine to inosine (A to I) and mRNA methylation (44). On the other hand, we have the resistance mechanisms related to miRNAs. miRNAs are short which are short (20-24 nt) non-coding RNAs that participate in the post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and the translation of the mRNAs. Recently, some miRNAs expression have been associated to chemopharmaceuticals resistance, these miRNAs are expressed by cancer cells and it has been seen that these are independent of the type of cancer. For example, miR-100, miR-222, miR-30a and miR-17 were found in breast cancer (32,45–47). Other non-coding RNAs such as long non-coding RNAs (LncRNA), circular RNAs (CircRNA) have also been associated with the development of resistance (44).

Genes and proteins related to chemo resistance

As we have already seen, there are certain mechanisms by which cells can evade the therapeutic effect of chemopharmaceuticals and these mechanisms may be due to gene-protein or protein-protein interactions, this interections could result in activation, inhibition or lost of function. These interactions can form positive or negative feedback loops, stimulation leading to an effect, which cyclically promotes the stimulus again.

The STRING database alone contains 67,592,464 proteins belonging to 14,094 organisms, of which it is estimated that they can form approximately 20,052,394,041 total interactions, including low-confidence interactions (48). Despite all these proteins and interactions that can take place, only some proteins are strongly related to chemoresistance, such as the ATP-binding cassette subfamily protein member three encoded by the *ABCA3* gene (gene *ID*: 21); the entire transporter encoded by this gene may be involved in xenobiotic resistance development and the programmed uptake during programmed cell death.

ATP-binding cassette subfamily B member one Protein is multidrug resistance-related protein, encoded by the *ABCB1 gene* (gene ID: 5243); this protein is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. Transporter 1 protein, a member of the ATP-binding cassette subfamily B encoded by the *ABCB2 gene* (gene ID: 6890), is involved in pumping degraded cytosolic peptides across the endoplasmic reticulum to the membrane-bound compartment where class I molecules are assembled. The ATP-binding cassette subfamily C member 1 protein encoded by the *ABCC1 gene* (gene ID: 4363), is a transporter member of the MRP subfamily that is involved in the multidrug resistance. The ATP-binding cassette subfamily C member 2 protein encoded by the *ABCC2 gene* (gene ID: 1244), this transporter is substrated with anticancer drugs such as vinblastine; therefore, this protein appears to contribute to drug resistance in mammalian cells. ATP-binding cassette subfamily protein G member 2, known as breast cancer resistance protein, functions as a xenobiotic transporter encoded by the ABCG2 gene (gene ID: 9429) . Cytochrome P450 enzyme family two subfamily D member 6, this protein is located in the endoplasmic reticulum and is known to metabolize up to 25% of commonly prescribed drugs; its substrates include antidepressants, antipsychotics, analgesics and cough suppressants, beta-adrenergic blockers, antiarrhythmics, and antiemetics, it is encoded by the *CYP2D6 gene* (gene ID: 1565). The cytochrome P450 has a family of three subfamilies. A member four enzyme encoded by the *CYP3A4 gene* (gene ID: 1576), has been implicated in the metabolism of approximately half of the drugs in use today, including acetaminophen, codeine, cyclosporine A, diazepam, erythromycin, and chloroquine. The enzyme glutathione S-transferase alpha one, encoded by the *GSTA1 gene* (gene ID: 2938), catalyze the glutathione addition to an electrophilic target, the targets include, carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress (32,38,42,44,45,49,50).

Figure 2 shows the intracellular localization of proteins related to resistance to chemopharmaceuticals, such as cytochrome P450 enzymes, some monooxidases, and enzymes from the uridine diphospho-glucuronosyltransferase superfamily (see Fig. 3).

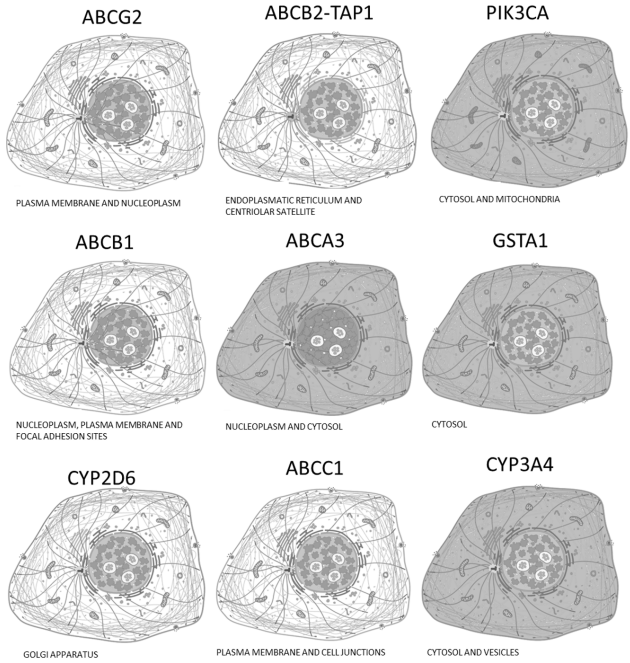
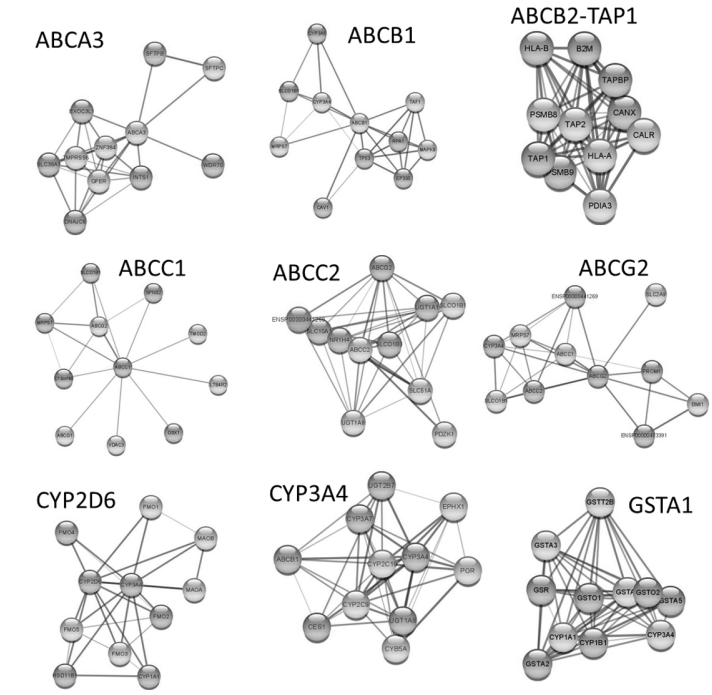


Figure 2. Cellular localization of the proteins encoded by the resistance genes. The gray colored sites are the cellular localization of the protein.



Gene	Main interaction gene	Score (0 al 1)
ABCA3	ADNJC6	0,844
ABCB1	CYP3A4	0,964
ABCB2	TAP2	0,998
ABCC1	ABCG2	0,932
ABCC2	PDZK1	0,956
ABCG2	ABCC1	0,932
CYP2D6	MAOA	0,955
CYP3A4	UGT2B7	0,981
GSTA1	GSTA2	0,963

Figure 3. Interactomes of the proteins encoded by the resistance genes with other proteins.

Recently there are studies that suggest other genes and proteins that are related to chemoresistance's, such as the transcription factors FOXO3 and FOXM1, which participate downstream in the PI3K-Akt, Ras-ERK, and JNK/p38MAPK signaling cascades. , these pathways are crucial for cell proliferation, differentiation, cell survival, senescence, DNA damage repair, and cell cycle control (50,51). Also, it has been seen that human aldo-keto reductase (AKR) enzymes catalyze the NADPH-dependent reduction of carbonyl groups to alcohols and participate directly in the metabolism of chemopharmaceuticals, contributing to chemoresistance (17). It has been found that S-phase kinase-associated protein 2 (Skp2) and E3 ligase are highly correlated with chemoresistance, and high expression of these proteins is associated with a poor prognosis in Her2-positive patients (52). Another important group that has been found to be related to chemoresistance is the group of heat shock proteins (HSPs), which may act as an upstream regulator of oncogenic pathways related to tumorigenesis, metastasis, and invasiveness in various types of cancer, cancer. HSPs proteins contributes to the progress of the disease and more specifically activate signaling pathways and genes related to chemoresistance (53). F-box proteins (FBPs) play a fundamental role in developing drug resistance through ubiquitination and degradation of substrates (54). *Snail* , *Twist1* , and zinc-finger E homeobox-binding 1 (*ZEB1*) genes orchestrate gene expression that mediates the transition from epithelial cells to mesenchymal cells, implicating cancer development and metastasis and are also related to resistance to medications (55). Intracellular cholesterol accumulation in cancer cells, promotes drug resistance and allows these cells to evade apoptotic signaling processes and maintain cell division and proliferation; also, lipid metabolism is implicated in resistance to chemopharmaceuticals (56,57). Insulin-like growth factor 1 (IGF-1) is known for its role in supporting cancer progression and metastasis through the promotion of neovascularization in transforming tissues and the promotion of proliferation, maintenance, and migration of malignant cells. A growing number of reports support that IGF-1 fuels tumorigenesis and induces the development of resistance to anticancer drugs (58). The solute transporter 1B3 (SLCO1B3) member of the organic anion transporters is a transporter normally expressed in the liver, transporting a variety of endogenous and exogenous compounds, including hormones and their conjugates. The extrahepatic expression of SLCO1B3 has been detected in different cell lines and cancer tissues. Recently, accumulating data indicates that abnormal expression and function of SLCO1B3 are involved in anticancer drug resistance (49,59).

It has been observed that resistance-associated proteins are highly related to proteins involved in drug metabolism, and

alteration in the sequence of these genes or overexpression is related to chemoresistance. Certain mutations in the CP540 superfamily enzyme-coding genes cause individuals to be slow, rapid, or super-rapid metabolizers of certain drugs, rendering therapies less effective for extensive metabolizers, and vice versa. Slow metabolizers are more susceptible to have a toxic effect, these effects depend on the type of drug being administered and the disease being treated (26). Table 1 describes some chemopharmaceuticals used in cancer therapy and the proteins related to their metabolism, the proteins involved in the metabolism of paracetamol are also shown . Paracetamol is an effective analgesic and antipyretic for the control of mild or moderate pain caused by joint conditions, ear pain, headaches, odontogenic pain, neuralgia, minor surgical procedures, among others. This drug is prescribed to people regardless of age or sex. There are proteins that may be involved in the metabolism of more than one chemopharmaceutical drug, such as the enzymes of cytochrome P450 3A4, and cytochrome P450 2B6; there are also efflux pumps, such as the protein of ATP-binding cassette subfamily G member 2. This multiple substrates for some proteins is not surprising; but; it is interesting the number of proteins involved in the metabolism of chemo pharmaceuticals and the metabolism of paracetamol. Due to its high medical prescription, it is believed that paracetamol is harmless in the body; however, as it is related to the response to a large number of genes related to chemoresistance, it suggests that this drug, and probably all the drugs we consume today every day in our lives, may contribute to resistance without realizing it, even knowing that sometimes we require higher doses to counteract a condition that we could previously alleviate with a low dose.

Table 1. Proteins involved in the metabolism of some anticancer drugs.

Etoposide(60)	Mercaptopurine(60)	Cyclophosphamide(60)	Teniposide(60)	Ifosfamide(60)	Capecitabine(60)
Multispecific canalicular transporter of organic anions 2	Adenosine kinase	Aldehyde dehydrogenase, dimeric with a preference for NADP	Cytochrome P450 3A4	Aldehyde dehydrogenase, dimeric with a preference for NADP	Cytidine deaminase
Cytochrome P450 3A4	Aldehyde oxidase	Cytochrome P450 2A6	Cytochrome P450 3A5	Cytochrome P450 2A6	Hepatic carboxylesterase 1
Cytochrome P450 3A5	ASmidophosphoribosyltransferase	Cytochrome P450 2B6	DNA topoisomerase 2-alpha	Cytochrome P450 2B6	Sodium/Nucleoside Cotransporter 1
DNA topoisomerase 2-alpha	Equilibrium nucleoside transporter 1	Cytochrome P450 2C19	Myeloperoxidase	Cytochrome P450 2C8	Thymidine phosphorylase
DNA topoisomerase 2-beta	Equilibrium nucleoside transporter 2	Cytochrome P450 2C8		Cytochrome P450 2C9	Thymidylate synthase

Etoposide(60)	Mercaptopurine(60)	Cyclophosphamide(60)	Teniposide(60)	Ifosfamide(60)	Capecitabine(60)
Multidrug resistance protein 1	GMP synthase [glutamine hydrolyzation]	Cytochrome P450 2C9		Cytochrome P450 3A4	
Myeloperoxidase	Hypoxanthine-guanine phosphoribosyltransferase	Cytochrome P450 3A4		Cytochrome P450 3A5	
Prostaglandin G/H synthase 1	Inosine-5'-monophosphate dehydrogenase 1	Glutathione S-transferase Mu 1		retinal dehydrogenase 1	
Prostaglandin G/H synthase 2	Protein 4 associated with multidrug resistance	retinal dehydrogenase 1			
UDP-glucuronosyltransferase 1-1	Protein 5 associated with multidrug resistance				
	Ras-related botulinum toxin C3 substrate 1				
	Sodium/Nucleoside Cotransporter 2				
	Solute transporter family 28 member 3				
	Thiopurine S-methyltransferase				
	Xanthine dehydrogenase/oxidase				

Irinotecan(60)	Doxorubicin(60)	Tamoxifen(60)	Gemcitabine(60)	Acetaminophen (Paracetamol)(60)
Multispecific canalicular transporter of organic anions 1	Alcohol dehydrogenase [NADP(+)]	Cytochrome P450 2B6	5'(3')-deoxyribonucleotidase, cytosolic type	ATP-binding cassette subfamily G member 2
Cholinesterase	Aldo-keto reductase family 1 member C3	Cytochrome P450 2D6	CTP synthase 1	Bile salt sulfotransferase
Cocaine esterase	ATP-binding cassette subfamily G member 2	Cytochrome P450 3A4	Deoxycytidine kinase	Cytochrome P450 1A2
Cytochrome P450 3A4	Multispecific canalicular transporter of organic anions 1	Cytochrome P450 3A5	Deoxycytidylate deaminase	Cytochrome P450 2A6
Cytochrome P450 3A5	Carbonyl reductase [NADPH] 1	Dimethylaniline monooxygenase [N-oxide former] 1	Equilibrium nucleoside transporter 1	Cytochrome P450 2D6
DNA topoisomerase 1	Carbonyl reductase [NADPH] 3	Dimethylaniline monooxygenase [N-oxide forming] 3	Nucleoside diphosphate kinase A	Cytochrome P450 2E1
Hepatic carboxylesterase 1	Multidrug resistance protein 1	Estrogen receptor	Ribonucleoside diphosphate reductase large subunit	Cytochrome P450 3A4
Multidrug resistance protein 1	Multidrug resistance-associated protein 1	Sulfotransferase 1A1	Ribonucleoside diphosphate reductase M2 subunit	Estrogen sulfotransferase

Irinotecan(60)	Doxorubicin(60)	Tamoxifen(60)	Gemcitabine(60)	Acetaminophen (Paracetamol)(60)
Multidrug resistance-associated protein 1	NAD(P)H dehydrogenase [quinone] 1	UDP-glucuronosyltransferase 1-10	Ribonucleoside diphosphate reductase subunit M2 B	Glutathione S-transferase P
Solute transporter member of the 1B1 family of organic anion transporters	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	UDP-glucuronosyltransferase 1-4	Sodium/Nucleoside Cotransporter 1	Glutathione S-transferase theta-1
UDP-glucuronosyltransferase 1-1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial		Solute transporter family 28 member 3	Multidrug resistance protein 1
UDP-glucuronosyltransferase 1-10	NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial		Thymidylate synthase	Multidrug resistance-associated protein 1
UDP-glucuronosyltransferase 1-9	NADPH: cytochrome P450 reductase		UMP-CMP kinase	Protein 4 associated with multidrug resistance
	Nitric oxide synthase, endothelial			Protein 5 associated with multidrug resistance
	RalA binding protein 1			Sulfotransferase 1A1
	Family of solute transporters 22 member 16			Sulfotransferase 1A3/1A4
	Xanthine dehydrogenase/ oxidase			UDP-glucuronosyltransferase 1-1
				UDP-glucuronosyltransferase 1-6
				UDP-glucuronosyltransferase 1-9
				UDP-glucuronosyltransferase 2B15

Therapies against resistance to chemo pharmaceuticals

Today there are a wide variety of cancer therapies; some could help evade resistance mechanisms such as intermittent treatment regimen and minimum dose necessary; this treatment is based on containing tumors at a fixed tolerable level to allow expansion of drug-sensitive cells at the expense of resistant ones. Another emerging therapy is polytherapy, which focuses on targeting a cancer-specific overactive transcription factor/regulator, in addition to containing the necessary chemo pharmaceuticals to fight cancer, trying to propose simultaneously targeting multiple dependencies that generate chemoresistance, hence hybrid drugs are born, which simultaneously target many points of signaling networks and various structures within a cancer cell (22,24,61). Also, monoclonal antibodies directed at the inhibition of signal transduction can be used to prevent the proli-

feration of cancer cells, such is the case of anti-HER2 antibodies such as pertuzumab (Omnitarg), panitumumab (ABX-EGF) and Cetuximab (Erbitux). , which are under evaluation in clinical trials (51). Tumor plasticity is a phenomenon in which cancer cells adapt to tumor microenvironment; it is possible to develop strategies directed to specific plasticity regulators in order to overcome or stop cancer progression (31). Today there is an enormous amount of information that can be used by mathematical models to make computational predictions using systematic and quantitative approaches, with the purpose of providing deeper insight into resistance mechanisms, generating new hypotheses, or suggesting promising treatment strategies . for future therapies (62).

Mitochondria are the central organelle for cellular energy supply, they can rapidly undergo dynamic changes and integrate cellular signaling pathways to provide bioenergetic and biosynthetic flexibility to cancer cells; thus, mitochondria contribute to multiple aspects of cell characteristics. tumor, including drug resistance. To combat this organelle, there are different therapies, such as intelligent drug delivery systems or the modification of traditional treatments. At present, mitochondrial-directed photothermal therapy (PTT), photodynamic therapy (PDT), and chemodynamical therapy (CDT), have attracted worldwide attention due to their advantages, such as wide therapeutic range, minimal toxicity, and excellent profile. non-invasive safety and low resistance (63–66).

On the other hand, deregulation of the ubiquitin-proteasome pathway is known to be associated with various diseases, particularly neoplastic ones; it is also related to late relapses, suggesting the development of acquired resistance. The use of proteasome inhibitors (PIs) is a therapy that has received attention from the pharmaceutical industry. Different classes and several inhibitors have been developed; however, only three were approved by the FDA and the EMA: bortezomib, carfilzomib, and ixazomib (67).

Regarding RNA therapies, the miR-200 family are tumor suppressors and are commonly decreased in cancer. The miR-200 family has been reported as a valuable diagnostic and prognostic marker. (47). Therefore, these miRNAs can be packaged into vesicles for cancer therapy and chemoresistance.

Conclusions

The emergence of drug resistance is a barrier to effective cancer treatment. Resistance develops during chemotherapy, radiotherapy, molecularly targeted therapy, and immunotherapy in most cancer patients and decreases survival. Despite the enormous amount of information about

resistance, we are still far from finding a solution to the problem since chemoresistance is a multifactorial phenomenon involving the interaction of multiple genes and proteins between them. For this reason, it is necessary to have a detailed understanding of chemoresistance and the mechanisms that develop it.

It is necessary to acquire new approaches related to cancer treatments, which must consider the resistence of chemopharmaceuticals not as a possibility of occurrence but as a fact of appearance at any stage of therapy. Therefore, it is extremely important to study the genes involved in drug resistance in cancer, as well as their coding proteins, with a focus of make more efficient and less harmful therapies and achieve more patients and longer survival.

Conflict of interest and financing

The Authors have no conflict of interest.

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Research article

Morphogenesis of penis and spongy urethra during human gestation

Alba Rocío Valencia[1], Manuel García Flórez[2]

Abstract

Background. Every year, approximately 500,000 children in the world are born with congenital abnormalities of the urinary system and kidneys. Therefore, pediatricians and urologists must understand the normal processes that lead to male sexual differentiation. **Objective.** The aim of this study was to describe in detail the process that occurs during masculinization of the fetus, which leads to the formation of male structures under normal conditions. **Methods.** Fifty-four fetuses with gestation periods between four and 18 weeks were collected, which were considered normal, did not have any signs of external anatomic abnormalities or any alteration in their development, and were a product of spontaneous abortions and tubal pregnancies. The urogenital sinus region was collected and prepared for scanning electron microscopy and high-resolution optical microscopy to observe the cellular characteristics of the urogenital fold during external development in male embryos. **Results.** This work shows the formation of the glans and spongy urethra in a detailed manner from the eighth week of embryonic development, carefully describing the role of the labioscrotal folds and the fusion of the walls of the urogenital fold during the subsequent stages of development to form the proximal part of the urinary tract. **Conclusion.** The formation of the penile urethra from the urethral fold and its posterior fusion have a probable role of ectodermal cells, in addition to the endodermal origin established previously.

Keywords: urogenital system, sry gene, sex differentiation, sex determination processes, gestational age.

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Introduction

The urogenital system in mammals, the bladder, urethra, and external genitals develop from epithelium and cloacal mesenchyme, which is a transient embryonic cavity at the tail end of the posterior intestine. Urogenital malformations are frequent in humans and are often seen with malformations of the genital tubercle, penis in men and clitoris in women, and defects in the separation of the urethral and rectal compartments (1). Each year, it is estimated that 6% of births worldwide (approximately 8 million infants) have serious genetic defects. Out of this 6%, approximately 1% of human fetuses show congenital anomalies in the urinary tract and kidneys (2, 3). Therefore, it is crucial for pediatricians and urologists to understand the normal processes that lead to sexual differentiation.

Even though the male external genital differentiation is a very detailed process, there are still migration and cellular differentiation processes that need to be studied further, since in many cases, most of the information has been obtained from studies in mice models (4-6). Despite the fact that, by studying murines, relevant information has been found and access to experimental manipulation has been given, it is important to note that they have some anatomical and morphological differences when compared to humans, which presents a difficulty when trying to use direct analogies (7) (8).

To elucidate this process, in this study were used specimens considered normal human males, from eight to fourteenth week of gestation, and a detailed description of the development of the external genitalia was performed using optical and scanning microscopy, showing the different processes of migration and cellular proliferation that result in the differentiation of male structures.

Materials and methods

Samples

For this research, the samples were collected over a period of a year and six months; and were obtained from spontaneous or frustrated abortions and ectopic pregnancies and were acquired from the Hospital Universitario del Valle de la ciudad de Cali, with the collaboration of the Department of Pathology and Obstetrics Gynecology from Universidad del Valle.

From the samples obtained, 54 embryos and fetuses that were between the eighth and fourteenth week of gestation and did not have any external morphological defects, such as low implantation of ears or some kind of mutilation in limbs, were selected (Table 1). The samples were placed in 10% buffered formalin. The entire body of the small specimens and pelvic blocks of the larger specimens were included.

Determination of embryonic age

Classification of the gestational age was performed with the help of a stereoscope, based on age tables (9), parameters of normal fetal growth, and information from the Department of Morphology of Universidad del Valle, Cali – Colombia (10).

From the embryos and fetuses obtained, the gonads were processed to differentiate the testicles from the ovaries to be associated with the external genitalia. The morphological analyses and age determinations were conducted by two experts from the Pathology and Obstetric Gynecology Department of Universidad del Valle.

Scanning Electron Microscope

A scanning electron microscope was used to examine detailed cellular development during the maturation of the external genitalia. We chose scanning electron microscopy (SEM) over transmission electron microscopy (TEM) to give detailed surface imaging of specimens. Because SEM generates a three-dimensional topographic image and can analyze a greater area than TEM of the specimen at once. For SEM, the tissue samples were fixed in 2% paraformaldehyde, 2% glutaraldehyde and 0.1M phosphate buffer (PH 7.4) for 48h at 4 °C. The tissues were then set on 2% tannic acid for 2h, washed with distilled water for 1h and treated with 1% osmium tetroxide for 2 h at room temperature, dehydrated with increasing concentrations of ethanol, the samples were taken to the dry critical point, and finally covered with gold and observed using a Jeol 5800 *scanning electron microscope* (JEOL Ltd.). Musashino, Akishima, Tokyo 196-8558, Japan.

Optical Microscopy

For optical microscopy, the samples were rehydrated with different concentrations of isoamyl acetate and mixtures of pure alcohol and isoamyl until decreasing alcohol concentrations were achieved, followed by washing with 0.1% phosphate buffer. Afterwards, they were passed through alcohols at increasing concentrations and then with propylene oxide. They were then infiltrated with histological resin and polymerized at 60 °C to be cut with a glass blade in an ultra-microtome, stained with toluidine blue, and H&E mounted (11) (12), and observed (13).

Ethical approval

This study was carried out in accordance with the ethical standards of the Universidad Surcolombiana institutional committee on human experimentation. (MEMORANDO No. 052, October 26, 2020).

Results

Morphological characteristics of the external genitalia development

Eighth week of development

In the eighth week of gestation, the main events included growth of the genital tubercle and formation of the epithelial appendix. The overlapping ectodermal cells at the distal end level, where the glans originate, and from that point forward, this tubercle is known as the phallus.

The urogenital fold is now longer and has less depth as a result of the growth of the phallus (Fig. 1, A y B); (Table 1)

Table 1. Discrimination of a sample of fifty-four embryos and fetuses between the eighth and fourteenth week of gestation used in this study. These samples had no visible external defects and were considered normal.

Number of Embryos and Fetuses	Age (weeks)	Cephalo-Caudal Length (mm)	Morphological Characteristics
5	8	23 - 31	* Flat nose * Separated eyes
6	9	32 - 44	* Eyes closing * Round head
6	10	45 - 56	* Intestines in the umbilical cord * No physiological hernia * Erect head * Molded extremities
9	11	57 - 69	* Head half the length CC. * External genitalia beginning to differentiate
11	12	70 - 81	* Head still dominant * Sex determined. * Length of definitive members
11	13	82- 97	* There are signs of hair
6	14	98 - 110	* Head upright * Nail development

Ninth week of development

Further development can be observed in ectodermal cells of the urogenital fold. The phallus continues to grow, and at this point is the structure with the most genital development. The glans is now more defined, and it lies at the end of the urogenital fold and conservation of the epithelial appendix. The labioscrotal swellings increase in volume and grow towards the anal region, in the direction of the middle line where they will fuse posteriorly; as a result of this growth, the anal fold disappears (Fig. 1C).

Changes in the phallus fold and mesoderm; the shape of the linear genitalia changes to rhomboid, showing proliferation of the cells on the outer part of the urogenital fold. This defined space allows for the organization of the penile urethra. The superficial ectoderm, which serves as a lining for the urogenital fold from the beginning, serves as an origin point for the epithelium of the urethra (Fig. 1D)

The phallus' mesoderm shows cellular proliferation that is related to the deepest region of the fold; these cellular clusters will give origin to cavernous bodies (Asterisks, Fig. 1D)

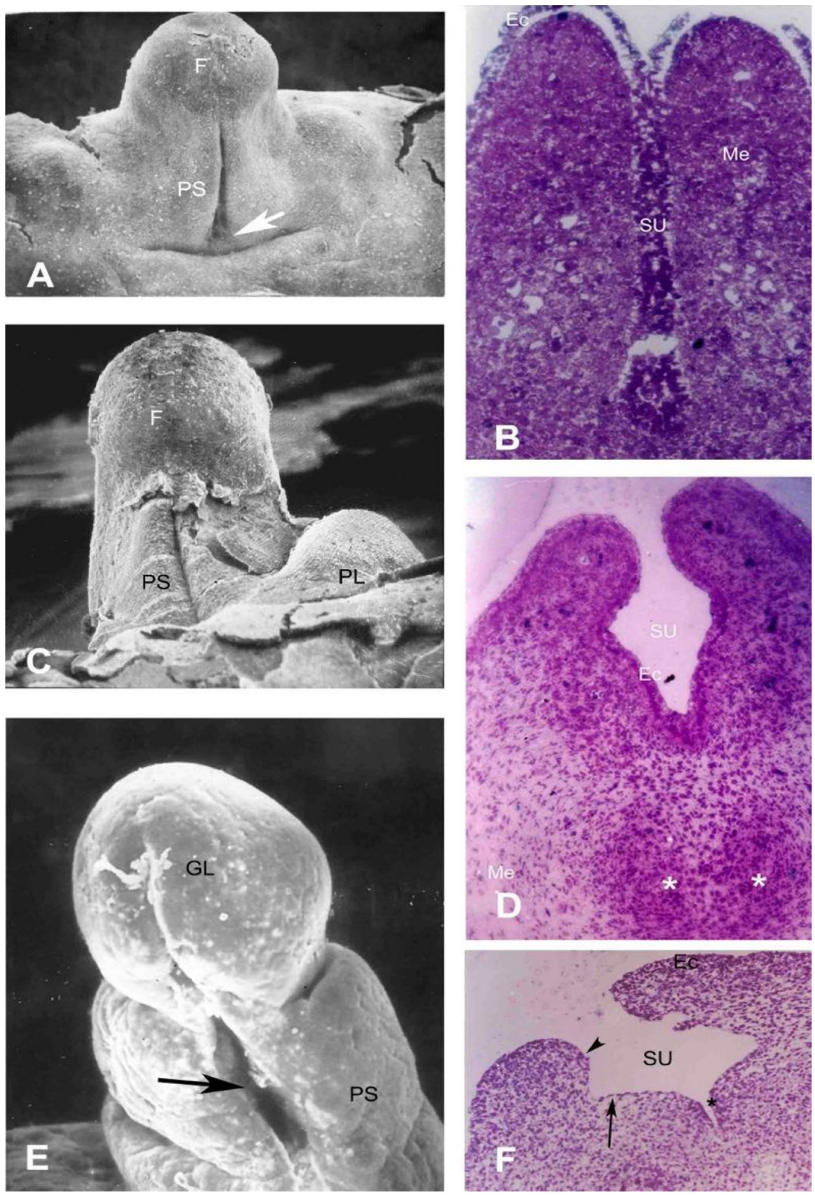

Figure 1. Microphotographs of the genital area of masculine embryos between eight and ten weeks of gestation (1A) Scanning electron microphotography of the genital area of an embryo at eight weeks of development, showing the phallus (f), fold of the urogenital sulcus (PS), and anal fold (arrow) (40X). (1B) During this phase, the superficial epithelium (Ec) can be observed covering the urogenital sulcus (SU) and mesoderm (Me) (400x). (1C) Detailed picture of the genital area of a nine-week-old embryo, labioscrotal folds (PL), and folds of the urogenital sulcus (PS) and phallus (f). Magnification 80X; (1D) also, the urogenital sulcus (PS) can be seen covered with epithelial cells (Ec) on its internal and external parts, and in the internal mesoderm (Me), internal conglomerates organized in a circular shape (asterisk) can be seen (150X). 1E Details of the glans (GL) of a ten-week-old fetus, where the urogenital sulcus (arrow) and folds of the urogenital sulcus (PS) can be observed. (1F) Likewise, the transformation of the urogenital sulcus (SU) can be witnessed because of the presence of external elevation (top of the arrow) and internal elevation (asterisk), which is surrounded by the superficial ectoderm (arrow) (80X).

Tenth week of development

The external genitalia continue to develop; the phallus is the predominant element with very defined glans and conserving the epithelial appendix (Fig. 1, E y F). At the end of the week, the balanopreputial sulcus appears as well as an ectoderm invagination at the middle line level of the glans, forming an ectothermic fold that carries on with the urogenital fold, which aids in the formation of the spongy urethra's glans. The labioscrotoral folds show an important development approaching the midline, causing the anal fold to disappear, resulting in a better appreciation of the anal opening. The folds of the urogenital fold show prominent to the sides of the fold, which has now acquired more depth (Fig. 1E y 1F)

The urogenital fold continues to change in shape and size, and its superficial borders continue to become closer as a result of the growth of the internal mesoderm. In the middle area, mesodermic growth that produces a couple of bulges that slightly change the shape of the groove light can be observed (Fig. 1F).

Eleventh week of development

In this week, the phallus has a defined glans and balanopreputial groove; however, the epithelial appendix has now disappeared, furthermore, an invagination of ectodermal cells at the glans level is observed causing internal cell proliferation, at the end of this invagination the area where the external urethral orifice is be located will be determined (Fig. 2, A y B)

The labioescrotoral folds bind at middle line level giving place to the scrotal bags and scrotal raphe; As for the urogenital sinus' folds they start to partially close during this week, showing as areas with a lot of closeness but without a real fusion.

The urethra is now completely closed, covered with epithelial cells, and has started to have a well-defined star shape (dotted line, Fig. 2B).

Twelfth week of development

The external urethral orifice is defined at the glans; the glans on the ventral side shows partial closure of the ectodermal invagination, specifically at the distal portion near the urethral orifice (Fig. 2C).

The medium raphe is not consolidated in the mesoderm and its proliferation forms a bulge in this area; this event is a result of the increase in cellular concentration at the midline level where the urogenital sinus folds have closed. The superficial ectoderm proliferated, and formation of the foreskin as an ectoderm fold was observed (Fig. 2D).

Thirteenth week of development

The internal area of the urethral orifice that forms the glandular portion of the spongy urethra is now covered by epithelium that

was previously invaginated around the tenth week of gestation (Fig. 2E) The urethra exhibited a characteristic star shape covered by the epithelium in the process of cellular differentiation (Fig. 2F)

The superficial ectoderm completely covers the closing, and it proliferates into a stratified epithelium with basal cells other than superficial cells. The raphe is now formed with a more ectodermal mesodermal bulge, and it can be seen that the mesoderm surrounding the urethra is filled with small blood vessels, whereas the vascularization of the peripheral mesoderm has larger blood vessels.

Fourteenth week of development

During this week of development, the external urethral orifice is observed, this orifice is very wide during its initial stages, but will eventually close (ou, Fig. 2G)

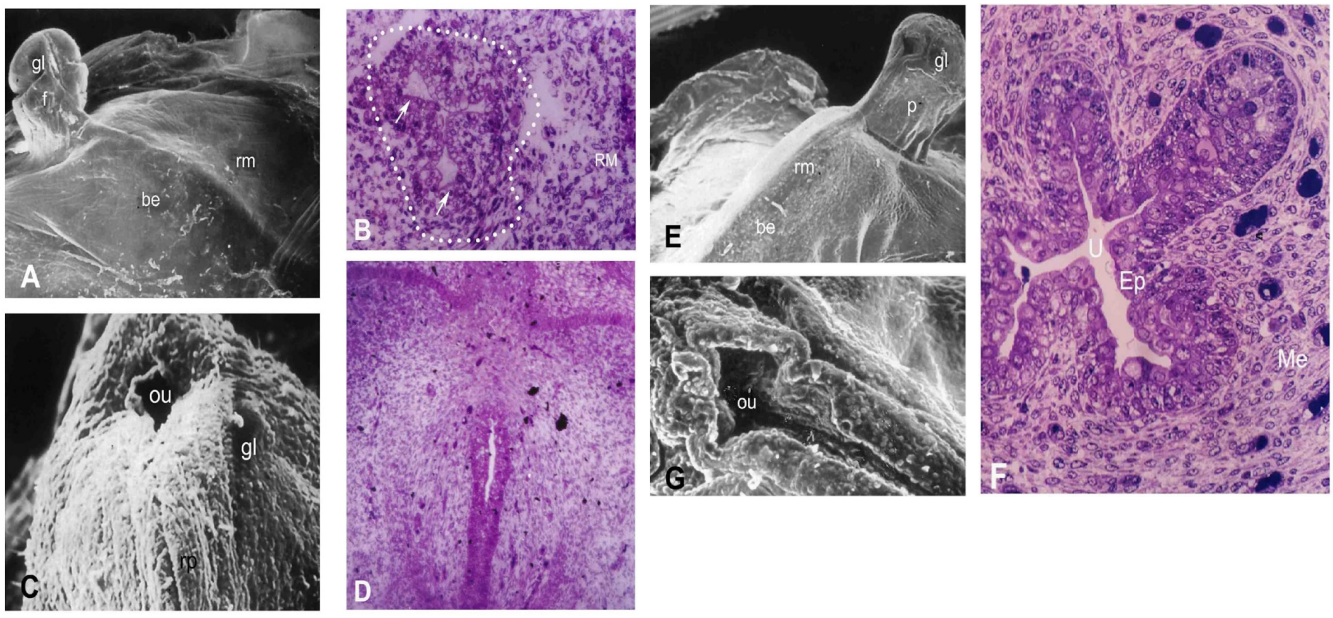

Figure 2. Microphotographs of the genital area of masculine embryos between 11 and 14 weeks of gestation (A-G). 2A At the eleventh week of development, the phallus (f) can be seen at the end of which the glans (gl) can be observed, and the closed medium raphe (rm) also shows laterally from the scrotal bags (be) (30X). 2B The cross-section of the phallus showing the medium raphe (RM) without being completely consolidated; the urethra (dotted lines) is covered by the ectoderm (arrows) (400X). 2C On the twelfth week of development, the glans and the external urethral orifice were clearly developed (150X). 2D The foreskin (pr), which is made up of the ectoderm (Ec), medium raphe (RM), mesoderm (Me), and urethral orifice (U), can be seen (400X). 2E After 13 weeks of development, the penis (p) is evident, and at the extreme of it, the glans (gl); at the inferior portion of the penis, the scrotal bags (be) and the medium raphe are located (30X). 2F The penile urethra (U) can be seen to have a stared shape during the 13 weeks; it is lined with an epithelium (Ep) that is being differentiated with prominent nucleus; the mesoderm (Me) can be seen surrounding the urethra (400X). 2G In the fourteenth week of development, a detailed external urethral orifice (ou) can be seen (200X).

Discussion

The male external genitals originate from structures formed during the ambisexual stage, which are differentiated, giving origin to their defined structure (14),(15, 16). Sexual differentiation is determined genetically following hormonal action: once the SRY gene is expressed, testosterone production increases, influencing the central nervous system as well as the internal and external genitals that have not yet been differentiated, which initiates the maturation process that induces masculinization of the fetus (17) (18),(19), (20), (21).

Earlier, at the level of the cloaca on its cephalic portion and at the middle line, an elevation known at the genital tubercle where the penis will originate appears (22), (23).

Sexual differentiation of the external genitalia occurs after differentiation of the gonads and expression of sexual steroid receptors on the genital tubercle. The three germinal layers play a role in the formation of the external genitalia. The genital mesoderm conforms to the stromal tissue of the phallus, the endodermally derived urethral plate forms the entire epithelium of the urethral tube, and an ectodermal epithelium envelope forms the skin that surrounds it (19), (24) (25).

The study of human embryos that are a result of spontaneous abortions can provide a lot of information to understand normal and altered embryological development (26). During the ambisexual phase, between the sixth and twelfth weeks of gestation, the genital tubercle is very similar in males and females, and they are almost the same size (23), making it difficult to identify any changes during this period (2)

In the sixth week of intrauterine life, the urorectal fold grows, dividing the cloaca into two parts: the anterior part that gives origin to the urogenital sinus and a posterior part that gives origin to the anal region. Simultaneously, the urogenital sinus originates from urogenital folds that form scrotal bags (15), (27). Two weeks later, the testicles differentiate and secrete testosterone, which acts over the genital tubercle and the urogenital folds, inducing masculinization of the external genitalia (6). For the development of the folds, the genital tubercle grows to form the penis, whereas the urogenital fold enlarges and closes, forming the spongy portion of the masculine urethra on the medium raphe, which starts in the eighth week of intrauterine life following a fusion that starts from the base to the tip of the phallus (28).

Between the eighth and ninth weeks of gestation, Leydig cells differentiate in the testicles and secrete testosterone (15), (29). Testosterone and/or dihydrotestosterone (a more potent androgen) masculinize the genital tubercle, Wolffian duct, and the urogenital sinus. As a result, the anogenital

distance increases, followed by elongation of the genital tubercle and eventually the formation of urethral plaque. The urethral plaque originates in the endoderm (confirmed by positive immunohistochemistry for Foxa1) (30),(31),(32); and extends from the base of the urethra to the proximal region of the future penis' glans (33), (34).

The genital tubercle that becomes the penis contains tissues derived from the three germ layers: the ectoderm gives rise to the skin of the phallus and foreskin (35), the cavernous bodies and connective tissue of the penis stroma are of mesodermal origin, and the endoderm originates from the penis urethra (4). The urethral plaque canalices distal to the proximal region (Fig. 1A, arrow) to form an open structure that simulates a zipper, the epithelial proliferation (identified for the expression of Ki67) (23), is abundant in the canalizing urethra and on the floor of the urethral sulcus, this event could be responsible for its lateral expansion.

The initial canalization of the urethral plaque extends distally to the coronal sulcus, indicating that the urethral sulcus does not extend inside the glans. The coronal sulcus now defined on the glans is prominent from the penis shaft at approximately ten weeks of gestation (Fig. 1E), becoming an important mechanism in the differentiation between the penis' body and the glans (35). As previously mentioned, the urethra of the penis' body forms as a result of the canalization of the urethral plaque, whose folds are initially open and eventually fuse to form the tubular structure. In contrast, inside the glans, the urethral lumen is formed by limited canalization that does not include the formation of urethral plaques (34) (36).

Interestingly, two morphogenetic mechanisms occur during the development of the urethra in humans. Direct canalization of the urethra in the glans region and formation of the urethra from the urethral sulcus open from its base. Merging events are related to the formation of the urethra inside the body of the penis (arrow, vertical space) (34), (35), (37).

The fusion of urethral folds during penile urethra formation is a complex process. As the medial edges of the urethral folds approach each other, longitudinally intertwined ridges are formed that initially approach each other without merging, leaving clean channels between the lumen of the developing urethra and the exterior, which are initially evident (Fig. 1E, black arrow) (37). With the progression of penile urethra development, the medial edges of the urethral folds fuse in the midline to form the median raphe of the penis. (closed zipper).

From a broad perspective, the process during the formation of the penile urethra (in the body) consists of three separate events: (a) the fusion of the whole epidermis com-

pletes the ventral skin of the penis. (b) Fusion of the endoderm forms the penile urethra. (c) Finally, after removal of the midline epithelial seam, a ventral confluence is established between the right and left mesenchyme of the urethra (23).

Anatomic and immunohistochemical studies reinforce the idea that in humans, the urethra's epithelium originates in the endoderm of the urogenital sinus (4) and that the urethral meatus represents an interphase between the ectoderm epithelium and the epithelium of the urethra's endoderm. The entire human urethra is formed as a result of the growth of the urethral plaque in the genital tubercle and the fusion of the urethral folds along the body of the penis.

Further evidence of the endodermal origin of the urethral epithelium is the expression of FOXA1 during urethral development (23),(35). In contrast, Glenister theory states that the glandular urethra forms from the skin (ectodermal intrusion) growing inside the gland and meets the endoderm-derived urethra at the junction of the shaft of the penis with the glans (37).

Conclusion

Taken together the results of this study, we identified the participation of cells with a possible ectodermic origin in different canalization processes and in the formation of the urethra; however, more specific data are needed to confirm the origin of these cells. Furthermore, information related to the origin and derivation of urethral epithelium based on data collected from developing penises requires validation using adult specimens.

Author contribution statements

A.R.V and M.G.F contributed to the design and implementation of the research, analysis of the results, and writing of the manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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Research article

ObRb, AdipoR1, and *CYP19* gene expression show significant association with obesity and overweight in healthy women

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Abstract

Obesity and overweight are diseases characterized by excessive accumulation of adipose tissue in the body and are defined as an increase in Body Mass Index (BMI) with values greater than 25 kg/m^2. Multiple causes of these increases in BMI have been reported. Some have been attributed to the expression of several genes involved in the leptin and adiponectin pathway as well as postmenopausal stage in women.

In this research, we focused on evaluating whether the expression of *ObRb, AdipoR1*, and *CYP19* genes have any association with the increase in BMI in the female population of Mexico City over 55 years of age. For this, we worked with a group of 45 women volunteers without the diagnosis of any confirmed pathology.

The study population was classified into three groups (average weight, overweight and obese) according to their BMI and was characterized according to their clinical data of blood cytometry and blood chemistry. At the same time, RT-PCR determined *ObRb, AdipoR1*, and *CYP19* gene expression.

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Obtained results showed a moderate correlation (r=0.648, *p*=0.043) of *ObRb* gene expression in women with obesity and a moderate negative correlation (r= -0.389, *p*=0.034) *AdipoR1* gene in women with overweight or obesity.

Based on BMI data, more than 70 % of the study population was overweight and obese. Also, it was found that 64 % of the study population exceeded 150 mg/dL of triglycerides which is the normal healthy range.

Keywords: *ObRb, AdipoR1, CYP19*, body mass index, obesity, overweight.

Introduction

Obesity and overweight have spread rapidly throughout the world due to different dietary changes and the lack of physical activity and have become a public health crisis [1-2]. In Mexico, and according to the ENSANUT-INEGI (National Health and Nutrition Survey and National Institute of Statistics and Geography) in 2018, the percentage of adults with obesity was 36.1 % and overweight 39.1 %, respectively, placing Mexico as the second country with the second-highest prevalence rate in obesity and overweight [3-4].

Obesity and overweight are defined as a disease characterized by the excessive accumulation of adipose tissue in the body, presenting itself as a variation in the Body Mass Index (BMI) [5-6]. The BMI provides the most useful measure to identify a state of obesity or overweight, since it indicates the relationship between weight and height of an individual, a person is considered with obesity when his BMI is equal or higher than 30 kg/m^2 and overweight when his BMI is equal or higher than 25 kg/m^2 [7-8].

BMI increase is favored by the presence of postmenopausal stage in women, In Mexico the average age in which this stage varies is between 41 and 55 years, due to different changes in hormones and slow metabolism, as a result of estrogens and progesterone deficits which increase the risk of developing cardiovascular and metabolic diseases, diagnosis and the development of different types of cancer [9-13].

Obesity and overweight are related to several adverse effects, including developing metabolic and cardiovascular diseases and diagnosing and developing various types of cancer [9-13]. Several environmental and genetic factors influence the development of this pathology[14- 17]. Studies have reported that 50-70 % of BMI variations are attributable to genetic differences specific to everyone[18]. Currently, more than 127 genes associated with obesity have been reported,

among which the long leptin receptor gene (*ObRb*) and the *CYP19* gene coding for the aromatase enzyme stand out[19-21].

The long leptin receptor gene (*ObRb*) belongs to the cytokine family and regulates fat metabolism. So far, six isoforms of this receptor have been reported, being *ObRb* the active form, which is associated with the JAK2-STAT3 system and with other intracellular signaling cascades. It has been reported that a mutation or variation can affect its functionality, preventing it from binding to leptin or that upon binding, it cannot be activated, resulting in excessive hunger and weight gain [22-28].

The adiponectin type 1 receptor gene (*AdipoR1*) is a ubiquitously expressed transmembrane receptor with a high affinity for globular adiponectin, abundantly expressed in skeletal muscle. It is binding to adiponectin activates adenosine monophosphate-dependent protein kinase (AMPK), PPAR-γ, and mitogen-activated protein kinase (MAPK). Adiponectin increases insulin sensitivity through different signaling pathways, reduces hepatic glucose synthesis, and promotes fatty acid oxidation [29-31].

On the other hand, the *CYP19* gene coding for the aromatase enzyme is involved in the biosynthesis of estrogens, and aromatase inhibitors, it may affect the distribution and regulation of body fat by modulating the ratio of androgens to estrogens in adipose tissue and has been associated with obesity-related phenotypes in postmenopausal women [32-33].

Therefore, the present study aimed to determine whether the expression of the genes long leptin receptor (*ObRb*), adiponectin receptor type 1 (*AdipoR1*), and the gene coding for the enzyme aromatase (*CYP19*) were associated with BMI in Mexican women and could be used as predisposing factors for obesity and overweight.

Materials and methods

A total of 45 healthy female volunteers, older than 55 years of age without a confirmed diagnosis of any pathology and with the prior signature of informed consent were recruited. Patients diagnosed with some infectious-contagious disease were excluded as well as those who had a dyscrasia and took drugs from 24 to 48 hours prior to sampling. Patients who decided to leave the study on demand and voluntarily were eliminated as well as non processable, insufficient or noncompliant with the requested criteria blood samples. Data collection was performed following the research ethics guidelines of the participating hospital and the WMA Declaration of Helsinki 2013 [34].

BMI (Body Mass Index).

The determination of BMI was established through the weight-to-height ratio using the formula:

$$BMI = \frac{Weight(kg)}{Heightratio(m^2)}$$

To classify the study population, the recommended criteria by the WHO were used: average weight (BMI < 25 kg/m^2), overweight (BMI ≥ 25 kg/m^2), and obesity (BMI ≥ 30 kg/m^2) [7, 8].

Clinical information of biometric parameters

The participant's blood chemistry and the biometric parameters were performed to evaluate the levels of glucose, urea, creatinine, uric acid, total cholesterol, and triglycerides using spectrophotometry on the Beckman colter ADR7000 platform. Through blood cytometry, the number of leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, erythrocytes, Hgb (Hemoglobin), Hto (Hematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin) was evaluated, MCHC (Mean Corpuscular Hemoglobin Concentration), RDW (Red Cell Distribution Width), HDI (Red Cell Dispersion Index), PWV (Platelet Volume) using ACTDiff Beckman colter automated analyzer. Everything was performed at the participating hospital following their internal procedures.

Total RNA extraction

5 mL for biometry and 10 mL for blood chemistry were extracted from peripheral blood in BD Vacutainer® tubes. For total RNA extraction, erythrocyte lysis was first performed using QIAamp RNA Blood Mini Producer kit (Quiagen, Cat. No. ID: 52304) according to the manufacturer's conditions. The total RNA obtained was quantified using the IMPLEN® NanoPhotometer P300 NP80 at 260/280 nm absorbance.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR)

According to the manufacturer, 500 ng of total RNA was used for cDNA synthesis, Revert Aid First Strand cDNA Synthesis kit (Thermo Scientific K1622, Cat. No.ID:10387979) instructions, using the GeneAmp* PCR System 9700 thermal cycler. For endpoint PCR amplification, the MasterMix 2X kit (Promega, Cat. No. ID: M750) was used according to the manufacturer's instructions. For primers, the sequences were used for *ObRb*: Fw: 5'-GATAGAGAGGCCCAGGCATTTTTTA-3' and Rv:5'-ACACCACTCTCTCTCTCTCTCTCTTTTTTTGATTGA-3', for *AdipoR1*: Fw:5'-AATTCCTGAGCGCTTCTTCTTTCCT-3' and Rv: 5'-CATAGAAGAAGTGGACAAAGGCTGC-3', for *CYP19*: Fw:5'-CAAGGTTATTTTTTGAT GCATGG-3' and Rv:5'-AATCCTTGACAGACTTCTCAT-3', as constitutive gene 18S was used: Fw:5'-GTCTGTGATGATGCCCTTAGA TG-3' and Rv: 5'-AGCTTATGACCCGCACTTAC-3' [35-36]. They were taken to

incubation on the GeneAmp* PCR System 9700 thermal cycler with the following protocol: denaturation at 94 °C /1 min, hybridization at 60 °C /1 min, and extension at 72 °C /1 min for 35 cycles.

Electrophoresis

Products obtained from endpoint PCR were loaded onto a 2.0 % agarose gel (Sigma, Cat. No. ID: 1001134274), and electrophoresis (C. B. S. Scientific, MGU-203T-B) was run at 90 Volts for 40 min. This using 1X TAE run buffer (40mM Tris-Base (Sigma, Cat. ID No. ID 10708976001)), 40mM Acetic Acid (Sigma, Cat. ID No. ID A6283), 1mM EDTA (Sigma, Cat. ID No. ID E7889) with 0.01 % ethidium bromide (Sigma, Cat. ID No. ID: 1239458) as an intercalating agent to visualize the obtained amplicon bands. They were developed under UV light with the aid of the Photodocumentator (UV Transilluminator ANT Technology CUV 40A). Subsequently, the optical intensity of the obtained bands was quantified by densitometry and normalized to arbitrary units corresponding to the positive control of the constitutive 18S gene. Image J 1.52a software (Wayne Rasband) was used for image processing.

Statistical analysis

BMI data were analyzed using descriptive statistics for general qualitative and quantitative variables, using IBM SPSS Statistic 25 2017 statistical software. The correlation analysis of BMI expression concerning each *ObRb*, *AdipoR1*, and *CYP19* gene was analyzed through Pearson's correlation test for those with a normal distribution and Spearman for non-normal distribution data using IBM SPSS Statistic 25 2017 statistical software, considered a *p*-value of 0.05. GraphPad Prism 7 statistical software was used to generate the graphs.

Results

Determination of obesity and overweight according to BMI.

A population of n= 45 volunteers were observed with an average age of 60.7 ± 6.8 years and an average BMI of 27.9 ± 4.1 kg/m^2. They were classified according to BMI into three groups: a) Volunteers with average weight or normal weight, b) Volunteers with overweight, and c) Volunteers with obesity. We observed that 71.1 % of the population presented a BMI higher than 25 kg/m^2 (22.2 % with obesity and 48.9 % with overweight), while the remaining 28.9 % were within the normal BMI.

Hematological and biochemical characteristics.

According to Table 1, the data obtained from the blood biometry for the three groups average weight, overweight and obese, no significant differences were found. For the blood chemistry results, no significant differences

were found between groups. However, it was observed that 64 % of the participating volunteers showed an increase of more than 150 mg/dL in triglycerides,

Table 1. Hematologic and biochemical characteristics of the study population.

	Normal weight (n= 13)	Overweight (n= 22)	Obese (n=10)	p
Age (years)	60.84 ± 7.78	60.50 ± 7.80	60.4 ± 5.23	0.986
Weight (Kg)	58.00 (55.5-61.00)	64.85 (60.87-64.85)	76.50 (69.25-89.00)	0.000
Height (m)	1.56 ± 0.05	1.52 ± 0.06	1.52 ± 0.06	0.227
Leukocytes (10e3/µL)	6.18 ± 1.57	6.02 ± 1.58	6.83 ± 0.89	0.349
Neutrophils (%)	59.50 (55.75-65.60)	54.10 (46.45-61.25)	54.40 (52.27-58.35)	0.225
Lymphocytes (%)	28.13 ± 6.20	32.99 ± 10.69	32.61 ± 7.99	0.290
Monocytes (%)	5.32 ± 1.52	5.17 ± 1.34	5.49 ± 1.53	0.840
Eosinophils (%)	1.90 (1.45-3.80)	2.35 (1.57-3.95)	3.45 (2.87-4.45)	0.829
Basophils (%)	1.0 (0.62-1.87)	0.8 (0.75-1.75)	0.9 (0.67-1.52)	0.783
Erythrocytes (10e3/µL)	4.84 ± 0.26	4.79 ± 0.33	4.83 ± 0.28	0.878
Hgb (g/dL)	14.8 (41.1-14.8)	14.8 (14.27-15.67)	14.8 (13.77-15.67)	0.633
Hto (%)	47.08 ± 2.67	47.50 ± 3.60	45.61 ±4.32	0.377
MCV (fL)	96.6 (93.5-102.0)	99.25 (95.32-103.37)	95.36 (84.57-97.67)	0.031
HCM (pg)	30.0 (29.9-31.45)	31.0 (30.30-32.70)	30.60 (9.47-31.45)	0.333
MCHC (g/dL)	37.70 (30.05-32.70)	31.90 (30.27-33.05)	32.10 (30.92-33.44)	0.933
RDW (%)	13.0 (12.85-13.60)	13.10 (12.6 -13.65)	13.65 (12.97-14.32)	0.223
HDI (g/dL)	2.53 (2.34-2.75)	2.54 (2.42-2.77)	2.56 (2.37-2.73)	0.766
Platelets (10e3/µL)	261.23 ± 62.68	236.31 ± 47.61	258.90 ± 60.56	0.355
VMP (fL)	11.18 ± 1.19	11.51 ± 1.80	11.11 ± 1.96	0.766
Glucose (mg/dL)	95.4 (85.6-106.6)	99.7 (87.8-114.9)	95.90 (87.2-96.3)	0.565

	Normal weight (n= 13)	Overweight (n= 22)	Obese (n=10)	p
BUN (mg/dL)	13.0 (12.0-18.0)	28.8 (23.5-31.7)	18.0 (13.0-22.5)	0.078
Urea (mg/dL)	28.1 (26.3-38.8)	13.0 (11.0-15.0)	37.6 (27.4-48.05)	0.098
Creatinine (mg/dL)	0.79 ± 0.09	0.79 ± 0.11	0.89 ± 0.12	0.498
Uric acid (mg/dL)	4.78 ± 0.81	5.39 ± 0.79	5.70 ± 0.61	0.345
Cholesterol (mg/dL)	182.22 ± 19.44	196.08 ± 20.54	196.26 ± 14.43	0.547
Triglycerides (mg/dL)	170.9 (132.4-259.2)	158.2 (132.4-272.1)	205.8 (138.4-283.3)	0.976

Abbreviations: BMI (Body Mass Index), Hgb (Hemoglobin), Hto (Hematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), RDW (Red Cell Distribution Width), HDI (Red Cell Dispersion Index), PWV (Platelet Volume). * Data out of range of the upper limit allowed.

Overexpression of ObRb and AdipoR1 genes in overweight and obesity

Endpoint RT-PCR detected *ObRb, AdipoR1*, and *CY19* genes. Fragments of each of them can be seen in Figure 1, which allowed us to detect differences in the genes' expression among the participating volunteers.

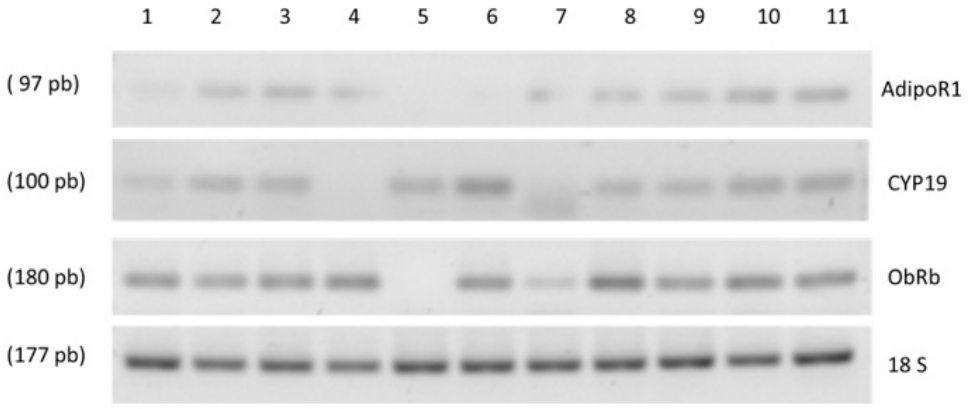

Figure 1. ObRb, AdipoR1, and CYP19 expression levels in postmenopausal women. Notes: The image of ObRb, AdipoR1, and CYP19 expression levels shows the bands obtained from the RT-PCR reaction, including the 18S positive control.

The optical intensity of the obtained bands normalized to arbitrary units concerning the positive control of the constitutive *18S* gene was quantified (Figure 2).

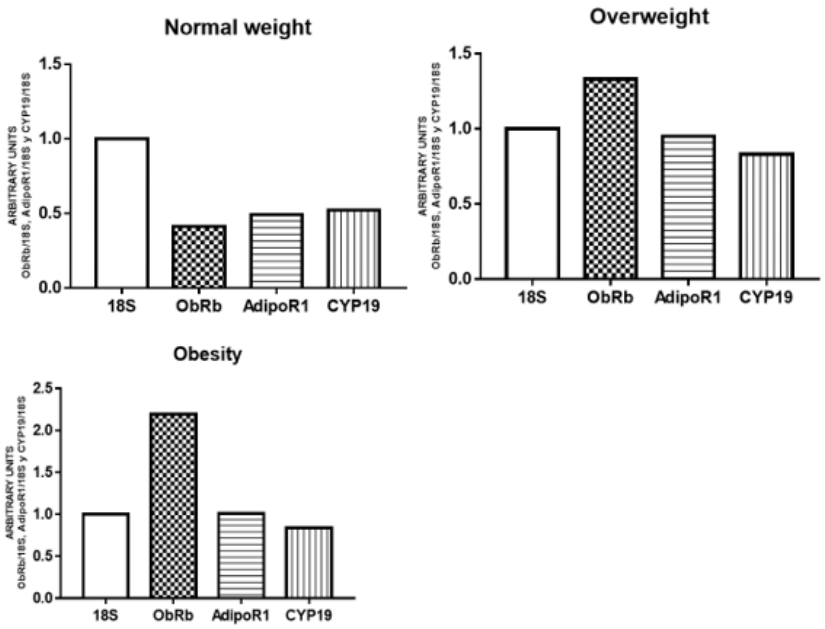

Figure 2. Effect of overweight and obesity on ObRb overexpression. Notes: Representative image of the effect of overweight and obesity on ObRb and AdipoR1 overexpression. The optical intensity of the bands obtained by RT-PCR was quantified and normalized to arbitrary units concerning the positive control of the constitutive 18S gene.

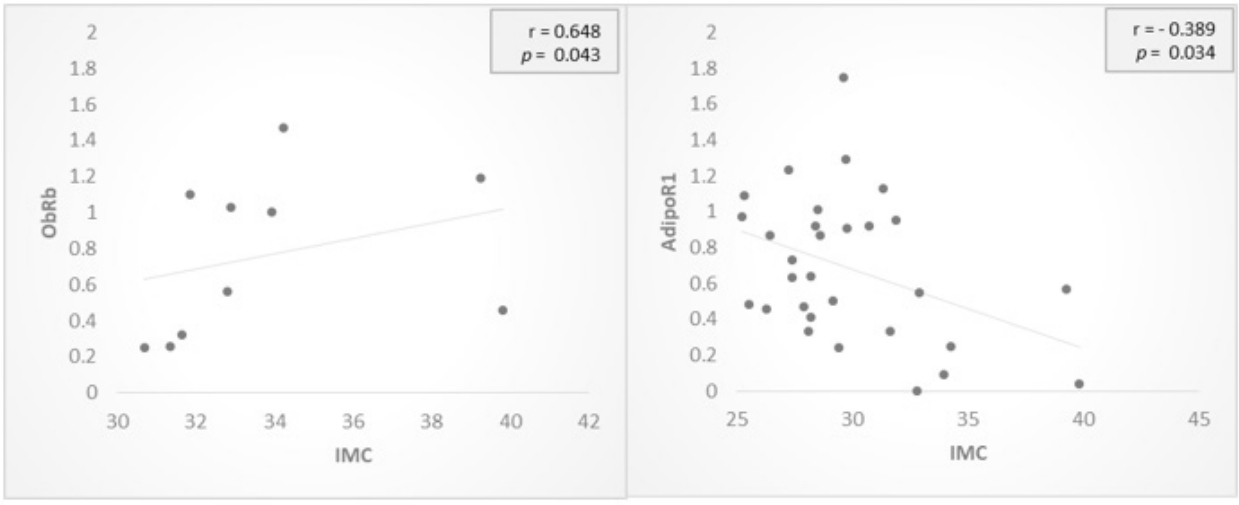

Figure 3. Correlation of ObRb, AdipoR1 gene expression for BMI. Notes: Scatter plot of the correlation of ObRb gene expression with BMI greater than 30 kg/m2 on the right (r=0.648, p=0.043) and for AdipoR1 gene with BMI greater than 25 kg/m2 (r= -0.389, p=0.034) on the left.

Table 2 shows the evaluation of the correlation of *ObRb, AdipoR1*, and *CY19* gene expression for BMI, considering the classification of the 3 study groups (average weight, overweight and obese). Significant differences were obtained for the *ObRb* gene with BMI higher than 30 kg/m^2 (p=0.043) and for the *AdipoR1* gene with BMI higher than 25 kg/m^2 (p=0.034), suggesting an association of the expression of these genes with increased BMI (Figure 2 and 3). We found no significant differences in control for the gene coding for the aromatase enzyme (*CYP19*).

Table 2. Correlation of ObRb, AdipoR1, and CYP19 gene expression coding for aromatase enzyme.

Gen/IMC	< 25 Kg/m2		> 25 Kg/m2		> 30 Kg/m2	
	r	*p*	r	*p*	r	*p*
ObRb	0.217	0.576	0.165	0.382	0.648*	0.043
AdipoR1	0.444	0.231	-0.389**	0.034	-0.464	0.177
CYP19	0.343	0.366	0.168	0.376	-0.115	0.751
* Moderate correlation * * Negative moderate correlation						
Notes: BMI less than 25 Kg/m2 (average weight), greater than 25 Kg/m2 (overweight), and greater than 30 Kg/m2 (obesity), respectively.						

Discussion

Our study obtained from the analysis concerning BMI showed a prevalence of up to 71.1 % of obesity (22.2 %) and overweight (48.9 %). Similarly, in the 2018 Health and Nutrition Survey (ENSANUT), a prevalence in women worldwide in Mexico was estimated at 78.1 % when BMI was higher than 25 kg/m^2 [3].

However, several studies have suggested using BMI as an anthropometric indicator to determine obesity and metabolic risk factors. Furthermore, they should also consider waist circumference values since it is necessary to distinguish between body fat and fat-free mass[37-39].

In this sense, the guidelines on Prevention, Diagnosis, and Treatment of Overweight and Obesity suggest the performance of clinical studies during the evaluation of patients with overweight and obesity, including blood biometry and other parameters [40]. It is important to emphasize that the evaluation of these clinical studies also allows the early recognition of some metabolic or cardiovascular diseases as recommended by the *Canadian Clinical Practice Guidelines on the Management and Prevention of Obesity in Adults and Children 2006* [41]. Notably,

we did not observe significant differences between the study groups.

Additionally, during the determination of some parameters, as in the case of triglycerides, 64 % of the studied population obtained an increase higher than 150 mg/dL, which is a normal range considered as healthy, which could indicate a possible association of increased BMI with high triglyceride levels as shown by Calleja and Sanchez (2012) [42]. It is essential to consider that the age of this study group was above 55 years of age, and it is suggested that they were close to the postmenopausal stage. Likewise, Escobedo *et al.* (2014) noticed that the older the age, the higher the prevalence of presenting increased triglycerides, and that the presence of the postmenopausal stage could be directly related to BMI [28, 43-46].

On the other hand, one of the causes of BMI variations is due to genetic differences, in this sense Tuomo Rankinen *et al.* (2005) presented the map of genes related to human with obesity, reporting an association between DNA sequence variation in specific genes such as *ObRb, AdipoR1*, and *CYP19* and phenotypes. However, these differ between populations [20].

Our analysis of the long leptin receptor expression known as *ObRb* showed a moderate correlation of expression in women with obesity (r=0.648, p=0.043). Several authors have studied the expression of this gene in various populations and have reported that it plays a fundamental role in the pathophysiology of obesity by favoring an increase in % body weight [47, 48].

In the same way, we found a moderate negative correlation of the *AdipoR1* gene with overweight or obesity (r= -0.389, p=0.034), coinciding with the finding of Rasmussen *et al.* (2012), where it decreased in the presence of obesity but increased in the presence of overweight [31].

Indeed, we could not determine an association with the *CYP19* gene, possibly due to some variations between individuals. However, we suggest studying the presence of genetic polymorphisms that provide more information about the association of this gene with BMI [33].

Conclusions

The present study demonstrated that.

1. There is a moderate correlation of *ObRb* gene expression for patients with BMI greater than 30 Kg/m^2 (r=0.648, p=0.043) and moderate negative correlation with the *AdipoR1* gene for patients with BMI greater than 25 Kg/m^2 (r= -0.389, p=0.034) in Mexican women from the State of Mexico and older than 55 years of age who are healthy. However, it is essential to ensure the data replication and extrapolate it to the entire Mexican female population, having more varia-

bles that can influence the diagnosis of this pathology and can be used as predisposing factors.

2. The increase of more than 150 mg/dL of triglycerides in our study population alludes to increased lipoproteins and, therefore, the possible presence of dyslipidemia associated with obesity is possibly caused by physical inactivity inadequate nutrition. However, it is essential to corroborate the behavior of this parameter and others such as cholesterol in different populations to confirm the diagnosis.

3. We suggest the importance of studying genetic polymorphisms or SNPs in the genes involved with variations in BMI and determining the circulating blood levels of certain adipokines such as leptin and adiponectin provide more information about the association with this pathology and the different phenotypes.

4. We also suggest the importance of promoting physical activity and providing help on balanced diets to maintain a BMI below 25 kg/m^2, thus mitigating the risk of developing obesity and developing metabolic and cardiovascular diseases.

Conflict of interests: There is no conflict of interest.

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Research article

Proposal for a model for the care of the sexual health of the young adult before HPV

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Abstract

Introduction. Sexually Transmitted Infections such as HPV mainly affect adolescents and young adults; Women are more susceptible to contracting them due to multiple factors. Promoting sexual health has increased knowledge about prevention, but it is still insufficient. **Objective:** to propose a model of sexual health care in young adults before HPV. **Methodology:** the focus of the study was qualitative, convenience sampling, with 13 sexually active key informants. A semi-structured interview was used in the data collection to identify the paradigm of the young adult against HPV in three categories: sexuality, risk of infection, and care. The theoretical proposal of the model reinterprets the concepts to build the framework of the proposal. **Results:** beliefs or myths prevail about sexuality limited to intercourse as the only source of pleasure; HPV is recognized as an STI, but it is unknown if there are symptoms and it is confused with another STI; there is a risk of infection mainly due to the number of sexual partners, the responsibility for care is left to the couple, and health services are not used as a preventive form. The care proposal emphasizes self-care according to this paradigm that integrates the physical, psychological, self-knowledge and spiritual areas. **Conclusions:** self-care is an interesting concept to address in promoting the sexual health of young adults in the face of the reality that they live concerning STIs.

Keywords: young adult, care, sexual health, HPV, self-care.

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Introduction

Caring is an innate characteristic of the human being; care is given to a sick person, to a child while it is growing up, and to a loved one, and this care implicitly involves providing well-being; it is a relationship between two people, one who cares and the other who requires care. Professional care is developed by nursing professionals with scientific knowledge in health, endowed with technical skills that help individuals, families, and communities to improve or recover their health (1, p. 75) from the care setting to the educational.

Based on these aspects, it is worth asking why care or care is important. Colliere (2) mentions that men have always needed care because this act of life allows it to continue and develop. On the other hand, Leonardo Boff (3) refers that "care is more than an act; it's an attitude. But who or who needs to be cared for? Waldow affirms that "all beings are vulnerable in their existence, but there are moments or circumstances in which vulnerability is more visible, and they need care" (4, p.235). In each stage of life, the human being requires care; in childhood, when one is an adult, and in old age, there are specific needs. From a demographic point of view, the proportion of adolescents and young people is the largest in the world today; there are 1.2 million young people between the ages of 15 and 24, representing 16% of the world population (5). As a stage of human development, in adolescence and youth, physical, emotional, mental, and social changes begin during adolescence, and a transition to adult life is experienced; decisions are made regarding a career, work, or starting a family. The expression of sexuality -which has begun during adolescence generally- is lived according to the norms and rules learned at home, with friends, or through own experience; this determines a particular pattern of behavior, which may or may not be healthy.

According to the United Nations Population Fund (6), young people face the main risks of unchosen, involuntary, and/or unprotected initiation of sexual life, exposure to unplanned, unwanted, or impending pregnancy risk conditions, and exposure to an STI, of the latter, HPV is the most prevalent. Its importance lies in the fact that it is primarily asymptomatic, and many women are unaware of the changes it generates at the cellular level; therefore, persistent infections are frequent and are precursors of cervical cancer. According to the General Directorate of Epidemiology in 2021 in Mexico, the incidence of HPV in the female population between 15 and 19 years of age is 6.8 new cases per 100,000 women of the same age. For the 20 to 24-year-old group increases to 21.3 cases. These data are not comparable with men, where only those between 15

and 24 years old represent 5.61 new cases per 100 people of the same age range (7); Another important fact is that according to the 2021 National Health and Nutrition Survey on COVID-19, only 36.9% of adolescent women reported having received the HPV vaccine (8).

Given this scenario, it is necessary to address the care needs for HPV among young people; Comprehensive sexual education is a sexual right of adolescents, as well as information about sexuality; these and other rights promote and protect health, well-being, and dignity by providing the necessary tools to have knowledge, attitudes, and skills (6) and is a condition for adolescents and young people to exercise their sexuality with autonomy and responsibility. In this sense, international programs focus on promoting sexual health, understood as a state of physical, mental, and social well-being that includes the experience of sexuality pleasantly and safely (9), primarily through education and information comprehensive, prevention of gender violence, HIV prevention, psychosexual orientation, contraceptive methods, among others (10). Returning to these guidelines, in Mexico, there are Specific Action Programs for Sexual and Reproductive Health, but they are directed mainly toward adolescents. However, it is said that fewer risky behaviors are incurred among young people; statistics show high rates of STIs and HPV in this age group, mainly among women. Given this, three risk behaviors related to the spread of HPV have been identified and their inclusion in educational strategies in health promotion: early initiation of sexual relations, not using condoms frequently, and having multiple sexual partners, in addition to preventive information related to the infection and the knowledge and application of the vaccine is integrated, obtaining favorable results to increase knowledge on these topics (11-13). Although there are advances, why does HPV persist in this population despite the attention in preventive matters?

According to Heredia and Rodríguez (14), in Mexico, there are two models in the academic field to approach sexual education: a model of education for reproductive sexual health, which prioritizes responsibility to acquire knowledge, attitudes and healthy and healthy styles, and the model of integrated sexual education, whose purpose is the learning of cognitive, physical, social, and emotional elements of sexuality. However, programs aimed at adolescents are dominated by biomedical content (15, 16). Nursing interventions in the field of sexual health have focused on sexual education, as shown by various studies (17-19). Still, it is also It is important to recognize who the young adult is and the behaviors that make them vulnerable to infection.

This reflection led to consider other aspects of the human being such as the perception or beliefs about the infection, as well

as the methodologies used to address this issue that have focused on the informative aspect and today must be expanded and oriented towards the experience of sexuality, the meanings they attribute to life, the body, care, among others (20). In this sense, Foucault shows a perspective towards self-care, which can be applied to sexual health care; Self-care is knowing, training, and improving oneself to assume certain established rules and principles (21). This is conceptualized as a set of activities that a person usually performs to satisfy their own needs; It is a basic attitude of the human being that responds to physical, spiritual, intellectual, psychic, and emotional needs of himself and others; it demands the cultivation of being integral: body, psyche, mind, and spirit (22).

Promoting self-care from this age is relevant for different reasons; Exposure to infection is determined by the beginning of sexual life, which occurs in the stage of adolescence and young adulthood to a lesser extent; young adults are the population group with the least approach to health services, the perception about of infection is low compared to cervical cancer, it takes more than knowledge to adopt preventive behaviors. Universities are spaces where they develop, learn and acquire habits; therefore, attention and care become key elements to dealing with the issue of STIs and their implications for health and using the means and resources available to create strategies. and alternatives that affect care in their lives. For this reason, the objective of this research is a proposal for sexual health care emphasizing self-care before HPV.

Methodology

The study has a qualitative approach; a Non-probabilistic sample made up of 13 young adults between 18 and 25 years old, men and women from the first period of the Nursing career at UAEMex in February-July 2018 with the beginning of sexual life. Data collection was through a semi-structured interview that began with a trigger question: What care do you take to prevent HPV infection? This question generated additional questions that provided valuable information in analyzing the care carried out by the young adult against this infection. The participation of the students was voluntary, informing previously and verbally about the objective of the study interview, and an informed consent letter was provided where the confidentiality of the data was emphasized.

The generation of a care proposal poses a series of steps or phases; It is a systematized process comprising a reference framework or theoretical framework and a methodology. Implicitly is the planning, development, implementation, and evaluation of the same as organizing the data. For the first phase of the proposal, the construction of the reference framework started with the

content analysis that investigated the meaning of infection for young adults and the care they take to prevent it, transforming unstructured textual data into structured ones. This analysis allowed us first to know young adults' paradigm regarding infection and care for its prevention, from their reality and from which the main concepts that include the proposal in self-care were identified. Considering basic elements already incorporated into educational strategies and care needs interpreted from qualitative data. The analysis generated three main categories: construction of sexuality, vulnerability, and self-care.

For the definition of these concepts, theoretical references such as Heidegger, Foucault, Colliere, and Leonardo Boff were taken up again, which respond to the objective of the proposal, that is, from an existentialist approach, propose a proposal that responds to the concerns and needs and care that the young adult lives and feels, as a person whose existence gives him at some point in his life the expression of his sexuality, the experience of care and the self-care for authentic existence, that is, living with the greatest possible freedom and responsibility.

For this study, the general principles of the Declaration of Helsinki are considered in sections 19 and 20, on vulnerable groups and the confidentiality of information. Regarding the General Health Law in force in Mexico, article 100 preserves respect for the dignity, protection of the rights and well-being of the individual and does not expose them to any risk during the development of the interview or for the investigation. Finally, an Ethics Committee approved the development of the research.

Results and discussion

The paradigm of young adults regarding HPV was organized into three main areas: sexual behavior, risk of becoming infected, and care (Table 1). Based on the WHO definition, sexuality is lived and expressed through various forms according to the context in which the young person lives and practices his sexuality, influenced by biological factors and social, cultural, ethical, and religious issues, to mention a few (10). This gives them a particular conception of sexuality while they are integrating their paradigm; the construction of knowledge about sexuality permeates the sexual behaviors they practice and how they perceive the presence of STIs, particularly HPV. In this paradigm, the biological part of sexuality still permeates the group of young people; It is already known that the beginning of sexual life is decisive for exposure to the genital HPV virus, but if added to this it is observed that young people see sexuality as only sexual contact with another person (with or without reproductive purposes), it implies that they do not recognize other forms of sexual pleasure or satisfaction and it is very likely that young people expose themselves to problems related to STIs sin-

ce they do not distinguish other forms of sexual expression that can protect them or at least reduce the risk of infections, even more so if these They do not show apparent symptoms such as HPV.

Table 1. The paradigm of young adults regarding HPV

Concepts	Young Adult Paradigm
Sexuality	● Sexuality equals reproduction. ● Physical differences between men and women. ● Sexuality focuses on genitals. ● Intercourse as the only form of pleasure. ● Meet the couple. ● There is a sexual morality that approves or disapproves of sexual behavior, it is related to the stigma of HPV (rejection from the family).
Risk of becoming infected	● Have an active sexual life. ● Multiple sexual partners. ● The lack of symptoms. ● It is an infection associated with cervical cancer. ● It is recognized as an STI and is a normal infection. ● Listen and learn about HPV infection. ● Confusion of symptoms with other STIs, mainly HIV. ● The woman is more at risk since the man is only a virus carrier. ● The risk of becoming infected is higher among men than women. ● The infection is preventable.
Careful	● Use of condoms as a safe method, but to prevent pregnancy, not STIs. ● Having only one sexual partner. ● The early start of sexual life is not an important care element. ● Little attendance at diagnostic studies (vaginal cytology). ● Low percentage of women with HPV vaccine application. ● Going to the doctor is the most visible form of care, but not until they observe a sign or symptom. ● The responsibility of caring for oneself falls on the couple (mainly from woman to man). ● There is a feeling of indifference to information as a way of taking care of yourself. ● Care is directed toward preventing pregnancy, not an STI. ● Poor communication with the couple. ● Desire to take care of yourself vs ways of taking care of yourself

Source: Semi-structured interview. Own elaboration

The risk of infection is another aspect that impacts health care in the face of an STI. Since HPV is a very frequent STI, it is to be assumed that young adults have information about it; in fact, it was identified that there are differences between hearing about the virus and being aware of it. And this could be observed when young people identify the main forms of contagion that have to do with sexual behavior (although for this study, the early age for the onset of sexual life was not recognized as a risk factor), which was included in educational strategies to prevent STIs. Knowledge also accounts for the tools that young people have to protect themselves against infection, and this is one of the most studied aspects in research on HPV; Risk behaviors and, therefore, the preventive part are identified, but they are unaware of its associa-

tion with cervical cancer and whether or not there are symptoms, which is why it is often confused with other STIs such as herpes, gonorrhea or HIV/AIDS. However, in addition to sexual behavior and knowledge, an aspect little studied was identified within risk; perception: it is recognized that the risk is greater among women since the man is a carrier (23), but in this study, it is men who are recognized as more susceptible to becoming infected, which was revealing and interesting data from analyze; and this can be related to how they provide care, an aspect that will be discussed later.Finally, how young adults take care of themselves starts from the transmission factors themselves. Still, with some particularities: of the three known risk behaviors, the one most related to HPV is that of multiple sexual partners; the use of condoms is referred to as a preventive method, being this more common and accessible, although more towards avoiding pregnancies (24) and, the age of onset is not considered as a conduit of transmission, neither risky nor much less preventive.

Health services are essential in preventing STIs and even more as a preventive measure; this was another relevant aspect within the young adult paradigm: attendance at screening programs such as Pap smears by women is low, and a minimal proportion of them have been vaccinated against HPV. Since exposure to the virus occurs from the beginning of sexual life, it represents one of the great challenges for working between institutions and society in general to destroy taboos and factors that impact attendance at health services; Young adults do not attend sexual health training or workshops as a way of taking care of themselves, but rather because they are educational programs at school or they respond to scheduled appointments at health centers as part of government program support.

An interesting aspect of addressing and analyzing is that they are not aware of self-care; that is, men and women know that they must take care of themselves so as not to become infected, but the care falls on the behavior and knowledge of the couple: they refer to the fact that they can take care of themselves by going to the doctor or using protection methods, but in the end, they do not know if their partner will have multiple partners or if they go to the doctor, which exposes them to contagion regardless of what each one does. Here it can be deduced that, on the one hand, they desire to take care of themselves and be healthy. Still, in their behavior, the lack of knowledge, couple care, seeking information, and approaching health services prevail.

For the second stage of the model proposal, the concepts that frame the care proposal were defined -where the person, the environment, health, and care are implicit as basic elements- to establish new meanings in the health-HPV relationship. that guide new care guidelines for HPV and other STIs.

Table 2. Conceptualization of the care proposal

Young Adult HPV Paradigm		Conceptualization of the care proposal	
Concepts	**Young Adult Paradigm**	**Concepts**	**Framework**
Sexuality	From the point of view of sexuality, it is important to point out that certain beliefs or myths about sexuality still prevail, referring only to sexuality. presence of intercourse (anal, vaginal, oral) as a source of pleasure.	Experience of sexuality	Rubio (25) refers that the experience of human sexuality focuses on 4 dimensions or holons: reproduction, gender, eroticism, and bonding. affective
Risk of becoming infected	It is related to the knowledge and perception of the infection: HPV is recognized as an STI and it is a normal infection because it is frequent; Although risk behaviors are recognized, having multiple sexual partners stands out, they do not have apparent symptoms, and the woman is vulnerable since the man is only a carrier of the virus.	Vulnerability	They are defined as vulnerable to a specific threat or, one is vulnerable to being in a situation of loss, which can be health, employment, etc. Being vulnerable can appear before the situation, which makes it more or less prone to a specific loss; that is, it is susceptible or, after an event, to how the individual deals with the situation (26). In this sense, being vulnerable to STIs has various aspects, one is vulnerable in various ways: individually and collectively. In the first, the stage of youth itself, which is full of physical, psychological, and emotional changes, makes young adults prone to acquiring STIs; on the other hand, the context in which they live make them vulnerable because it exposes them to risks, such is the case. the case of the beliefs, norms, or ethics under which it has been formed and creates patterns of more or less predictable behavior in this population.
Careful	The use of protection methods such as condoms and the age of sexual initiation is not considered important factors in care or first choice against HPV, there is little assistance to health services as a preventive form, and few women attended to receive the vaccine. This shows that there is little search for information, couple care is infrequent, and the responsibility is left to the couple.	Take care of existence	Being in coexistence with the world. The consciousness of being determines existence; it understands itself from its existence, from the possibility of being or not being. To exist is to always be on the way through the experiences encompassing consciousness, the way of living in the world with a particular meaning (21; 27). The young adult must become aware of his being, this implies realizing his existence, and his existence is knowing himself and the world where he is and from this, being free to make decisions.
		Take care of sexual health	Recognize that sexual health is part of a person's overall health and should consider removing barriers or prejudices, valuing their own body, seeking information about reproduction, establishing meaningful relationships, making informed decisions, and maintaining effective communication, characteristics of a person sexually healthy. It requires comprehensive education, training, and support for health professionals, comprehensive sexual health services, and promoting research on sexuality that generates new knowledge and new forms of care (28, 29).
		Self-care	Self-care is conceptualized as a set of activities a person usually performs to satisfy their needs. In addition to health care, it takes care of thoughts, attitudes, behaviors, emotions, values, and biopsychosocial needs, including goods and everything that generates well-being (22). This makes it possible to focus care on the individual's ability to seek care to prevent risk behaviors, prevent adverse situations, and promote a vision of promoting life rather than care for the disease and thus influence the perception of care that young adults have regarding HPV infection and seeking care

Source: Semi-structured interview. Own elaboration

This conception established the relationships between these concepts and the logical process guiding the proposal as shown in Figure 1. Being a young adult implies a stage of changes in many areas, preparing for academic or professional life, but rarely think about planning to take care of health and much less sexual health, and this is mainly because of the stage of youth where people perform multiple activities, and young people have a sense of well-being, which leads them to take risks without realizing it the consequences in the medium or long term because it is a "healthy population" and the disease is not "part of their life."

These behaviors are determined according to an established role and personal experiences, the experience of friends, acquaintances, or strangers, as well as information sources related to technology and health personnel, complement their knowledge about their sexual health and the behaviors that " must be carried out", although many times these are not healthy. In addition, there are beliefs, morality and ethics of the family and the community influence the behavior of the young person even without him being aware of this, however, social pressure most of the time points them out for not meeting expectations their role as men or women in the sexual realm. These situations place the young adult in a state of both physical and social vulnerability; Although physical vulnerability has been addressed more, it should be noted that since they are in a late adolescence stage, the growth phase has not yet ended and the lack of tissue maturation (for example in the cervical area) as well as other Cofactors such as the use of oral contraceptives or the abuse of tobacco use may favor the easy acquisition of the infection, aspects that should continue to be reported in educational programs (30-32). Social vulnerability on the other hand speaks of the context of the young adult; sexuality continues to be a taboo subject in Mexican society and this aspect should be considered when proposing a strategy to promote healthy sexuality because young adults cannot be isolated from their environment, or suddenly change the way they think and in what you think is right because you were taught that way or because experience has dictated it that way. Many times, you can't change the environment, but you can find tools that become an opportunity to want to take care of yourself. Faced with this reality but also perceived vulnerability, interventions in their health *care* become essential for their well-being; the health professional's role in this aspect must be guided by always listening to the young adult and his feelings about his sexuality and how he lives it. This is an important point because, for many young people, talking about this subject is overwhelming, and their first approach to health services is usually one of rejection or prejudice.

Based on these reflections, the proposal emphasizes *self-care* as a goal; This was an aspect identified as a prevailing need for care by young adults in their sexual health care, although not consciously; the majority of young people (men and women) leave the responsibility for their health care to their partner, so the risk of HPV infection is given "by what the other does or does not do" about care in your sex life; not only because of the relationship with one's own body but also with others and the environment.

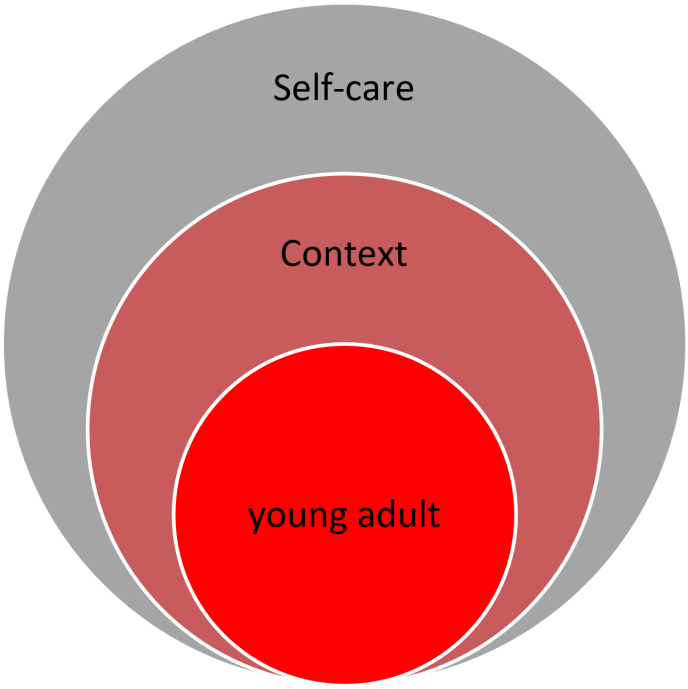

Figure 1. Care proposal based on the young adult paradigm and frame of reference.

This makes it possible to elucidate that taking care of sexual life is not only using the condom correctly and frequently, having only one sexual partner and having information, but also the awareness of human existence, valuing the body, communicating with people, their own pleasure, sexual satisfaction, mental and spiritual well-being that make sexual life more fulfilling at this stage of life; with informed decisions and with the responsibility to also care for the other, from the perspective of promoting sexual health.

For Heidegger, care seen from existentialism is before any attitude that the person may have or the situation in which they find themselves, it is the first root of the human being and it is an essential way of being; and this arises when the existence of the other person is important to me as a person, it represents an intrinsic action, but at the same time it is a conscious act born from the circumstances, caring and taking care of oneself is recognizing one's own existence in the first place (33). In accordance with Boff, caring is an essential characteristic of

the human being and lies in its own nature; this human condition reveals an evolution of their physical condition, thoughts and emotions in the interaction and adaptation with their environment. This must provide a sense of existence and, therefore, a value in caring for life, the other, and the world in which one lives (3).

Now, and returning to the existential part of care, self-care (Figure 2), according to Foucault, creates a consciousness that then allows the potentization of both individual and group strengths to care for oneself above all else, not because it is present in this world, but because his existence requires him to take care of himself so that this existence is authentic, and that is where well-being fits.

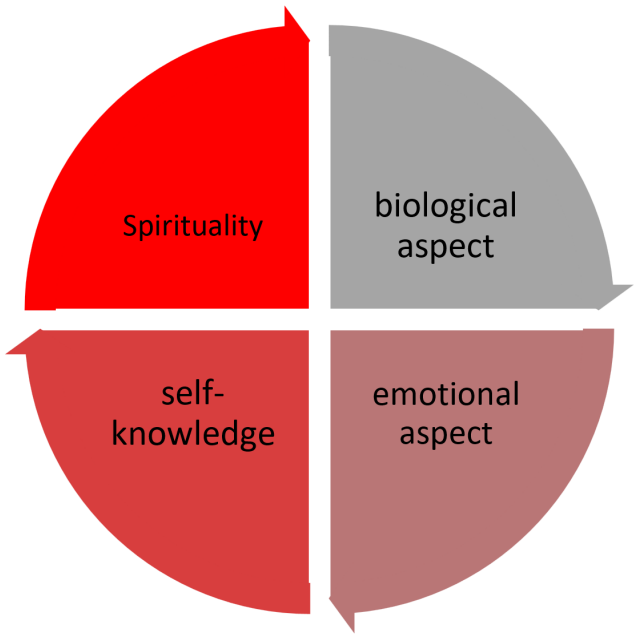

Figure 2. Base elements for self-care as a care model for HPV in young adults.

It is a way of caring that enables the person, it is an interpersonal relationship, it also represents an educational approach at the individual level, and it represents a transformative practice because it is a model of understanding, respect, sensitivity, and solidarity, which make up dimensions of care through accompaniment and monitoring in the first individual instance.

Self-care appeals not only for the assistance of urgent needs but guides the individual, family or community to take care of their health, a fact that implies a constant throu-

ghout their lives. Foucault also mentions that taking care of oneself is to know, train and improve oneself in order to assume certain rules and principles established in morality that led to the truth (21). It has to do with the relationship with others because it involves how one takes care of oneself and of caring for others, but self-care should always be above all else because it is ethically and ontologically the first philosophy of the relationship with oneself; this is related to the body constitution and the soul which are connected and represent the actions of people; that is, that the body is understood in addition to the biological aspect in the relationships and experiences that the person lives as well as the educational processes that make up this relationship between body and soul to seek virtues and cultivate them, in the formation and authentic valorization of the body itself (34).

Aspects that need to be taken up again within the physical aspect of caring for oneself are the assessment and knowledge of one's own body; most young adults are unaware of signs and symptoms not only of HPV infection but of other STIs, or there is confusion about it. This leads to the fact that in the event of an anomaly, men, and women see as normal what is some alteration, in addition to the feelings of shame that this can lead to, and therefore, they are not tended promptly. Although some infections can present symptoms, many others are asymptomatic, making their identification difficult. One of the proposals that at the educational level is integrated into the strategies is to provide information on STIs such as syndromes; that is, various STIs share signs or symptoms, and in the event of any of them, it is important to check oneself. This breaks with a traditional scheme of listing STIs and their characteristics, information that is little captured by young adults. They generally have information on the most frequent ones or those where more prevention is done, such as HIV or HPV.

Another aspect identified and that gains importance is that sexuality is seen in addition to reproductive purposes, such as only having coital relations. Undoubtedly, it is a way of experiencing pleasure, but it is not the only one. This perception of sexuality limits its exercise to the genitals (29), considering there are other ways of experiencing pleasure without necessarily having intercourse. This aspect is important because susceptibility can be reduced if you have other practices (whether with safe sex or protected sex) where the risk of becoming infected is lower. This information should be provided within educational programs on sexual health and contraception promotion.

Self-knowledge is another element to consider as a way of taking care of oneself, understood as the person's beliefs about the qualities that make them know themselves. This faculty of observing themselves (in-

trospection) accounts for their thinking and the meaning of their experiences. This includes both self-esteem and self-concept (35). According to Guevara (22), these aspects are key to developing favorable behaviors and is based on values that produce well-being and feeling good about oneself, which generates behaviors that protect health. Hence, strengthening these aspects can help to change the behavior of unhealthy lifestyles observed by the same young adult; behavior or behavior change is the goal to prevent STIs and is an aspect that takes a long time to achieve Because it is a process and often remains unfinished, it requires a commitment from both the young adult and the nursing professional (36).

Finally, one of the aspects that are left aside is spiritual needs, understood not as religiosity (which will undoubtedly be important in the life of the young adult if he considers it a significant aspect) but as those activities that, in addition to feeling a feeling of pleasure, have a meaning in my person, my life, with my family or in my social relationships or as a couple. This meaning creates a feeling of well-being and commits me to my person, my health, and its care not as a duty but with the responsibility and freedom of how I want to live and exercise sexuality.

Conclusions

Given the statistics that account for the constant incidence of STIs, health strategies promote sexual education focused on reducing risky sexual behaviors mainly, focusing on using condoms and emphasizing contraceptive methods in case of unplanned pregnancies desired. These strategies are focused on the adolescent population, which, although it is a vulnerable population, ranges from young people from 19 to 25 or up to 29 years of age also requires joining health programs related to sexuality and reproduction and considering beyond contraception, beliefs, perceptions, and thoughts about sexuality not only in the physical sphere but also in the social or spiritual sphere, aspects that, according to Rubio Aureoles, need to be in harmony for health education strategies to be more effective. Healthy sexuality is essential for these young people because it impacts the development of other spheres of life (social, psychological, etc.), and learning to take care of it brings well-being, security, satisfaction, fulfillment, or any other feeling that gives the young adult a pleasant sensation. Taking care of oneself is not learned overnight if it can be taught; it is a process and, as such, requires a path that must be guided by personnel who demonstrate empathy and the responsibility of wanting to know the other person to offer their aid; on the other hand, the young adult must have a willingness to know himself in all areas of his life, because in this way he will identify the strengths and weaknesses that he can use in his health care through being aware of his existence and the changes you

can make in your life that put your health at risk. Taking care of your own health by taking care of the other, of your partner, because it reflects what you do for yourself and exercising healthy and responsible sexuality by your own way of seeing life.

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Review article

Systematic review: Acute abdomen in paediatrics

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Abstract

Introduction. Abdominal pain is a frequent reason for consultation in the emergency department, being the result of various pathologies, mostly of benign origin. However, the lack of timely identification and treatment can lead to significant complications. **Objective.** The purpose of this review is to deepen in the etiology of acute abdomen in pediatric patients. Accurate identification of the underlying cause not only enhances early diagnosis, but also plays a crucial role in mitigating potential complications. A thorough understanding of the origin of this condition lays the foundation for the implementation of more accurate treatments, with a positive impact on medical care. **Methodology.** An exhaustive literature search was conducted in renowned databases such as Pubmed, Google Scholar, ScienceDirect and Springerlink, as well as in medical literature, during the period from June to December 2021. This systematic review addresses the essentials of the topic of Pediatric Acute Abdomen, a frequent clinical entity in both emergency settings and pediatric primary care. Of note is the lack of familiarity with this phenomenon, as well as the need for studies addressing the predominant causes according to different pediatric age groups. In addition, there is a notorious lack of specific data on this phenomenon in the context of pediatric health in Mexico. **Conclusions.** This analysis aims to fill this gap, providing a more complete and detailed view on acute abdomen in pediatric patients, underlining the urgency of further research to enrich our understanding of this pathology in the Mexican setting.

Keywords: abdominal pain, acute abdomen, pediatric patient, diagnosis, evaluation, medical education.

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Introduction

The International Association for the Study of Pain (IASP) defines pain as *"an unpleasant sensation and emotional experience in response to actual or potential tissue damage"*. Acute pain is primarily due to sudden tissue, parenchymal or organ injury due to a postoperative event, trauma or, among others. (1) "Acute abdomen" (AA) is characterised by a 48-72 hours progression, with pain which is continuous, severe, localised or generalised and painful at abdominal palpation, in addition to deterioration of general condition. "Acute abdomen" (AA) is characterised by a 48-72 hours progression, which is continuous and severe. In addition to deterioration of general condition and painful abdominal palpation. (2) Also, it constitutes a set of signs and symptoms; like nausea, vomiting and fever, that manifest the existence of an intra-abdominal disorder that may require surgical intervention. (3)

Within the emergency department and primary paediatric care, abdominal pain is usually one of the most frequent reasons for consultation and in most cases tends to originate from non-surgical causes, which is why an adequate differential diagnosis is necessary according to its presentation in acute episodes, and intermittent and recurrent abdominal pain. Diagnosis and therapeutic follow-up depend on the patient's age, as the causes are different with development.

Acute abdomen is a spectrum of diseases that have insidious and unbearable pain as a common feature; depending on its location in different quadrants of the abdomen, it can indicate the root of the etiology. (2) This spectrum requires an in-depth study of the causes, because despite being a common entity in the paediatric population, the symptomatology is ambiguous, not to mention that in this age group, infants manifest pain through crying, making early diagnosis difficult; this is often unnecessary because it is based on an erroneous examination (4) or because the peritoneal innervation is too extensive, which causes more intense pain than the real source. (5)

Acute appendicitis is generally one of the most common causes of surgical acute abdomen in children, although it can be difficult to differentiate from other aetiologies. (6) Based on acute abdomen, Villegas (October 2017) mentions: *"the most frequent surgical conditions in pediatric patients are appendicitis, and intussusception (the most common cause of intestinal obstruction, between three months and six years of age, 90% of cases occur before 24 months of age)".*

Despite efforts to make imaging a more accurate diagnosis, clinical manifestations, as well as physical examination, remain the cornerstone of diagnosis. (7) The aim of this review is to analyse and unify the existing information on acute abdomen in the paediatric population.

Etiology

There are multiple classifications to find the aetiology of acute abdomen in paediatric patients, for example, the location of the pain. Figure 1 shows a scheme that illustrates the most common aetiology of AA, in relation to the region where the pain is located:

FIGURE 1. ACCORDING TO: IVÁN BAUTISTA, IVÁN RIVAS, GERARDO BLANCO.

- Epigastric: initial stage of appendicitis, pancreatitis, acid peptic disease.
- Periumbilical: early stages of appendicitis, Meckel's diverticulum, gastroenteritis, constipation.
- Right flank: cholecystitis, cholelithiasis, hepatitis, irritable colon.
- Lower hemiabdomen: appendicitis, ovarian cyst, ovulation hemorrhage, urinary tract infection, adnexitis, testicular torsion.
- Right iliac fossa: appendicitis, ovarian cyst, ectopic pregnancy, ileitis, mesenteric adenitis, urinary tract infection.

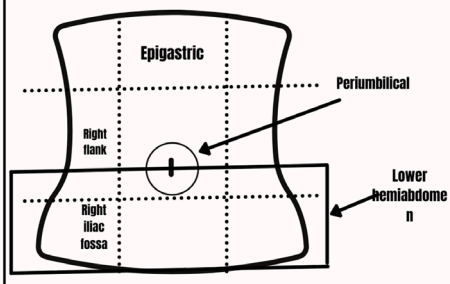


Figure 1. Own elaboration based on 8. Antonio J, Cadenas A, De La M, Espí T. Diagnóstico y tratamiento del dolor abdominal agudo (abdomen agudo) en Urgencias. Soc Española Urgencias Pediátricas [Internet]. 2020;1:197–213. Available from: www.aeped.es/protocolos/

The aetiology of acute abdomen varies according to the age of the patient, as observed in the study carried out by the Paediatric Emergency Department, Hospital Infantil Universitario Niño Jesús, Madrid, Spain, the aetiologies of AA most frequently found in patients under two years of age are: intestinal invagination, acute appendicitis, intestinal volvulus, incarcerated hernia; in children from 2 to 5 years of age: acute appendicitis, incarcerated hernia, Meckel's diverticulum, intestinal obstruction due to previous surgery, tumours; in children over 5 years of age: acute appendicitis, Meckel's diverticulum, intestinal obstruction due to previous surgery, tumours: acute appendicitis, incarcerated hernia, Meckel's diverticulum, intestinal obstruction due to previous surgery, tumours; in children older than 5 years: acute appendicitis, inflammatory bowel disease (IBD), cholecystitis, pancreatitis, diabetic ketoacidosis, testicular/ovarian torsion, nephritic colic and tumours; while in adolescents the following stand out: acute appendicitis, testicular/ovarian torsion, pelvic inflammatory disease, nephritic colic, cholecystitis, pancreatitis, hepatitis, IBD, tumours and ectopic pregnancy in the case of the female sex. (8)

Finally, and with a focus on the current perspective of the COVID-19 pandemic, a

study was conducted in the Department of Paediatric Surgery, Hospital Universitario Pediátrico Niño Jesús in Madrid, Spain in patients under 18 years of age with a clinical diagnosis of suspected acute abdomen with COVID-19. In all cases the reason for ED consultation was abdominal pain, mainly generalised or localised in the left iliac fossa, associated with fever, vomiting and diarrhoea. This retrospective observational study concluded that SARS-CoV-2 infection can cause digestive symptoms as the first clinical manifestation, simulating AA. It is therefore important to perform a good differential diagnosis, a good clinical history and a complete physical examination, not forgetting blood tests and imaging, to avoid paediatric inflammatory multisystemic syndrome. (9) With this evidence it can be said that a possible new cause of acute abdomen in the paediatric age group is derived from SARS-CoV-2 disease.

Pathophysiology

The pathophysiology of the acute abdomen (AA) is addressed by studying its main and most frequent expression: abdominal pain. The definition of pain according to the International Association for the Study of Pain (IASP) states that: *"Pain is an unpleasant sensory and emotional experience associated with or similar to actual or potential tissue damage"*, being abdominal pain as long as it is located in this specific anatomical region even if the damage is not located in any abdominal structure. (10)

To approach the type of acute abdominal pain, it was classified according to its origin and its pathophysiological mechanism. Depending on its origin, it is subdivided into abdominal and extra-abdominal pain, the former considers the damage in any segment of the digestive tract, either solid viscera or abdominal structure (spleen, pancreas, liver, gallbladder or mesentery), as well as damage to the genitourinary system, spine, skin or soft structures of the abdominal wall (2), while the extra-abdominal pain is that damage whose origin is in structures far from the abdomen, such as the thorax.

To understand the pathophysiological mechanisms of abdominal pain, we must be clear about some basic points about the receptors, the stimuli and their distribution in the organs involved, as well as their classification into three categories: visceral, parietal and referred.

Pain receptors (nociceptors) are free nerve endings located in various tissues that are sensitive to mechanical, thermal and chemical stimuli (11) with little or sometimes no adaptation to them; Regarding AA, their location in the wall of the hollow viscera, in the capsules of solid organs, in the parietal peritoneum (the visceral peritoneum lacks these receptors) and in the mesentery must be remembered, as well as the predominant painful stimuli in these structures, which

are mechanical and chemical, represented by distension, ischaemia and inflammation respectively, the latter mainly in the mucosa of the hollow viscera, which are especially sensitive to chemical stimuli. (12)

The receptors when excited will generate an electrical signal which travels along specific nerves depending on the damaged tissue and the type of painful stimulus involved, factors which together with the patient's own characteristics, such as pain threshold, will define the different characteristics of pain.

Symptomatology

An in-depth study of the patient's symptoms will allow the clinician to make a diagnosis and adopt an appropriate course of action to speed up patient care and avoid excessive requests for laboratory and imaging studies that would delay the resolution of the condition.

We divide the important symptoms to investigate in the course of the AA that accompany the pain into two categories: digestive and extra-digestive symptoms. (2) Digestive symptoms include: vomiting, diarrhoea and constipation, while extradigestive symptoms include: fever, voiding symptoms, cough, rectal bleeding and gynaecological symptoms in adolescents. (15)

Digestive symptoms include:

- Vomiting: It is very unspecific and can accompany almost any childhood pathology. Despite this, some of their characteristics point to the existence of an important organic pathology (surgical or otherwise). (15) Food vomiting, bilious vomiting, faecal vomiting that are persistent and follow pain are indicative of surgical pathology. (2)

- Alterations in bowel habits: It is important to know whether diarrhoea or constipation and the presence of mucus, pus or blood in the stool coexist with the pain, as this will indicate the cause. For example, the most frequent cause of pain with vomiting and diarrhoea is acute gastroenteritis, although it may also be the clinical presentation of appendicitis (stools of a soft and scanty consistency may appear), mainly retrocecal. (15,16) Inflammatory bowel disease (IBD) or haemolytic-uraemic syndrome (HUS) must always be considered. In the case of isolated constipation, it is a frequent finding in non-specific abdominal pain, but when it is associated with vomiting: a bowel obstruction syndrome must be ruled out. (12)

- Hematochezia: The presence of blood in faeces with a redcurrant jam - like appearance in an infant accompanied by colicky abdominal pain makes it necessary to look for intussusception, however, it is a late sign appearing in only 15-20% of cases. The most common cause in older children is IBD. (15)

Extradigestive symptoms are:

- Fever: if it appears concomitantly with alteration of the general condition, it is suggestive of surgical pathology. (2) In acute appendicitis it is moderate (38-38.5 °C) hours after the onset of pain, while in gastroenteritis, it appears from the beginning and may be high (39 °C) in enteroinvasive or low (37.5-38 °C) in viral aetiology. When fever is higher than 40°C from the onset, it suggests infectious pathology. (15)

- Urinary symptoms: Dysuria with pollakiuria and urgency should raise suspicion of urinary tract infection. It is important to investigate for polyuria and polydipsia because patients with diabetic ketoacidosis may report significant abdominal pain simulating surgical AA. (2,15)

- Respiratory symptoms: The presence of respiratory symptoms will force us to rule out lower lobe pneumonia, derived from the fact that children with respiratory distress or pneumonia may complain of abdominal pain. (2) The presence of cough, dyspnoea and fever should be questioned.

- Gynaecological symptoms (in adolescents): In adolescents who have already had menarche, the date of the last menstrual period should always be asked, and the possibility of ectopic pregnancy, miscarriage or dysmenorrhoea should be considered in case of acute abdominal symptoms. When the patient has a Tanner scale of less than IV and no menarche, imperforate hymen should be considered. (15)

Diagnosis

The diagnosis of AA is always a challenge for the clinician and is more complex in the paediatric age group, especially in the non-verbal child (neonate, intubated infant, infant with disabilities or of foreign origin) because he/she is deprived of the essential means to communicate his/her experience with the limitations that this entails. (1)

The clinical history and physical examination become an opportunity to build a solid relationship with the child's parents. (17)

This process is based on a thorough collection of anamnestic data, a methodical physical examination and a rational request for complementary tests, remembering that an adequate history and a thorough physical examination are the cornerstone for establishing the diagnosis. (1) Before starting the diagnostic process, it is essential to know the main pathologies that cause acute abdominal pain according to age groups and their frequency. (2)

Anamnesis

The history taking should allow both the parents and the child to describe the entire chronology of evolution. (17) It is important to carry out an anamnesis aimed at seeking oriented data that should include the following information:

Pathological history: it is important to know the presence of a history of abdominal surgery or similar previous episodes, medications received, accidental or intentional ingestion of drugs, as well as a history of trauma or underlying diseases. (2) In the specific case of adolescent women, it is of utmost importance to collect gynaecological history, including the date of the last menstrual period, as well as the presence of risky sexual behaviour with the use or not of contraceptive methods. (16)

When taking the history we must approach the semiology of pain carefully, as it poses a significant diagnostic challenge due to the wide variety of underlying causes and also the inability of the child or parents to describe it. (5)

Location: We can classify it according to where the pain is located; epigastric (pain originating in the liver, pancreas, biliary tract, stomach and upper intestine), periumbilical (originating in the distal part of the intestine or in the cecum and proximal colon), hypogastric or suprapubic (originating in the distal part of the large intestine, urinary tract or pelvic organs), generalised (found in cases of referred pain originating in other non-abdominal organs) and sacral (related to pain originating in the rectum). In general, the more distal to the umbilicus the location of the pain, the greater the risk of surgical pathology. (16)

Intensity: This is a key point, not only for diagnosis, but also for the correct treatment of pain and its subsequent reassessment. Commonly the subjective assessment of pain by the physician does not correlate reliably with the pain actually felt by the patient; this is an important limitation in newborns and infants, which reduces its difficulty in preschool children. Because of the above problem, objective tools have been proposed and established to assess pain in the paediatric age group. It is recommended to use validated scales, depending on the age of the child, to establish pain intensity. (16)

The purpose of using age-appropriate scales is to objectify the subjective. Both the reliability and validity of these tools have been regularly verified, allowing us to rely on these data in a scientific manner and thus to adapt our pharmacological treatment. (1) Although we can rely on the scales, we must always compare them with the patient's clinical history, evolution and associated treatments, and keep in mind the possibility of the child lying (preschool age and beyond). Table 1 summarises the tools used by age in the child.

Table 1. Own elaboration based on Annequin D, Ngo J. Assessment and management of pain in neonates, children and adolescents. EMC - Tratado Med [Internet]. 2019;23(1):1-6. Available from: https://doi.org/10.1016/S1636-5410(18)41701-1.

PAIN ASSESSMENT TOOLS BY AGE	
0 to 4 years old	Behavioral heteroevaluation by observation of the child. Neonatal pain and discomfort scale (EDIN) Neonatal lnfant Pain Scale (NIPS) Pediatric Pain Evaluation Scale (EVENDOL) for estimating pain in children under 7 years of age in the emergency department. Verbal Rating Scale (VRS)
4 to 6 years old	Faces Pain Scale-Revised
From 6 years of age	Visual analog scale (VAS) with a vertical slider Numeric Pain Rating Scale: «rating between 0 and 10»

Mode of presentation: It is classified as acute (usually indicating mechanical processes such as intestinal perforation, ectopic pregnancy, intussusception, testicular torsion or ovarian torsion); gradual (in inflammatory processes, such as appendicitis, pancreatitis or cholecystitis); or intermittent (in gastroenteritis or colic, usually corresponding to referred pain in the biliary tract or its ducts): appendicitis, pancreatitis or cholecystitis); or intermittent (in gastroenteritis or with colicky character it usually corresponds with referred pain of the bile ducts or their ducts, pancreatic ducts or urinary tract, uterus or fallopian tubes). (2,16)

Duration: Intense pain lasting more than six hours points to a surgical pathology. It is important to know the relieving and aggravating factors of pain, for example, abdominal pain in appendicitis tends to worsen with movement and subside with rest, while pain that worsens with urination should suggest a urinary origin. (16)

Type of pain: it is classified as colicky, stabbing (suggesting obstruction of the gastrointestinal or genitourinary tract) or continuous (related to acute inflammatory processes). (16) Diffuse pain usually occurs in evolved situations of the above processes. On the other hand, it is essential to know if there is irradiation of the pain.

Physical examination

Initial assessment

When the patient arrives at the ED, an initial assessment and stabilisation should be performed using the Paediatric Assessment Triangle and the ABCDE sequence (15), which can be applied to identify major physiological problems and initiate initial stabilisation, based on assessment of appearance, work of breathing and colouring. (16)

If there is Central Nervous System dysfunction (due to pain or poor cerebral per-

fusion) or in case of shock (compensated or decompensated), the patient should be stabilised before taking a detailed history and physical examination. Vital signs are measured and a peripheral venous line is cannulated for capillary glycaemia, intravenous fluids are administered and supplemental oxygen is provided if necessary. (15)

Assessment of pain intensity: It is a priority to assess pain using an age-appropriate scale to allow reliable determination of pain intensity in order to administer the most appropriate analgesic treatment. (15,16)

General physical examination

It is of utmost importance, whenever possible, to empathise with the child and gain their trust in order to facilitate an adequate examination. It should be thorough and complete with inspection of the abdomen in its entirety, including the otorhinolaryngological area (acute pharyngotonsillitis or otitis), chest, genitalia and skin inspection (15), which should always be performed since abdominal pain is commonly a non-specific symptom related to extradigestive pathology. (16)

It is important to assess the severity of the patient's condition through consciousness and activity. Vital signs should be taken on a case-by-case basis, with temperature (to assess the presence of fever) and weight in all patients, as well as hydration status. (15)

We must assess for signs of haemodynamic instability (the presence of tachycardia may suggest compensated shock due to sepsis or hypovolaemia, while hypertension is present in Schönlein-Henoch purpura or as a result of haemolytic-uraemic syndrome, and hypovolemic shock in an adolescent may be related to ectopic pregnancy). If dyspnoea is present, we should assess for oxygen saturation and respiratory rate (the presence of rapid breathing with abdominal pain suggests peritonitis or pneumonia; Kussmaul-type breathing may be related to diabetic ketoacidosis). (15)

Always look for signs of possible extra-abdominal causes such as pneumonia, urinary tract infections and meningitis. In the skin the presence of purpura suggests Schönlein-Henoch purpura, if erythema nodosum and granulosa pyoderma are present it suggests inflammatory bowel disease.

Abdominal examination

Physical examination of the abdomen

Inspection: This begins with observation of the child's behaviour and attitude on the examination table in search of scars from previous surgery, external signs of bowel movements, bultomas (incarcerated hernias), distension, haematomas (trauma and in unusual locations due to leukaemia, haemophilia or maltreatment), petechiae, purpura, jaundice (in haemolytic crisis, in

acute gallbladder pathology or hepatitis), external inflammation, tumours or masses (inguinal or scrotal) and skin lesions which may be exanthematous (rash) due to viral infections or anaphylaxis, or on the other hand, localised such as erythema nodosum or pyoderma gangrenosum. (15,16)

Auscultation: This is performed in all quadrants of the abdomen to assess whether peristalsis is increased, decreased or alternating. (16) If there is an increase in the hydro-aerial sounds, gastroenteritis is suspected; the presence of abdominal sounds with signs of struggle alternating with periods of silence suggest obstruction. A decrease in abdominal sounds suggests peritonitis, and a complete absence of abdominal sounds suggests ileus. (15) The cardiopulmonary system should be auscultated if it is suggestive of pulmonary pathology.

Percussion: We can assess the presence of tympanism. The presence of generalised tympanism will suggest meteorism or, on the contrary, if there are warning signs, it is associated with obstructive or peritonitis syndromes (intestinal obstruction or perforation). (15,16)

Palpation: Should be performed in a gentle and relaxed manner, assessing the patient's expression in order to obtain their cooperation while looking for rigidity and masses. (15) Starting with the quadrant furthest away from the pain in a gentle manner, and finally, we locate the area of greatest painful intensity, observing the child's expression to locate the area of maximum pain. (15,16)

Finally, we perform the different appendicular signs: Psoas, Rovsing, Blumberg or gallbladder pathology (Murphy). Signs of peritoneal irritation strongly suggest surgical pathology. (15,16)

Deeper palpation may reveal abnormal abdominal masses (intussusception, abscesses, tumours) and visceromegaly (splenic sequestration or haemolytic crisis in patients with sickle cell disease) which should be evaluated. (15,16)

Anatomical correlation of the nine quadrants into which the abdomen is divided is the mainstay of the physical examination, and a systematic approach pattern is required together with established anatomical knowledge so that vital details are not overlooked.

External genitalia: Inspection and palpation of the external genitalia is indispensable for the correct assessment of abdominal pain. In the male, the existence of balanitis or urethritis justifies the existence of acute abdominal pain. (16)

Testicular inflammation with hydrocele leads us to suspect possible involvement of the spermatic cord, testicular torsion or inguinal hernia. Gynecological examination should be performed in girls who report vaginal discharge, sexually active adolescents, as well as in all cases of suspected

sexual abuse. (15,16) The most common causes of abdominal pain of gynaecological origin in paediatrics are evolving clinical expressions of an imperforate hymen or vaginal atresia.

Rectal examination: This is an examination that is uncomfortable for the patient, and is not currently recommended unless necessary, as well as in suspected peritoneal syndrome, to determine whether there is lateralized pain at the level of the cul-de-sac of Douglas or a mass effect, to assess whether there is anal stenosis, sphincter tone, palpable masses and, finally, the size and presence of faeces in the rectal ampulla (useful in the assessment of faecal impaction). (16) It will be full of faeces in functional constipation and empty in Hirschsprung's disease. (15) It also helps to assess internal genitalia in girls, as it facilitates palpation of the cervix and corpus uteri, as well as adnexal swelling.

Active manoeuvres: Evaluate the following manoeuvres: sitting up from a lying position, lifting the legs or attempting to jump, as they are limited in the case of peritoneal involvement. If the clinical history and examination do not provide a clear diagnosis, the patient should be placed under observation and the manoeuvres repeated. (15)

Complementary explorations

The extensive aetiology of AA confronts us with multiple possible complementary tests and our conduct will be dictated by the clinical context of each patient so that we use them rationally.

They will be requested depending on the findings during the anamnesis and physical examination, as they allow us to orientate the cause of abdominal pain in most cases, so that a rational use of complementary explorations in the assessment of abdominal pain is recommended; it is relevant to mention that if there are no alarm signs or symptoms, no complementary test will be necessary. (15,16) If AA is suspected, radiological or laboratory tests should be performed to complete the evaluation.

Blood tests: In patients with incoercible vomiting or signs of dehydration on physical examination, the presence of water and electrolyte disorders, hypoglycaemia and impaired renal function should be ruled out (urea and creatinine levels will help in the assessment of renal function and hydration status). (16)

The presence of an elevated white blood cell count (with neutrophilia), as well as an elevation of acute phase reactants, suggests an infectious process as in the case of appendicitis, in some cases of cholecystitis and in about half of the cases of intestinal obstruction. (16) Anaemia points to pathology with blood loss. Examination of peripheral cells may reveal red blood cell destruction and thrombopenia, as in haemolytic-uraemic syndrome.

Liver and pancreatic enzyme studies are useful if the clinical history suggests liver or pancreatic pathology (serum levels of amylase, transaminases and lactate dehydrogenase will help in the diagnosis). (16)

Urinalysis: This should be performed whenever there is a voiding syndrome associated with abdominal pain and should be systematically indicated when abdominal pain affects infants, due to its relevance as a differential diagnosis. (16)

Pyuria and haematuria may be present in urinary tract infections and renal lithiasis, usually pelvic appendicitis may show isolated leukocyturia and/or microhaematuria associated with bladder irritation. If haematuria is associated with protheinuria, the possibility of Schönlein-Henoch purpura or haemolytic-uraemic syndrome should be evaluated. Ketonuria and glycosuria may be part of a diabetic debut. (16)

Pregnancy test: Should be considered in postmenarchal adolescent girls with acute abdominal pain. (16) A test to rule out the possibility of pregnancy should be performed prior to radiological studies. Secondly, it is used for the study of pregnancy situations, such as ectopic pregnancy.

Imaging in AA in paediatric patients: Due to the often nonspecific symptoms, imaging in the acute abdomen in childhood plays an extraordinarily important role, as it will guide the course of a surgical emergency towards surgical intervention or conservative treatment. The GOLD standard in these pathologies is abdominal ultrasound. (18)

Simple abdominal X-ray: Mainly in standing, supine or right lateral decubitus position. In most cases, it is not useful for diagnosis. (16) It is difficult to interpret and involves subjecting the patient to a non-negligible source of radiation.

Current recommendations are not to routinely perform abdominal radiographs in children with abdominal pain. The silhouette, size and location of the kidneys, liver and spleen should be assessed. The borders of the psoas should be sharp. Pathological findings include hydroaeric levels, dilated or thickened loops and the presence of pneumoperitoneum or intestinal pneumatosis (15).

Chest X-ray: Confirms the diagnosis of pneumonias that may simulate acute abdomen. It is recommended when alterations in respiratory auscultation are found, as well as in patients under 3 years of age with catarrhal symptoms and persistent fever associated with abdominal pain. It is diagnostic in intussusception (16) and can aid in the diagnosis of acute appendicitis. Occasionally, it can reveal the presence of mesenteric adenitis, help in the diagnosis of pancreatic and gallbladder pathology and hydronephrosis.

Abdominal ultrasound: Female genital ultrasound is very useful for the diagnosis of adnexal pathology (ovarian cysts, ovarian

torsion and presence of pregnancy); while testicular doppler ultrasound can be useful in certain cases of acute scrotum such as testicular torsion and epididymitis. (16)

Bedside abdominal ultrasound by trained clinicians is a tool increasingly used for the assessment of traumatic or non-traumatic abdominal pain, allowing early diagnosis, and thus speeding up treatment (16). It is widely useful in the assessment of acute abdominal pain and, compared to other scans, has the advantage of not subjecting the patient to radiation.

Abdominal computed tomography: This procedure has a high radiation exposure. Alternative imaging modalities will be our most common tools due to their greater diagnostic certainty without radiation exposure. If used, focused studies with low radiation dose are recommended.

Contrast CT is useful for the evaluation of patients with acute abdominal pain, when a wide variety of diagnoses are considered, it can help in tumours and abdominal trauma. In addition, it has a high sensitivity and specificity for diagnosing appendicitis and is the most sensitive imaging test for paediatric nephrolithiasis. (18)

Abdominal MRI: Not widely used for the assessment of acute abdominal pain in the child, because it is too time consuming and requires sedation, its usefulness in the diagnosis of acute appendicitis is similar to that of computed tomography, without subjecting the patient to irradiation (16), a fact reaffirmed in an article where according to Warner et al. the appendix can be identified by MRI in most children, which is similar to the results of computed tomography reports. (19) It is used diagnostically to clarify unclear ultrasound findings. (18)

Treatment

In paediatric patients presenting with AA in the Emergency Room, ABCDE manoeuvres are performed to stabilise them, followed by analgesia, which is the cornerstone of treatment, and in the event of nausea and vomiting, antiemetics are administered. (8,20)

Once the patient is stabilised, the cause is sought through clinical examinations and imaging to confirm it, but in the meantime, priority should be given to identifying warning signs and symptoms that indicate a surgical emergency, placing them on serum therapy. (2)

The definitive treatment is chosen on the basis of the aetiology found, which is why it is divided into conservative or non-surgical and surgical; the former refers to treating the symptoms with analgesia and the infection found with broad-spectrum antibiotics for gram-negative bacteria. (8,20) The second consists of performing a laparoscopy or laparotomy, usually as an emergency procedure. Currently, laparotomy is more commonly

used because the literature reports that no complications were found after laparotomy, while in laparoscopy adhesions were seen some time after surgery. (21)

In the COVID-19 times it was reported that PCR should be performed in patients arriving with abdominal pain as this virus causes symptoms similar to AA but should not be operated on. (9)

Prognosis

The prognosis depends on the aetiology and early diagnosis, however, it has been reported that males are more likely to have a higher chance of surgery. (20,22) For symptoms resembling AA caused by COVID-19 the prognosis is good. (9)

Material and method

A search was made for articles in Pubmed, Google Scholar, ScienceDirect, Springerlink databases; and medical literature, said search was carried out from June to December 2021, about Acute Abdomen in paediatric patients.

Inclusion criteria:

1. **Publication filter**

- Articles pertaining to health sciences, focused on paediatric patients and no older than 5 years.
- An exception was made for a book published in 2008.
- Included were: review articles, case reports, descriptive studies, cohort studies, case-control studies, conferences and prospective studies.

2. Word filtering:

- Taking as keywords: paediatric acute abdomen, acute abdomen, children, paediatric, kids, kids, children, pain and the Boolean operators used: AND and OR. As an example: "paediatric acute abdomen OR acute abdomen in children".

Exclusion criteria

- Texts whose focus was acute abdomen in adult patients were excluded.
- Texts that did not have paediatric acute abdomen as a central theme were discarded.
- Articles published before 2016.
- Articles on acute abdominal pain.

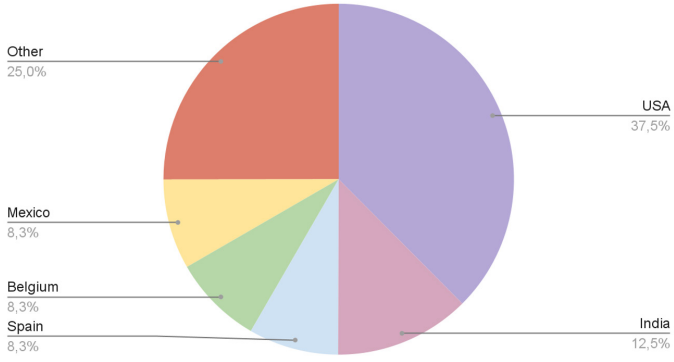
Results

Of the total number of articles found, those that met the exclusion and inclusion criteria were a total of 24, which were sorted into a database.

Based on this database, four columns were added to show the following characteristics:

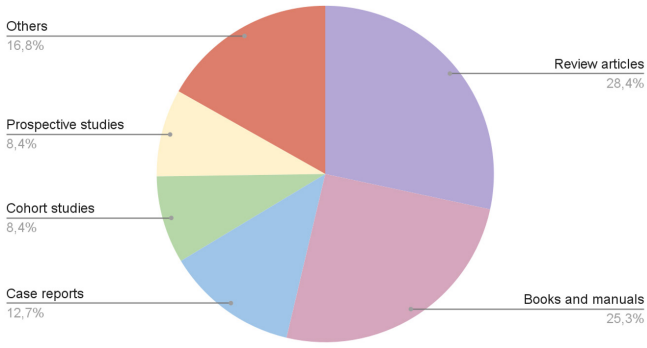
- Country of origin.
- Types of study/documents.
- Most common causes.
- Year of publication.

Of the 24 articles, 9 are from the United States of America, corresponding to 37.5%; 3 are from India, 12.5%; 2 are from Spain, 8.33%; 2 from Mexico, 8.33%; 2 from Belgium, 8.33%; leaving the remaining 6 from the following countries: Germany, Brazil, China, Ecuador, France and the Netherlands, which only included one article each, with a total percentage of 25%. The distribution is shown in graph 1.



Graphic 1

Review articles stood out in this systematic review with a total of 7.28%; within the literature (books, manuals), we found 6, 25%; 3 are case reports, 12.5%; 2 are cohort studies, 8.33%; 2 are prospective studies, 8.33%; and the remaining four are a narrative review, a case-control study, a descriptive observational study and a conference, 16.66%. (Graph 2)



Graphic 2

Of the information collected, 11 references (45.83%) did not provide conclusive information on the etiology of paediatric AA, while the remaining 13 references (54.16%) do mention etiology of paediatric AA. 16%) do mention aetiologies, where the main surgical cause of paediatric AA is acute appendicitis, only 7 references explicitly state that it is the main surgical etiology in older children (not infants, not neonates, not under one year of age), the others, although not excluding appendicitis as the main surgical aetiology in older children, do not mention it either. Intussusception corresponds to one of the main surgical causes in children under two years of age (infants, neonates) according to 5 references and one reports it in children older than one year. Within the non-surgical pathologies of AA, out of 5 references, 4 mention that gastrointestinal

diseases (gastroenteritis, constipation), followed by infectious diseases of the respiratory tract, correspond to the main non-surgical pathologies.

PubMed was the database with the most references, with a total of 10, 41.66%; sciencedirect gave us 4, 16.66%; NCBI gave us 3, 12.5%; AEPED, journals and pole of knowledge gave us 3 more, 12.5%; and the remaining 3 belong to literature, 12.5% and one conference, 4.16%.

Discussion

A review of the literature was carried out, incorporating different studies on the acute abdomen in paediatric patients, to gather information to promote correct medical action based on basic knowledge of definitions and concepts, as well as epidemiology, etiology, pathophysiology, and predominantly the clinical expression of acute abdomen in this age group.

The cardinal symptom in these patients is reported to be a pain; however, the most common concomitant symptoms were also found to be fever, vomiting, abdominal hypersensitivity, diarrhea, painful urination, and even recurrent airway infections.

It was found that, regardless of the cause of acute abdomen in paediatric patients, especially in neonates and infants, communication is a barrier to diagnosis and the physician must be prepared to perform an accurate history taking (usually with parents or primary caregivers), a complete physical examination supported by appropriate laboratory and laboratory tests, as well as the ability to offer treatment as quickly as possible. For the above reasons, it was gratifying to include pain assessment scales for paediatric pain useful in the ED as they are a great support for the physician faced with a neonatal or infant patient who does not yet have the linguistic ability to communicate.

This spectrum is variable and depends on each of the pathologies, however, it specifically encompasses only conditions affecting preschoolers, schoolchildren and adolescents (23). Newborns may present with necrotising enterocolitis. Midgut volvulus occurs 40% of the time in the first week of life, 50% in the first month and 75% in the first year. Intussusception usually occurs between 9 and 24 months of age. The most common cause of paediatric acute abdomen is appendicitis in children older than one year (20). Overall, acute appendicitis is the most common cause of surgical acute abdomen in all age groups and worldwide, although it can be difficult to differentiate from other causes of paediatric acute abdomen: morbidity in children is high, with an overall frequency of appendiceal perforation of 12.5% to 30%. Early and accurate diagnosis of acute appendicitis is important to avoid both a serious outcome and unnecessary surgery (6).

In a prospective observational study conducted between 2016-2017 by the department of Paediatric Surgery with the collaboration of Paediatric Emergency Department, Institute of Child Health, Sir Ganga Ram Hospital, New Delhi, where a total of 720 children were seen, and based on their final diagnosis they were divided into two groups: group I: 8% presenting with a surgical aetiology; leaving 92% of children with a non-surgical aetiology, it was concluded that: the cause of pain in more than 90% of children presenting to the paediatric emergency department with complaints of abdominal pain is non-surgical. Constipation was the most common diagnosis in these children, followed by acute gastroenteritis, other less common causes observed were: pain of uncertain aetiology and urinary tract infections and upper respiratory tract infections. In 8% of children whose aetiology of pain is surgical, acute appendicitis was found to be the most common surgical cause, although the aetiology will change according to the age of the patients. (5)

In newborns the most common causes reported were neonatal necrotising enterocolitis, intestinal atresia and intestinal malrotation: corresponding to surgical aetiologies; in the infant population, intussusception and incarcerated hernias were observed; in children older than 5 years and up to adolescence, appendicitis was reported as the most common cause; however, some other causes observed were trauma and idiopathic peritonitis.

Extensive information was obtained on the clinical context in which the paediatric patient develops as the correlation between clinical, laboratory and laboratory findings in the paediatric patient is well known and guides through the many differential diagnoses.

Limitations of the study

Confusion between acute abdomen and acute abdominal pain, as well as its use of synonymy with acute abdomen. Although epidemiological information on the most frequent diagnoses of acute abdomen in paediatrics exists, it comes from other countries and there is a clear lack of reported data on the subject in Mexico.

Conclusions

This systematic review has been carried out with the aim of touching on the key points of the subject of Paediatric Acute Abdomen, as this is a fairly common pathology both in the emergency department and in paediatric primary care, and one with which we are not very familiar. There is a lack of further studies on the analysis of the most common causes according to paediatric age group, as well as more data on this phenomenon in Mexico.

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Research article

The innovative methodology for teaching and learning about sexuality care in a population of young Mexicans

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Abstract

Introduction. Sex education in Mexico focuses on biological aspects, with traditional teaching-learning strategies, which do not encourage reflection on learning that impacts the care of sexuality, so it is urgent to propose a change so that the student becomes a subject and not an object of your learning. **Objective**. To report the experiences in developing the innovative methodology through the Maguerez Arch for the teaching and learning of the Care of the sexuality of the University Emerging Adult. **Method.** Qualitative study based on the Arco de Maguerez problematization methodology, developed on 12 Nursing students from 18 to 25 who attended 12 program sessions: "Taking care of my sexuality." The reports were analyzed by content analysis and technique of the speech. **Results.** According to the five moments of the Maguerez Arch: 1) Observation of reality: ignorance of contraceptive methods and sexually transmitted infections, 2) Identify the key points: An approach to sexuality to the biological aspect and non-invasive care it is part of their sexual life 3) Theorizing the problem: topics to be developed by the students: sexual education, communication, self-esteem, and empowerment, 4) Solution hypothesis: the care and knowledge of their sexuality if it is a priority in their life, 5) Reality application: they recognize the right to live sexuality with responsibility and care. **Conclusions**. The methodology was innovative, motivating, and significant, allowing the student to make conscious decisions regarding the care of their sexuality.

Keywords: learning, teaching, careful, sexuality, emerging adult.

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Introduction

Sexuality is an integral part of the personality of every human being; its development depends on the satisfaction of basic human needs such as the desire for contact, intimacy, emotional expression, pleasure, tenderness, and love. The development of sexuality is essential for individual, interpersonal, and social well-being (1). Sexuality education is a responsibility of society as a whole; it begins from birth within the family group and continues throughout life in the successive links that people establish when interacting with different groups. Today there are significant changes in behavior, attitudes, and values related to the sexuality of young people and adolescents (2).

Sex education should guide young people on the development processes of sexual maturity with high criteria about sex and lead them to consciously accept -not instinctively- the ways to satisfy their concerns at the appropriate age (3). In this way, the importance of the (3) discussion of sexuality becomes evident since it is still presented as a social taboo, surrounded by myths, stereotypes, and beliefs, restricting itself to superficial dialogues between parents and children or silence. On the other hand, within the family regarding these issues, in educational institutions, the biological approach of the school curriculum has prevailed, teaching concepts related to sexuality.

In Mexico, the teaching of sexuality lacks a comprehensive vision, favoring a traditional approach to education, emphasizing the transmission of biological knowledge of reproduction and the promotion of sexual abstinence, without a culture of prevention from the health care perspective. Sexual life, sexual and reproductive rights; The absence of information based on scientific evidence is also observed, since young people's access to information sources such as: friends, the Internet and magazines contribute to the lack of consistent guidelines, which makes this population more vulnerable (4).

The contents of sexual education have been present in the Mexican educational model since 1972, moving from the moralist or abstinence approach, the biological functionalist or good behavior, preventive or pathologist or well-called risk, the integrative and comprehensive and development and well-being, this last approach is the one that is in force in basic and upper secondary education and emphasizes aspects associated with sexuality, to strengthen self-knowledge, self-care, self-esteem and self-regulation (5). The foregoing reflects that for almost 50 years in Mexico, the educational model included topics on sexual education; however, the results are not very visible since emerging adults considered within an ageing period of 18 to 25 years (6) perform risky behaviors that expose them to sexually transmitted infections,

pregnancies and abortions, situations that denote the lack of care for their sexuality. These risk behaviors are not limited to the adolescent population; they continue to appear in the stage of emerging adulthood, which is when young people decide to enter the university, form families, and enter the labor field. Education is taught in the Bachelor of Nursing based on the transmission of information on contraceptive methods and sexually transmitted infections that is usually carried out in the classroom with a traditional approach, for which the awareness and effective knowledge of sexuality in the emerging adult has not been achieved, that allows them to carry out actions to care for their sexuality.

Risky sexual behaviors that lead to pregnancies, abortions and sexually transmitted infections continue to be a health problem in the life of emerging university adults; evidence of this problem is presented in studies such as the association between multiple sexual partners and the early initiation of coital sexual relations in university students, carried out in 2017 in 1854 university students between 18 and 24 years of age from the health area (medicine, nursing, dentistry and psychology) from two public universities, which concluded that university students from the health area also have a lack of care in their sexuality, since they have coital sexual relations at an early age, consider more sexual partners and carry out casual, transactional and group sex, so it becomes evident that studying a degree in the area health does not limit the vulnerability to acquire sexually transmitted infections, as well as the presence of unplanned pregnancies (7).

In the same sense, the study carried out at the Autonomous University of the State of Mexico in 2017 resumed, in 86 emerging adults with the Nursing Degree of 18 and 19 years of the second period reflected that 84.1% of this population maintains an active sexual life; of them, 47.7% began between the ages of 14 and 17 and 52.3% after the age of 18. 52.3% admit to having used emergency contraceptive methods, such as the morning-after pill, and 20.5% of this population have risky sexual relations by not using protection methods, which makes them susceptible to acquiring a sexually transmitted infection (STI) and unexpected pregnancies. 90.9% have had sexual relations with only one partner and 9.1% with two or more sexual partners; 97.7% report that they have not presented sexually transmitted infections, while only 2.3% say they have had an STI (8). The training of the nursing profession is based on professional care, understood as the essence of the nursing discipline, whose purpose is to comprehensively care for the human being in health and disease conditions through care teachings supported by scientific and theoretical-practical knowledge humanistic for the conservation and maintenance of life (9) During

the training it is vital that the student becomes aware of his care in an integral way, which includes the care of his sexuality.

In this context it is important to know: How do emerging adults learn to take care of themselves in relation to their sexuality? To identify absence or gaps in knowledge in emerging adults that support the planning of educational interventions for the care of sexuality. Education allows the development of skills that enable young people to make decisions and be responsible in health matters. Therefore, comprehensive sexual education is necessary that delves into the biological, emotional, and affective dimensions of sexuality, going beyond traditional approaches focused on risk as well as conservative visions of sexuality, based on scientific evidence, in values of respect for human rights, with gender sensitivity, and cultural relevance (10).

For this reason, it is important to develop prevention programs with innovative methodologies, which, added to the accessibility to health services, will be essential for emerging university adults to achieve an integral development of their person and favor an effective, sexual, conscious life. Prudent, with an optimal disposition and training toward the care of his person, allows him to transfer knowledge in his professional practice through his training.

Specifically, students trained in nursing will be a key element when entering the health sector as professionals. They will have the mission of transferring scientific knowledge regarding the forms and actions that care implies to promote sexual health in people's lives.

This research aims to report the experiences in developing the innovative methodology through the Maguerez Arch for teaching and learning the Care of the sexuality of the University Emerging Adult.

Methodology

This descriptive, analytical, and interpretative study uses a constructivist dialectical approach with experience reports based on the theory of problematization through the Maguerez Arch method by Juan Díaz Bordenave and Adair Martins Pereira in 1977 (11); this is made up of five stages: **1) Observation of reality:** in this stage, the student reflects on his reality and recovers his personal experiences to analyze a problem around his sexuality, **2) key points of the problem:** at this moment, a synthesis of what is important to investigate about the problem is made, **3) theorization:** the members of the group check what they already know and analyze what they need to know to investigate the answer to the problems raised, seeking explanations of the observed reality, **4) solution hypothesis**: it is built from the search for the resolution of the problem critically and creatively. **5) reality application:** the student

or the group applies the newly constructed knowledge to their practice, making it possible to solve the problem and thus carry out generalization exercises to other similar situations and other study topics. The research emphasizes the problematization of how they are taught and how they learned to take care of their sexuality. The problematization methodology rescues knowledge through direct investigation of reality, in an effort to build an understanding of their reality, linking theory with practice, that is, what they learn with what they do.

The subjects of the study were delimited among 35 emerging adults considering a population of 12 key subjects, who will be between the ages of 18 and 25, are studying for a nursing degree, both sexes, with an active sexual life, who attended the 12 sessions to delve into the history of their narrations, who signed the informed consent, attached to the General Health Law, in article 100 about "ethical aspects of research in human beings"; fraction II.

The method began with presenting the problem-solving methodology of the Maguerez Arch to the entire group, and the program "taking care of my sexuality" was made known; each of the moments was explained to them, clarifying doubts and detecting participation in the participating population. The use of the Maguerez Arch is considered relevant for developing critical and creative professionals when they are sensitized for a conscious, informed, and consistent action in congruence with their context. (11), the intervention using the Maguerez Arch method changes the traditional approach to learning about sex because the problematizing methodology stimulates reflective and critical thinking in the emerging adult student of the Nursing Degree.

Twelve sessions were held with the participants lasting two hours each, in a classroom of the Faculty of Nursing and Obstetrics; considering that group meetings are ideal for discussion and exchange of experiences with sexuality, the members of the group presented common characteristics associated with the central theme under study (12).

The story collection process was carried out through participant observation and log recording considering the following sessions described in Table 1:

Table 1: Activities in the sessions following the Maguerez Arch method

STAGES	ACTIVITIES IN THE SESSIONS
Observation of reality)	1st session: presentation and discussion of the problematization methodology. 2nd session: presentation of the case study for discussion. Reflect on reality and determine the problems.
Key Points **(PC)**	3rd session: select and define the key points for solving problems. 4th session: prioritization of problems.
theorizing **(T)**	5th session: theoretical foundation of the key points to solve the case. 6th session: presentation and discussion of the issues related to the key points. 7th session: presentation and discussion of the topic.
Solution Hypothesis **(HS)**	8th session: construction of the solution hypothesis through the identification of teaching-learning strategies 9th session: analysis and reflection on the strategies and define their application to the group context.
Application to reality **(AR)**	10th session: link the topics analyzed during the previous stages with the lived reality. 11th session: a sequence was elaborated where the awareness process of paulo Freire was integrated for the care of the sexual life of the emerging adult. 12th session: the scheme of the awareness process was socialized to the plenary session of the group and its use was proposed.

The results and discussion were structured through discourse analysis to rescue the stories following the order of the problematizing methodology.

Results and discussion

The phase of observation of reality for the recognition of the problem

It was commented that it was important to understand the teaching that they had regarding sexuality, how they learned to take care of themselves, the importance that it has occupied in their life, or the feeling of their sexual experiences by exposing the experience of one of the 19-year-old female participants, without children, when experiencing a consensual sexual relationship with a consensual partner, as described below:

"…I obtained the information on sexual education at school with lectures and talks in middle and high school, with topics of contraceptive methods, for example, the condom, the pills that must be taken before having sexual relations, and the patch that is used in the arm, but I don't know how it works ..." "People can get HIV from lack of hygiene…. For example, you bathe daily, but perhaps your partner does not, and when you have sexual intercourse, the secretions from the penis and the vagina combine, and you can get an infection. I know that I must protect myself because if there is no protection, I can have a pregnancy. Desired, if it is not with the right person at that moment, it would be the worst thing that could happen to me..."

"…My family doesn't talk about sexuality; my parents only tell me that the school

is for that. Yes, I have an active sexual life, and my partner does not like using a condom; he says that he does not feel the same, sometimes I have felt pressured by my boyfriend to have sex, but I have not had a check-up to verify my state of health Perhaps because I am indecisive, for fear that they will deny me the floor, I feel humiliated and ashamed…."

When analyzing the exposed story, ignorance was identified in the use of contraceptive methods and sexually transmitted infections. It was difficult for the young woman to share her experience with the participants. In the first place, to reduce the tension, the 12 participants were placed in a circle, and the young woman began her story timidly and with a flushed face, with a bit of stuttering and moments of silence, as he continued his talk and when he observed that the other participants nodded their heads as a sign that they identified with his experience, he became more confident, his shyness decreased, and he continued expressing his experience, as Yances refers to it, in 2020, the Emerging adults increasingly find small spaces that they can take advantage of for the free expression of sexuality, in which it is possible for them to share expressions that reveal problems or gaps in the knowledge of their sexuality (13) and the care of it as they they affirm it

"…There is a deficient sexual education, sexual rights are generally unknown, ummm the term sexuality care is not considered an important part of life, or I could say that there is a lack of interest on the part of emerging adults to obtain information regarding the care of their sexuality, perhaps due to low self-esteem and indecision in contacting truthful sources of information, for which reason many times adequate decisions are not made about sexuality…."

The story presents a disturbing need to have information for the emerging adult to obtain knowledge regarding their sexuality; according to Saeteros in 2018, young people have perceived needs due to their age, the environment where they operate, and the lack of information because the one they have is considered irrelevant and unsatisfactory (14). It is also noted that they already reflect on care, that until this part of their training, they had only considered it as information to avoid a sexually transmitted infection and unexpected pregnancies; however, care as an act of life they are not associating it with their sexuality, as if taking care of it was not part of being human. Therefore, the group of participants generated reflections around different themes, considering the meanings they have regarding sexuality and the lack of knowledge, as well as the context in which the student has grown, allowing the identification of themes that, according to the case Having already presented their experience, emerging adults considered it important to know and analyze regarding sexual education. The

horizontal communication and active listening that was generated in the classroom allowed the expression of the participants through the posing of questions, generating greater confidence, reflection, and analysis of their sexual education, which gives the possibility of expressing themselves more freely. Their concepts raise their doubts about the subject.

Based on this reflection, the group was motivated to problematize by asking: What is the teaching-learning strategy to learn about my sexuality? Once the question was analyzed, it was agreed to use the Maguerez Arch as an active methodology in the study—the teaching-learning process of sexuality and its care.

Once this reflection was carried out, it was concluded that, as Trad Lab refers to it, problem situations are considered strategies to bring the student closer to reality, promoting the recognition of similar situations experienced in their life trajectory. (15) In this first stage, the objective of reflecting on the participants' experiences was achieved.

2nd Stage: Identify the key points of the problem

From the careful observation of the problem, the second stage continued to identify the "Key Points", the prioritization of the problem was carried out, and it was characterized in a more consistent way for its understanding. At this moment, the young people and the researcher defined what would be studied and identified the aspects they needed to analyze in depth to base the search for a solution to the problems. (16).

At this stage, the emerging adults reflected to identify the key points, and the main question was proposed: What problems did you identify about my sexuality? According to the case presented, this questioning was generated in consensus with the entire group. So the key points of the problem are narrated below:

> *"…. There is a lack of knowledge regarding contraceptive methods; we do not know the correct way to put on a male condom, we young people do not give importance to the risks of having unprotected intercourse, and many times, we do not identify that as women we have rights…."*

> *"...We associate the word sexuality with having sex, care is not considered when initiating coital sexual relations, sexual education is not received at home and at school, only contraceptive methods are generally reported..."*

> *"...As young people, there is no communication within the home, there is ignorance regarding the prevention of pregnancy, we do not have the concern and the decision to seek information when starting an active sexual life..."*

"…It is common for sex to be practiced without care, and when women are passive, calm and shy, they do not assert their decisions and do not dare to request information on contraceptive methods. Sex education in the family does not exist, there are parents who are very reserved about sexuality, many times they do not have emotional support…"

The students addressed the problems in a talk in the classroom; after listening to their stories, it can be deduced that they are aware of the lack of knowledge about sexual education issues, they agree in relating sexuality to sex, and to this, with a coital sexual relationship, an approach to sexuality reduced to the biological aspect is identifiable; likewise, it is evident that care is not part of their sexual life, it is also important that they identify that a cause of the little sexual education is the lack of communication of these issues within the family nucleus, they even report that it does not exist. Therefore, from this reflection, key points about the problem were determined through a greater synthesis and linking of possible solutions that adapt to the reality and context of the student of the nursing degree, located in the emerging adult life stage, where they are primarily exploring their identity, they are unstable, rarely take responsibility and find themselves at crucial moments in their lives when making personal and professional decisions(17).

In short, young people express that the problems that stand out and are key in the experience of their sexuality are related to the knowledge of the care they should have regarding the use of protection methods, there is no awareness regarding the risks involved in having life sexually active, within the family there is no talk of sexual education, so young people confuse the term sexuality with having sex and sex for them is not a biological connotation of the division between the anatomical and physiological characteristics that identify being feminine or masculine, for young people the word sex, is synonymous with having coital sexual relations, a result that coincides with what was described by Vasconez in his study carried out on Ecuadorian university students in 2022 where the perception of sexuality is reduced to a mere function intercourse (18).

3rd stage: theorize the problem to understand the problem.

At this stage, young people are oriented to seek a theoretical explanation of the problem to analyze and strengthen the discussion with the contributions of science to clarify the study (19). In the same way, at this moment, young people are investigating. Because of the problems identified and observed in the previous phases, analytical mental operations occur during theorizing that favor the intellectual growth of young people. The whole group should study the problems identified (20)

The participants determined the topics that had to be investigated and studied: sexual education, self-esteem, communication, and empowerment. Asking young people to decide to look for books and articles on the topics was not easy, perhaps because of the student's habit of presenting the contents already organized into agendas. The young people were asked to investigate the subject to learn about their needs. In this case, the key points that the group identified were the following:

Sexual education: it was the process in which young people knew to understand sexuality, not only in the teaching-learning process of the functions of the reproductive system but also about the sexual manifestations of the body itself, sexual rights, and care of *being* in its most holistic form, this is how they described it in order of importance:

"...Sex education from the family environment is important; in school, it is necessary to address issues such as the use of contraceptive methods, sexually transmitted infections, and information on the advantages and disadvantages of sexual practice..."

"…It is essential at all stages of life since individuals lack information about their sexuality because it is taboo. It is vital to educate everyone to have sexual health…."

"...It is important because there is more knowledge of the changes that human beings experience in the different stages of their lives, the advantages and disadvantages of having sexual activity are identified, and information is available to prevent sexually transmitted infections..."

"…Sex education helps to enjoy our sexuality, without risks, preventing unexpected pregnancies and sexually transmitted infections; having sex is not bad, it is normal, it is a basic need, the problem is when it is done irresponsibly…."

Communication: this process was carried out by the young people through dialogue about sexuality and school, since within the family, there is no openness and space to deal with the issue of sexuality, considering it a taboo subject loaded with prejudices. This important aspect of communication was reflected when they reported the following:

"…Get the information, tools, and motivation to make healthy decisions about sex and sexuality…"

"…Being informed is of great importance because it allows us to know the risks we face and how to prevent them…."

"…Because of the parents' beliefs and attitudes, they often do not have the confidence to discuss sexuality issues, doubts, and emotions are not expressed…."

"…Sex education is not received at home, these issues are not discussed there, parents

frequently say that is what school is for, and she is the one who has the responsibility of transmitting information on sexuality…."

Self-esteem: Confidence, respect for oneself, and development of abilities that each person must face life's challenges.

Students consider that learning about sexuality, giving importance to it, and exercising their sexual rights are closely related to emerging youth's self-esteem. The same thing was expressed in the following texts:

"…There are young people who feel very sad, do not trust themselves, and do not tell anyone what is happening to them…."

"…Young people start their sexual life active and later feel bad emotionally, guilt and shame…."

"…There is a lack of interest on the part of young people to find out about sexuality. They do not consider it important for their life…"

Empowerment: It is a process by which human beings have control of their decisions and actions related to their life, education, health and sexual life. Empowerment is related to power, which means control, which is the process of gaining control over the care needs of the sexually active life of emerging adults. Under this tenor, the young people identified that there is a lack of empowerment, which is shown in the following stories:

"...I can mention that sometimes due to lack of security, fear, insecurity and lack of confidence, we carry out actions that will not always favor the care of our body..."

"...Young people in many situations also show a lack of interest in being informed, since currently there are many means at our disposal to obtain this knowledge, for example about contraceptive methods..."

"...In the absence of having the power to make correct decisions about our active sexual life, relationships are allowed where even physical aggression is experienced..."

The development of this stage began with the delivery of articles and texts related to the themes of the key points; the young people were placed at a round table and began the commented reading to carry out a discussion of the reality lived in their sexuality and made a comparison with the aspects found in the literature. After this analysis, conclusions were drawn from the themes developed for their subsequent socialization among the group members through the exhibition. Using the problematization methodology implies a change in the position of the teacher and the young people to generate reflection and criticism of each one of the topics, for the young people to reflect on Who are they? What do they do? And how do I take care of my

sexuality? It was a challenge, the above associated with the traditional way in the teaching-learning process and the biological approach of their sexual education, as well as motivating the student to be the subject of their own learning and choose the theoretical aspects that are required. to solve a problem, makes him aware of his needs and his strengths in the knowledge and care of his sexuality.

4th stage: a hypothesis to solve the problem

In this stage, the hypothesis for the solution to the problem is formulated, and the previous moments are taken up again and re-evaluated to specify and elaborate possible solutions. Viable alternatives to solve problems critically and creatively arise from the confrontation between theory and reality, allowing young people to restructure knowledge and reflect on new possibilities with a more analytical look(20), therefore reflecting on the learning strategies that will allow the nursing professional in training to exercise their sexuality responsibly and take care of their sexual life, the group was asked to return to the term "consciousness" because the human being, when becoming conscious, takes with freedom their own decisions, autonomously and consciously, in the same way, awareness in sexual education was considered that allows through a process of action-reflection -an action that the student (21) carry out actions of care of his life sexual information that may be transferred to users.

The young people raised the possibility of carrying out a life plan where their objectives and goals included the care of their sexuality within the aspects that they identified to improve their sexual life; the following was rescued:

> *"...Young people require effective sexual education in educational institutions, where care is emphasized about their active sexual life..."*

> *"...Motivate decision-making about the educational and personal projects of each student...learn to identify when there is a risk when exposing themselves to sexual conduct...value themselves as a person..."*

> *"...Establish objectives and goals that allow us to complete our studies and improve our lives...."*

> *"...Conduct talks with young people about their sexuality, where shared responsibility is emphasized when leading an active sexual life. Through a relationship of trust and respect...."*

> *"...Talks with young people in small groups on knowing your body, your sexuality, improving your self-esteem...."*

The students raised hypotheses where care and knowledge about their sexuality are among the priorities in their life project;

they also stated that at no other time had they considered sexuality as an important aspect of their life.

5th stage: Application to reality

During this stage, the student and the teacher must return to the context of the group and reflect on the knowledge acquired and constructed to apply or propose interventions for the resolution of problems that will be applied to the reality of the group of participants and other groups with similar problems (22) when it is possible to gather previous knowledge in an action-reflection-action exercise, the construction of new knowledge is made possible to transform the observed reality, through the hypotheses raised, thus transforming the reality of the student (23) regarding their learning of sexuality

When the knowledge is transferred to the reality of each student, the following narratives emerge:

> *"…Now I can resume my active sexual life responsibly taking care of my body, choosing a contraceptive method, and having periodic medical check-ups…."*

> *"…We stop to think about the family, the school, but we don't think about how to take care of our sexuality; we focus on so many things, and we don't pay so much attention to the issue of sexuality and when we least realize it we can have a pregnancy without planned…."*

> *"…After my last experience, and after knowing how I should take care of my sexual life, I am already stronger and more determined and would not have coital relationships with anyone, I think I am maturing in that part… I learned that I must love myself…"*

> *"…Sexual health is important for me and my partner, learning to have a responsible, safe and pleasant sexual life…"*

> *"… Learning to better express my sexuality, now I know that it is important to enjoy my sexuality with my partner responsibly and safely…learning about my sexuality motivated me, and as a life guide, it helped me to meet myself I must be more responsible taking care of myself and my partner, exercising my sexuality with respect, freedom in a healthy way."*

By applying what they have learned through the active methodology, young people reflect and decide to take different actions regarding their sexuality. The important thing in this intention for change was to make them aware of the importance of caring for their sexual life to develop their projects. Personal and professional, but above all it stands out that now they report that they have the right to live a full, pleasant sexuality but with care, respect, and responsibility for their person and their

partner. Other studies conducted in Mexico regarding educational interventions show positive effects in increasing knowledge of sexual and reproductive health (24).

Conclusions

The problematizing strategy adapted to the learning process of the sexuality of the nursing degree student constituted a challenge that generated a student with feelings of autonomy and greater responsibility in the appropriation of their learning.

Applying the Maguerez arch through a program gives young people different possibilities to structure their knowledge autonomously and follow the problems detected and prioritized.

The Maguerez Arch constitutes an innovative practice in the teaching-learning process. He contributes to training reflective and critical professionals who will have the tools to continue these actions in their professional practice.

The development of innovative practices with the use of the Arco de Maguerez and the principles of problem-solving education in the classroom for learning about sexuality care requires the training of teachers to motivate students to reflect, the teacher needs to become as a facilitator, not as a transmitter of knowledge and indications.

During the application of the intervention, the students expressed the difficulty of carrying out their teaching-learning process based on questions, because it implied a process of reflection, deep analysis, and conscious decision-making. However, they considered a perfect strategy for the development of topics such as sexuality and other topics that are found in their Nursing degree curriculum. Therefore, applying this innovative strategy is very significant for the students and the researcher; it moved the consciences of the participants.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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Research article

Importance of the dilution test in the dosage of coagulation factors XII and XI in plasma with positive lupus anticoagulant

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Summary

Introduction. Thrombosis is associated with acquired risk factors or hypercoagulable states. Antiphospholipid antibodies are found in infectious processes or associated with arterial or venous thrombosis. Among these is the lupus anticoagulant, which is considered an interference inhibitor because it prolongs phospholipid-dependent tests in vitro. **Objectives.** To relate the activity of factor XII and XI in patients with positive lupus anticoagulant. **Methodology.** Of 55 plasmas, 34 met the inclusion criteria. Factors XII and XI were dosed by metric clot methods. Samples less than 50 Ul/dl are considered low factor activity, to which the dilution test (parallelism) is performed. If a recovery greater than 15% of the factor is evidenced, it is considered interference. If, on the contrary, the result remains with little variation, it is confirmed. factor deficiency. **Results.** Of 34 dosages, 79.4% (27/34) presented a decrease in factor XI. A dilution test was performed, and 100% recovery of factor XI was observed; Regarding the dosage of factor XII, 29.4% (10/34) presented values below 50 IU/dl and 70.6% (24/34) normal, did not recover in 22.2% (2/9) presenting deficit. **Conclusions.** The study of a prolonged PTT is carried out due to suspicion of lupus anticoagulant or factor deficiency. The importance of the laboratory is that every time a decreased coagulation factor is found, plasma dilution must be done to determine if there is recovery of the factor or is a deficit of this.

Keywords: syndrome antiphospholipid, anticoagulant lupus, dilution, factor XII, factor XI

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Introduction

Thrombosis is considered an alteration of multifactorial origin secondary to alterations in hemostasis, platelet function, leukocyte activation, and the vascular wall, which leads to clot formation and blood vessel obstruction, causing changes in blood flow. quality of life of people who suffer from it, such as paralysis, memory loss, and speech loss, among others. The disease can be caused by habits such as smoking, a sedentary lifestyle, dyslipidemia, obesity, or by diseases of genetic and/or acquired origin. The maintenance of the disease and the costs make it a public health problem.

Investigations carried out in experimental models have confirmed that plasmatic coagulation factors are also altered by different antiphospholipid antibodies. This type of antibody can recognize some epitopes of coagulation factors, evidencing the decrease in these proteins when functional activity is determined. As in vitro tests dependent on phospholipids, mainly PTT, are prolonged, the main factors to investigate are factors XII and XI because they are contact factors and normally when factor XII is decreased there are no clinical manifestations of bleeding, whereas when factor XII is decreased, there are no clinical manifestations of bleeding. XI is increased, and there is a hypercoagulable state. then the interference of these antibodies manages to demonstrate the decrease in the level of these factors. The important point to highlight is the role that the laboratory plays in being able to determine if there is indeed a decrease in the factors or if the decrease is due to the interference of the antibodies and that is where the plasma dilution test should be performed, making the dilutions depending on how low the factor is 1/2 or 1/4, 1/8 or 1/16 if there is recovery of the factors it indicates that it is due to interference of the antiphospholipid antibodies with the reagents used in the test. Among these antibodies, the most relevant are anticardiolipin antibodies, anti-phosphatidylserine antibodies, anti-Beta 2 glycoprotein antibodies, and lupus anticoagulants. Antibodies with persistent high titers are associated with arterial and venous thrombosis and recurrent miscarriages.

In the study, factors XII and XI were dosed because the variation in their activity may be an additional risk factor for thrombosis in patients with positive lupus anticoagulant.

Decreased factor XII concentration causes prolonged in vitro testing and may lead people to trigger thrombosis. This process is due to mutations in the F12 gene, located on the long arm of chromosome 5 (5 q 35.5), This gene codes for the protein factor XII or Hageman, and this factor is in the bloodstream in an inactive form until it is It encounters the walls of the injured vessels and is activated and initiates coagulation, interacting with factor XI. Factor XII also plays an important role in stimulating in-

flammation, a normal response of the body to infection, irritation, and other injuries. In addition, it interacts with another protein, prekallikrein, this interaction initiates chemical reactions that lead to the release of bradykinin that promotes inflammation, additionally, factor XII activates the fibrinolytic system, activating plasminogen to its active form plasmin, which destroys fibrin clots. and fibrinogen.

More than 20 mutations leading to factor XII deficiency have been identified, most of the mutations substitute amino acids in factor XII, altering its structure. The deficiency is inherited in an autosomal recessive pattern. This factor XII abnormality may predispose affected individuals to developing blood clots (thrombi) at an early age with a higher risk than the general population of developing deep vein thrombosis or bleeding disorders and unexplained repeated miscarriages in some affected women. Researchers are studying drugs to block (inhibit) factor XII as a potential therapy for people who are prone to developing blood clots.

Factor XI is encoded by the F11 gene, on the long arm of chromosome 4 (4q32-35), like many other coagulation factors, it is a serine protease. In humans, factor XI is activated by factor XIIa and thrombin, factor XIa activates factor IX by selective cleavage of peptide bonds. It is an autosomal recessive disorder; that leads to bleeding. Elevated levels of factor XI have been implicated in cases of thrombosis generating a procoagulant state.

Antiphospholipid Syndrome (APS) is classified as an autoimmune disease with antibodies (Acs) isotype IgG / IgM/, IgA, it is characterized by presenting a clinical picture of arterial or venous thrombotic events and recurrent abortions, hemocytopenias, hemolytic anemias, neurological alterations with titers elevated and persistent antibodies. This antiphospholipid syndrome occurs in two forms: primary where no other autoimmune disease is evident and secondary where it is associated with an underlying autoimmune disease, the main one being Systemic Lupus Erythematosus. (1-4)

Antiphospholipid antibodies (aPL) are very heterogeneous immunoglobulins against cellular components or against coagulation factors. These antibodies act on both procoagulant and anticoagulant mechanisms at the membrane level of endothelial cells, platelets, and trophoblasts, among others, stimulating these cells to increase the expression and secretion of different molecules that favor pre-thrombotic states. (1-4)

In antiphospholipid syndrome, several pathophysiological mechanisms are proposed in which antiphospholipid antibodies alter the procoagulant and anticoagulant homeostatic reactions that occur on the cell membrane, blocking the binding of the protein with the negatively charged phospholipids, also blocking the ac-

cess of other proteins to these. phospholipids, therefore phospholipid-dependent coagulation reactions are inhibited, such as inhibition of protein C, protein S, Antithrombin (AT), Annexin 5 A, inhibits the anticoagulant activity of B2 GPI, alters the fibrinolytic system, decreases the PGI2 production, increased TXA2 in platelets, all these events are associated with pre- thrombotic states (5)

Elevated levels of coagulation factor XI predispose to thrombosis in patients with antiphospholipid syndrome, a relationship between altered factor XI activation and the syndrome has been discovered. Factor XI is a proenzyme that is activated to XIa by the action of activated factor XII or thrombin, factor XIa is responsible for the activation of factor IX, which leads to other enzymatic reactions for thrombin generation. (6-10)

There is evidence that the disulfide bonds in Factor XI are reduced to free thiols by the action of oxidoreductase enzymes on factor XI; The activation of factor XI by the action of thrombin or factor XIIa and treated with TRX1, significantly increased the reduced XIa, compared to the oxidized To verify the aforementioned, the ELISA test was performed to measure reduced factor XI in patients with antiphospholipid syndrome, it revealed that these patients have higher plasma levels of reduced FXI than normal controls, this contributes to understanding the predisposition to thrombosis when there are elevated levels of reduced FXI (11,12)

Positive lupus anticoagulants with anti B2GPI or anti- Cardiolipin are risk factors for thrombosis and for a pregnancy after 12 weeks. In a study carried out in people under 70 years of age with a first episode of thrombosis, it was shown that 3.1% of the people with deep vein thrombosis were positive for lupus anticoagulants. In a case study in women younger than 50 years, 17% of stroke patients were positive for lupus anticoagulant, and the risk was increased in women taking oral contraceptives. Lupus anticoagulant with the presence of aB2GPI correlates with a higher risk of thrombosis than lupus anticoagulant due to antithrombin autoantibodies, asymptomatic patients positive for lupus anticoagulant, antiphospholipid antibody and aB2GPI can be found with risk of a first event, they are called triple positives, these patients has high titers of antibodies, which bind to the LB major epitope in domain I of B2GPI; domain I aB2GPI, associated with a high risk of thrombosis due to the action of autoantibodies. Tests have also detected autoantibodies to the phosphatidylserine-prothrombin complex, this can help diagnose antiphospholipid syndrome and the association with high risk of thrombosis. On the other hand, in studies carried out, tissue factor (TF) could contribute to the prothrombotic state of patients

with persistent lupus anticoagulant and a history of thrombosis. (13,14)

Antiphospholipid antibodies require the presence of B2GP to bind to cardiolipin, aB2GPIs are of low affinity which is increased when bound to the B2GPI protein. Fibrinolysis is also affected due to the decrease in protein C, it alters the activity of thrombin-activated fibrinolysis inhibitors, as it is well known, this molecule is a plasminogen activator inhibitor, it indirectly prevents the clot from forming. degrade, on the other hand, the platelet is activated by the aB2GPI-B2GPI complex, increasing the production of TXA2, a powerful vasoconstrictor and platelet aggregator, it also inhibits Antithrombin that inhibits factor IXa , Xa and Thrombin. (fifteen).

The lupus anticoagulant depends on phospholipids, is responsible for prolonging coagulation time in vitro, and is associated with anticardiolipin antibodies, B2GPI, causing thrombosis and abortions. Oral direct thrombin and Xa anticoagulants, heparin, and vitamin K antagonists are used to prevent venous and arterial thrombosis but may be responsible for false-positive laboratory test results for lupus anticoagulants. (16)

The importance of factor XII is to participate in the activation of the intrinsic pathway of coagulation and plasminogen to generate plasmin. Deficiency of this factor is more associated with arterial and venous thromboembolic complications leading to life-threatening myocardial infarction and pulmonary embolism, miscarriages, and cerebrovascular accidents, among others. (10)

The additional treatment of direct anticoagulants (DOACs) against the mentioned factors and to reduce aggregate thrombotic events in patients with APS refractory to conventional treatments who present nephropathy, should be administered with care, the anticoagulant action begins 2 hours after the first dose at Unlike vitamin K antagonist anticoagulants, if the anticoagulant is discontinued it rapidly loses its action, mostly eliminated in the urine (17-22).

The laboratory tests recommended by the subcommittee for scientific standardization for Lupus Anticoagulant (LAC) / antiphospholipid syndrome, are three lupus anticoagulants and anticardiolipin anticoagulant, aB2GPI, for a good diagnosis by the laboratory. IgG is more related to thrombosis than IgM, but in abortions it is IgM. Laboratory detection of lupus anticoagulants in anticoagulated patients is important because they can interfere with the result, causing false positives or negatives. For this, some reagents have heparin neutralizers capable of inhibiting unfractionated or low molecular weight heparin, and vitamin antagonists K may affect the detection of lupus anticoagulant. (23,24)

Taking into account that in some patients with Catastrophic antiphospholipid syn-

drome (CAPS), a rare and potentially fatal disease, is a systemic coagulopathy related to antiphospholipid antibodies that must be rapidly diagnosed and treated. It is characterized by clot formation in multiple organs high production of cytokines in a short time and high titers of antiphospholipid antibodies (25).

Among the recommendations for the management of antiphospholipid syndrome, the profile of high-risk antiphospholipid antibodies associated with thrombotic and obstetric antiphospholipid syndrome is important. Low-dose aspirin is recommended for asymptomatic carriers of antiphospholipid antibodies, patients with lupus erythematosus without thrombotic or obstetric antiphospholipid syndrome, and non-pregnant women with a history of obstetric antiphospholipid syndrome. Patients with antiphospholipid syndrome and first venous thrombosis should be treated with vitamin K antagonists with an INR 2-3. Rivaroxaban should not be used in patients with triple antiphospholipid antibody-positive antiphospholipid syndrome. (26)

Materials and methods

This research is descriptive, cross-sectional, correlational, and non-experimental. The project is endorsed by the Research Committee of the Universidad Colegio Mayor de Cundinamarca.

55 plasmas were selected, of which 34 plasmas met the inclusion criteria: samples from patients with prolonged TTP with positive lupus anticoagulant and exclusion criteria: samples from patients anticoagulated with direct inhibitors such as Rivaroxaban and Apixaban, direct factor X inhibitors and the inhibitor Dabigatran direct from thrombin

For the statistical analysis, a descriptive analysis of the continuous variables was carried out, the Shapiro-Wilk test was applied to define the type of distribution of the variables, in the variables of non-normal distribution the median, the Interquartile range (IR) and the maximum and minimum values. Proportions are presented in the categorical variables. For the bivariate analyses, the Pearson's Chi-square difference test, the Mann-Whitney test, the Kruskal-Wallis test, the Kruskal-Wallis test, and the binomial test were used.

A value of $p \leq 0.05$ was considered a significant difference. The statistical program SPSS 25 was used.

Coagulation factors XII and XI are measured in plasma by coagulometric methods using the CA 1500 and CS-2100i equipment. The test is performed by mixing plasma deficient in the factor to be quantified with the patient's plasma. A TTP is carried out for the case of factors XI and XII. The second data are interpolated into the specific calibration curve for each factor and the results are expressed in UI/dl.

The calibration curve is made with the dilutions defined for each factor, following the protocol of the programming manual of each equipment. The equipment automatically takes the coagulation times of each of the dilutions and graphs the curve with its corresponding factor activity.

Samples with results less than 50 Ul /dl are considered to have a low factor activity value, due to the possibility that it may be due to interference from the lupus anticoagulant, the parallelism test is performed, which was carried out by diluting the plasma ¼ and dosing the factor again. If a recovery of more than 15% of Factor is evident, it is considered that there is **recovery of the factor when diluting (parallelism).** If, on the other hand, the result of the factor remains with little variation in the different dilutions, that is, close to the initial value and is validated with the note: **No recovery of the factor is observed when performing the dilution (parallelism), which confirms factor deficiency.**

Results

Description of demographic characteristics with positive lupus anticoagulant

Of the total of 55 samples analyzed that were positive for lupus anticoagulant, 58.2% (32/55) had prolonged PTT and 3.6% (2/55) had normal PTT.

Table 1. Result of lupus anticoagulant.

		Negative	Positive	Total	p
Male	No.	11	18	29	0.968
	Percentage	52.4	52.9	52.7	
Female	No.	10	16	26	
	Percentage	47.6	47.1	47.3	
		21	3. 4	55	
p: Chi-squared difference					
There is no difference in the type of lupus anticoagulant according to sex					

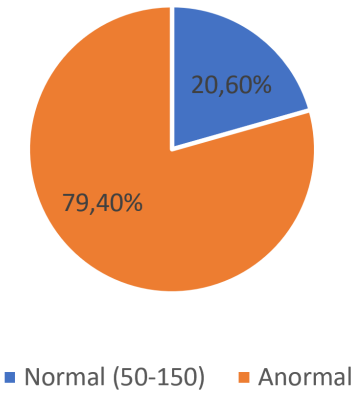
Of the total of 34 samples positive for lupus anticoagulant, 18 (52.9%) corresponded to men and 16 (47.1%) corresponded to women. (Table 1)

Table 2. Relationship between age and gender

			Men	Women	Totals	p
Age	8 to 13 years	Number	8	1	9	NA
		Percentage	44.40%	6.30%	26.50%	
	14 to 23 years	Number	3	5	8	
		Percentage	16.70%	31.30%	23.50%	
	24 to 40 years	Number	4	6	10	
		Percentage	22.20%	37.50%	29.40%	
	> 40 years	Number	3	4	7	
		Percentage	16.70%	25.00%	20.60%	
	Total	Number	18	16	3. 4	0.552
P: Chi-squared difference						

There is no difference in the distribution of the type of lupus anticoagulant according to age groups.

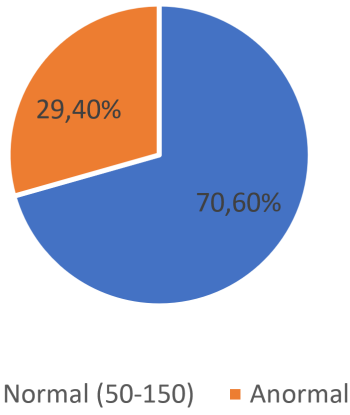
It is noteworthy that in the ranges from 8 to 13 years, the number of men is greater than women, unlike the other ranges in which the values are very similar for both men and women.



Graph 1. Factor XI according to UI/dl

The distribution of factor XI is not normal, median of 33.85 UI/ dL, interquartile range of 31.5, minimum of 33.2 and maximum of 88.1. A normal value of 50 to 150 IU/ dL of factor XI is considered.

As can be seen, 20.6% (7/34) were normal and 79.4% (27/34) were below 50 IU /dl.



Graph 2. factor XII according to UI/dl

The factor XII distribution test is normal. Mean: 64.91. Standard deviation 29.19. Minimum: 4.5 Maximum 122.0. A normal range of 50 to 150 IU/dl is considered.

As can be seen, 29.4% (10/34) below 50 IU/ dl and 70.6% (24/34) normal.

Table 3. Relationship between Factor XI and Factor XII

			Factor XII		
			Average (50-150)	Abnormal	Total
Factor XI	Average (50-150)	Number	7	0	7
		% of the total	20.60%	0.00%	20.60%
	Abnormal	Count	17	10	27
		% of the total	50.00%	29.40%	79.40%
	Total	Count	24	10	3. 4
		% of the total	70.60%	29.40%	100.00%

Of the total the 34 samples, 20.6% were found with both factors between normal limits, and 29.4%, both with abnormal factors. The factor with the greatest alteration is factor XI.

Parallelism, ¼ dilution for factor XI

Of the 27 samples with factor XI, less than 50 IU/dl, parallelism (¼ dilution) was made to 20, which corresponds to 74% (20/27).

Table 4. Frequency of Factor XI recovery with 1/4 dilution

	No	%
He recovered	20	100
Total	20	100

Of the 20 samples of factor XI to which parallelism was performed, 100% recovered

Of the 10 samples with factor XII less than 50 IU/ dL, parallelism was performed (¼ dilution) to 9, corresponding to 90% (2/9), not recovered.

Table 5. Frequency of Factor XII recovery with 1/4 dilution

	No	%
I do not recover	2	22.2
He recovered	7	77.8
Total	9	100

Of the nine samples to which parallelism was performed, 22.2% (2/9) did not recover, which suggests factor deficiency and 77.8% (7/9) did recover.

Of the 10 samples to which parallelism was performed for factor XI and XII, 7 recovered in both factors.

Discussion

The results of this study of factors XII and All samples with positive AL were dosed with Factor XI and Factor interference of antiphospholipid acs in 79.4%, in which 100% recovery of the factor was observed. The data observed in the samples analyzed reflect the importance that in all hemosta-

sis laboratories where these factor dosage tests are performed and these are decreased, dilution tests are performed to evaluate interference by possible antibodies, and in this way the results issued are used by doctors to follow the best clinical conduct for the benefit of patients. As recommended by the WFH guidelines (27)

It should be noted that when determining factor XII of the samples positive for lupus anticoagulant, interference from antiphospholipid antibodies was found in 29.4%, for which reason the dilution test (1/4) was performed, in which recovery was observed in 77.8% and 22.2% did not recover, confirming the factor deficiency. These data make clear the importance of carrying out the dilution in hemostasis laboratories, since samples with real factor XII deficiency can be found, but also others that are simply caused by the interference of antiphospholipid antibodies. When we receive samples with prolonged PTT we can find ourselves in different scenarios: samples with real deficiency of some coagulation factor (Factor XI and XII), samples with positive lupus anticoagulant accompanied by a deficiency of some contact factor, or only samples with lupus anticoagulant positive with normal factor dosage after performing dilution tests.

This experience with this work makes clear the importance of the clinical laboratory in carrying out these tests and that the responsible professionals can carry out the complementary studies that are required to give a correct and clear interpretation of the results. Well-performed and interpreted tests will facilitate clinical correlation and thus the treating physician will adopt the best behaviors, especially since these patients will receive chronic anticoagulation. On the other hand, it must be remembered that the laboratory must always have excellent communication with the treating physician to confirm any finding of interest. As recommended by the WFH guidelines (27)

When carrying out the comparative study between factors XII and XI, it was found that 29.4% of the two factors were altered, there is a higher incidence of alteration in factor XI, as well as a higher frequency in male children.

A notable finding is that the longest PTT values were found in children from 8 to 13 years of age compared to the other age groups.

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Conflict of interests

The authors express that there are no conflicts of interest.

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Review article

Systematic review and bibliometric analysis of the metabolome found in human breast milk from healthy and gestational diabetes mellitus mothers

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Abstract

Introduction. Human breast milk is considered the gold standard of nutrition, given that thanks to the diversity in the metabolome it manages to meet the individual needs of each infant by providing essential metabolites that contribute to and intervene in optimal growth and development. Few factors can modify the composition of breast milk and, simultaneously, its benefits. However, the increase in maternal metabolic diseases such as gestational diabetes mellitus raises the question of whether it can be one of the factors that condition the quality and quantity of metabolites contained in breast milk. **Objective.** To identify the metabolome of breast milk from healthy mothers, its influence on the growth and development of the infant, and to recognize those that are altered because of gestational diabetes mellitus. **Methodology.** A systematic review was carried out using multiple databases. For the bibliometric analysis, we used the results of Web of Science and Scopus and the Tree of Science and Bibliometrix software.

Keywords: metabolome, breast milk, diabetes mellitus.

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Introduction

Metabolomics is a branch of the omics sciences responsible for studying the set of metabolites present in biological systems known as the metabolome (1,2). In it, low molecular weight molecules can be identified in each matrix at a given moment in time, generating an overview of the sample that determines the physiological or pathological state of the sample, which can contribute to the detection of risk factors in populations (3).

From the metabolic and nutritional point of view, one of the populations of special care are infants between 0-6 months; during this stage of life, it is proposed that feeding should be exclusively through breastfeeding. This is defined as a physiological act that provides the baby with specialized and individualized nutrition, by containing the optimal distribution and quantity of nutrients that are adapted to the nutritional and energetic needs of the infant, It is considered the gold standard of nutrition in this first stage of life and this is how metabolomics in neonatology offers an important approach to investigate the link between nutrition and infant health. (4) The first study to analyze the metabolome of breast milk, according to researchers, was conducted by Cesare Marincola et al. in 2012 (5)(6).

Breastfeeding is the main protective factor against infant health; therefore, its promotion, protection, and support have been a priority for all countries. In Colombia, breastfeeding is initiated from the very instant of birth and currently, 43% of mothers use it as exclusive infant feeding according to a report published in 2017 by Unicef, the World Breastfeeding Collective, and the World Health Organization.

The world is currently undergoing a public health problem generated by epigenetic conditions in people. This problem encompasses metabolic diseases, which include diseases such as obesity, type 2 diabetes, heart and/or metabolic disease, and non-alcoholic fatty liver disease (7,8).

The metabolic diseases with which the mothers arrive at pregnancy or develop during pregnancy can influence the quality of the nutrients in breast milk and in the same way have repercussions on the health of the infant, mainly leading to metabolic disorders in the short and medium term (9). Recently, the variation in the metabolome of breast milk in diabetic mothers in relation to healthy mothers was published, finding a high variation in amino acids and lipids that are fundamental in the development of the child (10).

Gestational diabetes mellitus (GDM) is understood as "an intolerance to carbohydrates that is discovered or manifests itself for the first-time during gestation" (11). In Colombia, the prevalence is currently estimated to be between 10.3% and 15% of which 5% are type II diabetics, 7.5% are type I

diabetics who become pregnant, and the remaining 87.5% are true diabetes of pregnancy (12). All these figures are on the rise due to poor eating habits, the predominant sedentary lifestyle and environmental conditions that generate even more metabolic diseases such as gestational diabetes mellitus (GDM), so it is vital to know the impact of this disease on the first protective factor of childhood, which is breast milk.

The objective of this review is to identify the different metabolites in breast milk from healthy mothers, their influence on the growth and development of the infant and to recognize those that are altered because of GDM.

Methodology

Systematic research was carried out by means of a literature search in databases such as Web of Science, Scopus, Scielo, PubMed, and Google Scholar using the keywords "Metabolome", "Gestational diabetes mellitus", "Human Breast milk metabolome", "metabolomics profile" "growth and development" "neonatal health". Using the connectors "AND" and "IN".

Articles whose content dealt with the keywords were chosen, as well as epidemiological studies on prevalence rates in this geographical area and associated factors.

The reviewed publications were selected with a maximum age of 6 years, except for root articles.

For a complete understanding of the studies, the criteria considered for inclusion were: (i) published primary research (original), (ii) systematic reviews, (iii) reviews, and (iv) research or clinical trials. The literature search was conducted in a period between January 2020 and July 2021.

Search equation

A bibliometric analysis was performed using the packages "tosr" and "bibliometrix" (13,14) for this, the search was performed in Web of Science (WoS) using the following equation: Topic: (metabolomic* OR metabonomic*) and Topic: ("breast milk" OR "human breast milk") in a period from 2001 to May 15, 2023, in the indexes SCI-EXPANDED, SSCI, A&HCI, ESCI. Likewise, a search was performed in Scopus using the same equation in the fields Title, Abstract, and Keywords. From these searches, 165 results were obtained in WoS and 272 in Scopus. All the results were downloaded with the references cited in each of the articles, to construct a citation network for the prioritization of the documents. The WoS and Scopus results were merged into a single data file using the R package "tosr".

Citation analysis

Each of the references and citations of each document were analyzed by means of a citation network using the SAP algorithm of Tree of Science, which is an open

code for prioritizing publications (15). This systematic search methodology is based on the prioritization of documents using indicators of the topology of the citation network explained in detail by Zuluaga et al. (16) and making an analogy with the tree of knowledge. Briefly, the methodology consists of identifying the classic articles (roots), the articles that gave growth to this area of knowledge (trunk), and the recent articles with the greatest impact (leaves). The citation network is used to extract the documents with a high degree of entry, the documents with high intermediation, and the most recent ones that are linked to the root and the trunk. Likewise, the modularity algorithm (17) is applied to select different clusters or perspectives in the knowledge area. The number of clusters is selected using the tipping point algorithm; (13,18). All the code for this citation analysis is available through the following link: https://github.com/coreofscience/tosr. Additionally, 104 articles found in the PubMed database that were not prioritized in the methodology described above were manually added (Figure 1).

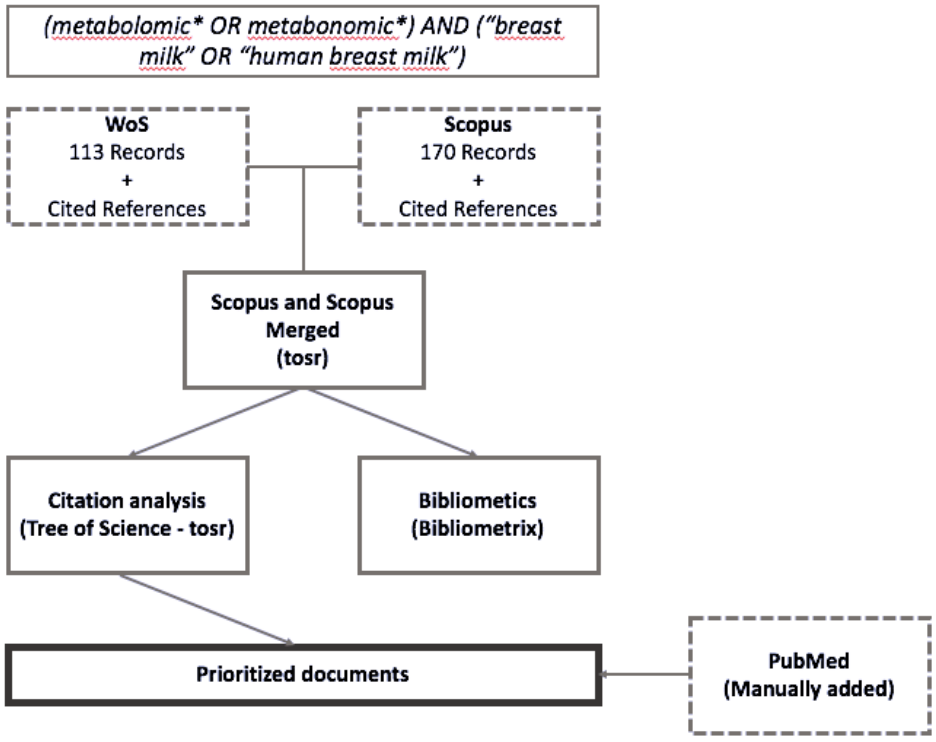

Figure 1. Methodology for the search and selection of articles. Own source.

Bibliometric Analysis

Bibliometric analysis was performed using the Biblioshiny tool, an application of the R package Bibliometrix (14), from which indicators such as annual scientific production, number of citations per journal and per author, publications per author per year, trend of keywords per year and the collaboration network were extracted.

Results

Search equation and citation analysis

The literature review was carried out in WoS and Scopus since between these two databases they integrate more than fifty thousand scientific journals (19) and additionally allow the user to download the metadata and references cited within each article. The search equation resulted in 272 references in WoS and 165 in Scopus, which were downloaded in plain text with all the metadata and citations reported in each of the articles resulting from the search. These results generated an initial network of 805 nodes (articles) and 1878 links (citations); after using the network indicators and filters to extract the groups or perspectives, a final network of 347 nodes and 582 links was obtained (Figure 2).

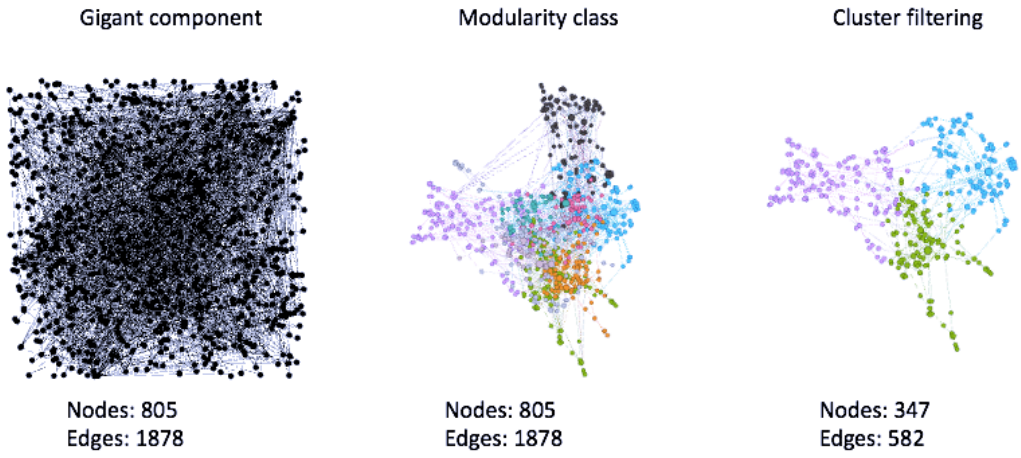

Figure 2. Prioritization of documents by citation analysis. Own source.

The title of each group was extracted to generate the word cloud for each perspective (Figure 3). Likewise, the articles were organized according to their importance by means of the root, trunk and leaf indicators as explained in the methodology section:

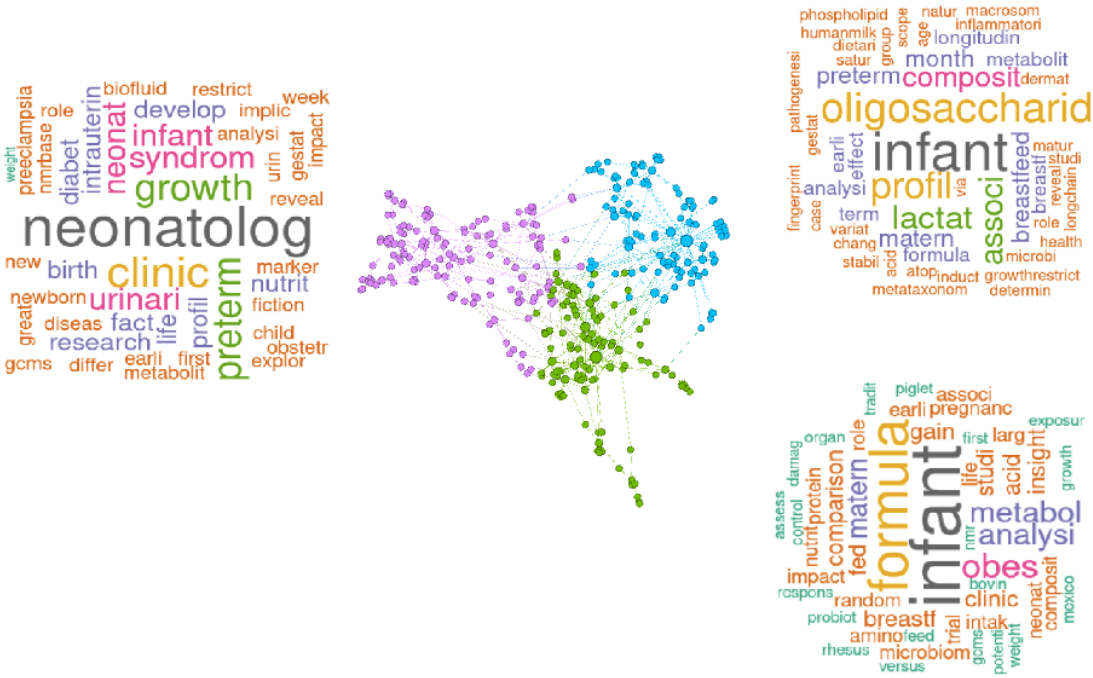

Figure 3. Citation network and word cloud. Own source.

The first perspective represented in Figure 3 by the purple cluster represents 16.89% of all the articles in knowledge. According to the word cloud, it can be inferred that the articles are related to the influence of breast milk on growth, and the clinical behavior of neonates in situations such as preeclampsia, diabetes, and preterm birth. The second perspective determined by the green cluster represents 13.54% of the total number of articles and refers to the study of formulas in different animal models and metabolomic analysis by NMR. The third cluster represents 12.67% and is represented by the blue color, and is related to the oligosaccharide profile, lactation studies, and longitudinal studies of newborn feeding.

Bibliometric analysis

The bibliometric analysis was divided into four different aspects, the first related to publications and journals, the second related to authors, the third to the documents with the highest impact and the fourth to the social structure. The metabolomic studies of breast milk show according to the number of publications per year an increasing trend, which during the year 2022 presented the highest number of publications (Figure 4a). Figure 4b. The five articles with the highest number of citations are shown. Marincola et al., (2012) and Marincola (2015). Spevacek (2015). Sundekilde (2016).

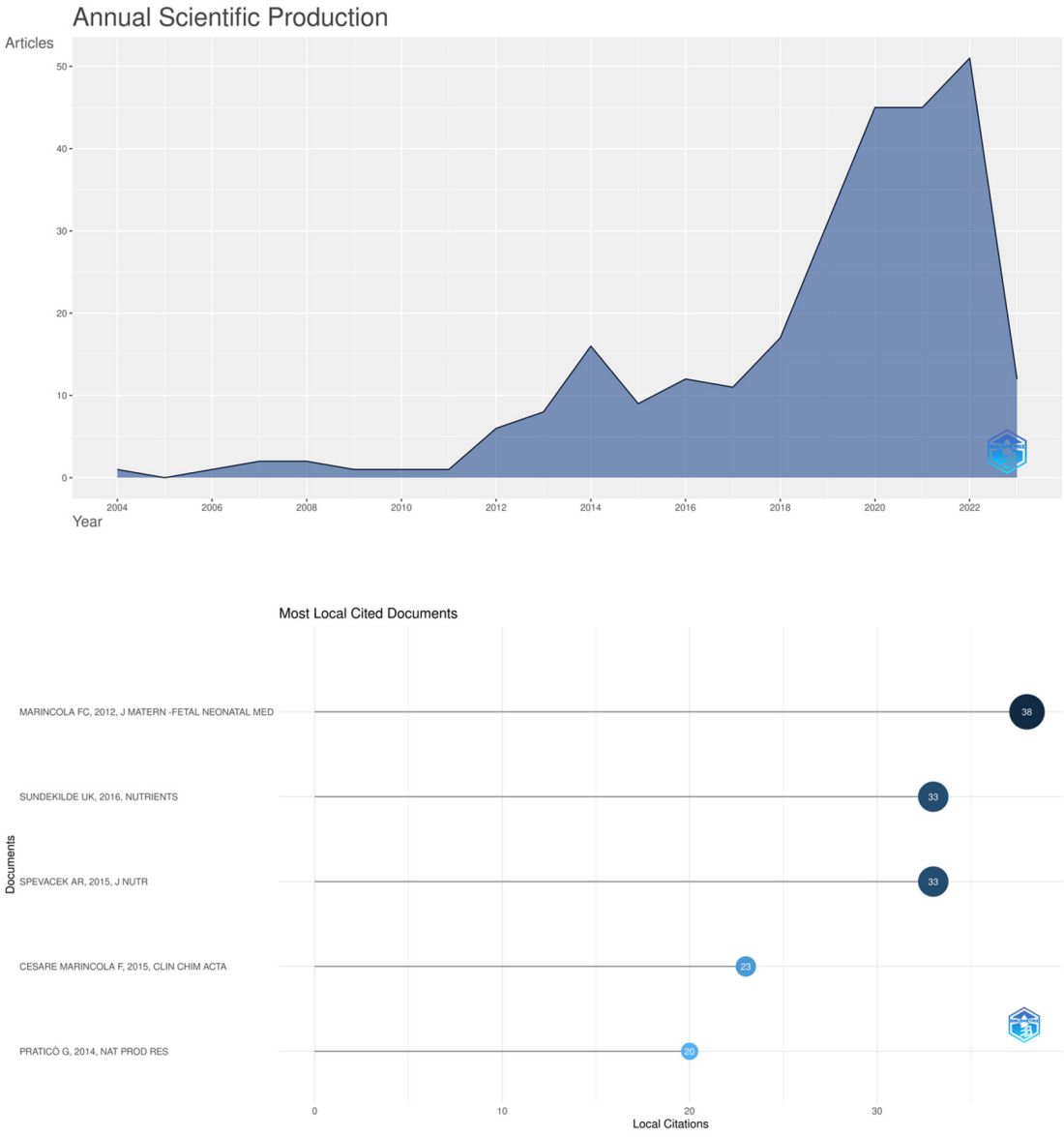

Figure 4. a. Annual scientific production. b. Papers with highest citations. Own source using bibliometrix software.

On the other hand, the five authors who have published the most in metabolomics and breast milk are shown in Figure 5a, where the total number of published documents related to the area of knowledge is indicated. Figure 5b shows the frequency of publication of each of the five authors, with the number of documents published per year.

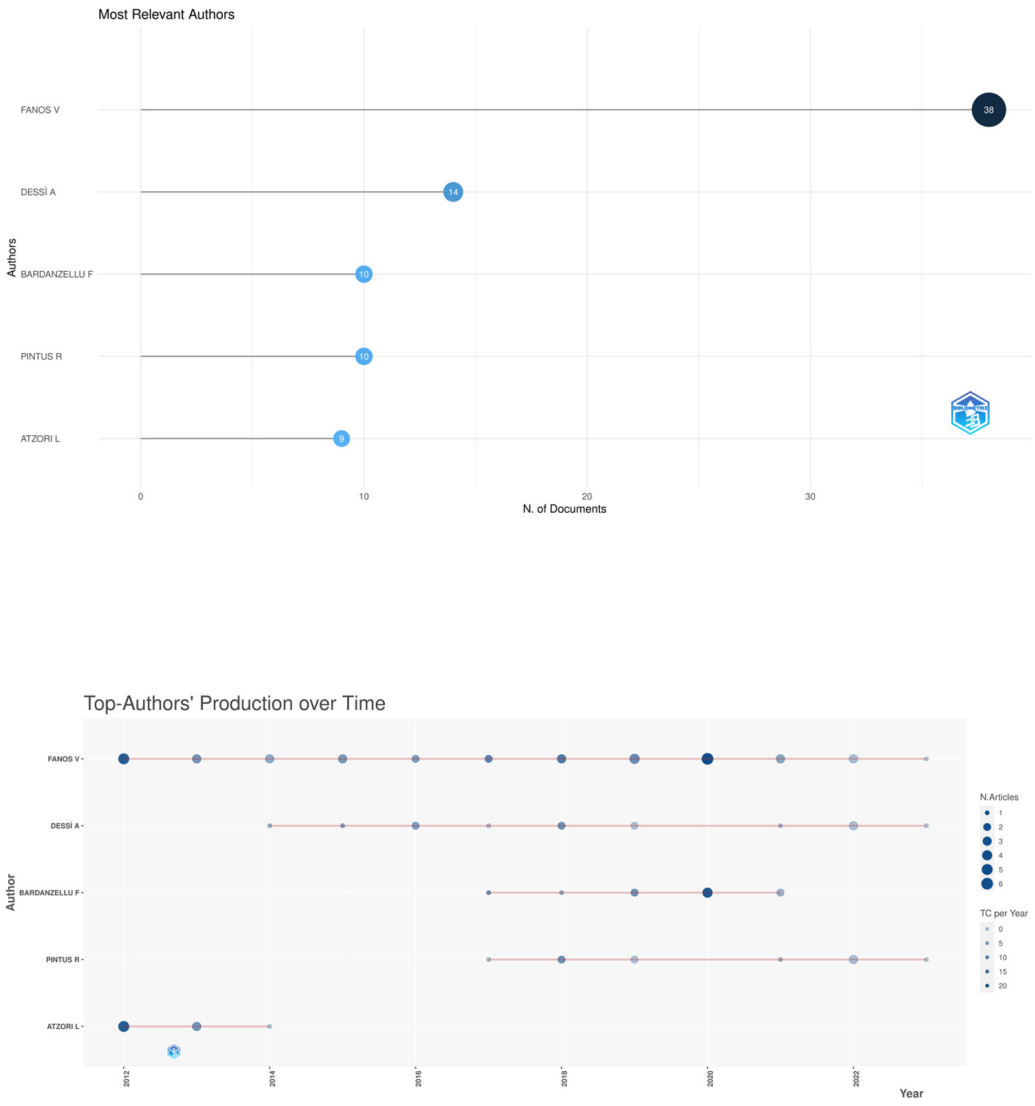

Figure 5. a. Most relevant authors. b. Authors' production over time. Own source using bibliometrix software.

The research of Fanos and collaborators has been based on the study of the components of breast milk, their bioactives and their interaction with the development of the infant. Atzori and coworkers delved into the application of metabolomics in maternal-fetal medicine and the recognition of metabolome changes in mothers with gestational diabetes mellitus. The focus of Bardanzellu et al. is on the characterization of the metabolome and microbiome of breast milk and its comparison with formula milk, where the influence of each nutrient on neonatal metabolism is evaluated. Dessí et al. focused on the clinical impact of metabolites contained in breast

milk, their intra- and intervariability; and Reali et al. investigated the metabolomic differences between colostrum and mature human milk and their functions at each stage of development.

Collaborative networks are of co-authorship analysis where clear evidence and results are shared that allow a greater association and deepening of the present subject studied.

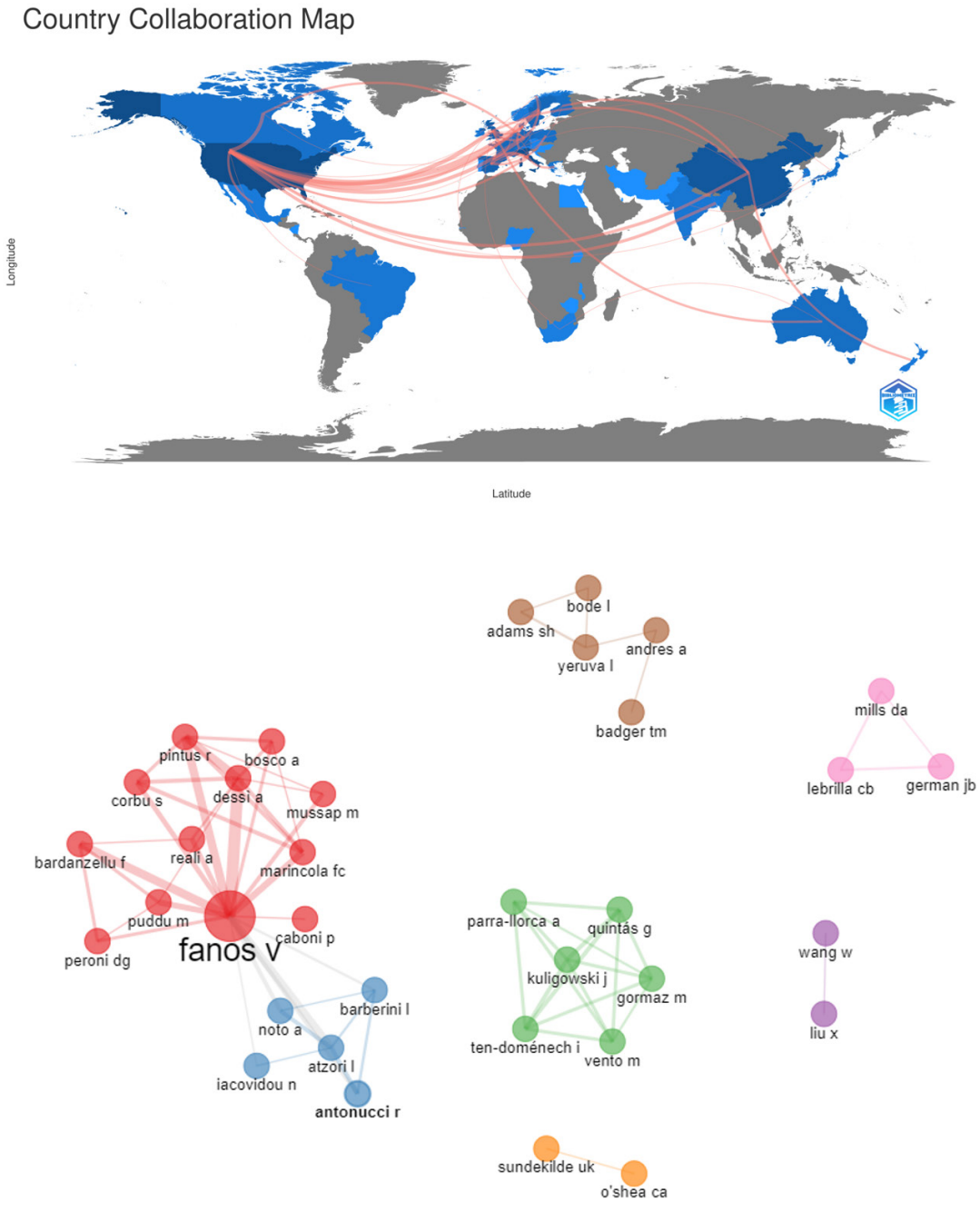

Figure 6. a. Collaboration networks. b. Contribution between countries. Own source using bibliometrix software.

Analysis of the documents

The multiple properties and components of breast milk in the neonatal stage are crucial for optimal growth and development since this is one of the most critical periods of life (20).

However, breast milk contains about 600 species and it is stipulated that a breastfed infant ingests up to 10 million live bacteria per day (6), in addition, this liquid has special characteristics that allow the neonate to adapt to extrauterine life and influence the rest of it, thanks to its capacity in the activation of metabolic processes such as growth, development of the immune, nervous and cognitive systems, colonization, intestinal microbiological maturation, among many others in which the bioactive compounds of breast milk intervene (21,22).

Although the industry has made efforts to create formula milk similar to breast milk, it has been identified that this objective has not been achieved so far, in view of the fact that there are marked differences both qualitatively and quantitatively in relation to the metabolites that compose it, being amino acids and fatty acids the ones with the greatest variability (23-26).

In a comparative study of the year 2021 between the metabolites of human milk, goat's milk, equine milk and cow's milk, 37 metabolites were found to be significantly different among the four samples, and complementarily, seven main metabolic pathways were identified in the milk of these animals that allow a global analysis of their metabolome (27). However, in the study conducted by PHAN M and collaborators, it is evident that after an analysis of 261 metabolites, 151 of these, present in formula milk, are closely related to human breast milk (28). This is evidence that the efforts made by the industry to achieve formula milk similar to breast milk are progressing more and more, but, even so, an exact similarity has not been achieved, so it cannot replace it.

The composition of breast milk is not stable; on the contrary, it undergoes a series of transformations according to the biological age and physiological needs of the infant (5,29,30). It has even been shown that the external environment (geographical location), the internal environment (mode of delivery), the state of mind, and the dietary intake of the mothers can alter the composition of the milk (31)(32)(33).

When the lactation process begins, the liquid coming from the mother between the first and fifth or sixth day is known as colostrum, characterized by its thick and yellow appearance, contains a large number of proteins and minerals, as well as many immune active substances, such as macrophages and lymphocytes, complement systems, lysozyme, oligosaccharides and antimicrobials such as lactoferrin, which give an immunoprotective action to the newborn and

decrease the production of inflammatory cytokines (34,35).

After the first six days of lactation, some authors describe the existence of transition milk, in which nutrients and caloric content stabilize to give way to mature milk with a completely different metabolomic composition to meet the requirements of this stage of the infant (36). After the first month of lactation, breast milk reaches its maximum point of maturity in metabolites and energy which tends to optimal development and growth in this first stage of life (35,37).

It should be clarified that human milk metabolites are derived from three sources: (i) nutrients from synthesis in the lactocyte, (ii) those of dietary origin where the concentrations in the milk from the mammary gland are strongly related to the mother's intake (38) and (iii) those from maternal reserves regardless of the state of the milk (39). The mother's health and nutritional status, all this to guarantee one hundred percent of the caloric and nutritional intake demanded by the infant (29,30,39).

Mature milk is the food that is provided for a longer period in infant lactation compared to the other phases of milk, which are transitional and of short duration. In fact, it has been suggested that the metabolites and microbiome of mature milk remain stable for up to 24 months (40). Therefore, it is of vital importance to characterize this biofluid and the relationship it has with the growth, and development of the infant and its physiological functions (41).

For an adequate metabolomic analysis of breast milk, several aspects must be considered, from its extraction, preparation, storage, and study. The most critical parts of the process are storage since this step must be carried out quickly and at appropriate temperatures, and study since it identifies polar and non-polar elements (42).

One of the techniques for the analysis of these compounds that provides a complete view of the dynamics of breast milk is liquid chromatography adapted to mass spectrometry, since it guarantees a wide coverage of the metabolome of breast milk, especially for its fatty part (glycerophospholipids and sphingolipids of low abundance), in addition, it allows monitoring short-term changes and the composition of variable metabolites. However, it should be clarified that the analysis to quantify HMO can only be performed in those that have a reference standard (26,43,44).

Currently, there are new study techniques such as mass spectrometry with capillary electrophoresis that allow an evolution in the study of the metabolome of diverse fluids, given that it identifies highly ionic and polar elements such as organic acids, sugars, and amino acids that cannot be appreciated

through liquid chromatography, however, being a recent technique, it is difficult to access and presents a higher cost (45).

Considering that this liquid is stable throughout the months, its classic distribution of metabolites is highlighted. The main component and the one which is present in greater quantity is water with 88% - 90%, as quality it presents an osmolarity like that of the blood plasma which allows the infant an optimal hydroelectrolytic balance without having additional water requirements (41).

Lipids are the most abundant solid fraction during this stage of breast milk, contributing 40% to 55% of the total energy of the milk, followed by carbohydrates, considering that the most abundant in mature milk is lactose (6.7 g/100 ml). This disaccharide provides the infant with a high energy demand for brain function and when metabolized, it is the most important source of galactose for the maintenance and development of the central nervous system (30,41).

The protein contained in mature milk is produced in 80% to 90% by the lactocytes of the mammary gland, making it the third most abundant solid in breast milk (41,46). In addition, breast milk is characterized by the presence of different compounds, not nutrients, which together with the macronutrients will be essential in the infant's nutrition, fulfilling physiologically specific functions.

Although the systems of the term neonate are complete, many of them are immature. There is a variety of evidence correlating the metabolites in breast milk and the influence it has on maturation.

Growth and development is an event that occurs during the stage of conception and ends with adolescence, having one of its highest peaks during the first year of life, since it influences the exponential maturation of all organs and tissues to ensure proper functioning in the rest of the stages of life (47).

There are specific metabolites for the different adaptations of the infant's systems; however, the growth factors in breast milk play a transversal role in all the developmental processes of the child, since among their fascinating characteristics is their ability to reach the target organs intact, such as the nervous, gastrointestinal, respiratory, epithelial, and circulatory systems (48). Metabolites in the gastrointestinal tract

The infant's stomach has a reduced gastric capacity, and the intestine has not yet reached its potential in size. This is where the importance of feeding with breast milk lies since its composition contains growth factors such as vascular endothelial growth factor (VGEF), which interacts with specific receptors of the intestinal mucosa, particularly with those of the epithelial lineage. This generates hyperplasia and hypertrophy favoring the absorptive capacity of nu-

trients (22,49) It should be noted that the newborn has been exposed to microbial flora since the uterus, however, there is no established symbiosis (50).

Breast milk from the early stages of lactation provides the amount of enterobacteria necessary to enhance the immune response and metabolic processes. For these bacteria to survive, the metabolites that function as prebiotics are mainly HMOs, which are the third most abundant solid unit in breast milk, presenting up to 200 different HMOs, which makes it the mammalian milk with more of these compounds in a concentration of 16 g/L in milk (51,52).

On the contrary, they are used by non-pathogenic bacteria (53), especially by those belonging to the bifidobacteria genus, providing substrates which, when metabolized, confer beneficial effects (52), such as the generation of short-chain fatty acids (SCFA), which create a stable intestinal ecosystem (30,54).

The growth and maturation of the neonatal intestinal mucosa play a primordial role in the capacity of nutrient absorption and confer health and immunity to the infant. Certain growth factors and cytokines present in breast milk by mammary secretions such as TGF- β 2, EGF or FGF21, TGF β 2, and IL-10 promote intestinal maturation, configuring a uniformity in the microbiome by means of particular interactions between probiotic bacterial strains and these elements, thus guaranteeing diversity in the microbiota (55,56).

The same happens in pre-term neonates, who need to increase the rate of early growth and brain development due to their physiological conditions, so the milk of pre-term mothers is adapted to these rapid growth needs (57) thanks to its content of insulin-trophic and branched-chain amino acids, lacto-N-fucopentaose, choline and hydroxybutyrate, which are fundamental metabolites in energy utilization, protein synthesis, oxidative status and maturity of intestinal epithelial cells so important in prematurity (58).

Breast milk is the main exogenous source in the infant of compounds called polyamines. Among them, spermine and spermidine stand out in this biofluid, which generates growth, mucosal maturation, and cellular proliferation in the neonate (59). The tissues lining the baby's intestine are labile grow at high rates and have a very high turnover activity, which is why breast milk offers high concentrations of these molecules (30).

The enterocyte is the cell that makes up the intestinal parenchyma and its optimal function, maturation, and activity are directly related to the substrates that reach it (60). The free amino acids that reach the intestine through the ingestion of breast milk are, with the greatest abundance, glutamine,

and glutamate, comprising 70% (61). Glutamine is a precursor of intestinal health, so its absence as an exogenous supply will limit the functioning of enterocytes, due to the fact that its endogenous synthesis is limited (62). It has the capacity to be oxidized by intestinal cells and immune tissue present in the intestine, which makes it an energetic substrate for the periods of rapid and exponential growth that this organ undergoes in the first months of the life of the infant (61,62). Additionally, glutamine in the company of microbiota supports the intestinal barrier function by modulating certain specific intracellular pathways and decreasing the permeability of the wall (62).

Glutamate, an amino acid that increases throughout lactation, contributes to the necessary production of energy to support intestinal functions, and neurodevelopment, in addition to promoting the microbiota in the infant's intestine and regulating appetite (30,63,64).

For an adequate absorption and facilitation of the metabolism of nutrients, there are substrates that intervene in this task, such as lactoferrin, osteopontin, milk fat globule membrane (MGFM), and palmitic acid, this helps the absorption of calcium and magnesium, and jointly decreases constipation in the newborn (30,65-67).

The maturation and integrity of the gastrointestinal tract are of vital importance since this system performs the uptake and absorption of nutrients, directing them into the bloodstream to reach the other sites in the body, in seeking to satisfy the needs of the organs for optimal development (68).

Metabolites in the immune system

At birth, the infant is directly exposed to all the pathological factors that previously protected it in the amniotic sac, placenta, and intrauterine environment (69). The newborn presents a complete immune system, however, portrays some specific deficient functions such as "physical and chemical barriers, deficient function of innate effector cells, limited and delayed production of secretory immunoglobulin A (IgA), complement cascade function, and insufficient anti-inflammatory mechanisms of the respiratory and gastrointestinal tracts" (66).

The main immunological protectors of breast milk are immunoglobulins, including IgG, IgM, and IgA. The latter is the most prevalent and inhibits the binding of pathogens to the intestinal epithelium by trapping them in layers of mucus. IgM is responsible for binding identified pathogens and thus activating the complement factor. IgG transports, by phagocytosis, the antigens to the lamina propria, and has the capacity to activate the complement factor (66).

One of the fundamental components in this stage for the strengthening of the immune system is the growth factors since they

have an immunoregulatory function due to the action they exert on the cells of the mesenteric lymph node, thus allowing a notable increase of the natural killer (NK) cells, maturation of lymphocytes and reduction of cytokines which are directly related to allergic reactions (56). The function of both epidermal growth factor (EGF) and transforming growth factor (TGF) are involved in modulating the inflammatory response and improving the response to pathogens thanks to the production of B lymphocytes, immune cells of the thymus and lymphoid tissues (12,34,70). Macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor (G-CSF) contribute to the survival of macrophages in breast milk (69).

The latter together with neutrophils, T cells, stem cells, lymphocytes, antimicrobial factors, immunoglobulins, and cytokines operate in networks and orchestrate the functions of the infant's immune system (71). Speaking especially of stem cells, they trigger the beginning of the immune system response in the newborn, since they have the capacity to migrate to different organs and differentiate, among which is the thymus which, being a lymphoid and specialized organ of the immune system, its development will be directly related to the cellular immune response of the neonate's organism (72)(73).

Breast milk contains cytokines which are pluripotent peptides that have an immunostimulatory and immunomodulatory effect, inducing phagocytosis and antigen presentation, growth, and differentiation of immunoglobulin, and suppressing the production of immunoglobulin E (IgE). In summary, they are not only responsible for passive protection but also intervene in the immunological development of the recipient infant (52,69). Within the mammary gland, IL-6 participates especially, generating, in an increased manner, IgM and IgA immunoglobulins and decreases IL-1 and TNF α (74).

The metabolite responsible for iron deprivation, alteration of membranes of pathogenic microorganisms, microbial receptor analogs, and favoring the growth of epithelial cells is lactoferrin and its derivatives which have antimicrobial and fungicidal functions (65,75).

Lipids also modulate the immune system. For example, phospholipids, although they are in smaller quantities compared to colostrum and transitional milk, play an indispensable role in immune and anti-inflammatory responses of the infant together with omega 3 and omega 6 fatty acids since they are fundamental in anti-inflammatory and inflammatory processes respectively (76-78) Other lipids involved in the immunity of the infant are short chain fatty acids from the metabolism in the microbiota of HMO. Their mechanism is to acidify the intestinal lumen and thus prevent bacterial growth (52).

Other influential metabolites are osteopontin which performs immune regulation by interacting with cell surface integrins and CD44 receptors (65,79) and simultaneously, glutamine and glutamate are immunomodulators preventing neonatal allergies and infections (61).

Many pathogens, to infect, adhere to the glycocalyx, which is a layer that covers epithelial cells and is formed by glucans, proteins, and lipids. HMOs are structurally like glucans, which is how pathogens and toxins identify them as sites of adherence and thus transit the gastrointestinal tract without causing disease (29,52,80). Metabolites in neonatal metabolism.

At this stage of life, the newborn requires precise amounts of energy from breast milk nutrients for proper organ function and thus promotes constant growth and development. The lipids in breast milk are the main metabolites in the production of energy that the newborn needs to fulfill and maintain the body's functions. About 98 to 99% of these are composed of triacylglycerols, whose properties are derived from the fatty acids of which they are composed (67,81).

The lipid fractions in breast milk are distributed as follows: "34% -47% Saturated fatty acids being mainly palmitic acid with 17% -25%; 31% to 43% are monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) with 12% -26% omega 6 (n-6) and 0.8% -3.6% omega 3 (n - 3)" (30). Concluding that the ratio of unsaturated versus saturated fats is higher than in other mammals (67).

Mature human milk stands out for containing a higher concentration of lipids, which form the structure of the cell membrane, are responsible for signal transmission and cell recognition in signaling pathways, as well as influencing lipoprotein metabolism, being transporters and sources of fat-soluble vitamins (81).

Some of the fatty acids that make up breast milk are palmitic acid, which, in addition to providing energy, has a surfactant function within the respiratory system, preventing the collapse of the alveolus and, at the same time, contributes to the absorption of magnesium and calcium (30,67). The long-chain polyunsaturated fatty acids involved in the growth and maturation of various organs in the infant are n-3 (α-linolenic acid) and n-6 (linoleic acid). However, not all lipids have an impact on adiposity gain. On the contrary, many of them will fulfill other tasks within the newborn's organism. The only fatty acid that has been directly related to adipose gain is linoleic acid (82).

There are endogenous lipid mediators derived from long-chain polyunsaturated fatty acids called endocannabinoids. Although their function has not been extensively studied or understood, Gaitan et al. established that mature milk contains lower

levels of arachidonoylglycerol, which plays an important role in the sucking pattern of the infant (83). An important metabolite in energy generation is carnitine, since it promotes lipid utilization by controlling the entry of fatty acids into the mitochondria, facilitating the oxidation of pyruvate and large amounts of branched-chain amino acids (67).

Another large group of metabolites favoring energy production in the neonate are carbohydrates. Their total amount is related to the infant's length, weight gain, and percentage of fat-free mass (84). Lactose is positively associated with weight gain and adipose reserve in the period from 3 to 12 months of the infant (82), this is closely related to the increase of this disaccharide in human milk as the lactation time passes; and to maintain the osmotic state in balance, citrate, which is part of the intermediates in the energetic metabolism of the cells and in tricarboxylic acid, in this phase its quantity decreases (23). Its influence on infant growth is fundamental since it allows the absorption of nutrients that are directly related to bone health and growth such as calcium and others such as copper and magnesium involved in the generation of energy through phosphorylation (85).

Amino acids that promote energy generation by providing nitrogen necessary for growth in the newborn are alanine, glutamate, glutamine, isoleucine, threonine, valine, methionine, and the organic compound creatinine, the latter of which transports energy to sites of ATP synthesis (36). Glutamine participates in growth by enhancing the effects of growth factors including insulin-like growth factor and epidermal growth factor, while glutamate acts on weight gain (22,30,36,61).

Metabolically active proteins that are synthesized in the adipocyte have important metabolic functions, these are called adipokines formed by leptin that produces satiety, leads to high energy consumption, and increases serotonin availability; adiponectin increases insulin sensitivity; ghrelin favors regulation of energy metabolism and stimulates the secretion of growth hormone (GH) and obestatin modulates cell proliferation (86,87).

The growth factors play a fundamental role in the formation and maturation of various organs that consequently avoid any alteration in the baby's organism and promote adequate growth, the main ones are: vascular endothelial growth factor (VEFG), hepatocyte growth factor (HGF), epidermal growth factor (EGF) and insulin growth factors (IGF) (22) At the same time, polyamines that regulate development and stress response are necessary for cell growth and proliferation (59,77).

Each organ or system within the newborn's body uses specific metabolites for healthy development and within normal parameters. Thus, micronutrients such as zinc,

iron, and copper are used for the functions of most of them because they are part of enzymes, biocatalysts, and cofactors of different reactions (88). On the other hand, vitamins B5, B6, B9 and B12 perform biological activities in nucleic acid production and influence metabolic pathways such as glycolysis, gluconeogenesis, and amino acid metabolism. Together with other cofactors, they can influence DNA methylation by carbon metabolism (89). Organs such as the brain, pancreas, thymus, spleen, kidney, and liver mature mostly due to stem cells contained in breast milk (22,73).

Visual development is strongly influenced by the contribution of DHA and taurine (90) and the circulatory system benefits from choline and betaine for the prevention of cardiovascular diseases that may affect the health of the newborn (91). Hence lies the importance of the contribution of breast milk, since it has been shown that infants fed with this biofluid have a higher excretion of choline than those fed with formula milk, which implies better coverage (36,92).

Metabolites in bone development

Ossification in infancy is dependent on calcium and phosphorus (in a ratio of 1.2 to 2 respectively) and on vitamin D which is found in its original form in very low levels in breast milk but is sufficient to perform the functions of calcium homeostasis and bone metabolism accompanied by high concentrations of parathormone (PTHrP) which reduces the risk of hypocalcemia due to rapid accumulation in the bone (93,94).

Metabolites in the nervous and cognitive system

The brain is one of the organs that requires specific nutrients and metabolites for its development. The neonate needs high demands to fulfill the 4 stages of maturation that may not be consecutive, but simultaneous, they are cell proliferation; migration; organization and lamination of the brain; and finally, myelination. These are affected by internal or external factors so that an inadequate supply of nutrients would delay optimal maturation (95).

Neural myelination allows an adequate transmission of nerve impulses. During the lactation period. The baby receives sphingomyelin through the mother's milk which promotes the coating of neurons and improves neurobehavioral development in infancy (96). With the help of B vitamins, brain plasticity, and neuronal differentiation are regulated, promoting other brain functions (71). Particularly vitamins B9 and B12 are involved in homocysteine metabolism and myelin preservation. Their deficiency may lead to neurodevelopmental disorders (89).

Lipids and HMO are the metabolites with the greatest incidence in brain health, both in its development and in good neuronal functioning, because the brain is the organ

that houses a large amount of lipids after adipose tissue, the difference being that brain lipids are mainly polyunsaturated fatty acids and structural phospholipids of the membranes (97).

This is demonstrated by linolenic acid by the contribution of DHA which particularly targets the gray matter areas involved in motor control, sensory integration, and tension. In turn, arachidonic acid (ARA) derived from omega 6 is responsible for the plasticity of the hippocampus (89) and together with linoleic acid promotes brain development, neurotransmission, and normal functions of the cell membranes of the central nervous system (CNS); as well as HMO, in view of the fact that when fermented in the intestine they induce a regulation in brain signaling and inflammation that can generate some irreparable lesion (48,53,71).

Palmitic acid, although it is not a polyunsaturated fatty acid, is part of the lipids that play an essential role in the transport of proteins throughout the nervous system by a process called palmitoylation (98).

The main source of energy in the brain is attributed to glucose, which is obtained from various carbohydrates, and the normal processes of synapses and nerve impulses depend on its constant supply (89).

One of the oligosaccharides in human milk is 2'-fucosillactose (2'FL) and together with a metabolic resultant of them, sialic acid, which participates in the synthesis of gangliosides and glycoproteins to which important roles in neural growth are attributed, have evidenced notable improvements in later ages in memory, attention, and cognitive results (52,53,97). However, for these processes to occur, brain maturation must be properly established (53).

A large group of diverse metabolites stimulates the above-described, such as creatine, betaine, choline, lactoferrin, osteopontin, fat globules, leucine, isoleucine, and valine. Any error in the metabolism of these triggers direct neurological disorders. It should be noted that the amino acids, together with others such as methionine, participate in the regulation of the sleep-wake cycle in infants (22,36,67,99). While taurine allows a balance in terms of neural cell volume (41,100). The organization and distribution of the nervous system (NS) are modulated by the brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), S100B, and neurotropin (NT-3) which are part of the growth factors and are highlighted as they help vascularization, maintenance and development (101-104). Vitamin D, although not part of the growth factors, its deficiency may cause behavioral and learning disorders later, because it directly induces the activation of nerve growth factors and, additionally, promotes neurite outgrowth and protects hippocampal neurons from undergoing apoptosis (89).

There is much evidence correlating breast milk metabolites and their influence on the growth, development, and health of the infant, but it is even more important to evaluate whether there are maternal factors or conditions that may alter this composition and at the same time its benefits.BF in gestational diabetes mellitus (GDM)

GDM is a pathology defined as "carbohydrate intolerance that is discovered or manifests itself for the first-time during gestation" (12). Metabolic diseases condition maternal metabolism, which could affect the quality of breast milk during the lactation period since it is made from maternal substrates (9,39,105). GDM brings sequelae to both the mother and the newborn, which consequently can alter the composition of human milk and the effects of lactation such as delayed lactogenesis (103).

Some of the metabolites that are affected in mature milk will be described below, considering that 17 metabolites are altered at this stage of the 28 in total that are altered throughout lactation because of GDM (10).

Although energy does not correspond to a single metabolite, but is the contribution of macronutrients comprised in breast milk, according to Shapira and collaborators, the energy content in maternal mature milk was shown to be higher in healthy mothers when compared to mothers with GDM. While another study conducted in 2017 mentioned that in women with high pregestational BMI and GDM presented increased concentrations of fat and energy levels (27,106,107). It is speculated that the hypoglycemic diets that mothers with GDM undergo may be related to the decrease in energy content in this group, however, the mechanism has not been defined and the correlation is not established (106).

Regarding protein, it should be clarified that the literature is not yet so specific. However, Dritsakou and collaborators made significant findings regarding reduced levels of protein in mature milk in mothers with a controlled diet in GDM (107). When evaluating the quantity and concentration of lactoferrin and IgA in the milk of these mothers, it was demonstrated that GDM did not affect these metabolites, therefore, there were no changes, even if they were insulin dependent. It should be clarified that when a survey of the studies was made, no significant changes were found in terms of protein in mature breast milk, although it is important to report that GDM generates modifications in the proteins of human colostrum (103).

Six of the lipids suspended in the breast milk of mothers with GDM showed alterations. Five of them decreased in quantity, being 1 a saturated fatty acid (pimelic acid) and 4 unsaturated fatty acids (9-heptadecanoic acid, 10-pentadecanoic acid, 2-hydroxyglutaramic acid, and nervonic acid), while only one of saturated character increases its concentration (lignoseric acid)

(10). Lipid changes are considered significant in the study of Dritsakou et al. and Shapira et al. in terms of increased concentrations (106,107).

It has been shown that infants breastfed with milk from a mother with GDM lead to excessive weight gain at one year of life, directly related to the high lipid content. It is for this reason that the importance of an adequate intake of breast milk from mothers with GDM together with specialized and modified formula milk especially for these infants has been described to optimize results in body composition in later stages of lactation (27). The carbohydrate content is modified since 3 metabolites corresponding to this group are increased, they are iditol, galactitol, and sorbitol (27). In contrast, the study "The effect of gestational diabetes mellitus on human milk macronutrient content" found a lower concentration of all saccharides in the mature milk of GDM.In parallel, the study "The impact of maternal- and neonatal-associated factors on human milk's macronutrients and energy" reports no evidence of significant changes in these (106,107). In general, studies found no modifications in total carbohydrate levels between milk from healthy mothers and mothers with GDM, including insulin-dependent mothers (103). As for the hormones in the mature milk of mothers with this pathology, the variability found in insulin was related to an increase, especially in those who received exogenous insulin doses, while adiponectin was decreased, but in a specific period of time (day 90 of mature milk supply) (108). The hormone responsible for heat production in the neonate and for the conversion of white adipose tissue to brown adipose tissue is irisin, which according to Fatima and coworkers, is reduced in the BF of mothers with this disease (109).

Metabolites related to anti-infectious activity such as quemerin and dermicin, which stimulate the action of macrophages in inflammation, show higher amounts in the milk of mothers with GDM than in healthy mothers. Their importance in the prevention of diabetes in the offspring is highlighted (110).

The metabolomic profile of breast milk in GDM is varied and very broad; however, there are no recent studies that confirm the changes and associations of this metabolic alteration of the mother with the metabolites in the milk produced postpartum, especially in the mature milk stage. Although there is evidence of an incidence of alteration of the microbiota that can modify this profile (111,112).

Conclusion

As a result of an exhaustive and detailed search of recent studies on breast milk and the effects on the metabolome that it can suffer due to a metabolic alteration such as

GDM, it is concluded that breast milk continues to be the gold standard of nutrition in the first months of life, which is constantly ratified in research on the benefits in growth, development, and health of the neonate and in later stages due to its specialized metabolite content.

Perinatal physiology prepares the mother to respond to nutritional needs after birth, which will be satisfied through breastfeeding regardless of a pathological condition such as GDM. In short, the mammary gland and maternal metabolism generate adaptations to provide the infant with all the necessary metabolites without significant changes that could demonstrate alterations in the growth and development of the offspring of mothers with GDM. Despite this, there is no clear evidence to certify that GDM breast milk induces the development of metabolic diseases in infants at future ages.

In view of the above, more clinical trials are needed to corroborate in a broad and deep way the variations in the metabolome of breast milk in this pathological condition, its relationship with being a conditioning factor for the development of future metabolic diseases in the newborn and whether they are influenced by external factors, mainly the mother's diet, which has heterogeneous characteristics such as culture, geographic region, socioeconomic situation and other social determinants; In addition, to inquire about the metabolism and adaptations of the mammary gland that responds to the needs of the infant even in this pathological condition.

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Conflict of interest

The authors declare that they have no conflict of interest.

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