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ORIGINAL ARTICLE

Investigating the possible protective effect of caffeic acid phenethyl ester on aquaporin-2 changes in renal ischemia-reperfusion injury in rats

Investigación del posible efecto protector del éster fenetílico del ácido cafeico sobre los cambios de acuaporina-2 en la lesión por isquemia-reperfusión renal en ratas

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Abstract

Objective: *We aimed to investigate the changes in renal aquaporins (AQP) of rats in renal ischemia-reperfusion (I/R) injury and the protective effects of caffeic acid phenethyl ester (CAPE) against these changes.* **Methods:** *Forty-five adult rats were divided into six groups: control, sham (right nephrectomy), I/R (right nephrectomy + left kidney I/R), I/R+CAPE (I/R procedure after i.p. CAPE), sham + CAPE, and sham + dimethyl sulfoxide. Blood urea nitrogen, Cr, and K^+ levels were measured in the sera. Tissues were stained with hematoxylin and eosin for histopathological examination. For immunohistochemical analysis, AQP2 was applied using the streptavidin/biotin/peroxidase system. AQP2 gene expression in kidney tissues was examined by polymerase chain reaction (PCR).* **Results:** *In the I/R, congestion, inflammation, and necrosis were found to increase compared to the control. In the I/R+CAPE, improvement was observed in necrosis compared to the I/R. There was a decrease in AQP2 expression in the I/R. In PCR, no significant difference was observed in AQP2 gene expression between the I/R and control and between the I/R+CAPE and I/R.* **Conclusion:** *Renal I/R inhibits the production of AQP2 in the kidney and causes histological and biochemical damage. CAPE administration before I/R has a protective effect on the kidney.*

Keywords: *Kidney. Caffeic acid. Antioxidant. Renal ischemia. Phytochemical. Aquaporin-2.*

Resumen

Objetivo: *Investigar los cambios en las acuaporinas renales (AQP) de ratas con lesión por isquemia-reperfusión renal (I/R) y los efectos protectores del éster fenetílico del ácido cafeico (CAPE) frente a estos cambios.* **Métodos:** *Se dividieron 45 ratas adultas en seis grupos: control, simulado (nefrectomía derecha), I/R (nefrectomía derecha + I/R renal izquierda), I/R + CAPE (procedimiento de I/R tras CAPE i.p.), simulado + CAPE y simulado + dimetilsulfóxido. Se midieron los niveles de BUN, Cr y K^+ en suero. Los tejidos se tiñeron con hematoxilina y eosina para el examen histopatológico. Para el análisis inmunohistoquímico se aplicó AQP2 utilizando el sistema estreptavidina/biotina/peroxidasa. La expresión del gen AQP2 en los tejidos renales se examinó mediante PCR.* **Resultados:** *En I/R se observó un aumento de la congestión, la inflamación y la necrosis en comparación con el control. En I/R + CAPE se observó una mejoría de la necrosis en comparación con I/R. Se observó*

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una disminución de la expresión de AQP2 en I/R. En la PCR no se observaron diferencias significativas en la expresión del gen AQP2 entre I/R y control ni entre I/R + CAPE e I/R. **Conclusión:** *La I/R renal inhibe la producción de AQP2 en el riñón y causa daño histológico y bioquímico. La administración de CAPE antes de la I/R tiene un efecto protector sobre el riñón.*

Palabras clave: *Riñón. Ácido cafeico. Antioxidante. Isquemia renal. Fitoquímico. Acuaporina-2.*

Introduction

Water, which is a major component of human cells and all living things, passes through membranes through membrane channel proteins called aquaporins (AQP)[1,2]. More than 10 AQPs have been identified in mammals. AQP2 is expressed in the renal collecting ducts and is localized in the apical membrane of principal cells[2,3]. AQP2, which shuttles between the plasma membrane and the intracellular stores, passes from the stores inside the cell to the plasma membrane and plays a role in the passage of water through the membrane[2]. In healthy individuals, it is released from the apical plasma membrane under the action of antidiuretic hormone (ADH) and allows the reabsorption of water from the filtrate[1]. The presence of mutations in AQP2, which plays an important role in concentrating urine, results in diabetes insipidus, characterized by polyuria and polydipsia[2,4]. It was shown that AQP2 expression decreases in a compensatory manner in experimentally induced hypertension and consequently increases urine volume[5].

Ischemic damage to the kidney occurs in cases such as kidney transplant surgery, partial nephrectomy, aortic aneurysm surgery, hydronephrosis, sepsis, and trauma[6-10]. Ischemia refers to a decrease in blood flow to an organ, thus reducing the oxygen and nutrients carried by the blood. Restored blood supply after ischemia, called reperfusion, increases tissue damage and organ dysfunction more than ischemia. Renal ischemia/reperfusion (I/R) causes acute renal failure and has a high morbidity and mortality rate[11].

Various agents have been used as preventive or therapeutic agents in renal I/R[12-15]. Honey has been known to have health benefits for many years. Propolis, which is produced by honey bees using extracts collected from many plants in the environment, is a resin that protects the hives from external factors[16]. Honey contains mainly glucose and fructose along with many vitamins, minerals, flavonoids, phenolic acids, and carotenoids. Some of the phenolic and flavonoid contents found in high amounts in honey are phytochemicals called caffeic acid, caffeic acid phenethyl ester (CAPE), chrysin, and quercetin[17]. These substances have anticarcinogenic and antioxidant properties[17,18]. It was reported that CAPE, one of the important components of propolis, reduces kidney damage and serum oxidant levels[19,20]. It was also shown to be protective against renal damage due to myocardial I/R[21].

The aim of this study was to investigate the effect of renal I/R injury on renal AQP2, tissue morphology, and serum parameters in rats and the possible protective effects of CAPE against these changes by real-time polymerase chain reaction (PCR), immunohistochemical, histopathological, and biochemical methods.

Methods

Animals and experimental protocol

For the study to be conducted, the ethics committee approval numbered 2012/A-19 was obtained from the Experimental Animal Ethics Committee of İnönü University Faculty of Medicine. Our study was supported by İnönü University Scientific Research Projects Coordination Unit (Project no: 2012/77). In the study, 45 adult female rats of Sprague Dawley lineage weighing 250-300 g obtained from İnönü University Experimental Animal Production and Research Center were used. The rats were kept in an air-conditioned room with automatically controlled temperature (22 ± 1°C) and lighting (07.00-19.00 h). The animals were randomly divided into six groups. The control group (n = 7) was not given any medication, and no application was made. Right nephrectomy was performed in the Sham group (n = 8). In the I/R group (n = 8), right nephrectomy was performed, followed by 1 h of ischemia to the left kidney and a subsequent 24 h reperfusion. For the rats in the I/R+CAPE group (n = 8), 10 μmol/kg CAPE (Sigma-Aldrich, Chemie Gmbh, Steinheim, Germany) was administered intraperitoneally (i.p.) in dimethyl sulfoxide (DMSO) 30 min before the surgery. Then, a right nephrectomy was performed. The left kidney underwent 1 h of ischemia followed by 24 h of reperfusion[14]. In consistent with the literature, CAPE was given at a dose of 10 μmol/kg, which was shown to inhibit the xanthine–xanthine oxidase system and the formation

of free oxygen radicals[22]. The Sham + CAPE group (n = 7) was given 10 μmol/kg CAPE i.p. in DMSO. A right nephrectomy was then performed. The Sham + DMSO group (n = 7) was given DMSO as i.p. Then, a right nephrectomy was performed.

Surgical procedure

Anesthesia was performed using 100 mg/kg ketamine and 10 mg/kg xylazine i.p. A midline incision was made, the abdomen was opened, and right nephrectomy was performed. Then, the left renal vessels were clamped for 1-h of ischemia, followed by removal of the clamp and reperfusion for 24 h. Left kidney tissues were taken for examination and cut in half longitudinally, one half for histopathologic and the other half for immunohistochemical examination. The blood sample was taken to measure the biochemical parameters of blood urea nitrogen (BUN), creatinine (Cr), Na^+, K^+, and Cl^- levels.

Immunohistochemical and histopathological analysis

The tissue samples were first fixed in a 10% buffered formaldehyde solution for 48 h and followed up. After 5-μ thick sections were taken by embedding in paraffin, Hematoxylin and Eosin dyes were applied histochemically, and specimens were examined under a light microscope. For immunohistochemical analysis, 5-μ sections were taken from paraffin blocks, and AQP2 was applied using the streptavidin/biotin/peroxidase system. The obtained preparations were examined under a light microscope.

In histopathological evaluation, cortical, juxtacortical, and medullary areas of kidney preparations were examined for parenchymal and tubular damage. Congestion, inflammation, and necrosis were examined to determine parenchymal and interstitial damage. In addition, tubule dilatation, vacuolar degeneration of tubule epithelial cells, peritubular edema, and intratubular plug formation were examined for tubular damage, and scoring was performed on a semiquantitative scale. Accordingly, no damage was rated as 0, light and single focus damage as 1, moderately severe damage in several foci as 2, severe and widespread damage as 3. Slides that underwent immunohistochemical staining were evaluated for the intensity of chromogen staining and the distribution of stained cells.

BUN, Cr, and K were measured by spectrophotometric method on a Roche Cobas auto-analyzer (Roche Diagnostics, Mannheim, Germany).

Real-time PCR

Total RNA isolation

For the detection of AQP2 mRNA levels, kidney tissues of the study groups were cut into small pieces under sterile conditions on ice and were stored in RNA storage solution in a −35°C deep freezer until the day of analysis. Total RNA purification was performed on these tissues using the Qiagen RNeasy mini kit. Total RNAs isolated from kidney samples were run on 1% agarose gel at 100 mV in electrophoresis. RNAs samples in which 28S and 18S sharp ribosomal RNA bands were observed and no degradation was observed were used in the cDNA production.

cDNA synthesis protocol

For cDNA synthesis, the SuperScript III reverse transcriptase enzyme kit produced by Invitrogen was used as recommended by the producer. Briefly, 1 μg of total RNA, 1 μL of primer (4 pmol gene-specific primer or 100 pmol PoliT-18 primer), 1 μL of dNTP (10 mM), and ddH_2O were mixed to a total volume of 13 μL in a 100 μL PCR tube, mixed, and heated in a thermal cycler at 65°C for 15 min. To this mix was added 4 μL of ×5 First strand buffer, 2 μL of DTT, 1 μL of ddH_2O, 1 μL of SuperScript III reverse transcriptase enzyme, mixed and heated in a thermal cycler at 50°C for 60 min and at 70°C for 15 min, then stored at −20°C until analysis.

Real-time PCR protocol

Roche real-time PCR device (model LC480) and Roche PCR kit mix (Roche 04707516001) containing ROCHE real-time ×2 “syber green” dye were used. The data were analyzed according to the 2-ΔΔCT method. The reactions were performed at a total volume of 20 μL. For this, 10 μL of SYBR Green I master mix (enzyme, dNTP, Mg, buffer, and water) was prepared as 1 μL of cDNA, 1 μL of forward and 1 μL of reverse primers (10 pmol/μL) and 7 μL of double-distilled water. After optimization, the PCR conditions were as follows: initial denaturation for 10 min at 95°C, denaturation for 20 s at 95°C, annealing for 40 s at

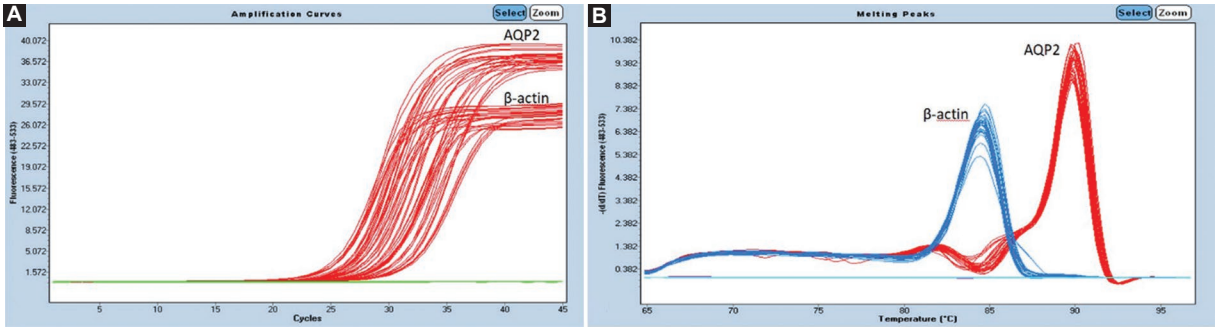

Figure 1. *Analysis of Aquaporin 2 (AQP2) gene expression by real-time PCR. Replication* **A:** *and melting* **B:** *curves of cDNAs synthesized from AQP2 and β-actin mRNAs of the experimental and control groups by real-time PCR using "SYBR Green" chemistry. PCR: polymerase chain reaction.*

60°C, and polymerization for 30 s at 72°C. A total of 45 cycles were used. All real-time PCR studies were carried out in triplicate on the same day to ensure its quantitative accuracy.

cDNA synthesis was performed in all samples using the Poly-T18 primer. In the next step, using the primers specific to the *AQP2* and β-*actin* genes, changes in *AQP* gene expression were determined in proportion to the β-*actin* gene by real-time PCR. Then, the AQP/β-*actin* gene expressions of the control group and the experimental group were compared (Fig. 1).

Primers, whose sequences and sizes are given in table 1, were used in the analysis of *AQP2* gene expression[23,24].

Statistical analyses

Data distribution models were tested using Kolmogorov-Smirnov test. Normally distributed data were expressed as average ± SD. Categorical and ordinal variables were presented as numbers and percentages. Of the quantitative data, those which did not have normal distribution were compared using Mann-Whitney U test, while analysis of variance was used for normally distributed parameters and the Tukey honestly significant difference test for multiple comparisons. The correlations between the parameters were analyzed by Spearman correlation analysis. A correlation coefficient was considered a strong correlation when r was ≥ 0.6 and a moderate correlation when r was between 0.3 and 0.6. Categorical variables were expressed as frequency and percentage, and were compared using the X^2 test. $p < 0.05$ was considered statistically significant. Statistical Package for the Social Sciences (SPSS) version 18 (SPSS Inc., Chicago, USA) software was used for statistical analyses.

Table 1. Primer sequences and product size for AQP2 and β-Actin

Genes	Primer squences	Product size (bp)
AQP2-F	5'-TTGCAGGAACCAGACACTTG-3'	174 B
AQP2-R	5'- GCGGAGACGAGCACTTTTAC-3'	
β-*Actin-F*	5'-CATCACTATCGGCAATGAGC-3'	159 A
β-*Actin-R*	5'-GACAGCACTGTGTTGGCATA-3'	

AQP2: aquaporin 2; F: forward primer; R: reverse primer.

Results

Congestion, inflammation, and necrosis were significantly more common in the I/R group than in the control group ($p < 0.05$). There was an improvement in necrosis in the I/R+CAPE group compared to the I/R group ($p < 0.001$) (Figs. 2 and 3; Table 2).

In immunohistochemical staining, it was determined that AQP2 expression in renal tubules in the I/R group was less than the control and sham groups (Fig. 4).

AQP2 gene expressions were lower in the I/R group than in the other groups ($p > 0.05$). *AQP2* gene expressions increased in the I/R+CAPE group compared to the I/R group ($p > 0.05$) (Fig. 5 and Table 3).

After real-time PCR using AQP 2 and β-actin cDNAs, the specificity of primer binding was checked by running the samples in an agarose gel. As a result of the analysis, it was seen that a single DNA band of the desired length was created for both genes (Fig. 5).

In terms of the biochemical parameters, BUN, Cr, and K^+ levels were significantly higher in the I/R group compared to the control group ($p < 0.05$) (Table 3).

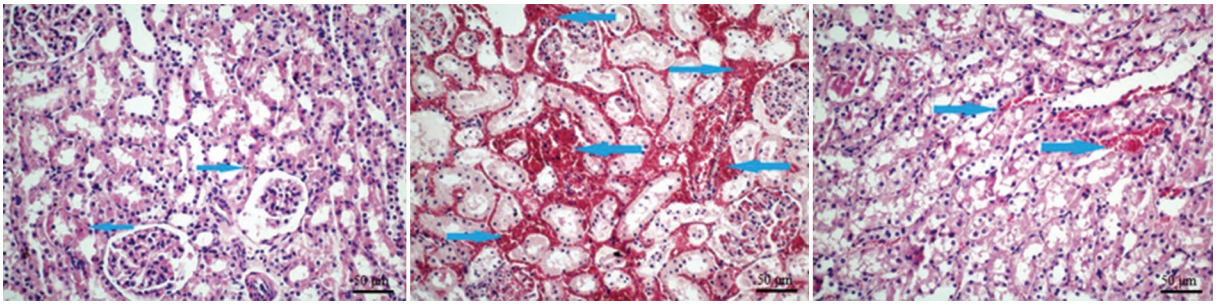

Figure 2. *Histopathologic evaluation of parenchymal and interstitial damage. Blue arrows indicate congested vascular areas.* **A:** *mild congestion (Control group).* **B:** *severe congestion (I/R group).* **C:** *moderate congestion (I/R+CAPE group). I/R: ischemia-reperfusion; CAPE: caffeic acid phenethyl ester; Mag x20; H&E staining for all images.*

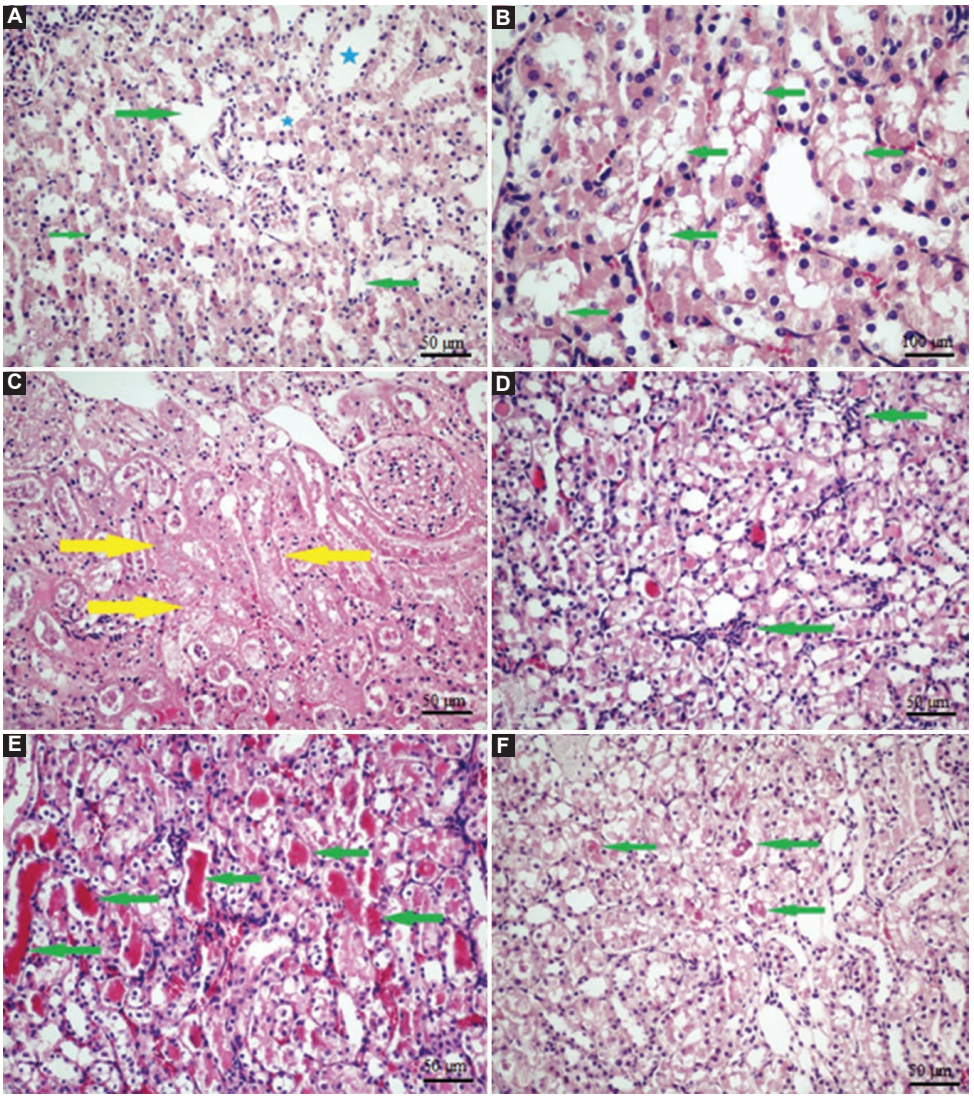

Figure 3. *Areas of edema and vacuolization* **(A and B)**. **A:** *interstitial and intertubular edematous areas (green arrows) and dilated tubules (blue stars) are seen.* **B:** *findings of vacuolar degeneration in proximal tubule cells (green arrows), this microphotograph was taken under x40 magnification to emphasise belp formation (control group). Necrosis and inflammation* **(C and D)**. **C:** *development of coagulation necrosis in a juxtacortical focus (yellow arrows).* **D:** *focal inflammation and leukocytic aggregation in medullary areas (green arrows) (I/R group). Tubular plug development* **(E and F)**. **E:** *dense eosinophilic plugs in proximal tubules (green arrows).* **F:** *development of pale eosinophilic protein plugs in proximal tubules (green arrows) (sham group). I/R: Ischemia-reperfusion. Mag x20. H&E staining for all images.*

Table 2. Comparison of the frequencies of histopathological findings between groups

Damage severity	Control n (%)	Sham n (%)	I/R n (%)	I/R+CAPE n (%)	Sham+CAPE n (%)	Sham+DMSO n (%)
Congestion*						
Absent	2 (25.0)	5 (62.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)
Mild/single focus	5 (21.7)	3 (13.0)	1 (4.3)	3 (13.0)	5 (21.7)	6 (26.1)
Moderate/a few foci	0 (0.0)	0 (0.0)	7 (53.8)	4 (30.8)	2 (15.4)	0 (0.0)
Severe, extensive	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Inflammation**						
Absent	3 (18.8)	1 (6.3)	2 (12.5)	1 (6.3)	5 (31.3)	4 (25.0)
Mild/single focus	4 (16.7)	5 (20.8)	3 (12.5)	7 (29.2)	2 (8.3)	3 (12.5)
Moderate/a few foci	0 (0.0)	2 (40.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)
Necrosis*						
Absent	7 (18.4)	8 (21.1)	3 (7.9)	6 (15.8)	7 (18.4)	7 (18.4)
Mild/single focus	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)
Moderate/a few foci	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0
Severe, extensive	0 (0.0)	0 (0.0)	1 (100.0	0 (0.0	0 (0.0)	0 (0.0)
Dilation						
Absent	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mild/single focus	4 (18.2)	2 (9.1)	1 (4.5)	4 (18.2)	6 (27.3)	5 (22.7)
Moderate/a few foci	3 (13.6)	5 (22.7)	7 (31.8)	4 (18.2)	1 (4.5)	2 (9.1)
Edema						
Absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Mild/single focus	6 (17.6)	5 (14.7)	6 (17.6)	8 (23.5)	6 (17.6	3 (8.8)
Moderate/a few foci	1 (10.0)	3 (30.0)	2 (20.0)	0 (0.0)	1 (10.0)	3 (30.0)
Vacuolization						
Mild/single focus	4 (20.0)	4 (20.0)	1 (5.0)	3 (15.0)	3 (15.0)	5 (25.0)
Moderate/a few foci	3 (12.0)	4 (16.0)	7 (28.0)	5 (20.0)	4 (16.0)	2 (8.0)
Plug*						
Absent	5 (20.8)	2 (8.3)	2 (8.3)	2 (8.3)	7 (29.2)	6 (25.0)
Mild/single focus	2 (9.5)	6 (28.6)	6 (28.6)	6 (28.6)	0 (0.0)	1 (4.8)

*p < 0.001.
**p < 0.05 (X^2 test).
I/R: ischemia-reperfusion; DMSO: dimethyl sulfoxide.

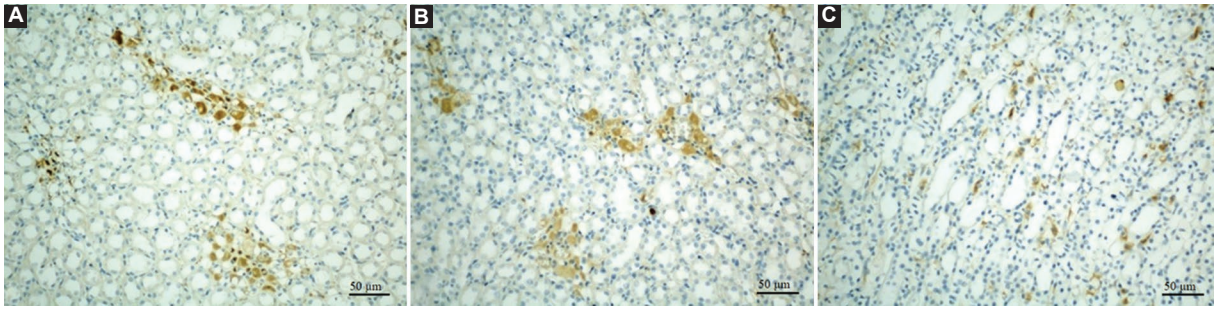

Figure 4. *Aquaporin 2 (AQP2) expression was performed immunohistochemically.* **A** *and* **B:** *regional cytoplasmic and membranous AQP2 expression in tubules in Control and Sham groups, respectively.* **C:** *a decrease in AQP2 expression was observed in the distal collector tubules in the I/R group. Mag x20.*

Discussion

Renal I/R injury is associated with impaired renal blood flow. This damage can occur due to direct kidney-related causes, such as kidney transplant surgery, as well as non-renal causes, such as trauma or aortic aneurysm rupture[12]. Our study was carried out to investigate the tissue damage and AQP2 expression changes in the kidney due to I/R in rats, as well as the possible protective effects of CAPE against these changes.

Table 3. Comparison of biochemical and RT-PCR values of the groups

Parameters	Control	Sham	I/R	I/R+CAPE	Sham+CAPE	Sham+DMSO	p
BUN (mg/dL)	19.28 ± 03.60[a,b]	22.63 ± 04.50[a,b]	127.13 ± 22.79[c,d]	102.00 ± 42.12[c,d]	41,57 ± 25.57	40.14 ± 23.21	< 0.001
Cr (mmol/L)	0.58 ± 0.03[a,b]	0.67 ± 0.04[a,b]	2.15 ± 0.63[c,d]	1.53 ± 0.72[c,d]	0.67 ± 0.06	0.66 ± 0.04	< 0.001
Na^+ (mg/dL)	142.29 ± 2.69	140.25 ± 1.38	137.00 ± 5.55	138.00 ± 7.25	140.57 ± 1.62	138.71 ± 1.38	0.178
Cl^- (mmol/L)	99.14 ± 1.68[b,c]	101.25 ± 0.89	101.00 ± 4.87	104.50 ± 3.50	104.00 ± 2.00	102.57 ± 0.53	0.006
K^+(mmol/L)	4.66 ± 0.24[a,b]	4.99 ± 0.27[a,b]	6.85 ± 1.55[c,d]	6.93 ± 1.76[c,d]	5.06 ± 0.45	4.77 ± 0.40	< 0.001
RT-PCR	0.72 (0.25-1.69)	0.63 (0.30-3.71)	0.11 (0.02-1.77)	0.77 (0.26-8.75)	2.45 (0.06-2.95)	0.71 (0.01-2.79)	0.182

[a]p < 0.05, different compared to I/R.
[b]p < 0.05, different compared to I/R+CAPE.
[c]p < 0.05, different compared to Sham+CAPE.
[d]p < 0.05, different compared to Sham+DMSO.
I/R: ischemia-reperfusion. Tukey honestly significant difference, ANOVA Multiple Comparisons (mean ± SD). ANOVA: analysis of variance; RT-PCR: real-time polymerase chain reaction; DMSO: dimethyl sulfoxide.

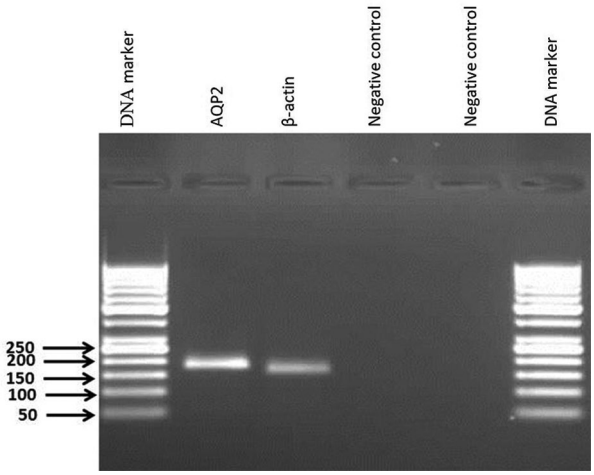

Figure 5. *Agarose gel (2%) electrophoresis of the PCR amplification of β-Actin and Aquaporin 2 (AQP2) cDNAs. The DNA size marker used was a 50 bp DNA ladder (Fermentas). PCR: polymerase chain reaction.*

Tasdemir et al.[14] observed that renal I/R induced tubular cell swelling, tubular dilatation, cellular vacuolation, medullary congestion, and necrosis in rats. Chen et al.[25] evaluated the kidneys in terms of tubular and glomerular anomalies as well as interstitial inflammation in acute tissue rejection after kidney transplantation in rats. They found an increase in histopathological score in rats with tissue rejection compared to the control group. Ozer et al.[21] observed tubular damage and periglomerular dilation along with dilatation, luminal cast formation, and involvement in some glomeruli in the renal tubules of rats with myocardial I/R. It was revealed that the histopathological damage was very mild in the group that was given CAPE before the procedure. In another experimental study, CAPE administration was found to prevent histopathological damage in rats with renal damage due to toluene[26]. It was reported that CAPE plays a protective role against tissue damage by alleviating oxidative stress and increasing antioxidant activity[27].

Yilmaz et al.[22] reported that 10 μmol/kg CAPE inhibits the formation of free oxygen radicals. It was reported in previous studies that histopathological damage occurs in rat kidneys with the administration of gentamicin, and administration of 10 μmol/kg CAPE for 2 days before gentamicin prevents tubular damage[28]. In the present study, CAPE was applied at a dose of 10 μmol/kg in accordance with the literature.

Various studies in the literature using the same[13] or similar[7] methodology to ours reported that serum BUN and Cr levels increased in rats treated with I/R protocols. However, it was shown that there was an improvement in BUN and Cr when different agents with antioxidant properties were given. It was determined that the deterioration of I/R was caused by the increase in oxidative stress. On the other hand, the administration of substances such as ivabradine and nesfatin-1, which are thought to be protective in renal I/R damage, reduced oxidative stress measured by the tissue oxidant (MDA), and antioxidant (SOD and catalase [CAT]) parameters[7,13].

In the literature, polyuria and an increase in serum BUN and Cr levels were reported to develop due to renal I/R[29-31]. This situation is explained by the deterioration of tubular functions in acute renal failure due to I/R. Reabsorption of water from the renal tubules is mediated by various sodium transporters. The decrease in sodium reabsorption in ischemic injury is due to the impairment in sodium transporters[30]. Low oxygen level and high oxygen demand in the renal tubules make the kidney vulnerable to hypoxi[29]. In the ischemic kidney, the ability to concentrate the urine is significantly reduced, and tubular reabsorption of

sodium is impaired. Leukocyte adhesion plays an important role in renal ischemia. Inflammatory cascades, which are activated during the period of reperfusion, cause the accumulation of neutrophils in the medullary vessels. Leukocyte-mediated increases in endothelial permeability lead to erythrocyte aggregation and medullary congestion. In addition, ischemic damage to the medulla continues even if kidney blood flow is restored[32]. Prevention of I/R-induced damage by antioxidant agents was shown by improvement in kidney function tests[29,30]. It was reported that BUN and Cr levels increased in rats given Gentamycin, while BUN and Cr levels returned to normal levels when CAPE was administered[33]. In line with the literature, BUN, Cr, and K^+ levels in the present study were high in I/R. It was observed that CAPE led to a slight improvement in kidney function tests. It was reported that the change in renal function tests in I/R occurs after histopathological change[12]. In the light of this finding, histopathological improvement must occur first to improve the biochemical values. Although the administered CAPE provided a slight improvement histopathologically, it may not have achieved a complete improvement in biochemical parameters. If the time between application and sampling increases in the experimental protocol, biochemical parameters could be restored.

The collector ducts that express AQP2 are located mainly in the renal medulla[2,3]. Cha et al.[31] observed a significant decrease of AQP2 expression in RT-PCR in the renal cortex and medulla in rats undergoing renal I/R. They reported that 1 week after surgery, AQP2 approached control group levels in the cortex, but there was no improvement in the medulla. Similarly, based on RT-PCR, Han et al.[29] detected decreases in AQP2 and vasopressin 2 receptor mRNA levels in the renal internal medulla of rats undergoing I/R. Besides, immunohistochemically, they also observed a significant decrease in AQP2 expression. In other studies, it was shown immunohistochemically that AQP2 expression was reduced in the collecting ducts of rats in renal I/R[30,32,34]. It was reported that there was a decrease in AQP2 mRNA and AQP2 protein expression levels in acute tissue rejection after the kidney transplant model in rats, and in another study, a decrease in vasopressin receptor mRNA levels was observed in addition to the decrease in AQP2 upon the administration of diuretic drugs[24,25]. Substances such as melanocyte-stimulating hormone, erythropoietin, and lithocholic acid, which have antioxidant or anti-inflammatory properties, were found to be protecting against AQP2 changes in renal I/R[29,30,32]. In our study, there was no significant difference in *AQP2* gene expression. We are of the opinion that a change in AQP2 could be observed after a complete histopathological change takes place. Increased ADH binds to receptors on the basolateral membrane of the principal cells in the collecting ducts, which in turn initiates a pathway that causes AQP2 to cross into the apical plasma membrane. AQP2, located in the intracellular vesicle, is phosphorylated by protein kinase A. The vesicles move toward and fuse with the plasma membrane, and AQP2 passes into the membrane, increasing the permeability of the membrane to water. It was reported that in acute tissue rejection after kidney transplantation, AQP2 passes into intracellular vesicles due to the decrease in ADH, thus reducing the permeability of the membranes to water. ADH maintains the water balance of the body by acting on the reabsorption of water from collecting channels by two different mechanisms, both of which involve AQP2. In the short-term mechanism, ADH-regulated AQP2 trafficking between intracellular vesicles and the apical membrane leads to an increase in acute ADH-induced water reabsorption from the collecting channel. In the long-term mechanism, the total amount of AQP2 is regulated depending on the change in the number of water channels in the cell[35]. In a case report in the literature, two patients with congestive heart failure were given ADH antagonists, and their kidney biopsy specimens were examined. In one of these patients, who had been newly diagnosed with diabetes mellitus, it was observed that the renal medulla was intact, with evidence of severe atrophy in the renal cortical tubules. In this patient, despite severe insufficiency in renal function tests, it was reported that the amount of urine excretion increased, and AQP2 expression was observed in the renal collecting duct. On the other hand, in the other patient who had diabetes mellitus and diabetic nephropathy for 8 years, mild atrophy in the renal cortex but inflammatory cell infiltration with severe atrophy in the renal medulla was detected. Absence of AQP2 was reported in immunohistochemical examination in this patient with low renal function tests and urine excretion[36].

Free oxygen radicals are blamed for tissue damage caused by reperfusion[11]. Reperfusion, which triggers energy-deprived and metabolically restless cells to produce reactive oxygen species (ROS), increases tissue damage and organ dysfunction more than ischemia. Some of the ROS are normally formed by mitochondria via xanthine oxidase and cyclooxygenase.

In ischemia, ATP is reduced to hypoxanthine, and xanthine dehydrogenase is converted to xanthine oxidase. When oxygen reenters the ischemic tissue by reperfusion, superoxide radicals are released as xanthine oxidase converts hypoxanthine to uric acid. The production of toxic agents such as superoxide, hydroxyl radical, and hydrogen peroxide exceeds the capacity of endogenous free radical scavengers and causes significant damage to ischemic tissues[37]. Overproduction of reactive oxygen products and lack of antioxidants are the main factors in the formation of I/R damage[7]. CAPE, which has antioxidant properties, suppresses lipid peroxidation. It inhibits the activities of nitric oxide synthase and xanthine oxidase. Thanks to its lipophilic properties, it interacts with mitochondrial membranes and reduces the formation of reactive oxygen derivatives[38]. It was reported that CAPE inhibits the production of free oxygen radicals by inhibiting xanthine oxidase, alleviating nephrotoxicity[28]. Ozer et al.[21] examined the renal damage caused by myocardial IR in rats and reported that preoperative administration of 50 μmol/kg (i.p.) CAPE corrected renal damage by scavenging free radicals and exerting antioxidant activity.

Unlike these studies, in the isoproterenol-induced myocardial infarction study of Oktar et al.[39], it was observed that MPO and MDA increased, and SOD and CAT activities decreased. However, no increase was observed in SOD and CAT activities while decreases were evident in MPO and MDA in rats given 10 μmol/kg (i.p.) CAPE, along with isoproterenol, for 7 days. It was argued that the increase in SOD and CAT activities should have been prevented by CAPE, albeit indirectly.

The strength of our study is that renal function tests, histopathological and immunohistochemical evaluations, and *AQP2* gene expression were performed in the same study, thus, our results were obtained with strong evidence. In addition, since the evaluations were made by creating different groups in our study, it was seen that the results obtained were clearly due to I/R and CAPE and were not changed by any other factor.

Conclusion

It was determined that AQP2 expression, kidney morphology, and kidney function were impaired in renal I/R, and CAPE was protective against the effects of I/R.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors declare that no patient data appear in this article. The authors have followed their institution's confidentiality protocols and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ORIGINAL ARTICLE

Characterization of human mesenchymal stem cell according to delivery route and its correlation with clinical-gynecological history

Caracterización de células troncales mesenquimales humanas según la vía de parto y su correlación con la historia clínica ginecológica

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Abstract

Objective: *To assess the pluripotency profile of human mesenchymal stem cells (hMSCs) as influenced by delivery mode and obstetric-gynecological background.* **Methods:** *Thirty-nine placentas were included. A fragment of the AM (5 cm) was utilized to obtain the AM-hMSCs. The cell cultures were monitored until 90% confluence, before carrying out expansion (passages). The cultures, cell morphology, adhesion, immunophenotyping characterization, and differentiation capacity were evaluated and compared with hMSC from bone marrow (BM-hMSC). T-student and Mann-Whitney U tests were carried out. Statistical significance was set at a $p < 0.05$.* **Results:** *No differences were observed in the proliferation and expansion of AM-hMSC obtained by vaginal or cesarean delivery route. Similar results were observed in the immunological characterization; however, CD105 was significantly lower compared with BM-hMSC. Nevertheless, cells from vaginal or cesarean delivery route showed great differentiation capacity to adipogenic, chondrogenic, and osteogenic lineages.* **Conclusions:** *The delivery route, clinical data, and obstetric and gynecologic histories are no limitations for using the AM to obtain hMSC and its possible application in Regenerative Medicine.*

Keywords: *Delivery route. Obstetric history. Mesenchymal stem cells. Amniotic membrane. Immunophenotyping. Cell differentiation.*

Resumen

Objetivo: *Caracterizar la pluripotencialidad de las CTMh de acuerdo con la vía de parto y los antecedentes obstétricos y ginecológicos.* **Métodos:** *Se incluyeron 39 placentas; las CTMh-MA fueron obtenidas de la MA. Los cultivos fueron monitoreados hasta el 90% de confluencia. La morfología, la adhesión, la caracterización inmunológica y la capacidad de diferenciación se evaluaron y compararon con CTMh de medula ósea (CTMh-MO). Se realizaron las pruebas t de Student y U de Mann-Whitney, y se consideró un valor $p < 0.05$.* **Resultados:** *No se observaron diferencias en la proliferación de las CTMh-MA obtenidas por vía vaginal o por cesárea. La caracterización inmunológica no mostró diferencias según la vía de parto; sin embargo, CD105 fue menor comparado con las CTMh-MO. No obstante, las células mostraron capacidad de diferenciarse al*

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linaje adipogénico, condrogénico y osteogénico sin importar su origen. **Conclusiones:** *La vía de resolución y los antecedentes obstétricos no son limitantes para el uso de CTMh de MA y su posible aplicación en medicina regenerativa.*

Palabras clave: *Canal de parto. Antecedentes obstétricos. Células troncales mesenquimales. Membrana amniótica. Inmunotipificación. Diferenciación celular.*

Introduction

Organ demand and regeneration strategies are currently a relevant topic, worldwide. Reports show many Mexicans on transplant waiting lists every year. Even though said transplantations are successfully carried out in Mexico, the shortage of organs remains constant[1].

Regenerative medicine (RM) and tissue engineering (TE) are a milestone in the replacement or regeneration of cells, organs, and tissues[2,3]. TE is mainly composed by three fundamental elements: autologous or allogenic cells with a multipotent or pluripotent regenerative potential, scaffolds as cellular support, and growth factors[4-8].

Human mesenchymal stem cells (hMSCs) are cells with the potential to differentiate into mesenchymal lineages (osteoblasts, chondroblasts, adipocytes), and under special culture conditions, they can transdifferentiate into endodermal (liver, lung, pancreas) and ectodermal (neuron-type) lineages[9-11]. The use of hMSCs and their application in organ restoration has attracted the attention of scientists and the clinical community[12-14]. In the past decade, the number of clinical trials related to hMSCs has seen a significant increase worldwide (1,138 records on clinicaltrial.gov). Several medical fields have shown promising results; for example, in cardiology, the administration of hMSCs has demonstrated improvements in dilated cardiomyopathy. Similar benefits have been observed in both ischemic and non-ischemic heart failure. Other medical specialties such as traumatology, pneumology, hepatology, nephrology, cartilage lesions, osteoarthritis, and immunomodulation of immune diseases (e.g., host versus graft reaction, rheumatoid arthritis) have also considered the use of hMSCs as a promising approach to restore tissue and organ function[14,15].

At present, hMSCs obtained from bone marrow (BM) are considered the gold standard. However, their retrieval through BM aspiration is an invasive procedure that must be performed in an operating room by an expert physician. In recent years, sources other than BM for obtaining hMSCs have been reported that include adipose tissue, muscle, dental pulp, amniotic membrane (AM), Wharton's jelly, and umbilical cord blood; even the number of cells from those sources has been described to be equal to or higher than the quantities directly retrieved from BM[16]. There are currently few standardized protocols for hMSCs collection. Moreover, the administration routes and the optimized number of cells are currently under evaluation in accordance with the pathology[17]. Furthermore, before its use as a clinically recommended procedure, pre-clinical and clinical studies need to be carried out.

The quality of the donated tissue must also be considered in relation to donor age, the timing of tissue collection, and tissue preservation, before collecting the hMSCs[18].

Controversial data regarding placenta-derived hMSCs and the association between the quality of the placenta and the age of the mother has been reported; placentas corresponding to mothers above 35 years of age are presumed to have poor vascular perfusion and delayed villous maturation[19].

In Mexico, a considerable number of pregnant women are overweight and obese. They gain even more weight per month during pregnancy, conditioning the presentation of hypertensive diseases associated with pregnancy. That pathophysiologic condition alters the blood flow and the exchange of gases (oxygen and carbon dioxide) and nutrients between the mother and fetus, not only affecting the product but also the epithelial and mesenchymal cells of the placental membranes[20-22].

The placenta tends to be thought of as biologic waste, with no ethical implications regarding its processing; it can be donated through signing a statement of informed consent. Different studies have considered the AM an alternative for retrieving hMSCs[23-25]. Nevertheless, at present, there are no studies associating the capacity of *in vitro* hMSCs proliferation with donor age and body mass index (BMI) or the delivery route of the pregnancy (vaginal delivery versus cesarean section). Therefore, the aim of the present work was to analyze the epidemiologic characteristics of the population seen at the Department of Obstetrics and Gynecology and the relation of those characteristics to the proliferation and

expansion, expression of multipotential markers, and the capacity differentiation of hMSCs obtained from placentas donated through signed statements of Informed Consent.

Methods

Patients

A cross-sectional study was conducted that included patients seen at the Department of Obstetrics and Gynecology of the Hospital General de México "Dr. Eduardo Liceaga." Inclusion criteria were primiparous or multiparous women between 20 and 36 years of age, at 38-40 weeks of gestation.

For the present study, some donors with comorbidities, such as arterial hypertension and diabetes mellitus (DM) were included, as well as donors with negative serology for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus. Placentas with a gestation period of > 40 weeks or < 38 weeks, membrane rupture of 12 or more hours of progression, patients with fever (> 38°C) or with diagnosis of fetal suffering, and positive serology test were excluded.

BMI was calculated, complete blood count, blood chemistry (BC), and prothrombin time were determined at the central laboratory of the Hospital General de México "Dr. Eduardo Liceaga." The AM of the placental tissue was processed for hMSCs retrieval. All the patients signed statements of informed consent The present study was approved by the research and ethics committees of the Hospital General de México "Dr. Eduardo Liceaga" (CI/315/15) and the School of Medicine at the Universidad Nacional Autónoma de México (DI/115/2015) and was conducted in accordance with the principles described in the 1975 Declaration of Helsinki.

AM collection and processing

Regardless of the delivery route (vaginal or cesarean) of the placenta obtained, the placental tissue was placed in a metallic sterile bowl to identify the periumbilical zone of the fetal surface. A circular fragment of approximately 5 cm^2 was obtained. The AM was placed in sterile phosphate buffer solution (PBS), pH 7.2 (1X, Gibco, USA), and then transferred to PBS + antibiotic-antimycotic (1X, Gibco, USA). After 10 min, the AM was recovered and the processing was performed, following the protocol reported by León-Mancilla et al., 2021[17].

hMSCs retrieval

To obtain the hMSCs, the AM was digested with Trypsin-ethylenediaminetetraacetic acid (EDTA) (0.05%) (Gibco, USA) at 37°C for 1 h. Once the digestion time was over, the tissue was incubated with Collagenase Type II (0.01%) (Gibco, USA) for 2 h at 37°C. The samples were then centrifuged at 2,000 rpm/10 min and the cell pellet was resuspended in 5 mL of DMEM-HG (high in glucose) culture medium (Gibco, USA). The cell count was carried out using an automated system (Bio-Rad, TC20™). A density of 2.5 × 10^4 cells/cm^2 was adjusted and harvested in culture bottles with 5 mL of DMEM culture medium, supplemented with 10% fetal bovine serum (Biowest, France), HEPES (Sigma Aldrich, Germany), and antibiotic-antimycotic ×1 (Gibco, USA). The cells were maintained at 37°C in a 5% CO_2 atmosphere. After 24 h, the culture medium was changed, to remove the cells that did not adhere to the culture flask.

The cell cultures were monitored, until reaching confluence above 80% (approximately 15 days), to perform the first culture passage (P1). Briefly, the cells were incubated with trypsin-EDTA (0.05%) for 5 min at 37°C, the cell suspension was centrifuged at 2,000 rpm/10 min, and the cell pellet was resuspended in 5 mL of DMEM medium. The cell count was performed, and repeat harvesting was carried out with 2.5 × 10^4 cells/cm^2. This procedure was repeated until the cells no longer had the capacity to proliferate.

Evaluation of hMSCs cultures through phase contrast microscopy

The hMSCs morphology was evaluated through phase contrast microscopy and scanning electron microscopy. For phase contrast microscopy viewing, the cells were observed directly on the culture bottles (T25), employing the Nikon Eclipse TE 2,000-S (Tokyo, Japan) inverted microscope. The images were obtained with a Nikon DMX 1,200 (Tokyo, Japan) camera and analyzed using the equipment software. In addition, adhesion capacity was evaluated through scanning microscopy.

Evaluation of hMSCs cultures through the collagen matrix (cell scaffold)

Approximately 2.5 × 10^4 cells/cm^2 were placed in a collagen scaffold obtained from Nukbone®. After 1 h

of incubation, the samples were placed in Zamboni (Newcomer supply, USA) fixative solution for 24 h. At the end of the fixation time, the samples were washed and fixed with 1% osmium tetroxide in 0.1M sodium cacodylate and covered with colloidal gold. The images were obtained using DSM-950 and EVO 10 (Zeiss, Germany) scanning electron microscope (SEM).

Immunophenotyping characterization

The AM-hMSCs membrane immune markers were analyzed by flow cytometry as was previously reported[17]. Briefly, AM-hMSCs from the third passage were selected and trypsinized. A total of 4×10^4 cells was incubated with the human MSCs analysis kit (BD Biosciences, Franklin Lakes, NJ, USA), following the supplier's recommendations. The kit contains antibodies anti-CD90, anti-CD73, anti-CD44, and anti-CD105, as multipotential markers. Whereas CD34, CD11b, CD45, CD19, and HLA-DR were used as negative immunophenotype markers (hematopoietic stem cells, HSC). The BM-hMSC immunophenotype characterization was used as a positive control. 20,000 events were analyzed by FACSCanto II flow cytometer and the data were processed using the FACSDiva software package (BD Biosciences, Franklin Lakes, NJ, USA). Three independent experiments were performed.

Differentiation of AM-hMSCs to specific lineages

Approximately, 4×10^4 cells were seeded in culture dishes (3 mm), after 21 days of culture the cells were incubated with the specific differentiation media,[24]. For chondrogenic differentiation was used chondrogenic medium (Cambrex Bio Science, NJ, USA) supplemented with transforming growth factor-β (TGF-β) (Cambrex Bio Science, NJ, USA).

The osteogenic medium was supplemented with ascorbic acid and β-glycerolphosphate and the adipogenic medium was supplemented with pre-mix ITS (Stem Cell Technologies Inc., Vancouver, BC, Canada). The specific differentiation was revealed using Alcian blue (Sigma-Aldrich, St. Louis, MO, USA) for chondrogenic lineage, von Kossa stain for the osteogenic phenotype, and red-O oil (Sigma-Aldrich, Germany) for the adipogenic differentiation. Images were obtained by Nikon Microphoto FDA, a Nikon DMX camera, and Nikon ACT software (Tokyo, Japan) Three independent experiments.

Statistical analysis

The quantitative variables were expressed as mean and standard deviation and the qualitative variables as median and percentage. The sample total was divided by age group and delivery route (vaginal versus cesarean). The comparisons were made using the t-test or the Mann-Whitney test for independent groups. The X^2 test was utilized to analyze the categorical variables. A 95% α significance level and statistical significance at a $p < 0.05$ were considered. All the statistical analyses were carried out using the IBM statistics package for Windows, version 21.0, Armonk, N.Y. IBM Corp.

Results

Demographic data of the placental tissue donors

Thirty-nine pregnant women were included in the study, 17 of whom were under 25 years of age (range from 18 to 25 years) and 22 of whom were above 25 years of age (range from 25 to 37 years). Under the selected age criterion, 11 placentas were recovered from vaginal deliveries and 28 from cesarean sections, with no differences between the age range and type of delivery route at which the placentas were obtained. The mean BMI of the two groups was in the obese range. The main occupation was housewife, with no differences in smoking or alcohol use, with respect to age (Table 1).

Regarding the biochemical data, there were only differences in uric acid between vaginal deliveries and cesarean sections, whereas the biochemical parameters, such as leukocytes, platelets, glucose, and others, were similar in the two groups (Table 2).

Obstetric and gynecologic data according to delivery route

In the obstetric and gynecologic history analysis, there were no differences between the criteria analyzed, with respect to vaginal delivery or cesarean section (Table 3).

Regarding the comorbidity analysis, no diagnosed clinical condition in the vaginal delivery donors was found, whereas 46.4% of the placenta donors that had a cesarean section presented with some type of comorbidity (Table 4).

Table 1. Demographic data according to age group

Delivery route and anthropometric data	Under 25 years of age, n = 17 (%)	Over 25 years of age, n = 22 (%)	p
Vaginal delivery, n (%)[ß]	4 (23.5)	7 (31.8)	0.568
Cesarian section, n %)[ß]	13 (76.5)	15 (68.2)	
BMI (kg/m^2)[†]	30.01 ± 5.0	32.7 ± 5.8	0.659
Normal[ß]	1 (5.9)	2 (9.2)	0.153
Overweight[ß]	8 (47.1)	4 (18.2)	
Obese[ß]	8 (47.1)	16 (72.7)	
Educational level			
Years (Q3 and Q1)	12 (6,16)	12 (6,16)	0.624
(Max and min)[¥]	(12,9)	(12,9)	
Occupation, n (%)[ß]			
Housewife	17 (100)	18 (81.8)	0.179
Businesswoman	-	2 (9.1)	
Student	-	2 (9.1)	
Drug addictions and smoking[ß]			
Alcohol use (no)	16 (94.1)	19 (84.6)	0.429
Smoking	15 (88.2)	17 (77.3)	0.376

[†]Significant t-test for independent samples.
[ß]Significant X^2 test for contingency tables.
[¥]Significant Mann-Whitney U test for independent samples. Significance was a $p < 0.05$ in all the statistical tests. BMI: body mass index.

Table 2. Biochemical values of the placenta donors, by delivery route (vaginal or cesarean)

Hematological and biochemical parameters	Vaginal delivery, n = 11 (%)	Cesarean section, n = 28 (%)	p
Leukocytes (10^3/µL)[†]	11.55 ± 2.32	11.47 ± 3.21	0.692
Neutrophils[†]	74.54 ± 16.84	73.83 ± 14.21	0.284
Hemoglobin (g/dL)[†]	11.65 ± 1.70	21.57 ± 55.18	0.237
[†]Platelets×10^3	258.3 ± 86.5	196.1 ± 62.8	0.181
Urea[†]	15.79 ± 2.66	17.2 ± 5.31	0.104
Creatinine[†]	0.64 ± 0.11	0.60 ± 0.14	0.626
Uric acid[†]	5.62 ± 0.59	5.35 ± 1.55	0.015
Glucose, n (%)[ß]			
< 80	6 (54.5)	15 (53.6)	0.713
80-90	2 (18.2)	8 (28.6)	
> 91	3 (27.3)	5 (17.9)	

[†]Significant t-test for independent samples. [ß]Significant X^2 test for contingency tables. Statistical significance, $p < 0.05$.

In vitro proliferation of the mesenchymal stem cells retrieved from the AM

The proliferation of the AM-hMSCs retrieved from the vaginal delivery and cesarean section placentas was evaluated through bright field microscopy. In general, all the cultures demonstrated similar behavior. Adaptation was apparent from 24 h and 85-90% confluence was reached at culture day 14 (Fig. 1).

The SEM results revealed hMSCs adhesion to a scaffold, as well as filopodium projection in the cells, confirming the adhesion capacity, and indirectly, the metabolic activity of the hMSCs (Fig. 2).

The cultures that did not progress to the subsequent passages were registered and categorized in groups by age and delivery route of the placentas obtained (Tables 5 and 6). The percentage of the P0-P3 cultures in the placentas obtained and organized by age group (under 25 years of age and over 25 years of age) was 88.2% and 72.7%, respectively, with no statistically significant difference between the two age groups (Table 5). Similarly, upon evaluating the culture passages, with respect to the delivery route of the placentas obtained, 81.8% of the cell cultures from vaginal deliveries and 78.6% from the cesarean section were between P0 and P3 (Table 6). Importantly, there were no statistically significant differences related to age group or delivery route of the placenta, with respect to the capacity of the cells to progress to the next passages.

AM-hMSCs from vaginal and cesarean through showed express similar levels of multipotential markers

After to observe that delivery route and the obstetric condition do not contribute on the cell proliferation of AM-hMSCs, we evaluated the expression of multipotential markers on cell obtained from vaginal and cesarean via. Interestingly, the multiple comparisons did not show differences in the expression of CD90+, CD73+, CD44+, and CD105+ in vaginal delivery versus cesarean (Fig. 3). However, hMSCs from BM display higher levels of endoglin (CD105+) compared with AM-hMSCs from both delivery routes. Moreover, the hematopoietic stem cell (HSC) markers showed an insignificant population in all the cases evaluated (Fig. 3).

AM-hMSCs can differentiated to adipogenic, chondrogenic, and osteogenic lineages

After observed that the delivery route does not show differences in the proliferation, adhesion, expression of multipotent markers, we evaluated the differentiation capacity. The data showed that each differentiation

Table 3. Obstetric and gynecologic history, according to the delivery route

Obstetric and gynecologic parameters	Vaginal delivery, n = 11 (%)	Cesarean section, n = 28 (%)	p
Pregnancy history (# pregnancies)[8]			
One pregnancy	4 (36.4)	15 (53.6)	0.200
Two pregnancies	6 (54.5)	7 (25.0)	
Multiple pregnancies	1 (9.1)	6 (21.4)	
Weight before pregnancy[†]	66.64 ± 13.12	66.22 ± 13.45	0.874
Weight after pregnancy[†]	78.27 ± 16.98	76.64 ± 15.34	0.786
Capurro[8]			
Pre-term	-	2 (7.1)	0.417
Full term	11 (100)	24 (85.7)	
Post-term	-	2 (7.1)	
Sex of the product (female), n (%)	5 (45.5)	10 (35.7)	
Weight of the product (g)[†]	3281.8 ± 448.8	2950.7 ± 544.7	0.249
Fetal pathology n (%)	5 (12.8)	0	
Prenatal control (yes), n (%)[8]	7 (63.6)	23 (82.1)	0.217
Multivitamin use (yes), n (%)[8]	8 (72.7)	22 (78.6)	0.697

[†]Significant t-test for independent samples. [8]Significant X^2 test for contingency tables. Statistical significance, $p < 0.05$.

Table 4. Comorbidities in the group of placenta donors, by delivery route

Condition	Vaginal birth, n = 11 (%)	Cesarean section, n = 28 (%)	p[8]
Absent	11 (100)	15 (53.6)	0.662
Gestational diabetes		2 (7.1)	
Cytomegalovirus		1 (3.6)	
Asthma		2 (7.1)	
Deep vein thrombosis		1 (3.6)	
Gestational thrombocytopenia		1 (3.6)	
Diabetes mellitus		1 (3.6)	
Intellectual disability		1 (3.6)	
Primary hypothyroidism		1 (3.6)	
Severe pre-eclampsia		1 (3.6)	
Left renal agenesia		1 (3.6)	

[8]Significant X^2 test for contingency tables. Statistical significance is a $p < 0.05$.

Table 5. Comparison of culture passages with donor age

Passage, n (%)[8]	Under 25 years of age, n = 17 (%)	Above 25 years of age, n = 22 (%)	p
P0	6 (35.3)	3 (13.6)	0.592
P1	5 (29.4)	6 (27.3)	
P2	2 (11.8)	3 (13.6)	
P3	2 (11.8)	4 (18.2)	
P4	-	2 (9.1)	
P5 or more	2 (11.8)	4 (18.2)	
P0-3	15 (88.2)	16 (72.7)	0.234
p > 3	2 (11.8)	6 (27.3)	

[8]Significant X^2 test for contingency tables. Statistical significance is a $p < 0.05$.

media allowed the correct differentiation to the three compromised phenotypes. In other words, AM-hMSCs obtained from the placenta of vaginal or cesarean delivery have the same capacity to differentiate at adipogenic, chondrogenic, and osteogenic lineages (Fig. 4).

Discussion

The amnion, or human AM, is the most internal of the fetal membranes, and so is in direct contact with the amniotic fluid and the fetus[23]. The AM is known to have an epithelial layer, a basal membrane, and a mesenchymal layer. Mesenchymal cells are multipotent and pluripotent cells that can differentiate into the three germ cell layers[26]. The hMSCs retrieval from the AM is advantageous, compared with hMSCs retrieval from BM, given that the placenta is obtained non-invasively and requires only the informed consent of the donor. In previous studies, our working group described the differentiation capacity and the expression of surface markers, as well as the genes (*oct-4, nanog, sox-2*) expressed by the hMSCs obtained from the AM but did not report the proliferation and expansion of the cells obtained or their relation to obstetric and gynecologic data[17].

Due to social, economic, educational, and medical factors, there has been an increase in recent years in

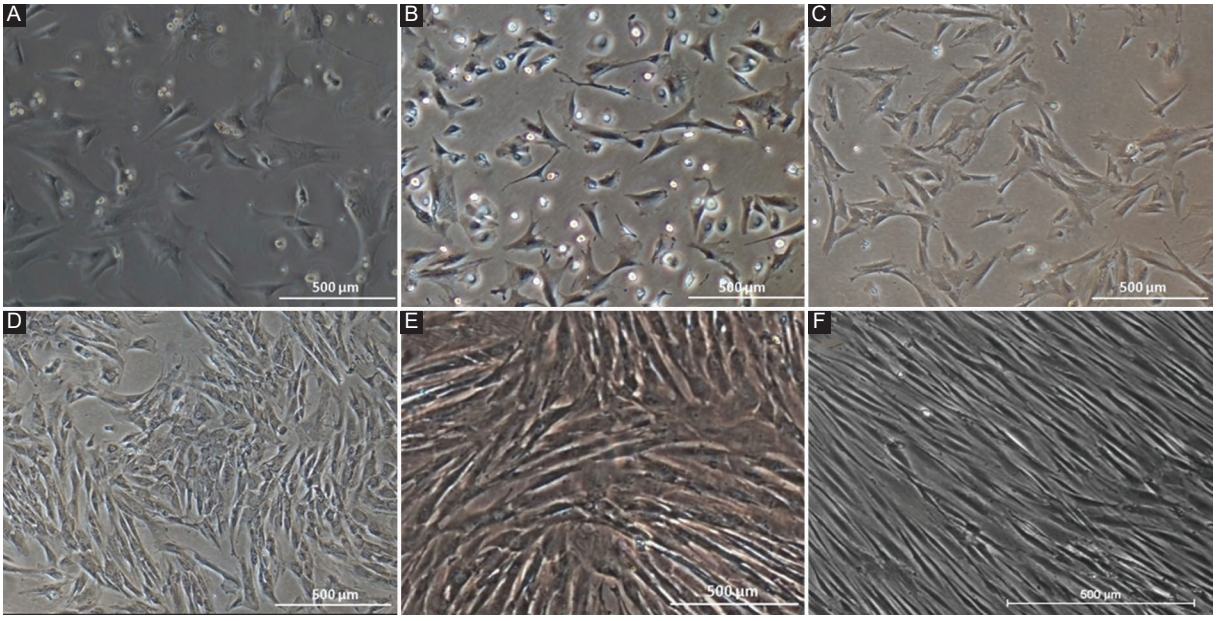

Figure 1. *Growth progression of mesenchymal stem cells from the placenta. Representative images of cell proliferation evaluated at,* **(A)** *24 h,* **(B)** *3 d,* **(C)** *7 d,* **(D)** *10 d, and* **(E)** *14 d from the cells retrieved from placentas at vaginal delivery, showing an increase in confluence. Similar pattern is observed in hMSCs from bone marrow at* **(F)** *day 14. hMSCs: human mesenchymal stem cells.*

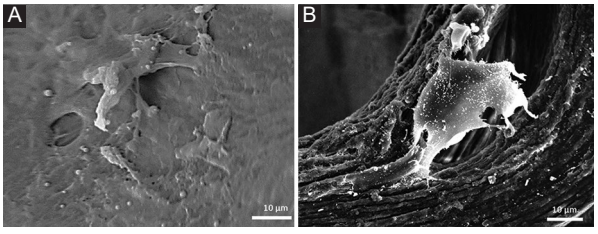

Figure 2. *Electron microscopy evaluation of hMSCs on a collagen structure of bovine origin. The adhesion capacity of the cells retrieved from placentas at cesarean section* **(A)** *and at vaginal delivery* **(B)** *is observed. Cytoplasmic projections and mesenchymal cell morphology type are also observed. hMSCs: human mesenchymal stem cells.*

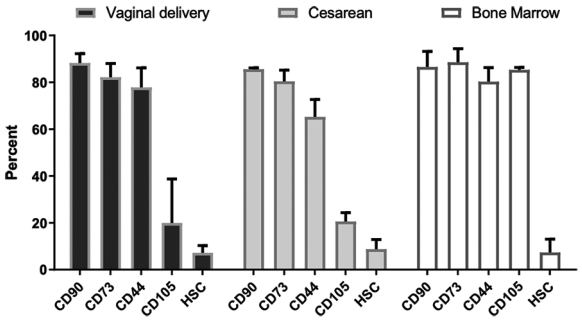

Figure 3. *Expression of pluripotential markers in AM-hMSCs according with the delivery route. Cells obtained from AM obtained from vaginal and cesarean route. The immunophenotyping was performed using CD90, CD73, CD44 and CD105. Hematopoietic stem cells (HSC), markers: CD34, CD11b, CD45, CD19, and HLA-DR were used as negative immunophenotype markers. Bone Marrow cells were used for multiple comparison of each marker. Data were expressed as the mean ± standard deviation. $p < 0.001$ for multiple comparison was consider. AM-hMSCs: amniotic membrane-human mesenchymal stem cells.*

the number of older pregnant patients[20]. In 2010, through multivariate analysis, the age of older placenta donors (above 35 years of age) was shown to be related to a significant decrease in the quantity of total protein, bFGF, HGF, KGF, NGF, and TGF-β1, compared with younger donors[27-29]. The same behavior was observed with respect to gestational stage, finding a decrease in the abovementioned factors, mainly from 280 gestational days to 294 gestational days in white women[27]. Contrastingly, in our study population, there were no differences in the women divided into groups of below 25 years of age and above 25 years of age, with respect to the demographic data or the proliferation and expansion capacity of the hMSCs in culture. We also found no statistically significant differences regarding weight between the two age groups (data not shown). In both the vaginal delivery and cesarean section groups, the BMI of the women revealed some degree of obesity. Those data are correlated with the predominant condition in the Mexican population[30,31]. In addition, the risk for presenting with maternal obesity and susceptibility to its associated diseases has been reported to increase during pregnancy[31].

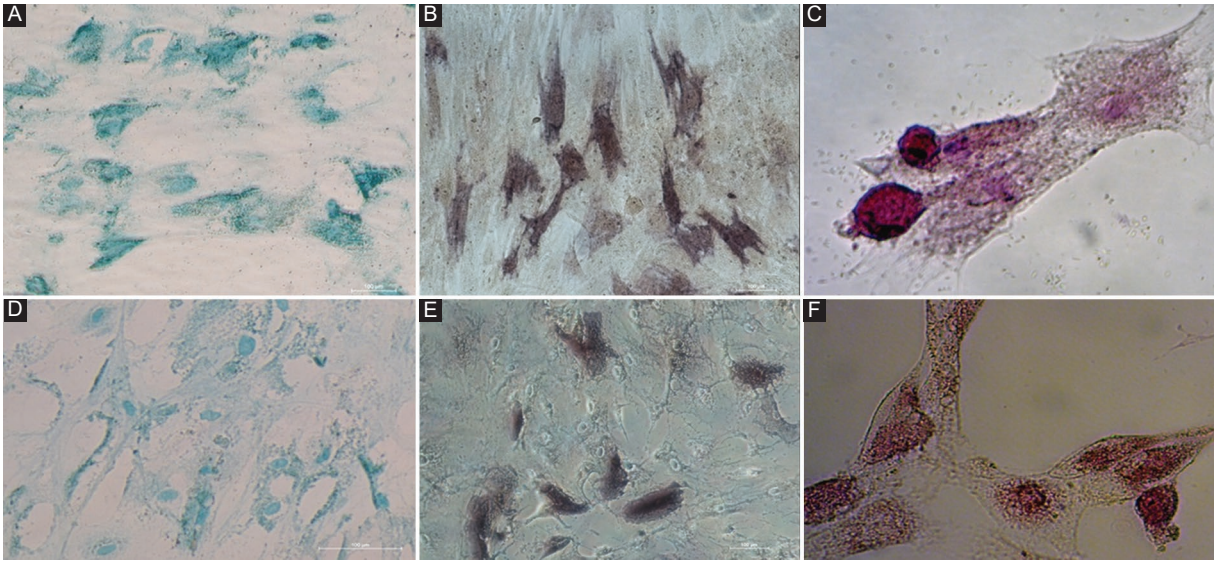

Figure 4. *Differentiation capacity of AM-hMSCs to chondrogenic, osteogenic and adipogenic in accordance with delivery via. Cells were stained using* **A** and **D:** *alcian blue,* **B** and **E:** *Von Kossa and* **C** and **F:** *oil red, to confirm chondrogenic, osteogenic, and adipogenic lineages, respectively.* **A:** *AM-hMSCs from placenta obtained of vaginal delivery incubated with chondrogenic,* **B:** *osteogenic and* **C:** *adipogenic differentiation media.* **D:** *AM-hMSCs from placenta obtained of cesarean delivery incubated with chondrogenic,* **E:** *osteogenic, and* **F:** *adipogenic differentiation media. AM-hMSCs: amniotic membrane-human mesenchymal stem cells.*

Table 6. Comparison of culture passages with the type of amniotic membrane mesenchymal stem cell retrieval

Passage, n (%)[ß]	Vaginal delivery, n = 11 (%)	Cesarean section, n = 28 (%)	p
P0	5 (45.5)	4 (14.3)	0.287
P1	3 (27.3)	8 (28.6)	
P2	1 (9.1)	4 (14.3)	
P3	-	6 (21.4)	
P4	1 (9.1)	1 (3.6)	
P5 or more	1 (9.1)	5 (17.8)	
P0-3	9 (81.8)	22 (78.6)	0.821
p > 3	2 (18.2)	6 (21.4)	

[ß]Significant X^2 test for contingency tables. Statistical significance is a $p < 0.05$.

A proteomic study on AM-hMSCs from donors that had a cesarean section revealed that obesity (BMI: 42.7 ± 7.7) produced the dysregulation of 62 proteins. Those proteins participate in the regulation pathways of oxidative stress and the regulation of the cytoskeleton and metabolic routes. However, the implications in the newborn or the mother are still being studied[31]. Despite the fact that our study groups of vaginal delivery and cesarean section did not have a BMI above 40, the possible alterations in AM-hMSCs proteins or genes are important to consider. We observed no apparent changes in proliferation or expansion of the cell cultures in either group. From the reported evidence and our results, we consider that to preserve good conditions in the hMSCs retrieved from the placenta, they should be collected from donors under 30 years of age, with a BMI no higher than 30 years. Similarly, in our study, we found that the obstetric and gynecologic variables analyzed had no effect on the proliferation or clonal expansion of the AM-hMSCs. Even though donors with comorbidities were included in the cesarean section group, and are intrinsic in that group, no direct role in the proliferation conditions was found in the AM-hMSCs cultures. Nevertheless, future studies with a larger population size need to be conducted, to determine possible changes at the cellular and genetic levels.

Even though the clinical indication for a cesarean section must be justified, the practice has become common worldwide in recent years, especially in low-income countries[32]. However, the fact that cesarean section has certain contraindications for both the mother and the product is important to consider[33,34]. Cultural implications and the promotion of reduced pain are the main reasons for choosing cesarean section versus vaginal delivery[35]. On the other hand, present health conditions worldwide have revealed an increase in the transmission of COVID-19 and other diseases through the vaginal delivery route[36].

At present, there are no studies on cellular and/or molecular changes with respect to AM-HSCs retrieved from placentas obtained at vaginal deliveries compared with those obtained at cesarean sections. The study most related to the type of delivery route and stem cell gene expression was conducted on cells retrieved from Wharton's jelly[37]. Those authors reported that there was higher expression of the *POU5F1* gene, (a gene associated with pluripotency that encodes for the OCT3/4 protein) in placenta donors that had vaginal deliveries (logRQ ± SE = 1.55 ± 0.31), compared with those that underwent cesarean section (logRQ ± SE = 0.66 ± 0.15) (p = 0.008). In addition, the working group reported that, with the progressive number of vaginal deliveries, there was reduced *POU5F1* expression[37]. Our working group is currently conducting studies at the molecular level for determining whether there are differences in the genes associated with pluripotency (*oct-4, nanog*, and *sox-2*) in AM-hMSCs, according to their collection method. However, in the present study, no alterations in the proliferative capacity, expression of pluripotential markers, and cell differentiation of the AM-hMSCs retrieved from placentas obtained at vaginal deliveries or cesarean sections were found.

The cell cultures revealed randomly distributed fibroblastoid morphology, but an organized arrangement like that observed in "marine currents" was apparent on day 14. The SEM analysis showed the adhesion capacity of the collagen matrix through the formation of lamellipodia or cytoplasmic extensions, which reflects their adaptation to the surrounding environment[38]. Our data are correlated with those already reported for hMSCs, regardless of origin[38,39].

Upon analyzing age, type of delivery route the hMSCs were obtained from, and proliferation and expansion capacity (passages), no differences with respect to the number of passages were found. Nevertheless, there was a higher percentage trend for cultures in the P0-P3 passages, whereas a lower percentage of cultures advanced beyond P3, which was observed in relation to age and type of delivery route. Pluripotent gene expression has been reported to be regulated differentially, according to culture passage, finding negative regulation in *nanog* gene expression in P4 and P5 culture passages[17]. In this context, we observed that cells from P3 express excellent levels of CD90, CD73, and CD44, which have been considered as multipotential markers; the expression was equal independently of the source that the cells were obtained. However, lower expression of CD105 was noticed in comparison with the gold standard source of hMSCs (BM). The difference in the expression of CD105 in AM-hMSCs versus BM-hMSCs, correlated with previous reports. Mark et al., observed and important reduction of CD105 expression in hMSCs obtained from BM, this phenotype was evident after that hMSCs were incubated in serum-free culturing condition, but those cells maintain multilineage potential to adipogenic, chondrogenic, and osteogenic lineages[40]. Endoglin plays an important role in angiogenesis and its expression in hMSCs has correlated with the regenerative potential in a murine model of myocardial infraction[41]. However, during the differentiation analysis we did not observe a correlation between CD105 expression osteogenic, adipogenic, and chondrogenic potential.

Our work is the first clinical study to present a relation between age, type of delivery route (vaginal delivery versus cesarean section), obstetric and gynecologic history, comorbidities, and AM-hMSCs proliferation, expansion, pluripotential markers, and differentiation capacity.

Conclusion

Our results showed no differences in AM-hMSCs in patients younger or older than 25 years of age, or in the vaginal or cesarean section delivery routes. The population seen at the Department of Obstetrics and Gynecology of the Hospital General de México is very heterogeneous with respect to age, BMI, and clinical history. We found no differences in AM-HSC, signifying that there are no limitations for using placental tissue from donors seen at the Department of Obstetrics and Gynecology, to obtain hMSCs and for their possible application in RM.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human

experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ARTÍCULO ORIGINAL

Índice de lactato/albúmina como predictor de mortalidad en sepsis abdominal

Lactate/albumin index as mortality predictor in abdominal sepsis

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Resumen

Objetivo: *Evaluar el valor predictivo del índice lactato/albúmina (ILA) para mortalidad en pacientes con sepsis abdominal ingresados a la unidad de terapia intensiva (UTI).* **Métodos:** *Se realizó un estudio transversal analítico de prueba diagnóstica. Se incluyeron pacientes mayores de 18 años con diagnóstico de sepsis abdominal ingresados a la UTI. Se calculó el ILA en las primeras 24 horas de su ingreso a la UTI para evaluar su valor pronóstico para mortalidad.* **Resultados:** *Se incluyeron 85 pacientes con diagnóstico de sepsis abdominal, cuyas principales causas fueron secundarias a trauma abdominal cerrado con lesión de víscera hueca en 22 (26%) y pancreatitis aguda grave en 21 (25%). Se calculó el ILA con un punto de corte de 1.04 como predictor de mortalidad para sepsis abdominal, con sensibilidad del 90.6%, especificidad del 72.5%, valor predictivo positivo del 66.5% y valor predictivo negativo del 92.75%.* **Conclusiones:** *El ILA puede ser útil en pacientes con sepsis abdominal como un marcador pronóstico de mortalidad rápido, fácil y económico, en comparación con otras escalas que requieren múltiples valores para su aplicación.*

Palabras clave: *Sepsis abdominal. Mortalidad. Índice lactato/albúmina.*

Abstract

Objective: *To evaluate the predictive value of the lactate/albumin ratio (LAR) for mortality in patients with abdominal sepsis admitted to the intensive care unit (ICU).* **Methods:** *An analytical cross-sectional study to validate the diagnostic test for abdominal sepsis. Patients over 18 years old with a diagnosis of abdominal sepsis admitted to the ICU. The LAR was calculated in the first 24 hours of admission to the ICU to evaluate its prognostic value for mortality.* **Results:** *We included 85 patients diagnosed with abdominal sepsis. The main causes of abdominal sepsis are secondary to closed abdominal trauma with hollow organ injury in 22 patients (26%) and severe acute pancreatitis in 21 (25%) patients. The LAR was calculated with a cut-off point of 1.04 as a predictor of mortality for abdominal sepsis, with 90.6% sensitivity, 72.5% specificity, 66.5% positive predictive value and 92.75% negative predictive value.* **Conclusions:** *The LAR may be useful in patients with abdominal sepsis as a quick, easy, and inexpensive prognostic marker of mortality, compared to other scales that require multiple values for their performance.*

Keywords: *Abdominal sepsis. Mortality. Albumin-lactate index.*

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Introducción

La sepsis grave y el choque séptico se encuentran entre las principales causas de morbilidad y mortalidad en todo el mundo. La estimación global del diagnóstico de sepsis por año es de 31.5 millones de casos. Esta patología sigue siendo una carga importante para el sistema de atención de la salud, responsable de aproximadamente el 30-40% de la mortalidad hospitalaria y con una tasa de mortalidad general en el mundo de casi el 25%[1,2].

La sepsis abdominal es la segunda causa más común de sepsis; la primera es un foco pulmonar. Se define como una disfunción orgánica que se manifiesta mediante la escala SOFA *(Sequential Organ Failure Assessment)* con ≥ 2 puntos, causada por una afección al tracto gastrointestinal o a una víscera hueca intraabdominal[3]. Los pacientes que sobreviven continúan representado un gasto de salud, debido a las diferentes secuelas físicas, psicológicas y cognitivas que presentan. A pesar de los avances en el diagnóstico y el tratamiento de la sepsis, su incidencia ha aumentado. Por lo tanto, los biomarcadores predictivos de mortalidad son importantes para el diagnóstico temprano y el tratamiento oportuno[4].

Existen diversas escalas y biomarcadores pronósticos, pero no hay ninguno que se haya establecido como referencia para establecer el pronóstico en pacientes con sepsis abdominal. Una de las escalas más utilizadas es la SOFA, la cual se usa para el manejo de la sepsis grave y predice la duración del ingreso y el riesgo de mortalidad. Con el paso del tiempo se ha buscado simplificar estas escalas con el objetivo de establecer un pronóstico de manera más rápida y eficaz, ya que el retraso en la identificación de esta patología incrementa su mortalidad[5-7].

El lactato es uno de los biomarcadores más estudiados para la sepsis y se ha demostrado que sus niveles elevados están asociados con un aumento de la mortalidad. A diferencia del lactato, la hipoalbuminemia se asocia con un incremento del riesgo de mortalidad y sirve como biomarcador en pacientes con sepsis grave.

Se cree que el índice lactato/albúmina (ILA) es un mejor marcador pronóstico, ya que está asociado a falla multiorgánica y mortalidad en la sepsis grave. Los niveles de lactato y albúmina deben ser inversos durante la sepsis, debido a que el descenso de la albúmina, al ser una proteína de fase aguda negativa, se correlaciona con la intensidad de la respuesta inflamatoria. Cuando se interpreta adecuadamente en un entorno clínico, tiene valores diagnósticos y pronósticos que guían el manejo del paciente y la toma de decisiones del médico. La identificación temprana de la sepsis es uno de los factores más significativos para un valor pronóstico, ya que se puede dar manejo adecuado para evitar el desarrollo de choque séptico con un terrible desenlace[8-10].

Este estudio fue diseñado para investigar la relación del ILA como marcador pronóstico de mortalidad en pacientes ingresados a la unidad de terapia intensiva (UTI) con diagnóstico de sepsis abdominal.

Métodos

Estudio transversal analítico que evalúa el valor pronóstico del ILA para la mortalidad. Se incluyeron pacientes ≥ 18 años con diagnóstico laboratorial y clínico de sepsis abdominal, con un promedio > 9 puntos en la escala de SOFA, ingresados a la UTI en el servicio de cirugía general durante el periodo de marzo de 2019 a febrero de 2021. Como criterios de exclusión se consideró que durante su estancia en la UTI hubieran tenido diagnóstico de COVID-19.

Se registraron como variables la edad, el sexo, el índice de masa corporal (IMC), la causa de la sepsis abdominal, la comorbilidad, el lactato sérico y la albúmina sérica.

De los pacientes que cumplieron con criterios de diagnóstico de sepsis abdominal se recopilaron datos de la historia clínica y parámetros bioquímicos séricos de lactato y albúmina, para poder determinar la puntuación SOFA en las primeras 24 horas de su ingreso.

El ILA se calculó dividiendo el valor de lactato sérico (mmol/l) entre el valor de albúmina sérica (g/dl). Los valores normales en la población adulta son < 2 mmol/l para el lactato sérico y 3.4-5.4 g/dl para la albúmina[11].

Se analizó la relación entre el ILA y la mortalidad asociada a sepsis abdominal. El seguimiento se realizó mediante el sistema de notas médicas del Instituto Mexicano del Seguro Social de forma individual, hasta el egreso del paciente de la unidad.

Se usó el programa estadístico Stalcalc dentro del programa Epi-Info 6.0. Se reportaron números crudos, proporciones y medidas de tendencia central y dispersión. Para evaluar la capacidad del ILA para predecir la mortalidad se utilizó la curva ROC (curva operativa del receptor) y se identificó el punto de corte óptimo. La validación de la prueba se estableció por

medio de la sensibilidad, la especificidad, el valor predictivo positivo, el valor predictivo negativo, la razón de verosimilitud positiva y la razón de verosimilitud negativa del ILA.

Resultados

Se incluyeron 85 pacientes con diagnóstico de sepsis abdominal que contaron con determinación de albúmina y lactato en las primeras 24 horas de su ingreso a la UTI. Del total de los pacientes, 60 (71%) fueron hombres y 25 (29%) mujeres. El promedio de edad fue de 43.18 ± 16.10 años (Tabla 1).

Solo a 3 (3.5%) de los pacientes no se les realizó cirugía durante su estancia; a 55 (64.7%) se les realizó una cirugía y a 27 (31.8%) dos. El promedio del IMC fue 30.1 ± 6.5 kg/m^2, lo que corresponde a obesidad de grado I.

El promedio del ILA fue de 1.37 ± 1.09 para pacientes que tuvieron sepsis abdominal. El promedio del lactato sérico en las primeras 24 horas fue de 3 ± 2.1 mmol/l y el de la albúmina fue de 2.3 ± 0.5 mmol/l.

El ILA como predictor de mortalidad en pacientes con sepsis abdominal mostró que el punto de corte en esta población fue de 1.04, con una sensibilidad del 90.6%, una especificidad del 72.5%, un valor predictivo positivo del 66.5%, un valor predictivo negativo del 92.75%, una razón de verosimilitud positiva de 3.2 y una razón de verosimilitud negativa de 0.12 (Fig. 1 y Tabla 2). De los 85 pacientes estudiados, 32 (37.6%) fallecieron. El tiempo de estancia intrahospitalaria fue de 20 ± 17.9 días.

Tabla 1. Características de los pacientes con diagnóstico de sepsis abdominal atendidos en la unidad de terapia intensiva

	Promedio ± DE	Min-Max
Edad en años	43.18 ± 16.10	18-83
	n	**%**
Sexo		
Hombre	60	71
Mujer	25	29
Etiología de la sepsis abdominal		
Trauma cerrado de abdomen con lesión visceral	22	26
Pancreatitis aguda grave	21	25
Perforación visceral por arma de fuego	14	16
Perforación de colon	9	11
Perforación de íleon	6	7
Perforación duodenal	8	9
Apendicitis complicada	5	6
Comorbilidad		
Tabaquismo	33	39
Alcoholismo	26	31
Diabetes tipo 2	24	28
Hipertensión arterial sistémica	23	27
Enfermedad renal crónica	1	1
Otra*	18	21

*Hipotiroidismo, cardiopatía, hepatopatía, neumopatía o enfermedad autoinmune.
DE: desviación estándar.

Tabla 2. Resultados del índice lactato/albúmina con un valor de corte > 1.04 como predictor de mortalidad en pacientes con sepsis abdominal

Punto de corte	1.04
Sensibilidad	90.6%
Especificidad	72.5%
Valor predictivo positivo	66.5%
Valor predictivo negativo	92.75%
Razón de verosimilitud positiva	3.2
Razón de verosimilitud negativa	0.12

Discusión

El choque séptico es actualmente la complicación más grave de la sepsis, el cual contribuye a una alta morbimortalidad en los pacientes hospitalizados. Este ocurre como respuesta a un agente infeccioso, con un incremento de la respuesta inflamatoria y antiinflamatoria alterada con afección endotelial, creando fuga capilar[11-13].

Diagnosticarlo a tiempo e iniciar un tratamiento apropiado puede cambiar el pronóstico, además de conocer qué pacientes requerirán medidas terapéuticas más avanzadas mediante escalas predictoras de morbimortalidad. En la actualidad existen múltiples escalas de mortalidad que se emplean en el día a día en la práctica médica, pero requieren múltiples valores que no se tienen a la mano al ingreso hospitalario del paciente[14-17].

Además, es importante mencionar que el 96.5% de los pacientes del estudio fueron sometidos a intervención quirúrgica, pudiendo cambiar esto la población microbiana causante del estado de sepsis, por lo que se manifiesta la importancia de la realización temprana de cultivos para establecer el tratamiento antibiótico específico. Cabe manifestar que en todos los pacientes con sepsis abdominal, a su ingreso a la UTI, se obtuvo el ILA, lo que fue de utilidad para predecir la mortalidad en nuestra serie.

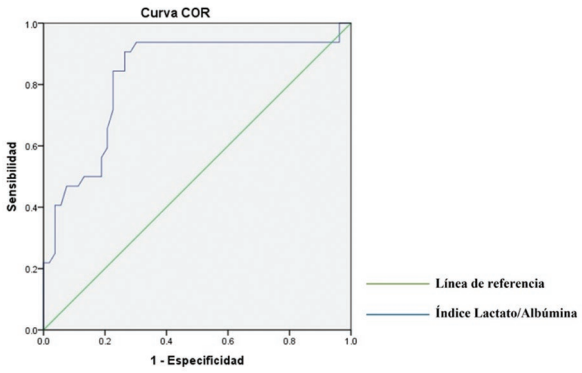

Figura 1. *Curva ROC para el índice lactato/albúmina.*

El lactato, al ser un indicador de perfusión tisular, aumenta significativamente en caso de isquemia celular e hipoxia, y conduce a trastornos metabólicos como resultado de una disminución en el volumen circulante efectivo de los tejidos. El nivel de lactato sérico se considera un marcador sensitivo para sepsis y choque séptico que refleja el metabolismo de la célula. Los niveles del lactato y albúmina predicen independientemente la mortalidad, y una combinación de ambos puede ser útil para aumentar aún más el valor predictivo. Un estudio realizado por Bou Chebl et al.[3] demostró que el ILA tiene mayor valor pronóstico para mortalidad que el valor inicial de lactato en pacientes adultos hospitalizados con sepsis.

Este trabajo intenta validar el ILA como predictor de mortalidad en pacientes con sepsis abdominal para calcular el pronóstico, al ser un método fácil de reproducir, rápido, práctico y de bajo costo para el manejo temprano. Sin embargo, será necesario evaluarlo prospectivamente y a mayor escala para definir su rol, y comparar su exactitud pronóstica con la de otras escalas ya determinadas. El ILA puede ser útil para predecir la mortalidad, al igual que otras escalas pronósticas[18-20].

En nuestros resultados, el punto de corte con mayores sensibilidad y especificidad fue 1.04 (90% y 72.5%, respectivamente), más bajo que el establecido en otro estudio prospectivo[11] en el cual se incluyeron 54 pacientes con sepsis y choque séptico, y se determinó un valor de corte > 1.735 fue 100%, una sensibilidad del 51% y un valor predictivo positivo del 17%. La diferencia en este caso podría ser explicada por las causas de la sepsis, la comorbilidad, el número de intervenciones y el tiempo de inicio de la sepsis y su ingreso intrahospitalario, ya que los pacientes acuden a valoración médica en varias ocasiones antes del ingreso. En el estudio de Gharipour et al.[11] se investigó la utilidad del ILA como marcador temprano de mortalidad en pacientes en la UTI, y se encontró que el rango de 28 días de mortalidad fue significativamente mayor cuando el ILA estaba arriba del valor de corte de 1.01, independientemente del nivel inicial del lactato. En cambio, un estudio observacional que comparó el ILA y el aclaramiento de lactato demostró que el mejor valor predictivo de mortalidad se relacionó con el ILA a las 6 horas en pacientes con choque séptico en la UTI[11,21,22].

En los estudios realizados por Yoo et al.[23] se encontró un punto de corte de 1.016 del ILA en las primeras 24 horas para predecir mortalidad. El valor de corte es similar al que se utilizó en el estudio de Chen et al.[14], en el que se incluyeron 4555 pacientes con sepsis, edad ≥ 60 años, IMC ≥ 24 kg/m^2, SOFA ≥ 2 puntos e ILA ≥ 0,16, los cuales fueron factores de riesgo independientes de muerte en pacientes con sepsis; sin embargo, en este estudio no se cuenta con la sensibilidad ni con la especificidad.

El valor de corte obtenido en nuestro estudio fue elevado en comparación con los estudios de Yoo et al.[23] y Chen et al.[14], lo cual podría ser explicado por el tipo de población incluida, que era de pacientes críticos en la UTI, y además la mayoría tenían una o varias intervenciones quirúrgicas, y esto puede modificar claramente nuestro ILA, pues una cirugía desarrolla una respuesta metabólica al trauma que exacerba la respuesta a la sepsis, y aumentan los marcadores de hipoxia tisular. Por otro lado, el ayuno entre cada intervención trae consigo una disminución considerable de la albúmina, lo que puede aumentar el ILA posterior a cada cirugía, explicando la diferencia tan marcada en el valor de corte establecido en cada estudio[24].

Pudimos encontrar un punto de corte del ILA estadísticamente significativo que se asocia con la mortalidad, el cual puede ser diferente a lo reportado en la literatura, ya que no existe un valor establecido de este índice[25]. No obstante, se requiere una evaluación prospectiva y a gran escala en población inespecífica para definir su rol, encontrar puntos de corte óptimos y comparar su exactitud pronóstica.

Existen limitaciones en nuestro trabajo al tratarse de un estudio retrospectivo, en el que solo se analizó el expediente clínico sin disponer de la totalidad de los datos necesarios para la comparación de las

diferentes escalas. Así mismo, el tamaño de la muestra fue pequeño, ya que los pacientes se captaron durante el periodo de pandemia de COVID-19 y, siendo nuestro hospital de referencia, la UTI siempre estuvo funcional para pacientes no COVID-19, pero tuvimos limitaciones por la gran demanda de pacientes COVID-19.

Existe una heterogeneidad sustancial entre los estudios incluidos. Las causas de sepsis abdominal son muy variadas, por lo que puede llevar a divergencias en los resultados, así como la diferencia en el número de intervenciones quirúrgicas en los pacientes.

Conclusiones

Nuestro estudio demuestra que el ILA tiene un adecuado papel en la identificación y el pronóstico de pacientes con sepsis grave y choque séptico, con altas sensibilidad y especificidad, y requiere pocos parámetros para su estimación. Se puede establecer que el ILA puede ser útil en pacientes con sepsis abdominal como un marcador pronóstico de morbimortalidad rápido, fácil y económico, desde el ingreso hospitalario, en comparación con otras escalas que requieren múltiples valores para su aplicación.

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Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.

Consideraciones éticas

Protección de personas y animales. Los autores declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

Confidencialidad, consentimiento informado y aprobación ética. Los autores han obtenido la aprobación del Comité de Ética para el análisis de datos clínicos obtenidos de forma rutinaria y anonimizados, por lo que no fue necesario el consentimiento informado. Se han seguido las recomendaciones pertinentes. El estudio se apegó a lo estipulado en la Declaración de Helsinski (1989) y las Guías Mexicanas de Salud. Fue aprobado por el Comité de Ética 1301 con el número de registro R- 2021-1301-130.

Declaración sobre el uso de inteligencia artificial. Los autores declaran que no utilizaron ningún tipo de inteligencia artificial generativa para la redacción de este manuscrito.

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ORIGINAL ARTICLE

Unmet health-care needs: a study based on Turkey health survey (2019)

Necesidades sanitarias insatisfechas: un estudio basado en la encuesta de salud de Turquía (2019)

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Abstract

Objective: *Reasons such as difficulty in payment, long waiting times, and the distance from health institutions and health-care needs of individuals may not be met. This study aims to determine the prevalence of health needs consisting of medical care, dental care, and prescribed drugs that are not met due to insolvency in Turkey and to evaluate whether features such as gender, age, education status, marital status, employment status, perceived health status, chronic disease status, and health insurance status affect these unmet health needs.* **Methods:** *The study data consist of the Turkey Health Survey dataset conducted in 2019 on a sample determined in the Turkish population by the Turkish Statistical Institute.* **Results:** *As a result of the analyzes carried out, it was found that the participants who were under 65 years of age, had a low level of education, had a poor perceived health status, had a chronic disease, were not covered by social security, were married, divorced and widowed had high medical care, dental care and prescribed medication needs that could not be met due to insolvency.* **Conclusion:** *In this context, it is recommended for policymakers to develop policies for individuals in disadvantaged groups.*

Keywords: *Health equity. Inability to pay. Health disparities. Turkey health survey.*

Resumen

Objetivo: *Por motivos como la dificultad de pago, los largos tiempos de espera y la distancia a las instituciones de salud es posible que no se satisfagan las necesidades de atención médica de las personas. Este estudio tiene como objetivo determinar la prevalencia de las necesidades de salud, incluyendo atención médica, atención dental y medicamentos recetados, que no se satisfacen debido a la insolvencia en Turquía.* **Métodos:** *Los datos del estudio proceden del conjunto de datos de la encuesta de salud de Turquía realizada en 2019 en una muestra determinada en la población turca por el Instituto de Estadística de Turquía.* **Resultados:** *Los participantes menores de 65 años, con un nivel educativo bajo, un estado de salud percibida pobre, alguna enfermedad crónica, sin estar cubiertos por la seguridad social, casados, divorciados o viudos, tenían altos niveles médicos y necesidades sanitarias, odontológicas y de medicación prescrita que no pudieron cubrirse debido a la insolvencia.* **Conclusiones:** *En este contexto, se recomienda que los responsables de la formulación de políticas desarrollen políticas para personas de grupos desfavorecidos.*

Palabras clave: *Equidad sanitaria. Incapacidad para pagar. Disparidades sanitarias. Encuesta de salud de Turquía.*

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Introduction

In the Universal Declaration of Human Rights, which is one of the primary documents on human rights, it is emphasized that health is a fundamental human right and need[1,2]. States have pledged to protect this right through international declarations, international conferences, domestic legislation, and policies[3]. It is also stated in the Constitution of the Republic of Turkey that health is a right and that citizens have the right to access health services[4]. Since health is seen as a fundamental right, health systems aim to improve society's health and provide access to the health services that the society needs, regardless of socioeconomic status and other non-need conditions[5,6].

However, there are some obstacles to this goal[7]. Unmet health-care needs, expressed as the lack of availability or limited availability of health services when and where they are needed, are seen as one of the biggest obstacles to accessing health services[8]. Unmet health needs include health needs such as medical care, dental care, mental care, and prescribed medications[9]. Among the main reasons for unmet health needs are some situations such as inability to pay, long waiting times, distance from the health institution, insufficient provision of the required health service or not providing them at all, and negative attitudes and thoughts of the person about the service[8,10]. Although the impact of these situations on unmet health needs differs from country to country, insolvency and lack of or inadequate provision of health services are seen as the most critical barriers to accessing needed health services[10,11]. A study conducted on Organization for Economic Co-operation and Development (OECD) countries states that approximately 7% of the population in the Netherlands, the Czech Republic, and the United Kingdom; and approximately 30% of the population of countries such as Estonia, Ireland, Latvia, Greece, and Portugal, delays receiving health-care services due to insolvency or cannot receive health care at all[12]. In a study conducted on approximately 230 thousand people in South Korea, it was reported that 35.8% of the participants could not access the health services they needed due to the lack of health services when they needed it, and 22.8% of them due to economic reasons such as payment difficulties[11]. Bagshaw et al.[13] conducted another study on 1277 people in New Zealand and found that one of the most important causes of unmet health needs is insolvency. A study conducted on approximately 20 thousand people in Serbia determined that inability to pay is one of the leading causes of unmet health needs[14].

There are many stakeholders in the Turkish Health System, including policy, administrative decision-making, financing, and health service delivery actors. Public institutions and organizations are dominant in providing health services, and there are foundations and private health institutions. Financing of health services is provided primarily by the Social Security Institution (SSI), the Ministry of Treasury and Finance, private insurance institutions, and international agencies, and through out-of-pocket payments[15]. The health transformation program implemented in Turkey in 2003 by former Health Minister Recep Akdağ and his team brought about significant changes in the management and organization, financing, resource management and service delivery functions of the health system and paved the way for the rapid expansion of health insurance coverage and access to health services for all citizens, including the poorest population groups. Access to and utilization of essential maternal and child health services have been improved to help significantly reduce under-5, infant and neonatal mortality, particularly among socioeconomically disadvantaged households, and Turkey has introduced Universal Health Insurance covering the entire population to improve equity and achieve the health system goals of improved health status and distribution, fairness in financing through reduced catastrophic health expenditures, and significantly improved population satisfaction with the health system[16]. General Health Insurance (GHI), which was put into practice in January 2012 within the body of the SSI and obliges all citizens to have health insurance, has an important place in the financing of health services[16]. Citizens insured with GHI and those who receive pensions from SSI and their dependents can benefit from health services. In addition, those who do not have any social insurance or those who have optional insurance are included in the scope of compulsory insurance, provided that their premiums are paid by themselves. Therefore, almost all of the population has been provided with health insurance[17]. For the financing of the system, premiums are collected for the employees based on their earnings within the scope of GHI. The income test is applied to those who do not have any job/seasonal work or are optionally insured. According to the results of the income test, the amount of premium to be paid is decided[18]. With this application, the premium to be paid is not

associated with income, but individuals who cannot pay their premiums still face the problem of not being able to access health services. However, to solve the problem mentioned above, decisions have been taken that enable individuals to receive health services from public hospitals even if they have premium debts[19]. Within the scope of the GHI, the examination contribution fee is not collected from the health services that individuals receive from the family physicians they are registered with. However, a fixed contribution is collected for outpatient treatment provided by secondary and tertiary health institutions and private health institutions. In addition, it has been stated that additional fees may be charged for some services determined by SSI in health service providers belonging to private health institutions and higher education institutions. For each drug prescribed for outpatient treatment, a contribution of approximately 20% of the drug cost is collected[18].

Out-of-pocket expenditures such as contributions and additional fees may prevent disadvantaged groups from accessing health services[20,21]. Out-of-pocket health expenditures have increased in Turkey over the years, and the per capita health expenditure in 2019, according to purchasing power parity, increased to $211, and its ratio in total health expenditure is 16.7%. The ratio of households with catastrophic health expenditure decreased steadily until 2012, when it fell to 0.14%, but after 2012 an upward trend began, rising to 0.43% in 2019[22]. Out-of-pocket expenditures and the resulting catastrophic expenditures can create a financial barrier to accessing health services, resulting in unmet health needs. In a study conducted in European countries, it was reported that the frequency of catastrophic health expenditures and unmet health needs is relatively high, and income inequality may be effective in unmet health needs[23]. In a study conducted on people aged 15 and over in Turkey, it was determined that the prevalence of unmet health needs due to insolvency decreased from 17% in 2006 to 9% in 2013, and it is reported that disadvantaged groups are at risk in terms of unmet health needs[24]. In a study conducted in Turkey by Yetim and Çelik[25], it was determined that the prevalence of unmet health needs in 2016 was 13.2%, and it was stated that the GHI was not successful enough to eliminate the problem of accessing health services. The reasons for this situation are out-of-pocket health expenditures and expenses incurred for accessing health services.

When the studies on unmet health needs were examined, it was determined that variables such as age, gender, educational status, marital status, employment status, perceived health status based on the person's statement, chronic illness, health insurance, income, region of residence and behavioral risk factors (smoking and alcohol use, physical lack of activity are among the essential determinants of unmet health needs[9,11,25-30]). Conditions that cause unmet health needs and determinants of unmet health services limit people's access to health-care services, resulting in an increased risk of mortality, deterioration in the quality of life, mental health, and health status[5]. In the light of this information, this study aims to determine the prevalence of unmet health needs, including medical care, dental care, and prescribed drugs in Turkey, and to determine whether the descriptive features of gender, age, education level, marital status, employment status, perceived health status, chronic disease status and health insurance status affect these unmet health needs.

Methods

Data and variables used in the research

The data set of the Turkey Health Survey (THS) conducted in 2019 on the sample determined in the population of Turkey by the Turkish Statistical Institute (TURKSTAT) constitutes the study data. Within the scope of the study, data from 17084 people aged 15 and over who participated in the research in question were used. The necessary legal permissions for the data used were obtained from TURKSTAT.

THS has been carried out regularly every 2 years since 2008, and the sample size is calculated to make estimations across Turkey. A questionnaire is used as a data collection tool in THS; the data are obtained through face-to-face interviews with the individuals reached and are based on the interviewees' statements. The THS questionnaire aims to reveal the general health profile of individuals, and many indicators are obtained[30]. Within the scope of the survey, questions are also asked about health needs, such as medical care, dental care, and prescribed medications that are not met due to insolvency. Regarding medical care, "In the last 12 months, have you been unable to afford medical care due to inability to pay?"; Regarding dental care, "During the last 12 months, have you ever needed dental care but could not afford it due to inability to pay?" and about the prescribed drugs, "During the last 12 months, have you ever been unable to afford the prescribed medicine because you needed

it, but could not afford it?" questions are asked, and the answers are "Yes" or "No." It is deduced that those who answer "Yes" to the question have unmet health needs, and those who answer "No" do not have unmet health needs. In this study, the dependent variable is medical care, dental care and prescription drugs that cannot be met due to insufficient ability to pay. It was also conducted to assess the impact of individual characteristics on the health care needs of the dependent variable.

In the study, the variables of gender, age, education status, marital status, employment status, perceived health status, chronic disease status, and health insurance status in the THS data set were used as independent variables.

Analysis of the data

It is stated that the most appropriate method to use is Logistic Regression Analysis if the dependent variable is binary and the independent variables are either categorical or continuous. In this study, in which the effect of the descriptive characteristics of individuals, which are the independent variables, on the unmet health needs, which is the dependent variable, is desired, Logistic Regression analysis was used because the dependent variables were binary and the independent variables were categorical[31]. Within the scope of the study, Statistical Package for the Social Sciences (SPSS) v22.0 software was used to provide descriptive statistics and conduct Logistic Regression Analysis.

Results

Table 1 shows the descriptive characteristics of the study participants. Accordingly, while 46.6% of the participants are men, 54.4% are women. 16% of the participants are in the 15-24 age range, 18% are in the 25-34, 19.9% are in the 35-44, 17.1% are in the 45-54, 14.7% are in the 55-64, and 14.4% consist of individuals aged 65 and over. When the education level of the participants was examined, it was determined that 12.8% of them did not finish any school or were illiterate, 50.2% were primary school graduates, 24.5% were high school or associate degree graduates, and 12.5% were graduates. It was detected that 68.6% of the participants were married, and 38.2% were working in a job. While 11% of the research participants described their health status as bad, 62.5% stated that they had a chronic disease. 7.9% of the participants do not have social security under the SSI.

Table 1. Descriptive characteristics of the participants

Sociodemographical characteristics	n	%
Sex		
Male	7,784	45.6
Female	9,300	54.4
Age		
15-24 years old	2,730	16.0
25-34 years old	3,070	18.0
35-44 years old	3,395	19.9
45-54 years old	2,918	17.1
55-64 years old	2,513	14.7
65 and over	2,558	14.4
Education		
No degrees of any kind	2,194	12.8
Primary education	8,577	50.2
High school and associate degree	4,180	24.5
Graduate and undergraduate	2,133	12.5
Marital status		
Single	3,610	21.1
Married	11,726	68.6
Divorced	574	3.4
Widowed	1,174	6.9
Employment status		
Employed	6,527	38.2
Unemployed	10,557	61.8
Health status		
Poor	9,988	58.5
Moderate	5,214	30.5
Bad	1,882	11.0
Chronic diseases		
Present	10,685	62.5
None	6,399	37.5
Social security		
Present	15,735	92.1
None	1,349	7.9
Total	17,084	100.0

When the health needs of the participants that they needed but could not meet due to insolvency in the last 12 months are analyzed (Table 2), it is seen that 8.6% could not receive the medical care they needed due to insolvency, 10.9% could not receive dental care, and 6.1% could not buy the prescribed drugs.

Table 3 shows the results of the Logistic Regression Analysis, showing the effect of various sociodemographic characteristics of the participants on the unmet medical care needs due to insolvency. When table 3 is examined, it is found that the percentage of explanation (Nagelkerke R^2) of the revealing model is 0.125, and the variables of age, education, marital

Table 2. Levels of medical care, dental care, and prescribed medication unmet due to inability to pay in the last 12 months

Items	n	%
Have you ever needed medical care that could not be met due to your inability to pay?		
Yes	1469	8.6
No	15615	91.4
Have you ever needed dental care that could not be met due to your inability to pay?		
Yes	1864	10.9
No	15220	89.1
Have you ever needed a prescribed medicine that could not be met due to your inability to pay?		
Yes	1046	6.1
No	16038	93.6
Total	17084	100.0

status, health status, chronic illness, and having social security in the model are statistically significant. When the age variable is examined, people who are 15-25 years (OR = 4.272), 25-34 years (OR = 5.005), 35-44 years (OR = 3.805), 45-54 years (OR = 2.808), and 55-64 years (OR = 1.685) old have more unmet medical care needs than participants aged 65 and over. Compared to the participants at the graduate level, those who did not complete a school (OR = 3.006), primary school (OR = 2.352), and high school and associate degree (OR = 1.425) graduates had more unmet medical care needs, and the unmet medical care needs increased with the decrease in education level. Married (OR = 1.348), divorced (OR = 1.749), and widowed (OR = 1.644) participants were found to have more unmet medical care needs due to insolvency compared to single participants. It has been determined that there are more unmet medical care needs due to insolvency when participants with poor (OR = 4.127) and moderate (OR = 1.884) health status compared with participants with good health status, participants with a chronic disease (OR = 2.023) compared with participants without chronic disease, and participants not covered by social security (OR = 3.155) compared with the participants who are covered by the insurance.

The results of the Logistic Regression Analysis, showing the effect of various sociodemographic characteristics of the participants on the unmet dental care needs due to insolvency, are shown in table 4. It was found that the percentage of explanation (Nagelkerke R^2) of the model was 0.079, and the variables of age, education, marital status, health status, presence of chronic disease, and having social security in the model had a statistically significant effect. When evaluated in terms of the age variable and compared to the participants aged 65 and over, which is the reference category, 15-25 years (OR = 3.837), 25-34 years (OR = 4.717), 35-44 years (OR = 4.250), 45-54 years (OR = 3.266) and 55-64 years old (OR = 2.064) participants were found to have more unmet dental care needs due to payment difficulties. In terms of the education variable, it was determined that those who did not complete a school (OR = 1.749), primary school (OR = 1.605), and high school and associate degree (OR = 1.218) graduates had more unmet dental care needs than graduates. In terms of marital status, married (OR = 1.416), divorced (OR = 1.750), and widowed (OR = 1.464) participants had more unmet dental care needs than single participants, and participants with poor (OR = 2.414) and moderate (OR = 1.495) health status have more unmet dental care needs than participants with good health status. It was seen that the levels of unmet dental care needs of the participants with chronic disease (OR = 1.968) and those who were not covered by social security (OR = 2.132) were higher.

Table 5 shows the results of the Logistic Regression Analysis showing the effect of the sociodemographic characteristics of the participants on the unmet prescription medicine needs due to insolvency. While the percentage of explanation (Nagelkerke R^2) of the model is 0.122, it is seen that the variables of age, education, marital status, health status, presence of chronic disease, and having social security in the model have statistically significant effects. Compared to participants aged 65 and over, it is seen that other age groups have more unmet prescription drug needs due to insolvency, and that unmet prescription drug needs decrease as the age of the participants increases. When evaluated in terms of education level, it was determined that the unmet prescription drug needs of the participants at other education levels were higher due to payment difficulties, and the unmet drug needs decreased with the increase in education level. In terms of the marital status variable, while married (OR = 1.342) and divorced (OR = 1.689), participants had higher unmet prescription drug needs than single participants. Participants with moderate (OR = 1.558) and poor (OR = 3.739) health status had more unmet prescription drug needs. It is seen that the participants with a chronic disease (OR = 1.926) have more unmet prescription drug needs due to insolvency than those who do not have it, and the

Table 3. Logistic regression analysis results on the effect of participants' sociodemographical characteristics on unmet medical care needs

Sociodemographical characteristics	β	OR (%95 CI)	p
Sex			
Male (reference)			
Female	0.103	1.109 (0.971-1.266)	0.127
Age			
15-24 years old			
25-34 years old	1.452	4.272 (3.109-5.869)	< 0.001
35-44 years old	1.610	5.005 (3.883-6.451)	< 0.001
45-54 years old	1.336	3.805 (2.997-4.832)	< 0.001
55-64 years old	1.032	2.808 (2.232-3.533)	< 0.001
65 and over (reference)	0.522	1.685 (1.336-2.124)	< 0.001
Education			
No degrees of any kind			
Primary education	1.101	3.006 (2.276-3.970)	< 0.001
High school and an associate degree	0.855	2.352 (1.845-3.000)	< 0.001
Graduate and undergraduate (reference)	0.354	1.425 (1.095-1.853)	0.008
Marital status			
Single (reference)			
Married	0.298	1.348 (1.086-1.673)	0.007
Divorced	0.559	1.749 (1.246-2.455)	0.001
Widowed	0.497	1.644 (1.198-2.257)	0.002
Employment status			
Employed (reference)			
Unemployed	0.056	1.057 (0.920-1.215)	0.433
Health status			
Good (reference)			
Moderate	0.633	1.884 (1.629-2.179)	< 0.001
Poor	1.418	4.127 (3.438-4.955)	< 0.001
Chronic diseases			
Present			
None (reference)	0.705	2.023 (1.721-2.378)	< 0.001
Social security (SSI)			
Present (reference)			
None	1.149	3.155 (2.691-3.699)	< 0.001

$p < 0.05$.
Nagelkerke R^2: 0.125.
Hosmer-Lemeshow: $\chi^2 = 5.175$; $p = 0.739$.
Correct classification percentage: 91.4%.

participants who are not covered by social security (OR = 3.022) compared to the participants who are covered by social security.

Discussion

The rate of unmet health needs is increasing worldwide[8]. In addition, unmet health needs, which are seen as an important public health problem, are an essential consideration for health system planners and policymakers to ensure equitable access to health services[32]. The most common causes of unmet health needs are: difficulty in paying, long waiting time for a planned visit/medical examination, physical distance, and transportation problems to the health-care provider[27]. Effective health policies that will be developed to eliminate unmet health needs can increase patients' quality of life with serious diseases, prolong their life expectancy, and improve the course of the disease[5,27]. This study aims to make international comparisons by revealing the prevalence of unmet health needs in Turkey and its relationship with various sociodemographic variables.

When the studies investigating the level of unmet health needs are examined, according to the study conducted by Chaupain-Guillot and Guillot[33] on 29 European countries, the level of unmet health needs in the European Union (EU) is 6.3%. Looking at other

Table 4. Logistic regression analysis results on the effect of participants' sociodemographic characteristics on unmet dental care needs

Socio-demographic characteristics	β	OR (%95 CI)	p
Sex			
Male		1.010 (0.900-1.134)	0.860
Female (reference)	0.010		
Age			
15-24 years old			
25-34 years old	1.345	3.837 (2.862-5.146)	< 0.001
35-44 years old	1.551	4.717 (3.714-5.990)	< 0.001
45-54 years old	1.447	4.250 (3.391-5.326)	< 0.001
55-64 years old	1.183	3.266 (2.622-4.067)	< 0.001
65 and over (reference)	0.725	2.064 (1.655-2.575)	< 0.001
Education			
No degrees of any kind			
Primary education	0.559	1.749 (1.391-2.198)	< 0.001
High school and associate degree	0.473	1.605 (1.335-1.929)	< 0.001
Graduate and undergraduate (reference)	0.197	1.218 (0.999-1.484)	0.048
Marital status			
Single (reference)			
Married	0.348	1.416 (1.173-1.710)	< 0.001
Divorced	0.560	1.750 (1.297-2.363)	< 0.001
Widowed	0.381	1.464 (1.085-1.975)	0.013
Employment status			
Employed			
Unemployed (reference)	0.013	1.013 (0.898-1.143)	0.831
Health status			
Good (reference)			
Moderate	0.402	1.495 (1.320-1.693)	< 0.001
Poor	0.881	2.414 (2.038-2.859)	< 0.001
Chronic diseases			
Present			
None (reference)	0.677	1.968 (1.721-2.251)	< 0.001
Social security (SSI)			
Present (reference)			
None	0.757	2.132 (1.829-2.484)	< 0.001

$p < 0.05$.
Nagelkerke R^2: 0.079.
Hosmer-Lemeshow: $\chi^2 = 6.350$; $p = 0.608$.
Correct classification percentage: 89.1%.

studies, it is 12.7% in Montenegro, 10.8% in Macedonia, 7.5% in Croatia, and 0.4% in Slovenia[27]. According to Chaupain-Guillot and Guillot[33], the highest level of unmet health needs in the EU was found in Bulgaria and Latvia. In these two countries, more than 15% of individuals aged 16 and over are reported to have unmet medical care/dental examination, or treatment needs in the past 12 months. In a study conducted on OECD countries; in the Netherlands, the Czech Republic, and the United Kingdom, approximately 7% of the population; and in countries such as Estonia, Ireland, Latvia, Greece, and Portugal, approximately 30% of the population delays receiving health-care services due to insolvency or cannot receive health care at all[12]. This study revealed that 8.6% of the participants could not get the medical care they needed due to payment difficulties, 10.9% could not get dental care, and 6.1% could not get the prescribed drugs in the last 12 months. Yardim and Üner[24] state that the level of unmet health needs due to insolvency shows a downward trend from 2006 to 2013, and this rate was 9% in 2013. Yetim and Çelik[25] state that Turkey's level of unmet health needs due to insolvency is 13.2%, and the level of unmet health needs in Turkey may be higher compared to developed European countries.

Research on sociodemographic factors associated with barriers to access, accessibility, and acceptability of health services revealed that variables such as

Table 5. Logistic regression analysis results on the effect of participants' socio-demographic characteristics on unmet needs for prescribed medicines

Sociodemographic characteristics	β	OR (%95 CI)	p
Sex			
Male (reference)			
Female	0.072	1.074 (0.919-1.256)	0.367
Age			
15-24 years old			
5-34 years old	1.941	6.963 (4.802-10.099)	< 0.001
35-44 years old	2.028	7.602 (5.593-10.334)	< 0.001
45-54 years old	1.651	5.210 (3.883-6.990)	< 0.001
55-64 years old	1.278	3.589 (2.698-4.774)	< 0.001
65 and over (reference)	0.642	1.900 (1.417-2.548)	< 0.001
Education			
No degrees of any kind			
Primary education	1.661	5.266 (3.701-7.492)	< 0.001
High school and an associate degree	1.197	3.311 (2.405-4.558)	< 0.001
Graduate and undergraduate (reference)	0.590	1.804 (1.282-2.540)	0.001
Marital status			
Single (reference)			
Married	0.294	1.342 (1.052-1.711)	0.018
Divorced	0.524	1.689 (1.141-2.502)	0.009
Widowed	0.351	1.421 (0.971-2.078)	0.070
Employment status			
Employed (reference)			
Unemployed	0.146	1.158 (0.984-1.362)	0.078
Health status			
Good (reference)			
Moderate	0.463	1.588 (1.340-1.882)	< 0.001
Poor	1.319	3.739 (3.030-4.613)	< 0.001
Chronic diseases			
Present			
None (reference)	0.655	1.926 (1.926-1.604)	< 0.001
Social security (SSI)			
Present (reference)			
None	1.106	3.022 (2.533-3.605)	< 0.001

$p < 0.05$.
Nagelkerke R^2: 0.122.
Hosmer-Lemeshow: $\chi^2 = 3.386$; $p = 0.908$.
Correct classification percentage: 93.9%.

gender, age, and education level, region of residence, household income, insurance status, smoking status, and poor health status are factors related to unmet health needs[27,35]. Within the scope of this research, the effects of sociodemographic factors on health needs that are not met due to insolvency were revealed and, in this context, those who are under 65 years old, have a low education level, have a bad health status, have a chronic illness, are not covered by social security, are married, divorced and widowed had higher needs for medical care, dental care and prescribed medication that could not be met due to insolvency. Within the scope of this study, it was determined that individuals aged 65 and under have unmet health needs. However, when the literature was examined, it was found that there were different findings on this issue. For example, according to a study conducted in Barcelona with 1315 elderly individuals (aged 65-97 years) in which the relationship between unmet health needs and mortality was examined, 10- 25% of elderly individuals could not benefit from health services and it was revealed that mortality rates were high in those with unmet health needs[36]. Another study investigating the unmet needs of people in need of long-term care in China, the country with the world's largest population of older people, and examining the 3-year mortality rate of 3,089

Chinese adults aged 65+ in need of long-term care, found that older adults with unmet health needs had an approximately 10% higher risk of death than those with met needs, controlling for demographic characteristics. Risks were particularly elevated among older women and urban older adults[37]. Similarly, there are many studies examining the relationship between unmet health needs and mortality and morbidity[38-40]. On the other hand, studies have found that unmet health needs have serious effects not only on mortality but also on morbidity or health status. For example, in a study conducted in Korea with the data of 7717 individuals, it was found that there was a relationship between unmet health needs and health outcomes, and it was determined that unmet health needs decreased quality of life by 1%[40]. Within the scope of this study, it was determined that the gender factor did not have an effect on unmet health needs. Similarly, Yardim ve Üner[24] found that adult men and women in Turkey are less likely to report unmet health needs due to insolvency. Another study conducted by Yetim and Çelik[25] in Turkey determined that women have more unmet health needs due to economic reasons.

In a study conducted by Lim[35] in Korea, it was revealed that gender has an effect on unmet health needs. According to Lim[35], women have more unmet health needs than men. This is also supported by other studies, and it is revealed that women have more unmet health needs than men[41,42]. Pappa et al.[5] explain this situation with the dual role of women; that is, women's responsibilities at work and home are an important predictor of unmet health needs.

According to the results of this research, it was revealed that the working status did not have an effect on the unmet health needs. This finding is also supported by the study of Yetim and Çelik[25]. The authors found that the levels of unmet health needs in working and non-working individuals were close to each other. However, different results may emerge in some international studies. For example, a Swedish study reports that 37% of unemployed respondents, including those who are economically inactive, have unmet health needs. This rate is 12% higher than that of working participants[43]. The unemployed are more likely to perceive the need to seek care for psychological problems than the employed. Lack of employment may be related to unmet care needs, especially among the unemployed who experience psychological symptoms[44].

Within the scope of this research, it was determined that married, divorced, and widowed participants have more unmet health needs due to payment difficulties than singles. The findings of this study are supported by various studies[25-27,45]. It is stated that divorced individuals communicate more with professional health-care providers (general practitioners, psychiatrists, and psychologists) due to social or emotional problems, and they perceive unmet health needs more frequently[46]. Women living alone are more likely to have difficulty finding available health-care resources, given the fact that they lack social support and social capital compared to men. Therefore, their limited knowledge of where and how to access the services they need can be seen as the reason for their unmet health needs[47].

When evaluated in terms of age, it was revealed that participants under the age of 65 in this study had more unmet health needs. According to Lim[35] research, the rate of unmet medical needs of individuals aged 60 and over is significantly lower than those aged 19-29. Yetim and Çelik[25] report that individuals between the ages of 35-54 have more unmet health needs. Therefore, it is seen that unmet health needs increase as age decreases. On the other hand, the study's findings conducted by Pappa et al.[5] in Greece also support this situation. According to the study in question, people in the 25-34 age group are twice as likely to have unmet health needs as the elderly (65+ years). The findings of another study conducted in Korea also support this situation[42]. Among the possible reasons youth have more unmet health needs are poor knowledge of health resources and different self-assessments of diseases[5,48].

In terms of education level, it was determined that participants with low education levels have more unmet health needs. Mitrasevic et al.[27] obtained similar results in their study. According to the study's findings, it has been shown that lower education levels increase the probability of the unmet need for health services. Another study conducted in Korea shows that those with low education levels have more unmet health needs[42]. It is seen that the need for health that cannot be met increases with the decrease in the level of education. It can increase the unmet health needs of people with lower and secondary education levels (representing lower and middle social classes) compared to those with a university education[5]. A previous study found that people with higher education use private health services more by paying out-of-pocket for the health services they need, and therefore, they have fewer unmet health needs[49]. Considering that a low education level is associated with low quality and low-paid jobs, it may

be difficult for them to meet their health needs out of pocket[5]. Those with low levels of education work in precarious employment, low-paying jobs with little or no benefits, and high job insecurity. In this case, it may be difficult for these people to meet their health needs, and they may delay addressing these needs due to payment difficulties.

It was found that the unmet health needs are higher in participants with poor health status. A study carried out in Turkey by Yardim and Üner[24] supports this situation. Among the health status variables, groups with poor health status have the highest level of unmet health-care needs. Looking at other studies, in a study conducted with 9205 people in Canada, it was reported that unmet health needs are higher among those with poor health status[50]. Another study conducted in Greece stated that poor health status is associated with increased unmet health needs[5]. Again, in the study carried out with 14,800 young participants in the USA, it was revealed that 19.2% of the youth had poor health status, and this situation increased their unmet health needs[51]. It is thought that several of the pathways to poor health and unmet health-care needs may be related to income and housing insecurity[52]. It is an important fact that those with poor health status cope with more diseases, and at the same time, lag behind in working life and cannot earn an income. Based on this fact, it is possible that while coping with the disease, on the one hand, the health needs that they cannot meet due to payment difficulties, on the other hand, increase.

Previous research shows that those with chronic diseases have more unmet health needs. A national study of 11,620 participants in Korea shows that those with at least one chronic disease have more unmet health needs[42]. Again, the findings of a study conducted with 360,105 adults in Canada showed that those with at least one chronic disease reported more unmet health needs than those without the chronic disease[53]. The findings of this study support this situation. Those with chronic diseases are likely to face catastrophic medical expenses[42]. Out-of-pocket expenses, which may lead to higher medical costs, may cause delays in addressing their health needs and increase unmet health needs.

This study determined that those who are not covered by social security have more unmet health needs. Lack of health insurance is associated with unmet health needs, according to the results of a study by Tumin et al.[54] covering 88 counties of Ohio. According to the information obtained from the SSI data, Yardim ve Üner[24] draws attention to the fact that 9% of the population of Turkey has premium debts and that these people are faced with the dilemma of spending more out-of-pocket in case of using health services or giving up seeking care.

Conclusion

It was revealed that those under 65 years of age, those with low education level, those with chronic diseases, those without health insurance, those with poor perceived health status, and those with marital status who are married, divorced or widowed, are in the high-risk group in terms of unmet health needs due to insolvency. Since unmet health-care needs are a critical indicator of a country's health system, it is very important to remove all barriers that prevent access to health services or limit the use of health services[26]. Unmet health-care needs due to insolvency may lead to various problems, including worsening health status and quality of life of citizens, mortality, and morbidity. In this context, it is recommended that policymakers develop policies for individuals in this disadvantaged group. Considering that certain groups are riskier than others as a result of the study, it is recommended to ensure and support these risky groups' social and economic development. Contribution exemptions or reductions may be provided to disadvantaged groups with unmet health needs due to insolvency, as this will increase out-of-pocket expenditures. The depth of the GHI can be increased in order to overcome the negative situations that will lead to out-of-pocket health expenditures and even catastrophic expenditures, and to facilitate access to health services. Future studies recommend investigating the leading causes of unmet health needs due to insolvency and examining which health-care needs cannot be met.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The study does not involve patient personal data nor requires ethical approval. The SAGER guidelines do not apply.

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ORIGINAL ARTICLE

Subcutaneous venous port catheter insertion through subclavian vein on 770 patients: do the catheter type and the placement technique matter?

Inserción de catéter venoso subcutáneo a través de la vena subclavia en 770 pacientes: ¿importan el tipo de catéter y la técnica de colocación?

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Abstract

Objective: *The aim of this study was to present our clinical experience in patients undergoing subcutaneous venous port catheter (SVPC) placement through subclavian vein for chemotherapy.* **Methods:** *We retrospectively investigated 770 patients undergoing SVPC placement. Two different catheters were used (polyurethane [n = 100, 13%] and silicone [n = 670, 87%]). Port reservoir (PR) was placed by removing subcutaneous fatty tissue equivalent to the resevoir size (n = 220, 29%), or buried directly under fatty tissue (n = 550, 71%). Results and complications according to catheter types and placement techniques were investigated.* **Results:** *There were 59 complications (7.7%). Port-site infection and wound dehiscence were higher when the reservoir was placed after removing subcutaneous fatty tissue (p < 0.05). Port-site infection, wound dehiscence, subclavian vein thrombosis, and catheter occlusion were common in polyurethane catheters (p < 0.05). Of 192 patients who were followed-up (mean 18 months), SVPC was removed in 25% due to the death of the patients (n = 100), completion of treatment (n = 87), and development of complication (n = 5).* **Conclusion:** *During SVPC insertion, the placement of PR under the adipose tissue and preferring silicone catheters may reduce the complication rates.*

Keywords: *Central venous access device. Chemotherapy port. Complications. Outcomes.*

Resumen

Objetivo: *Presentar nuestra experiencia clínica en pacientes sometidos a colocación de catéter venoso subcutáneo (CVS) a través de la vena subclavia para quimioterapia.* **Métodos:** *Investigamos retrospectivamente a 770 pacientes a los que se colocó un CVS. Se utilizaron dos catéteres diferentes: de poliuretano (n = 100; 13%) y de silicona (n = 670; 87%). El reservorio se colocó eliminando tejido graso subcutáneo equivalente al tamaño del reservorio (n = 220; 29%) o enterrado directamente debajo del tejido graso (n = 550; 71%). Se investigaron los resultados y las complicaciones según el tipo de catéter y la técnica de colocación.* **Resultados:** *Hubo 59 complicaciones (7.7%). Ocurrieron más infecciones del catéter y dehiscencias de la herida cuando el reservorio se colocó tras retirar tejido graso subcutáneo (p < 0.05). La infección del catéter, la dehiscencia de la herida, la trombosis de la vena subclavia y la oclusión del catéter fueron más comunes con los catéteres de poliuretano (p < 0.05). De los 192 pacientes con seguimiento (media: 18 meses), en el 25% se retiró el CVS por muerte del paciente (n = 100), finalización del tratamiento (n = 87) o desarrollo de complicaciones (n = 5).* **Conclusiones:** *Durante la inserción de un CVS, la colocación del reservorio debajo del tejido adiposo y la preferencia por catéteres de silicona pueden reducir las tasas de complicaciones.*

Palabras clave: *Dispositivo de acceso venoso central. Puerto de quimioterapia. Complicaciones. Resultados.*

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Introduction

Short- or long-term central venous catheter is the standard of practice for various central venous therapies, including chemotherapy, fluid administration, antibiotic therapy, and parenteral nutrition. Its first insertion on a human was reported in a self-experimentation attempt in 1929. Later, Seldinger technique was described facilitating catheter placement into vascular system over a guide-wire in 1953. In the 1970s, the first long-term central venous catheters were designed[1].

Subcutaneous venous port catheter (SVPC) or simply called "port" is a small reservoir connected to a venous catheter positioned in the subcutaneous tissue, and its first usage was reported in 1982[2]. It remains an integral part of chemotherapy in oncologic patients reducing patient discomfort. Lower risk of infection and thrombosis are among its advantages in patients necessitating a continuous intravenous line compared to peripheral vein access[1,3]. Yet, this technique has various complications including pneumothorax, hemothorax, hematoma, catheter-related infection, venous thrombosis, catheter occlusion or rupture, pain, and skin disorders[1,4,5]. This study aimed to present our clinical experience in patients undergoing SVPC placement.

Methods

This retrospective study consisted of 770 patients (427 males, and 343 females; mean age 54 ± 13 years; range 11-82 years) undergoing SVPC placement for adjuvant or neoadjuvant chemotherapy from January 2012 to December 2022. Patients' demographic/clinical features, surgical results, duration of catheter stay, reasons for catheter removal, and complications were investigated. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Local Ethical Committee (date: October 10, 2023, No: 4121) and written consent was obtained individually.

Before implantation, bleeding and coagulation times, and basic biochemical parameters of each patient were controlled. The intervention site was examined in terms of infection, swelling, mass, and previously received radiotherapy. The same thoracic surgeon implanted all the catheters under operating room settings. Right subclavian vein was mostly preferred (n = 747, 97%) due to its anatomical convenience. Left subclavian vein was used in patients with prior mastectomy, radiotherapy/head-neck surgery, or structural anomaly. Laryngeal mask airway and general anesthesia were used in the first 50 patients so that the principal surgeon should gain experience. Then, the next 50 patients received combination sedoanalgesia with propofol and dormicum. However, since it was remarked that patients felt pain and were unable to remain still during the procedure, sedoanalgesia using dormicum and fentanyl was preferred for the remaining 670 patients. Propofol was added just before the placement of port reservoir (PR). All patients were provided with continuous monitoring using pulse oximetry, electrocardiography, and a non-invasive blood pressure.

For the first 220 patients, polyurethane catheters (n = 100) and silicone catheters (n = 120) were used. Since it was observed that the rates of complications including port-site infection, skin dehiscence, and venous thrombosis were higher in patients with polyurethane catheters, only silicone catheters were preferred for the remaining 550 patients. Overall, silicone catheters were used in 670 patients (87%), and polyurethane catheters in 100 patients (13%).

Implantation was performed using Seldinger method, as previously described[3]. No superficial Doppler ultrasound was used. Needle was inserted 1 cm under the clavicular angle at the midclavicular area. A pocket of 3 cm was created 3-4 cm under the needle aspiration point for the port insertion. While creating a pocket, a piece of subcutaneous fatty tissue equivalent to the size of the PR was removed and the PR was placed in this pouch for the first 220 patients (29%). However, since we detected that skin dehiscence and port-site infection rates were higher, we did not remove any fatty tissue for the last 550 patients (71%), and buried the PR directly under the adipose tissue. No additional port suture was used in any patient.

No fluoroscopy was used to control the place of the catheter. Peroperative chest X-ray was taken for the first 100 patients. After gaining experience, the catheter was inserted over the guidewire according to the calculated length on the patient before the procedure. The length was 15-17 cm for thin patients, 17-19 cm for normal body type patients, and 19-22 cm for obese patients. Then, after 100 patients, we stopped having peroperative chest X-ray. During the procedure, the catheter was checked for proper working and probable leakages with preventing thrombus formations by aspiration blood through and giving saline solution

containing 100U/mL heparin inside. The skin incision was closed with 3/0 taper monofilament propylene. Skin sutures were put in a way not to overlay the punctured area over the PR.

Post-operative chest X-ray was routinely taken. Patients were discharged after 4-6 h of follow-up in the ward. First-generation cephalosporin and analgesics were administered orally for 5 days. The oncologists of the patients were advised to wait about 4-5 days to ensure the proper wound healing before beginning the chemotherapy.

Patients were called to follow-up visit 1 week after the procedure. Skin sutures were removed 10 days after implantation. Catheters were flushed monthly if patients were given monthly chemotherapy or had catheter left *in situ*. Complications and their management were noted. Complications were defined as early and late when they occurred within 7 days of implantation and 7 days after the procedure, as previously described[5]. Complications were analyzed according to the procedure of PR insertion (whether a piece of the adipose tissue removed to place the PR or it was buried under the adipose tissue) and the type of catheters (polyurethane versus silicone).

Descriptive statistics were used in number and percentage, including mean ± standard deviation, median and range, frequencies, and proportions. Continuous variables were compared using student t-test. Categoric variables were compared using a X^2 test or Fisher's exact test, as applicable. $p < 0.05$ was considered to be statistically significant.

Table 1. Indications for SVPC placement (n = 770)

Type of malignancy	n	Percentage
Colorectal cancer	420	54.6
Gastric cancer	80	10.4
Lung cancer	74	9.6
Bladder cancer	70	9.1
Breast cancer	32	4.2
Esophageal cancer	27	3.5
Pancreatic cancer	25	3.2
Head and neck cancer	21	2.7
Others*	21	2.7

*Including ovarian cancer, prostatic cancer, plasmocytoma, osteosarcoma, and retinoblastoma.
SVPC: subcutaneous venous port catheter.

Table 2. Complications of SVPC placement

Complication	n	Percentage
Port-site infection	22	2.9
Wound dehiscence	13	1.7
Pneumothorax	8	1.0
Catheter occlusion	8	1.0
Subclavian vein thrombosis	5	0.7
Port-catheter breaking off	3	0.4
Total	59	7.7

SVPC: subcutaneous venous port catheter.

Results

Table 1 outlines the indications for SVPC placement. Gastrointestinal system tumors predominated (n = 527, 68.5%) followed by lung cancer (n = 74, 9.6%). Most patients (n = 440, 57%) had metastatic tumors. Among them, 39 patients had received previous chemotherapy through standard venous access, and offered SVPC placement due to several reasons by their oncologists. The remaining 401 patients were not given chemotherapy before. Three hundred-and thirty patients (43%) necessitated SVPC placement for adjuvant (n = 247) and neoadjuvant (n = 83) chemotherapy.

Table 2 demonstrates the complications after the implantation. Fifty-nine complications developed (7.7%). Twenty-one complications (36%) including wound dehiscence and pneumothorax were early complications, while the remaining 38 (64%) including port-site infection, catheter occlusion, subclavian vein thrombosis, and port-catheter breaking off were late complications.

Port-site infection was the commonest complication (22 patients, 2.9%), managed by administering empirical antibiotics for 2 days. Fifteen patients unresponsive to treatment necessitated wound debridement and replacement of the PR 3-4 cm laterally. Wound dehiscence developed in 13 patients (1.7%). Simple suturing was enough for five patients, but the remaining eight patients necessitated wound debridement and replacement of the PR. Pneumothorax occurred in 8 patients (1.0%). Tube thoracostomy was performed for 2 patients, while the other six patients were given short-term oxygen support therapy. Catheter occlusion was detected in 8 patients (1.0%) which resolved by the catheter flushing using the

heparinized solution (1 mL/100 unit heparin). Five patients presented with neck pain, and arm pain and swelling. Ultrasound imaging demonstrated thrombotic obstruction of the subclavian vein (0.7%). These patients received low-molecular-weight heparin (enoxaparin sodium 1 mg/kg) for treatment. However, despite the therapy, port catheter had to be removed in two patients.

Complete transection and embolization of the catheter occurred in 3 patients (0.4%). A 39-year-old female patient with breast cancer developed sudden chest pain in the post-operative 6th month. Computed tomography of the chest showed the broken catheter fragment at the left pulmonary artery lying down from truncus. An 11-year-old male patient with retinoblastoma admitted a year after the SVPC placement and planned for catheter removal since his treatment ended. During the removal, a piece of the catheter transected and remained in the right ventricle. Another female patient (37 years old) with breast cancer developed sudden chest pain after vigorous physical effort (traditional dancing with hands and arms shaking for a long time). She had SVPC placement 3 months ago. Chest X-ray demonstrated the broken catheter in the left pulmonary artery. PR was taken out in these three patients, and interventional radiology team removed the broken parts of the catheter through Snare technique using the right femoral vein.

When complications were analyzed according to the technique of the PR insertion (whether a piece of fatty tissue was removed to place the PR or it was buried under the adipose tissue), we found that the rates of port-site infection (18 vs. 4 patients, 8.2% vs. 0.7%, respectively, $p < 0.05$) and wound dehiscence (10 vs. 3 patients, 4.5% vs. 1.4%, respectively, $p = 0.0004$) were higher in cases in the former technique. We also detected that the usage of polyurethane catheters increased significantly the rates of port-site infection (13 vs. nine patients, 13.0% vs. 1.3%, respectively, $p < 0.05$), wound dehiscence (six vs. seven patients, 6.0% vs. 1.0%, respectively, $p = 0.003$), subclavian vein thrombosis (four vs. one patient, 4.0% vs. 0.1%, respectively, $p = 0.001$), and catheter occlusion (four vs. four patients, 4.0% vs. 0.6%, respectively, $p = 0.01$).

The follow-up data of 413 patients (54%) were missing since they were coming from different cities of the country and went back home after the SVPC placement procedure. The remaining 357 patients (46%) were followed-up with a mean duration of 18 months (range 6-41 months). Of them, port catheters were removed in 192 patients (25%) due to the death of the patients during treatment (n = 100), the completion of treatment (n = 87), and development of complication (n = 5).

Discussion

This study reflects the results of SVPC placement of a tertiary referral center. We have been performing SVPC placement since 2012. In the first 5 years, we had 20-50 patients/year. However, in the recent 5 years, we had nearly 100 patients/year. Yanik et al.[3] reported the highest number of cases (n = 3000) in Turkey, and there are several national reports up to 225 patients[6-8]. Our study is the second national study with higher number of cases (n = 770).

Most patients undergoing SVPC placement had gastrointestinal system tumors (colorectal, gastric, and esophageal) and breast tumors (26-56% and 20-47%, respectively)[3,9-12]. Patients with gastrointestinal system tumors predominated in our study (n = 527, 68.5%). The mean age and gender of the patients vary according to the type of cancer; the mean ages were between 53 and 61 years, and 16-61% of the patients were male[3,5,12,13]. The mean age of the patients in our study was 54 ± 13 years, and 55% of them were male. SVPC can be placed in subclavian vein, and external or internal jugular vein. The laterality (left or right) is chosen according to surgeon's preference and patient's physical and medical history characteristics[14]. We commonly used right subclavian vein (97%), and preferred left subclavian vein in patients with prior mastectomy, radiotherapy/head-neck surgery, or structural anomaly.

The use of ultrasound is advised to secure the venous puncture[10-14]. We did not use ultrasound since the puncture could also be done without[3,6]. Besides, it was mentioned that the insertion of a catheter by a physician who had performed more than 50 catheterizations results lower rates of mechanical complication compared to by a physician who had performed < 50 catheterizations[15]. As mentioned above, all catheters were implanted by the same thoracic surgeon who had performed enough subclavian venous catheterization before beginning SVPC placement.

Fluoroscopy may be used to check the catheter position[10-13]. We did not use fluoroscopy, since the catheter could be positioned by calculating the length on the patient before the procedure, as described[3,14,16]. We obtained a post-operative chest X-ray to confirm the correct positioning of the port catheter and

documentation of pneumothorax and hemothorax, as suggested[3,11,14].

More than 15 million patients/year undergo central venous access in the United States with a complication rate of 5-19%[17]. The complication rate varies between 3 and 21%[3,4,12,18,19]. In our study, complications developed in 59 patients (7.7%). Mechanical complications including arterial puncture, nerve injury, hematoma, wound dehiscence, hemothorax, and pneumothorax occur during and directly after the placement and are called early complications. Late complications are infection, thrombosis, catheter occlusion, and break-off[20]. Although Fazli et al.[3] reported a high incidence for early complications (53%), late complications predominate with a rate between 54 and 92%[7,12,18]. Similar to the literature, we observed late complications more frequently in our study.

SVPC-related infections include localized (port-site) and systemic (bacteremia) infections. Shim et al.[13] reported the rate of infectious complications as 2.3% in 1747 patients. In general, the rate of infection varies between 1.3% and 9%[3-5,7,10,12]. Port-site infection was the commonest complication in our study (n = 22, 2.9%). None of our patients had systemic infection. Second common complication in our series was wound dehiscence (n = 13, 1.7%). The rate of this complication has been reported between 0.4% and 5%[3-5,12], so our result is consistent with the literature.

The movement of the needle at the wrong angle during the placement of SVPC might cause pneumothorax, which occur in 0.5-2%[3,4,7]. Eight patients (1%) in our study developed pneumothorax. If the physician prefers landmark-guided subclavian vein puncture (without ultrasound), the anatomic landmarks of the midpoint of the clavicle, the junction between the middle and medial border of the clavicle should be used, and the angle between the needle and clavicle must be < 30°. This reduces the rate of pneumothorax.

The catheter was occluded in our 8 patients (1%), which is a complication occurring in 0.1-2% of the patients[3-5,12]. To reduce the occlusion rate, we performed port washings with 1000U of heparin after each chemotherapy session, or every 4 weeks if no chemotherapy was given, or advised to the patient's caretaker to do so, as previously recommended[21]. Subclavian vein thrombosis rate was 0.7% in our study (n = 5). The incidence of venous thrombosis varied from 0.3 to 3.5%[3-5,10,14]. Piran et al.[9] reported a higher rate (8.5%), but their cases included also patients with deep venous thrombosis.

Port fracture and catheter embolization are rare complications resulting when the catheter within the subclavian vein is pinched within the thoracic outlet between the clavicle and the first rib, so-called pinch off syndrome. It occurs in 0.06-0.5% of the patients[3,14,22]. Port should be removed and fragmented part should be extracted by endovascular approach[22]. We observed this complication in 3 patients (0.04%), and the fragmented parts were extracted through Snare technique. To prevent this complication, the catheter entry point to the subclavian vein should not be more medially[22]. We recommend to make a puncture 1 cm laterally and inferiorly to the clavicula arch.

When the management of the complications fails, the port catheter must be removed. The removal rate varies between 1.6% and 17%[4,5,7,12]. SVPC-related infection is the most common complication necessitating the removal[12,19], but it may be required due to catheter occlusion, venous thrombosis, wound dehiscence, and catheter fracture and embolization[3,9,22]. In cases where there was no response to initial treatment, we replaced the PR 3-4 cm laterally instead of removing the port and continued broad spectrum antibiotics. In our study, 5 patients (0.6%) necessitated port removal due to catheter fracture in 3, venous thrombosis in 2. Our rate of port removal is less than the literature.

In our study, the PR was placed either by removing a piece of adipose tissue similar in size of PR and placing it there, or by burying it directly under adipose tissue. We detected significantly higher rates of port-site infection and wound dehiscence in the former technique ($p < 0.05$). The reason may be that patients undergoing chemotherapy are immunosuppressed and are prone to lose weight due to side effects of the treatment. Thus, skin erosion leading to infection is more common in these patients[10].

The usage of polyurethane catheters resulted in significantly higher rates of port-site infection, wound dehiscence, subclavian vein thrombosis, and catheter occlusion in our study ($p < 0.05$). Kartsouni et al.[12] reported that the catheter material did not affect the development of thrombosis or infection. However, Gallieni et al.[23] reported a higher incidence of thrombosis in polyurethane catheters. Polyurethane catheters were more susceptible to catheter-related infections and showed a higher thrombogenicity[11]. More thrombotic catheter occlusions in silicone catheters, and more venous thromboses in polyurethane catheters were reported[24]. Besides, the risk of catheter fracture is higher in silicon catheters[25].

There are controversies about when to administer chemotherapy after SVPC placement. Narducci et al.[19] reported that early use of the port within 7 days increased the risk of complications. However, it was demonstrated that immediate administration of chemotherapy within the 1st or 2nd days after the placement may not increase the complications[5,8]. We did not have comparative results in our study, but as a clinical opinion, we advise to wait 4-5 days to begin chemotherapy.

The main limitation of this study was the lower rate of follow-up. More than half (54%) of the patients in our study had missing follow-up data. The reason of this lower rate can be explained by the fact that our clinic is a part of a tertiary referral center. Most patients admitted from different cities, and returned back after the SVPC placement to continue their treatment. Although we tried to collect follow-up data either by their physicians or by the patients/relatives through e-mail or phone calls, this was not possible all the time. The second limitation was that this study mainly described our clinical experience and results in brief. We did not evaluate in details about the risk factors of each complication in details (such as gender, age, body mass index, tumor type, and previous chemotherapy). We only investigated the effects of two different PR placement procedure and two different types of catheter and obtained statistically significant results.

Conclusion

SVPC placement is a safe choice for long-term intravenous access. The complication rates are acceptable. To prevent or reduce the risk of complications, SVPC placement should be performed by surgeons with adequate experience and in qualified well-equipped centers. Furthermore, the placement of PR under the adipose tissue and the usage of silicone catheters may reduce the complication rates.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The authors have obtained approval from the Ethics Committee for the analysis of routinely obtained and anonymized clinical data, so informed consent was not necessary.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ARTÍCULO ORIGINAL

Características clínicas, imagenológicas e histopatológicas de desórdenes benignos de mama en hombres

Clinical, imaging and histopathological characteristics of benign breast disorders in men

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Resumen

Objetivo: *Evaluar las características clínicas, imagenológicas e histopatológicas en lesiones benignas de mama en hombres en un periodo de 3 años en un centro oncológico de referencia.* **Métodos:** *Estudio descriptivo de cohorte retrospectiva efectuado en pacientes varones atendidos en la Unidad Médica de Alta Especialidad No. 4 Luis Castelazo Ayala entre los años 2021 y 2023, que acudieron por alguna alteración de la glándula mamaria, confirmándose por imagen e histopatología un diagnóstico de benignidad. Se utilizó estadística descriptiva para el análisis de los hallazgos clínicos, imagenológicos e histopatológicos.* **Resultados:** *Acudieron 20 pacientes al servicio de oncología mamaria y se excluyeron cuatro por no cumplir con los criterios de inclusión establecidos, con lo cual se obtuvo una muestra poblacional de 16 pacientes. El principal hallazgo histopatológico benigno fue el miofibroblastoma en el 25% de los pacientes. El principal motivo de consulta fue la presencia de un nódulo palpable.* **Conclusiones:** *La patología mamaria en hombres es poco conocida debido a su baja prevalencia. En la actualidad no existen protocolos establecidos sobre su abordaje diagnóstico y terapéutico. Es importante contemplar los factores de riesgo y los hallazgos obtenidos en la clínica, por imagen y por histopatología, para realizar un adecuado tratamiento.*

Palabras clave: *Mama. Mama masculina. Mastografía. Ecografía. Lesión benigna. Histopatología.*

Abstract

Objective: *To evaluate the clinical, imaging and histopathological characteristics in benign breast lesions in men over a three-year period in a reference oncology center.* **Methods:** *Descriptive retrospective cohort study carried out on male patients treated at the Unidad Médica de Alta Especialidad No. 4 Luis Castelazo Ayala between the years 2021 and 2023, who came for some alteration of the mammary gland, confirming a diagnosis of benignity by imaging and histopathology. Descriptive statistics were used for the analysis of clinical, imaging and histopathological findings.* **Results:** *20 male patients attended the breast oncology service; four patients were excluded for not meeting the established inclusion criteria, to obtain a population sample of 16 patients. The main benign histopathological finding was myofibroblastoma in 25% of patients. The main reason for consultation was the presence of a palpable nodule.* **Conclusions:** *Breast pathology in men is little known due to its low prevalence. Currently, there are no established protocols on its diagnostic and therapeutic approach. It is important to consider the risk factors and the findings obtained in the clinic, by imaging and histopathology, to treat them well.*

Keywords: *Breast. Male breast. Mammography. Echography. Benign lesion. Histopathology.*

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Introducción

La patología mamaria masculina es poco frecuente y por ello poco conocida, aunque hay una gran variedad de afecciones. Ciertamente, el principal temor al momento de consulta es que se trate de un proceso maligno. Sin embargo, la mayoría de las lesiones de la glándula mamaria en los hombres son benignas, siendo su etiología más común la ginecomastia[1].

Ante cualquier lesión mamaria en un hombre hay que ser cauto en la evaluación y no subestimar. Si bien el cáncer de mama en hombres es raro y representa tan solo el 1% de todos los cánceres y el 0.2% de las muertes secundarias a estos, su incidencia ha aumentado un 26% en las últimas décadas, pero se considera un diagnóstico de exclusión[2-4].

Entre los factores de riesgo para el desarrollo de lesiones mamarias en los hombres se encuentran la edad, la criptorquidia, las lesiones testiculares, el síndrome de Klinefelter, la disfunción hepática, la historia familiar de cáncer de mama y los traumatismos torácicos[5].

Es importante comprender morfológicamente su anatomía, ya que la mama, al igual que la femenina, está conformada por tejidos glandulares y adiposos, pero en los hombres las unidades glandulares están compuestas solo por conductos que normalmente están circunscritos debajo del complejo areola-pezón. Por ello, las lesiones de glándula mamaria en los hombres son raras[6].

La presentación clínica más frecuente suele ser un nódulo palpable, con dolor focalizado que puede estar o no acompañado de aumento del volumen mamario. Es imprescindible solicitar estudios de imagen para iniciar el abordaje diagnóstico. La mastografía, el ultrasonido y la resonancia magnética son las principales modalidades de imagen[7]. Cabe señalar que la mastografía no ha demostrado diferencia estadísticamente significativa en su sensibilidad y especificidad sobre el ultrasonido en las lesiones mamarias en los hombres[8]. No obstante, resulta válido iniciar el protocolo diagnóstico solicitando una mastografía en pacientes mayores de 25 años o una ecografía mamaria en los menores de esta edad. No existe un consenso universalmente aceptado sobre el abordaje inicial con herramientas de imagen en los hombres, sino que este ha sido extrapolado de las experiencias en las mujeres[7,9].

En la mastografía existen condiciones, como la ginecomastia, que pueden simular condiciones inflamatorias crónicas. Ciertamente, la ginecomastia y el cáncer mamario no se sobreponen, pero la presencia de un nódulo y la ginecomastia pueden coexistir; sin embargo, no es recomendable realizar biopsia si únicamente este último se encuentra presente. En general, las lesiones no visibles en la glándula mamaria masculina suelen ser benignas, a diferencia de los hallazgos radiotransparentes, que en las mujeres en su mayoría resultan benignos y en los hombres ocurre lo contrario[5].

Ante un examen de imagen sospechoso es necesaria la toma de biopsia, que puede ser por aguja fina, escisional o con aguja de corte, prefiriéndose esta última guiada por ecografía debido al menor volumen de la glándula mamaria masculina y a las dificultades técnicas que puedan resultar[7]. De la misma forma, se debe contemplar no caer en un sobrediagnóstico y tomar en cuenta las repercusiones físicas y psicológicas, así como las posibles complicaciones que pueden aparecer, por lo que es importante optar por herramientas menos invasivas, ya que la mayoría de las lesiones en los hombres resultarán benignas[10,11].

El objetivo del presente estudio es evaluar las características clínicas, imagenológicas e histopatológicas de lesiones benignas de mama en hombres, en un periodo de 3 años, en un centro oncológico de referencia.

Métodos

Estudio descriptivo de cohorte retrospectiva efectuado entre los años 2021 y 2023 en pacientes atendidos en la Unidad Médica de Alta Especialidad No. 4 Luis Castelazo Ayala. Los criterios de inclusión fueron pacientes varones que acudieron al servicio de oncología mamaria por alguno de los siguientes motivos de consulta: nódulo palpable, mastalgia, telorrea o aumento de volumen mamario. A los pacientes se les protocolizó con algún estudio de imagen, como ultrasonido o mastografía, y se les realizaron biopsia y estudio histopatológico en el servicio de patología de la misma institución. Como variables del estudio se incluyeron la edad, los antecedentes familiares, el motivo de consulta, la densidad mamaria, las características de la lesión (lateralidad, tipo, localización, diámetro, densidad o ecogenicidad), la presencia de calcificaciones, la retracción del pezón, el engrosamiento cutáneo, la presencia de adenopatías, la clasificación BI-RADS *(Breast Imaging Reporting and Data System),* el tipo de biopsia y la tratamiento. Los criterios de exclusión

fueron ser mujer, tener reporte histopatológico obtenido de otra institución o tener reporte positivo para malignidad. Los criterios de eliminación fueron pacientes con expediente clínico incompleto, con seguimiento fuera de la unidad de estudio o que no hubieran otorgado su consentimiento informado para la utilización de los datos.

Se estudiaron los siguientes hallazgos: ginecomastia, ectasia ductal, miofibroblastoma, lesión papilar con atipia, fibroadenoma, mastitis, hiperplasia ductal usual y tuberculosis.

Para el análisis estadístico se utilizó el programa JASP versión 0.17, empleando estadística descriptiva. Todas las variables categóricas se expresan en frecuencias y porcentajes.

Resultados

De los 20 pacientes varones que acudieron al servicio de oncología mamaria en el periodo 2021-2023, fueron excluidos uno que contaba con reporte histopatológico de otra institución y no fue posible realizar la revisión de laminillas, otro que recibió tratamiento fuera de la unidad y dos por contar con expediente clínico incompleto.

El promedio de edad de la población de estudio fue de 61.5 años y la mayoría (62.5%) no contaba con antecedentes familiares para cáncer de mama. El principal motivo de consulta en nuestra unidad fue la presencia de un nódulo palpable en el 37.5%. Otros motivos de consulta fueron mastalgia, telorrea, aumento del volumen mamario, nódulo palpable con mastalgia y nódulo con presencia de telorrea. En cuanto a la lateralidad, la glándula mamaria más involucrada fue la derecha, en el 50%, mientras que la izquierda correspondió al 37.5% y la afectación fue bilateral en el 12.5% (Tabla 1).

El índice de masa corporal promedio fue de 28 kg/m^2, estando la mayoría de los pacientes (62.5%) dentro del rango normal. Las enfermedades crónicas reportadas fueron diabetes *mellitus* tipo 2 en el 18.7% e hipertensión arterial sistémica en el 43.8%; el 37.5% de los pacientes no presentaban comorbilidad (Tabla 1).

En cuanto al abordaje por imagen, la mayoría de los pacientes (62.3%) recibieron una categoría BI-RADS > 4, lo que justificó la toma de biopsia para descartar algún proceso maligno. El hallazgo mayormente reportado fue la presencia de algún nódulo (62.5%). Los patrones de densidad mamaria que predominaron fueron los tipo A (25%) y B (56.2%), es decir, aquellos con menor densidad mamaria. La principal localización de las lesiones fue retroareolar, en la mitad de los casos. Otros hallazgos menos reportados fueron calcificaciones, retracción del pezón, engrosamiento cutáneo y adenopatías (Tabla 2).

Tabla 1. Características demográficas de la población de estudio

Variables	Porcentaje (n = 16)
Edad promedio 61.56 años	
Antecedentes familiares	
Sí	37.5
No	62.5
Motivo de consulta	
Nódulo palpable	37.5
Mastalgia	6.2
Telorrea	12.5
Aumento de volumen	6.2
Nódulo y mastalgia	25
Nódulo y telorrea	12.5
Lateralidad	
Derecha	50
Izquierda	37.5
Bilateral	12.5
Índice de masa corporal (promedio 28 kg/m^2)	
Normal	62.5
Sobrepeso	12.5
Obesidad	25
Comorbilidad	
Ninguna	37.5
Diabetes *mellitus* tipo 2	18.7
Hipertensión arterial sistémica	43.8

Discusión

La mayoría de las lesiones de la glándula mamaria masculina son de etiología benigna, siendo la más frecuente la ginecomastia. Los principales motivos de consulta son el incremento del volumen mamario, la presencia de un nódulo palpable y la mastalgia[1]. Una de las mayores preocupaciones en el momento de consultar es que se trate de un proceso maligno; si bien es raro (menos del 1% de todos los cánceres en varones)[12], es pertinente protocolizarlos.

Entre los factores de riesgo más importantes para la presencia de patología mamaria masculina se encuentran la edad, el sobrepeso y la obesidad, la exposición a radiación, el desbalance hormonal y la historia familiar para cáncer de mama, incrementando significativamente el riesgo en quienes presentan mutación en el gen BRCA2[13].

Tabla 2. Características imagenológicas de las lesiones de mama en los hombres

Variable	Porcentaje (n = 16)
Densidad	
A	25
B	56.2
C	18.7
D	0
Localización de la lesión	
Periareolar	18.7
Retroareolar	50
Cuadrante superior externo	25
Cuadrante inferior interno	6.2
Tipo de lesión	
Nódulo	62.5
Asimetría	37.5
Ecogenicidad	
Hipoecoico	62.5
Isoecoico	31.2
Hiperecoico	6.2
Densidad	
Hipodenso	18.7
Isodenso	31.2
Hiperdenso	50
Calcificaciones	
Sí	6.2
No	93.7
Retracción del pezón	
Sí	6.2
No	93.7
Engrosamiento cutáneo	
Sí	12.5
No	87.5
Adenopatías	
Sí	31.2
No	68.7
Diámetro máximo de la lesión 19.2 mm	
Categoría BI-RADS	
2	31.2
3	6.2
4A	31.2
4B	6.2
4C	18.7
5	6.2

Es importante correlacionar las lesiones mamarias en los hombres tanto por estudios de imagen como por la clínica, con el fin de reducir las tasas de biopsias innecesarias. En pacientes varones, la ecografía ha demostrado ser una técnica de imagen útil, que ha permitido reducir la tasa de falsos positivos, aunque cabe mencionar que cuenta con ciertas limitaciones, como en la evaluación de las características acústicas posteriores al tratarse de tejidos más delgados[14].

En un estudio realizado por Yuan et al.[8] en 125 pacientes, se destacan las características ecográficas de lesiones tanto benignas como malignas. Las características más llamativas de las lesiones malignas fueron la excentricidad de la lesión, un componente mixto en su ecotextura (sólido y quístico), la forma irregular de la lesión, los márgenes mal circunscritos y la orientación no paralela[8]. La mayoría de las lesiones en nuestro estudio coinciden con lo descrito por Yuan et al.[8] en las características de benignidad como lesiones periareolares, hipoecoicas y nódulos bien definidos (Tabla 2).

Se debe reservar la toma de biopsia ante un escenario clínico que se corrobora por algún estudio de imagen, así como tomar en cuenta los factores de riesgo intrínsecos del paciente, con el fin de descartar un proceso maligno[14].

En un estudio realizado por Bicchierai et al.[11] se demostró que la biopsia por aguja de corte tiene mayor precisión al caracterizar las lesiones mamarias en hombres en comparación con las biopsias por aguja fina, por lo que se prefiere como primera herramienta diagnóstica. Se ha reportado que hasta el 15.3% de las biopsias por aguja fina resultan inadecuadas en los hombres. En nuestra serie se realizaron siete biopsias por aguja de corte y ocho por aguja fina; en los pacientes que presentaban como único dato clínico un nódulo palpable se prefirió la toma de biopsia por corte (Tabla 3).

La ginecomastia es la alteración más frecuente en la población masculina, cuya etiología es desconocida, aunque se asocia a desórdenes metabólicos, endocrinos, neoplásicos o inducidos por fármacos[15]. Fentiman et al.[16] reportaron que es un hallazgo frecuente, observado en el 40-55% de las autopsias, aunque en el presente trabajo no fue el hallazgo más frecuente. Clínicamente se presenta con hipertrofia mamaria y piel redundante en pacientes que suelen presentar sobrepeso[16] (Fig. 1).

En los exámenes radiológicos se pueden identificar tres patrones: nodular, dendrítico y glandular difuso. Se prefiere la ecografía Doppler como herramienta de imagen para iniciar el abordaje diagnóstico, ya que permite identificar la vasculatura incrementada típica en esta condición inflamatoria, a diferencia de la mastografía, en la que es posible observar una capa adiposa homogénea radiotransparente[17]. Histológicamente se caracteriza por la presencia de proliferaciones benignas de los conductos subareolares y periductales, así como

Tabla 3. Motivo de la primera consulta y tipo de biopsia realizada

Variables	Nódulo palpable (n = 6)	Mastalgia (n = 1)	Telorrea (n = 2)	Aumento de volumen (n = 1)	Nódulo y mastalgia (n = 4)	Nódulo y telorrea (n = 2)
Tipo de biopsia						
Biopsia por aguja de corte	5	1	0	0	1	0
Biopsia por aspiración	1	0	2	1	2	2
Escisional	0	0	0	0	1	0

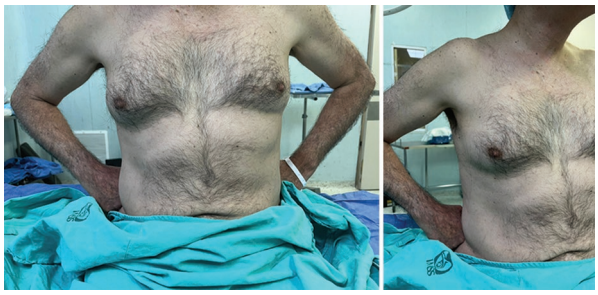

Figura 1. *Varón que clínicamente presenta ginecomastia en la mama derecha.*

por una ramificación de las células lactíferas. Se trata de un tejido hiperplásico e hipercelular[18]. Es una afección que tiene repercusiones psicológicas debido a la apariencia estética que presentan los pacientes, repercutiendo en su autoestima al generar inseguridad sobre su cuerpo[19].

En las últimas décadas se ha incrementado el número de pacientes varones que buscan atención para la corrección de la ginecomastia. Si bien es válido mantener en vigilancia a estos pacientes ante lesiones mamarias, algunos optan por la corrección mamaria, como reportaron Klinger et al.[18] en una serie de 148 pacientes a los que se realizaron desde procedimientos menos invasivos, como la liposucción, hasta mastectomías subcutáneas, la mayoría con fines estéticos. En nuestro estudio, todos los pacientes con esta alteración se mantuvieron en vigilancia (Tabla 4).

Los miofibroblastomas son tumores mesenquimales derivados de fibroblastos estromales que se encuentran comúnmente dentro del parénquima mamario (Fig. 2). Su prevalencia es baja y existen pocos reportes de casos, pero en nuestro estudio fue la lesión más prevalente, presentándose en cuatro pacientes (Tabla 5). Clínicamente se manifiestan como nódulos palpables indoloros. No presentan un patrón imagenológico específico; en la mastografía se aprecian

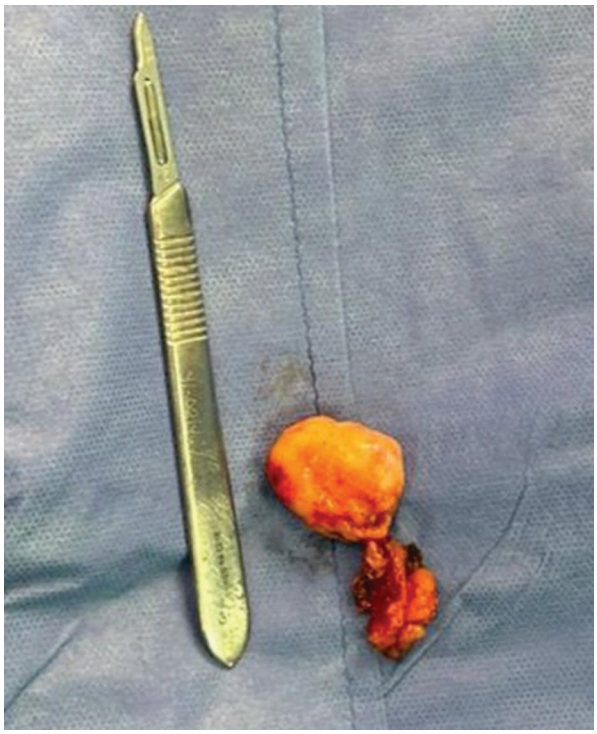

Figura 2. *Lesión benigna de mama de un paciente varón, compatible con un miofibroblastoma.*

como nódulos bien circunscritos, redondos u ovales, densos y no calcificados, y por ecografía Doppler es posible observar una mayor vasculatura periférica[20-22] (Figs. 3 y 4).

Histológicamente, esta lesión se compone de haces de células delgadas, uniformes, dispuestas en grupos, separadas por amplias bandas de colágeno hialinizado (Fig. 5). Es importante que se realice un análisis inmunohistoquímico para diferenciarla de otros tumores de células fusiformes, como los lipomas de células fusiformes y el tumor fibroso solitario, así como de procesos malignos, especialmente el carcinoma de células fusiformes. La mayoría serán inmunorreactivos para CD34, actina, CD10 y desmina[21,23]. El pronóstico de estas lesiones suele ser excelente,

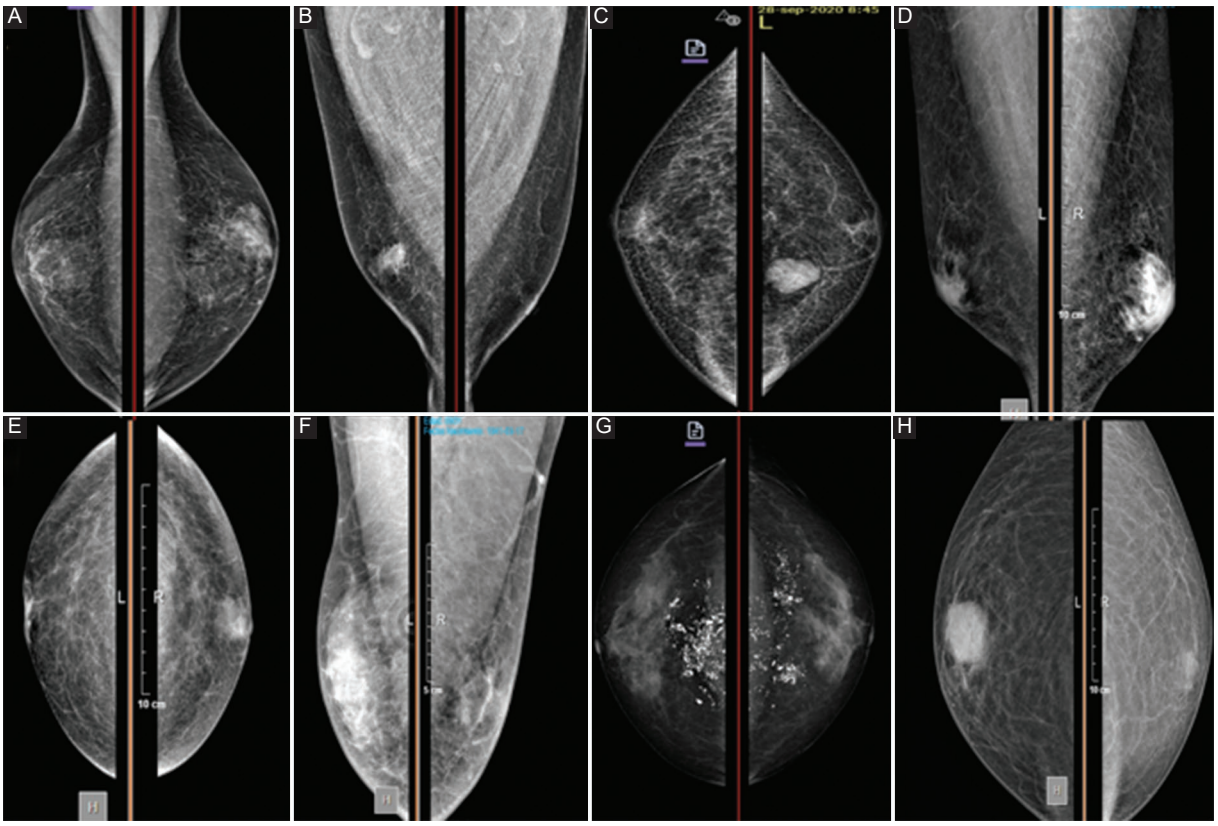

Figura 3. *Características mastográficas de lesiones benignas de mama en hombres.* **A:** *proyección craneocaudal. Se aprecia un aumento de volumen mamario bilateral con distorsión de la arquitectura. Se realizó biopsia, la cual reportó ginecomastia bilateral.* **B:** *proyección craneocaudal. En la mama derecha se observa un nódulo hiperdenso de características irregulares, cuyo reporte histopatológico indicó mastitis.* **C:** *proyección craneocaudal. En la mama izquierda se observa un nódulo hiperdenso, con bordes bien delimitados hacia los cuadrantes internos y el tercio posterior. El reporte histopatológico indicó miofibroblastoma.* **D:** *proyección mediolateral oblicua. En la mama izquierda se observa un nódulo hiperdenso, mal delimitado, retroareolar, que compromete los cuadrantes superiores e inferiores. El reporte histopatológico indicó hiperplasia ductal usual.* **E:** *proyección craneocaudal. En la mama izquierda se aprecia un nódulo mal definido, retroareolar, el cual fue compatible en el reporte histopatológico con fibroadenoma.* **F:** *proyección mediolateral oblicua. En la mama derecha se observa una zona hiperdensa que abarca los cuadrantes superiores e inferiores en el tercio medio, de forma irregular, en la cual se reportó lesión papilar.* **G:** *proyección craneocaudal. En la mama izquierda se observa una zona de sistematización, con distorsión de la arquitectura que abarca los cuadrantes externos e internos. En ambas mamas se aprecian múltiples calcificaciones de aspecto benigno. El reporte histopatológico indicó ectasia ductal.* **H:** *proyección craneocaudal. En la mama derecha se observa un nódulo hiperdenso, con bordes bien definidos, retroareolar, que en la biopsia por aguja fina fue drenado y el estudio histopatológico indicó tuberculosis.*

Tabla 4. Tratamiento empleado según los hallazgos histopatológicos

Tratamiento	Vigilancia (%)	Biopsia amplia (%)	Cirugía de Addair (%)	Mastectomía total (%)	Antibiótico (%)
Hallazgos					
Miofibroblastoma	-	75	-	25	-
Ginecomastia	100	-	-	-	-
Fibroadenoma	50	50	-	-	-
Lesión papilar	50	-	50	-	-
Ectasia ductal	50	50	-	-	-
Mastitis aguda	100	-	-	-	-
Hiperplasia ductal tipo usual	-	100	-	-	-
Tuberculosis	-	-	-	-	100

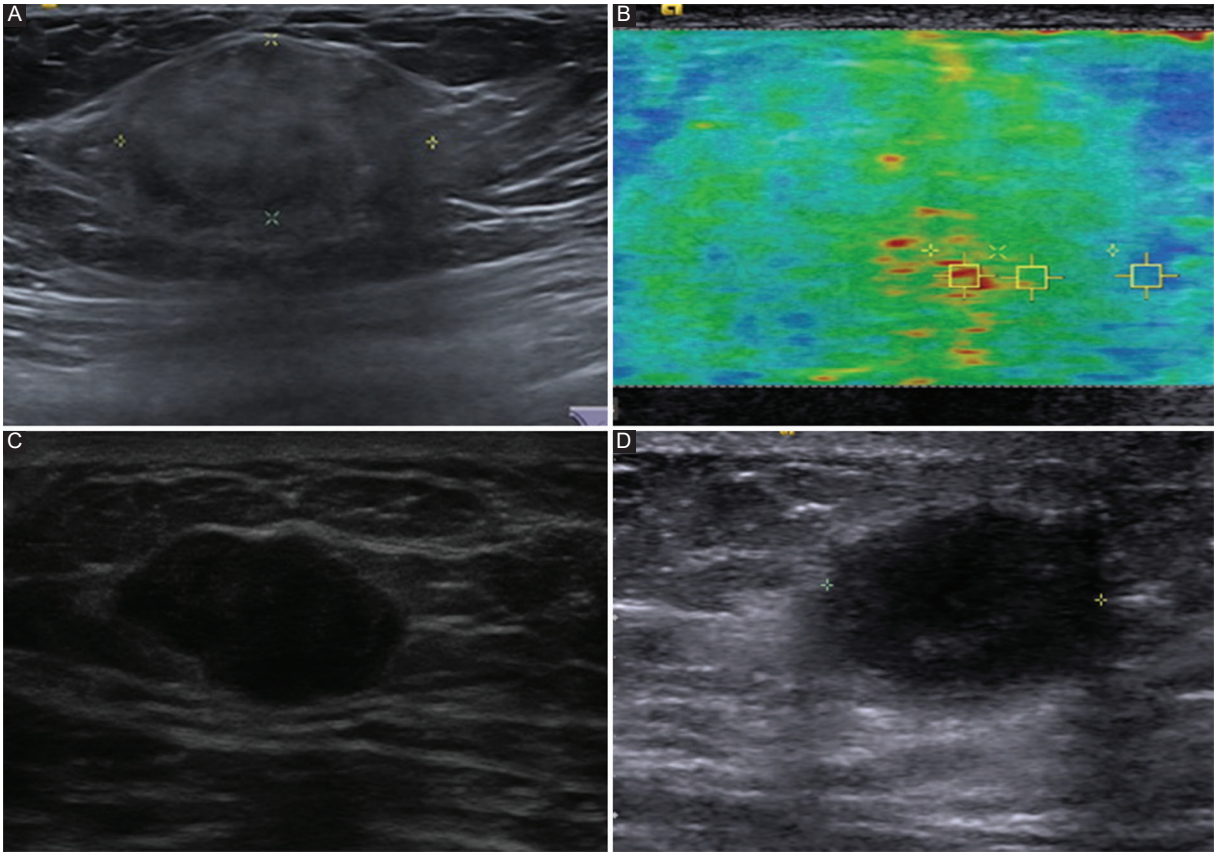

Figura 4. *Características ultrasonográficas y por elastografía de lesiones benignas de mama en los hombres.* **A:** *ultrasonido que muestra una lesión con características de nódulo isoecoico con bordes bien delimitados. El reporte histopatológico fue compatible con un miofibroblastoma.* **B:** *elastografía que muestra la solidez del tejido, el cual fue auxiliar para integrar el diagnóstico junto al reporte histopatológico final que indicó un miofibroblastoma.* **C:** *ultrasonido que muestra una lesión en la mama con características de nódulo hipoecoico con bordes irregulares, más ancho que alto, el cual resultó ser un fibroadenoma.* **D:** *ultrasonido que muestra una lesión en la mama, con presencia de un nódulo hipoecoico de bordes bien delimitados, cuyo estudio histopatológico reportó como fibroadenoma.*

Tabla 5. Frecuencia de casos por cada diagnóstico histopatológico

Hallazgos	Casos (n)	Porcentaje
Miofibroblastoma	4	25
Ginecomastia	3	18.7
Fibroadenoma	2	12.5
Lesión papilar	2	12.5
Ectasia ductal	2	12.5
Mastitis aguda	1	6.2
Hiperplasia ductal tipo usual	1	6.2
Tuberculosis	1	6.2

por lo que el tratamiento de primera línea es la resección quirúrgica completa y no es necesario complementarlo con otras medidas como radioterapia u hormoterapia[24].

Los fibroadenomas, a pesar de ser las lesiones benignas más frecuentes en las mujeres, no son tan habituales en los hombres[25]. La ecografía es la modalidad por imagen de preferencia, en la que se pueden apreciar como nódulos hipoecoicos, con bordes bien delimitados, sin mostrar vasculatura en el Doppler color (Fig. 3). Las recomendaciones para su resección son el rápido crecimiento del tumor, la persistencia sin regresión espontánea, la sintomatología como mastalgia y los antecedentes familiares para cáncer de mama[26]. En el presente estudio se decidió dejar a un paciente en vigilancia y a otro se le realizó resección de la lesión por petición propia (Tabla 4).

Las lesiones papilares en los hombres no son frecuentes, pero la importancia de su protocolización radica en que el segundo subtipo de cáncer más común en los hombres es el papilar, con un 4%, después del carcinoma ductal. La presentación clínica más habitual es un nódulo palpable acompañado de

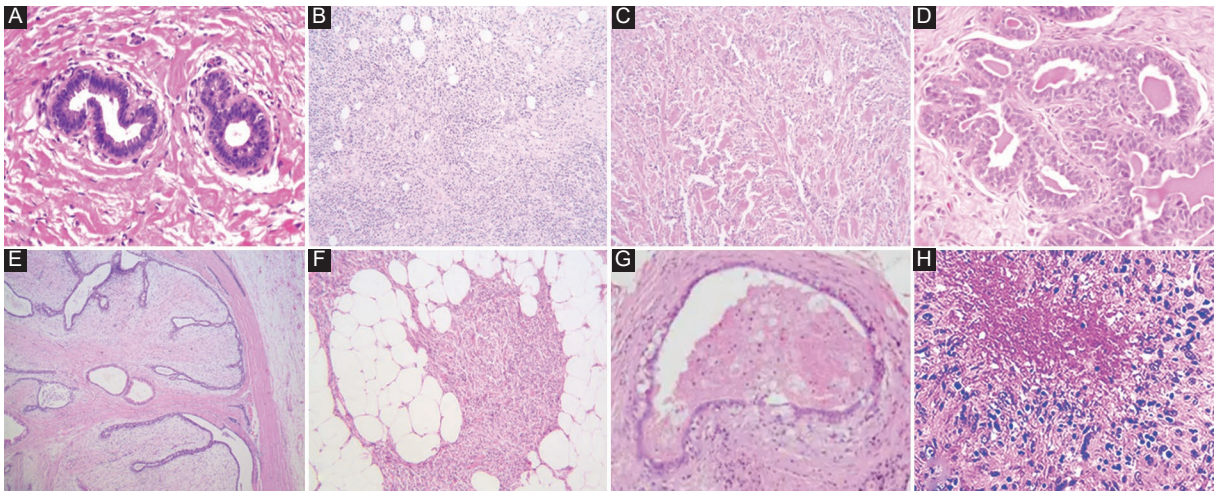

Figura 5. *Características histológicas de las lesiones benignas de la glándula mamaria masculina.* **A:** *en los conductos se aprecia cierto grado de hiperplasia epitelial, rodeada de estroma mixomatoso hipocelular. Estroma edematoso que contiene grandes cantidades de mucopolisacáridos. Se reportó como ginecomastia.* **B:** *se puede observar una respuesta inflamatoria, con linfocitos y células plasmáticas. Se reportó como mastitis.* **C:** *haces de células mesenquimales uniformes, delgadas y en forma de huso, dispuestas en grupos separados por bandas de colágeno hialinizado. No se observa atipia citológica y actividad mitótica. Se reportó como miofibroblastoma.* **D:** *células con núcleo normocromático, ovales, sin actividad mitótica. Se concluye hiperplasia ductal usual.* **E:** *neoplasia bifásica con componente epitelial y fusocelular benigno bien delimitado. Se reportó como fibroadenoma.* **F:** *lesión papilar sin atipia que presenta metaplasia apocrina.* **G:** *infiltrado inflamatorio alrededor del conducto, con macrófagos y células espongiformes. Se reportó como ectasia ductal.* **H:** *en la tuberculosis mamaria se pueden observar granulomas con células gigantes de Langhans acompañadas de necrosis caseosa.*

telorrea hasta en un tercio de los casos. Zhong et al.[27] reportan, en su experiencia con la cohorte más grande de lesiones papilares en hombres, que es recomendable la toma de biopsia por aguja fina como procedimiento diagnóstico inicial. En su cohorte, todos los pacientes con lesiones papilares benignas recibieron tratamiento escisional. En nuestra serie, a los dos pacientes que presentaron un nódulo mamario con telorrea se les realizó biopsia por aguja fina. Sin embargo, uno recibió tratamiento escisional y el otro se decidió mantener en vigilancia al no aceptar el tratamiento quirúrgico. El pronóstico de estas lesiones suele ser favorable en la mayoría de los casos (Tabla 3).

La tuberculosis mamaria ha sido denominada «la gran simuladora» debido a sus multifacéticas presentaciones. Farrokh et al.[28] describieron 32 casos de tuberculosis mamaria, en los que solo hubo un hombre, lo que refleja la rareza de esta enfermedad en los varones. En las mujeres se ha descrito principalmente en aquellas que se encuentran lactando, por lo que existe una predisposición a esta infección debido a la hipervascularidad y la dilatación ductal existente[28].

Clínicamente suele presentarse como un nódulo palpable solitario que puede acompañarse de cambios cutáneos, como retracción del pezón o piel de naranja, por lo que es imperativo descartar un carcinoma. En la mastografía se puede apreciar como un nódulo bien circunscrito, que en ocasiones simula un fibroadenoma[29]. Sin embargo, la herramienta de imagen más valiosa es la ecografía, debido a que permite no solo evaluar correctamente la lesión, sino también la toma de biopsia dirigida, prefiriéndose por aguja fina ya que a su vez es posible el drenaje percutáneo[30]. En el presente trabajo se encontró un caso de tuberculosis mamaria, en el cual se realizó biopsia por aguja fina dirigida por ecografía, se drenó y se instauró manejo antibiótico, que es el tratamiento más empleado en los casos reportados en la literatura internacional[28,29] (Tabla 4).

La hiperplasia ductal se caracteriza por una proliferación de células epiteliales intraductales que pueden presentar o no atipia. En los hombres suele aparecer en aquellos que clínicamente presentan ginecomastia. En la mastografía, los hallazgos suelen ser inespecíficos y puede expresarse como un nódulo o como microcalcificaciones amorfas (Fig. 3). En nuestro estudio solo hubo un caso, lo cual se puede deber a la falta de un patrón específico que oriente a su diagnóstico[7] (Tabla 5).

Por otra parte, las mastitis son procesos secundarios a infecciones del tejido mamario, que en ocasiones pueden llegar a formar un absceso. En la

mastografía se puede apreciar como un aumento unilateral en el volumen mamario, con engrosamientos trabeculares y cutáneos. Es importante correlacionarlo con la clínica para establecer el diagnóstico certero. Las ectasias ductales afectan a los conductos retroareolares y en ocasiones se acompañan de descargas a través del pezón, como ocurrió en nuestros pacientes. En la mastografía se pueden apreciar estructuras densas que convergen con el complejo areola-pezón y puede haber calcificaciones asociadas. En la ecografía pueden observarse residuos celulares, centrales o periféricos[4].

Conclusiones

La patología mamaria en los hombres sigue siendo un terreno desconocido debido a su baja prevalencia. La mayoría de las lesiones están relacionados con procesos benignos, pero es importante interrogar sobre los antecedentes del paciente e identificar los factores de riesgo para malignidad, además de realizar una buena anamnesis de la sintomatología y una adecuada exploración física.

Los estudios de imagen son herramientas auxiliares en el diagnóstico de la patología mamaria, pero a diferencia de lo que ocurre en las mujeres, la información obtenida es más limitada debido a las características anatómicas de la glándula mamaria masculina. Hasta el día de hoy no existe un consenso sobre las indicaciones para realizar un estudio de imagen en los hombres, pero ante la sospecha de una lesión son de gran ayuda para la toma de biopsias dirigidas por imagen.

Ciertamente, el cáncer de mama en los hombres representa menos del 1% de todos los casos; sin embargo, se debe protocolizar y ante la sospecha imagenológica, y tomando en cuenta los factores de riesgo, es pertinente la toma de biopsia para integrar un diagnóstico histopatológico, y a su vez un adecuado triple test (clínica, imagen e histopatología), del cual dependerá el manejo, evitando sobretratar las lesiones benignas, que serán las más prevalentes.

Financiamiento

Los autores declaran no haber recibido financiamiento para este estudio.

Conflicto de intereses

Los autores declaran no tener conflicto de intereses.

Consideraciones éticas

Protección de personas y animales. Los autores declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

Confidencialidad, consentimiento informado y aprobación ética. Los autores han seguido los protocolos de confidencialidad de su institución, han obtenido el consentimiento informado de los pacientes, y cuentan con la aprobación del Comité de Ética. Se han seguido las recomendaciones de las guías SAGER, según la naturaleza del estudio.

Declaración sobre el uso de inteligencia artificial. Los autores declaran que no utilizaron ningún tipo de inteligencia artificial generativa para la redacción de este manuscrito.

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ORIGINAL ARTICLE

Injectable vaginal bio-adhesive gel activated by hard nano ceramic particles: *in vitro* and *in vivo* properties

Gel bioadhesivo vaginal inyectable activado por partículas nanocerámicas duras: propiedades in vitro e in vivo

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Abstract

Objective: *This study aimed to synthesize, characterize, and evaluate the histopathological and biochemical efficacy of vaginal gels (VGs) in healing lacerations resulting from vaginal trauma. The bioadhesive gel containing nanoparticles (n-HAp) represents a novel application in this field.* **Methods:** *VGs were synthesized using n-HAp and characterized by field emission scanning electron microscopy (FE-SEM) and X-ray diffraction (XRD). High-resolution FE-SEM images confirmed the presence of n-HAp, while XRD verified its structural properties. Four experimental groups were established: Control, Sham, VG, and VG/n-HAp. Tissue and blood samples were collected on days 7, 14, and 21.* **Results:** *Biochemical parameters such as tumor necrosis factor-alpha (TNF-), interleukin-1 (IL-1), and vascular endothelial growth factor (VEGF) were evaluated in serum samples. Histological evaluations included inflammation, granulation tissue formation, collagen and reticular fiber density, re-epithelialization, and neovascularization. The VG/n-HAp group exhibited significantly faster healing and more pronounced inflammatory responses over time compared to the other groups.* **Conclusion:** *Overall, the addition of n-HAp to VGs accelerated both biochemical and histopathological recovery in a rat vaginal wound healing model.*

Keywords: *Bioadhesive gel. Vaginal wound model. Tissue repair. Nanomaterial.*

Resumen

Objetivo: *Este estudio tuvo como objetivo sintetizar, caracterizar y evaluar la eficacia histopatológica y bioquímica de los geles vaginales (GV) en la cicatrización de laceraciones causadas por traumatismos vaginales. El gel bioadhesivo que contiene nanopartículas (n-HAp) representa una aplicación novedosa en este campo.* **Métodos:** *Los GV fueron sintetizados a partir de n-HAp y caracterizados mediante microscopía electrónica de barrido de emisión de campo (FE-SEM) y difracción de rayos X (XRD). Las imágenes de alta resolución obtenidas por FE-SEM confirmaron la presencia de n-HAp, y las pruebas de XRD validaron sus propiedades estructurales. Se establecieron cuatro grupos experimentales: Control, Sham, GV y GV/n-HAp. Se recolectaron muestras de tejido y sangre en los días 7, 14 y 21.* **Resultados:** *Se evaluaron parámetros bioquímicos como el factor de necrosis tumoral alfa (TNF-), la interleucina-1 (IL-1) y el factor de crecimiento endotelial vascular (VEGF) en muestras séricas. Las evaluaciones histológicas incluyeron inflamación, formación de tejido de granulación, densidad de fibras colágenas y reticulares, reepitelización y neovascularización. El grupo GV/n-HAp mostró una cicatrización significativamente más rápida y procesos inflamatorios más marcados a lo largo del tiempo en comparación con los demás grupos.*

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Conclusiones: *En general, la adición de n-HAp a los GV aceleró la recuperación tanto bioquímica como histopatológica en un modelo de cicatrización de heridas vaginales en ratas.*

Palabras clave: *Gel bioadhesivo. Modelo de herida vaginal. Reparación tisular. Nano material.*

Introduction

Vaginal and perineal trauma, particularly during vaginal childbirth, represents a significant clinical challenge affecting approximately 75% of women. These injuries result in both short-term and long-term morbidity[1]. The impact of such trauma on women's quality of life can be substantial, potentially disrupting newborn care and breastfeeding. In addition, postpartum perineal pain, dyspareunia, and esthetic concerns may lead women to avoid normal vaginal delivery and opt for elective cesarean sections. The increasing rates of cesarean deliveries raise concerns in obstetric care, as high cesarean rates are considered indicators of poor quality. Health-care providers should strive to minimize the potential short- and long-term effects of perineal trauma and pain through evidence-based interventions[2].

Perineal lacerations can lead to pelvic pain, decreased sexual quality of life, and psychological trauma. Approximately 30% of women experience discomfort within the first 2 weeks after childbirth, and maternal pain and discomfort are common in first- and second-degree perineal tears[3].

Novel therapeutic approaches are needed to contribute to perineal wound healing, enhance tissue strength, and facilitate mothers' quicker return to normal activities.

Bioadhesive gels, with their ability to adhere to tissue surfaces, provide sustained drug release and support tissue regeneration, and have gained attention as potential therapeutic agents for wound healing. Nano-hydroxyapatite (n-HAp)-based bioadhesive gels show promise in various medical applications, including bone repair and tissue engineering. However, their efficacy in vaginal wound healing remains relatively unexplored[4-7].

In this study, we investigate the histopathological and biochemical effects of a novel bioadhesive gel prepared with nano hydroxyapatite particles in a rat model of vaginal lacerations. Our goal is to assess its potential as an adjunctive therapy for enhancing wound healing in vaginal trauma.

Furthermore, the usability of nano ceramic particles, which are frequently used in hard tissue applications, in soft and delicate tissue applications has been investigated in detail for the 1st time in this study.

We hypothesize that the incorporation of n-HAp into the gel formulation will accelerate the healing process, reduce inflammation, and improve tissue repair. Understanding the mechanisms underlying the effects of n-HAp-based bioadhesive gels on vaginal wound healing may provide valuable insights for clinical applications. By elucidating the cellular and molecular processes involved, we aim to contribute to the development of novel therapeutic strategies for managing vaginal trauma and improving patient outcomes.

Methods

Chemicals and reagents

Nanoparticles and vaginal gels (VGs) synthesis were performed with analytical grade, sodium hypochlorite (NaClO), EtOH, (CH_3CH_2OH, 190 proof, ACS spectrophotometric grade, 95.0% non-denatured ethanol), *isopropanol* (propan-2-ol, 70% in H_2O, $CH_3CH(OH)CH_3$), carboxymethyl cellulose sodium (CM-cellulose; CMC-Na, Viscosity: 1000-1400 mpa.s, standard deviation [SD] = 1.2), glycerol (Pharmaceutical-grade), acetic acid, (CH_2COOH, glacial, ReagentPlus®, ≥ 99%) and ammonium polyacrylate dispersing agent (DARVAN 821-A) were purchased from Sigma-Aldrich (St. Louis, USA). The Milli-Q50 SP Reagent Water System (Millipore Corporation, MA, USA) was employed to produce ultrapure water, which was subsequently used in the sample preparation process. All other *in vitro* and *in vivo* experimental supplies and reagents were of analytical grade and were purchased from Merck KGaA (Darmstadt, Germany), Thermo Fisher Scientific (Massachusetts, USA), and Bayer AG (Leverkusen, Germany).

Production of hydroxyapatite nanoparticles (n-HAp)

As per our previous research[8,9]. n-HAp was synthesized from natural bovine bones (femur) through a detailed method outlined below. Initially, freshly

collected bone segments were thoroughly cleansed and subjected to six cycles of boiling in dH_2O. To remove any residual fats, they were then degreased using 70% ethanol (EtOH). Subsequently, the bone pieces were immersed in a solution of NaClO (30% v/v) and left to dry for a minimum of 48 h before further processing. The dried bone segments underwent a calcination process at a temperature of 850°C, with a gradual heating rate of 5°C/min, and were maintained at this temperature for 4 h in the presence of air to ensure the elimination of any prions, such as Creutzfeldt-Jakob disease or Bovine Spongiform Encephalopathy. Following calcination, the bovine femoral bone parts were finely crushed into small pieces and then subjected to planetary ball milling using the Fritsch Planetary Micro Mill Pulverisette 7 premium line from Germany. This milling process was carried out in isopropyl alcohol using ZrO_2 balls at a speed of 300 cycles/min for a duration of 144 h, maintaining a ball-to-powder ratio of 10:1. Subsequently, the milled powder samples were dried in a pyrex pan. Ultimately, the resultant natural hydroxyapatite nanoparticles (n-HAp) were stored at RT in a desiccator for further use[10,11]. Figure 1 shows the experimental steps followed in obtaining n-HAp. This rigorous method ensures the production of high-quality n-HAp from bovine bones, providing valuable biomaterials for various biomedical applications.

Pharmaceutical compositions of n-HAp loaded bioadhesive VGs

Hydrogels, which are composed of gel formers, have been the subject of previous investigations and applications for drug delivery or administering other injectable substances. While certain hydrogels have demonstrated promising characteristics, such as degrading and dissipating from the injection site within a few days, there remain challenges to address. Some existing hydrogels lack the desired cohesiveness, resulting in dispersion after injection. In addition, certain hydrogels tend to degrade relatively rapidly when introduced into living organisms. In cases where these hydrogels incorporate hard ceramic particles to facilitate soft and/or hard tissue augmentation, the presence of bioceramic particles in the hydrogel can lead to ion release and potential contamination[12,13]. To achieve optimal outcomes, it becomes crucial to mitigate these concerns and ensure the bioceramic particles' influence on the hydrogel remains adequately controlled. Consequently, further research and development are needed to improve the performance and biocompatibility of hydrogels or biomaterials when combined with hard ceramic particles for tissue augmentation purposes[14,15]. By addressing these challenges, we can advance the potential of such hydrogel-based systems for various medical applications. A homogeneous aqueous solution was prepared using distilled water (dH_2O) by adding 0.5% v/v of acetic acid (CH_2COOH) to 100 mL at RT with vigorous stirring (pH ~4.8-5.1). Subsequently, 1.5% w/v of CMC-Na and 0.75% w/v of SA powder were carefully introduced into the solution and stirred at 37°C until complete solubilization was achieved. To facilitate the carboxymethylation reaction, the reagents were combined, and 6.5% v/v of glycerol was slowly added to the mixture, resulting in the formation of a uniform sticky solution. The reaction was allowed to proceed at 37°C for 6 h in a water bath to ensure complete carboxymethylation[16].

To prepare the n-HAp-loaded bioadhesive VG, the prepared n-HAp was first dispersed in 1 mL of dH_2O using an optimized quantity of ammonium polyacrylate solution (0.5% v/v), which served as a dispersing agent (DARVAN821-A). This dispersion was achieved through 15 min of sonication using an ultrasonic homogenizer (SONICS, VCX/750, Ultrasonic processors, Newtown, U.S.A.) at a concentration of 0.75% (w/v). The choice of 0.75 wt.% concentration was primarily to prevent clustering or agglomeration of nanoparticles, as observed at higher concentrations. After surface modification of the n-HAp with ammonium polyacrylate, the n-HAp solution was blended with the gel solution using low-shear mixing for 1 h to create the n-Hap-loaded bioadhesive VG/nHAp polymer solutions, maintaining a pH of approximately 4.5[17]. Subsequently, the prepared solutions were sealed and kept at RT for 24 h to allow the hydrolysis and polycondensation reactions to take place, leading to the completion of the gelation process. The same experimental procedure was applied to prepare the unloaded n-HAp gel (VG). Both resulting bioadhesive VGs were subjected to gamma irradiation in 10 cc syringes to achieve both sterilization and cross-linking, ensuring their sterility and stability[18]. The prepared calcium phosphate salts have been adjusted to a pH of 4.5 to ensure that the healthy vaginal environment falls within the pH range of 3.5-4.5[17].

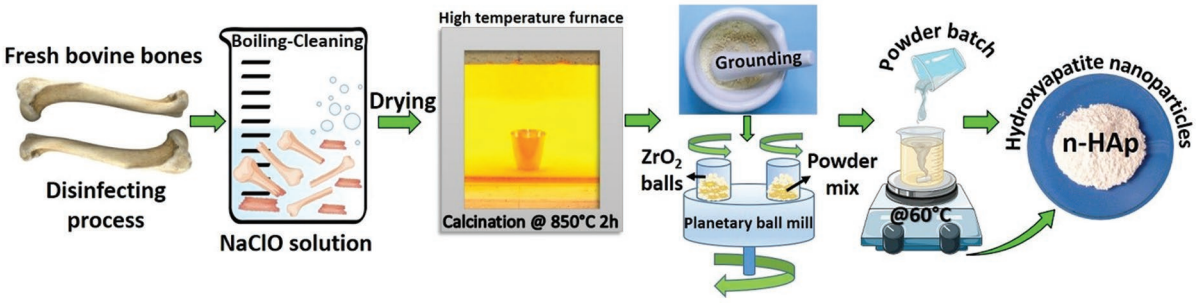

Figure 1. *The experimental steps for preparing n-HAp.*

Characterization of n-HAp by field emission scanning electron microscopy (FE-SEM), scanning transmission electron microscopy (S-TEM), and X-ray diffraction (XRD)

The microstructural analysis of n-HAp was conducted using field emission scanning electron microscopy (FE-SEM, Tescan Mira3 XMU, Brno, Czechia) to investigate particle morphology, shape, size, and phase formation. Before observation, the n-HAp samples were sputter-coated with gold for 2 min at 20 mA and then examined at an accelerating voltage of 10-20 kV. To further examine the detailed morphology of the resulting particle, S-TEM was employed. For S-TEM imaging on the FE-SEM, a transmission electron microscope (TEM) module was integrated onto the FE-SEM stage. The operating voltage utilized ranged from 15 to 30 kV, while the working distance was set to 3 mm to ensure a more precise evaluation of the sample on the Cu grid. The crystal structure analysis of the produced n-HAp was performed using an X-ray Diffractometer (Rigaku D/MAX/2200/PC) equipped with monochromatic Cu-K_α radiation (λ = 1.5408 Å). The instrument operated at an accelerating voltage of 40 kV and a current of 40 mA, and data were collected within a 2θ range spanning from 10° to 90°. To interpret the obtained data and characterize the crystal structure of the calcined and milled n-HAp, computer software (Jade; Materials Data Inc., Livermore, CA) was employed. By comparing the diffractograms with the standards provided by the joint committee of phase diffraction standards (JCPDS), a thorough analysis of the n-HAp crystal structure was achieved.

Cell culture analysis

In our cell culture experiments, we used L929 ATCC (NCTC clone 929@CCL-1) cells. These cells were grown in DMEM medium containing 10% FBS and 1% penicillin-streptomycin at 37°C with 95% humidity and 5% CO_2 in an incubator. The hydrogels were dissolved in 1 mL DMEM by stirring for 24 h at room temperature (150 rpm). We applied VG/nHAp gel to the cells at different concentrations (1%, 2.5%, 5%, and 10%). Cell viability (%) was calculated and plotted.[19].

Experimental animal analysis

For this research project, we used female Wistar albino rats (n = 60) that were newly adult, 16 weeks old, and not bred. The animals were housed under controlled laboratory conditions, including temperature, humidity, and a light–dark cycle. All experimental procedures conducted in this study were reviewed and approved by our university's Animal Research Ethics Committee under permit number 65202830-050.04.04-479. The care and handling of animals adhered to the European Communities Council Directive (86/609/EEC) for the protection of animals used for experimental purposes.

Vaginal injury model

A total of 60 rats were assigned randomly to four independent groups: Control Group (Control): No intervention. Laceration Group (SAM): Vaginal lacerations were induced. VG Group: VG was applied to the wound area. VG/nHAp Group: VG containing hydroxyapatite (n-HAp) was applied to the wound area. Each group was divided into 7th, 14th, and 21st day groups among themselves. There were 15 rats in the subgroups. All rats were fasted for 12 h before surgery. Animals were anesthetized with 2.5% isoflurane and placed on a heated table to maintain a body temperature of 37°C. After anesthesia was established, an 8-mm midline incision was performed in the posterior

vaginal wall, included the epitelial and posterior vaginal fibromuscular layers, sparing the anal sphincter and rectum.[20,21]. Postoperatively, the rats were monitored daily for signs of infection. Wounds were left to heal spontaneously in the sham group. In VG and VG/nHAp groups, bioadhesive gels were applied daily for 7, 14, and 21 days. The rats were housed in ventilated rooms and allowed to eat and drink ad libitum after surgery. The animals were sacrificed 7, 14, and 21 days after implantation samples were collected for biochemical and histological examinations. No antibiotic prophylaxes were administered, and no mortality occurred during the experiment. All surgical procedures were performed by the same surgeon under the same experimental conditions.

Biochemical analyses

In this study, on the 7th, 14th, and 21st days, blood samples were collected from all animals through intracardiac puncture into EDTA tubes. After centrifugation at 3000 rpm for 15 min, the serum was separated. Biochemical tests, including tumor necrosis factor alpha (TNF-α) (Cat No: E-EL-R2856, Elabscience), interleukin (IL)-1 (Cat No: E-EL-R0012, Elabscience), and vascular endothelial growth factor (VEGF) (Cat No: E-EL-R2603, Elabscience), were performed on the collected serum samples using the enzyme-linked immunosorbent assay (ELISA) method following the manufacturer's guidelines.

Histochemical analysis

The vaginal tissues obtained were fixed with 10% neutral buffered formalin. After fixation, the tissues were processed for tissue tracking and then embedded in paraffin. 4-5 μm-thick sections were obtained from the paraffin blocks using a Leica microtome[21]. The sections were stained with Hematoxylin and Eosin to assess epithelialization in the vaginal wound, inflammation in the lamina propria, and the amount of granulation tissue. In addition, Masson's trichrome staining (Masson's trichrome with aniline blue) was used to determine collagen fiber content, and the Silver impregnation technique was employed to assess reticular fiber content. The stained preparations were examined and photographed under a microscope (Olympus BX51, Japan). All histological examinations were conducted at the Department of Histology and Embryology, Faculty of Medicine, Sivas Cumhuriyet University. A blinded histologist evaluated the differences between groups based on tissue type and duration of injury. The main histological outcomes included the amount of inflammatory infiltration, granulation tissue, accumulation of collagen and reticular fibers, re-epithelialization, and neovascularization. These parameters represent the average values of five different histological images taken from vaginal tissue samples in each group. In this study, we used the histological scoring system proposed by Greenhalgh et al.[22] and Abramov et al.[2], as shown in table 1, with each parameter scored on a scale of 0-3.

Table 1. Wound-healing histologic scoring system (22, 2)

Variable	Score			
	0	1	2	3
Inflammation	None	Scant	Moderate	Abundant
Granulation tissue amount	None	Scant	Moderate	Abundant
Collagen deposition	None	Scant	Moderate	Abundant
Reticular fiber deposition	None	Scant	Moderate	Abundant
Re-epitelization	None	Partial	Complete but immature or thin	Complete and mature
Neovascularization	None	Up to five vessels per HPF	6-0 vessels/ HPF	More than 10 vessels per HPF

HPF: high-power field.

Statistical analysis

The mean values of biochemical data were calculated and plotted with ± SD using SigmaPlot 15.0. The two-tailed Friedman test was used for repeated measurements, and the Kruskal-Wallis test was used for comparison of independent groups. $p < 0.05$ was considered statistically significant. The Friedman test is a non-parametric alternative to one-way analysis of variance with repeated measures. It is used to test differences between groups when the dependent variable being measured is ordinal.

In the experimental vaginal wound model, vaginal healing criteria were scored according to table 1 and Friedman test was used to test whether there was a statistical difference between the scores obtained on the 7th, 14th, and 21st days for all variables in each

group and the significance level was set as α = 0.05. Another hypothesis of the study was whether there was a significant difference between the semi-quantitative score values on the 7th, 14th, and 21st day between the groups for each vaginal wound healing parameter. In order to test this hypothesis, the Kruskal-Wallis test, a non-parametric test, was applied at α = 0.05 significance level, and the results were obtained using the Statistical Package for the Social Sciences 25 statistical package program.

Results

Microstructural evolution of bone fragments and n-HAp

High-resolution FE-SEM photographs of bone fragments subjected to calcination, FE-SEM photographs of n-HAp obtained after calcination and ball milling of bone fragments, and S-TEM photographs taken for a more detailed examination of n-HAp are provided in figure 2.

When examining the FE-SEM photograph is shown in figure 2A, the morphology of the calcined bone pieces, which become quite fragile and brittle after the calcination process, is evident. In figure 2A, it was determined that after the applied calcination process, organic substances were effectively eliminated from the bone structure, and a distinct pore structure was formed with an average size of approximately 316 ± 130.25 μm.

From the microstructure photograph of the prepared n-HAp in figure 2B, it was observed that the structure, distributed in a uniform and homogeneous manner, consisted of a single phase. This homogeneity in morphology was noted as a positive physical feature in terms of high density and high performance. From the FE-SEM photographs, by counting at least 50 particles with distinct grain boundaries using the Image J analysis software program[23], the particle size of n-HAp was calculated to be approximately 460 ± 156 nm. In general, it was determined that the obtained n-HAps were irregularly shaped and partially in hemispherical form.

In the S-TEM analyses performed to visualize the n-HA crystals in more detail, the prepared powders were dispersed in isopropyl alcohol and passed through 200 nm filters[24]. After this process, large particles in the structure were removed from the environment, and S-TEM analyses were conducted. In the detailed photograph is shown in figure 2C, it was observed that the particles were below 500 nm in size and tended to aggregate. It was determined from the analysis that long-term grinding of n-HAp increased this phenomenon and resulted in particles with a high surface area.

Phase evolution of n-HAp by XRD analyses

Figure 2D displays the XRD patterns of n-HAp. The dominant crystal phase identified in the XRD patterns is hydroxyapatite (HAp) – [$Ca_5(PO_4)_3(OH)$] with varying peak intensities. In all analyses, the diffractograms confirm the presence of [$Ca_5(PO_4)_3(OH)$], which predominantly matches n-HAp (JCPDS card number 84-1998).

However, in the XRD results, in addition to the main phase, a small amount of minor β-tricalciumphosphate (TCP) (JCPDS card number 09-0169) peaks were detected. These small TCP peaks indicate the presence of β-TCP as a secondary phase next to the main HA phase in n-HAp. The β-TCP phase is a result of the decomposition of the HA mineral and represents stable polymorphs of the TCP mineral at various temperatures[25]. In the context of tissue engineering applications, when used/applied as a scaffold or filler, β-TCP is known to have significant potential in facilitating tissue regeneration, promoting angiogenesis (formation of new blood vessels), and increasing cell proliferation in both soft and hard tissues[26]. β-TCP is likely to play an increasingly important role in the development of medical therapies and treatments to improve patient outcomes and quality of life. X-ray analyses confirm the successful production of n-HAp as a filler nanoparticle for bioadhesive VGs (Fig. 2).

Cytotoxicity analysis

We applied VG/nHAp gel to the cells at different concentrations (1%, 2.5%, 5%, and 10%). Cell viability was calculated and plotted in figure 2. VG/nHAp applied on healthy L929 fibroblast cells did not result in significant differences in cell viability at different concentrations. VG/nHAp applied on healthy L929 fibroblast cells did not alter the nuclear morphology of cells at different concentrations (Fig. 3).

Biochemical analysis

In our experimental study, IL-1β, TNF-α, and VEGF levels in blood samples taken from all groups on days

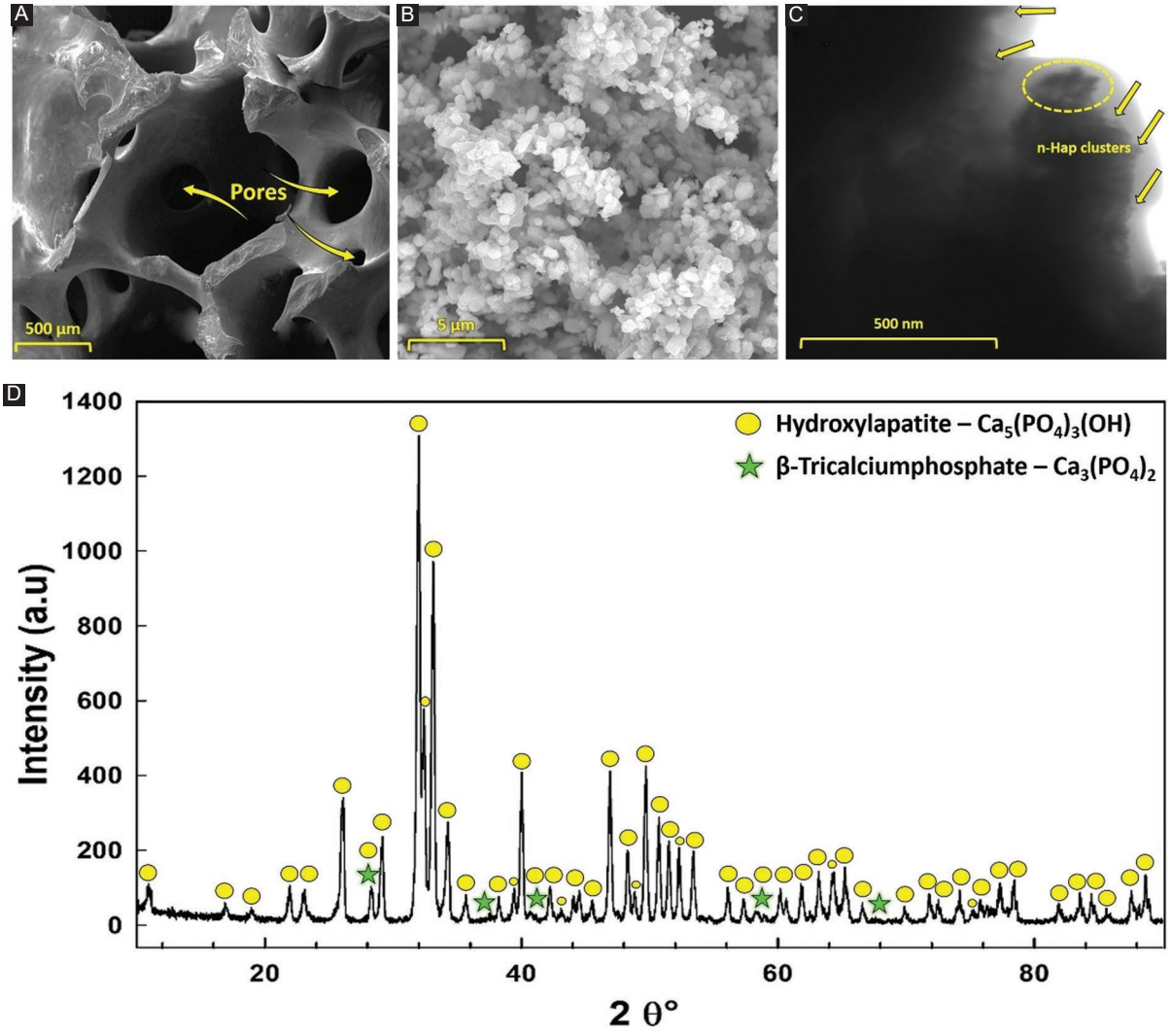

Figure 2. A: *high-resolution field emission scanning electron microscopy (FE-SEM) photographs of femoral bone fragments,* **B:** *after calcination at 850°C for 2 h,* **C:** *FE-SEM photographs of n-HAp after ball milling, and* **D:** *the X-ray diffraction patterns of the n-HAp.*

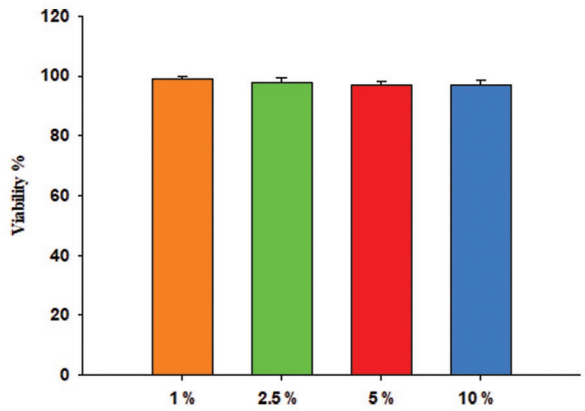

Figure 3. *Cell viability (%) results of vaginal gel/nHAp gel on L929 cell line at different concentrations.*

7, 14, and 21 were analyzed by the ELISA method. IL-1β and TNF-α, which are proinflammatory cytokines, regulate the mechanism by upregulating in the inflammatory phase of the wound healing process. IL-1β, TNF-α, and VEGF levels gradually decreased in Control, Sham, VG, and/nHAp groups on days 7, 14, and 21. However, as seen in Fig. 4A-C, this decrease is especially evident in the VG/nHAp group.

Histological analysis

On the 7th, 14th, and 21st days of the experiment, the typical multilayered squamous keratinized epithelium

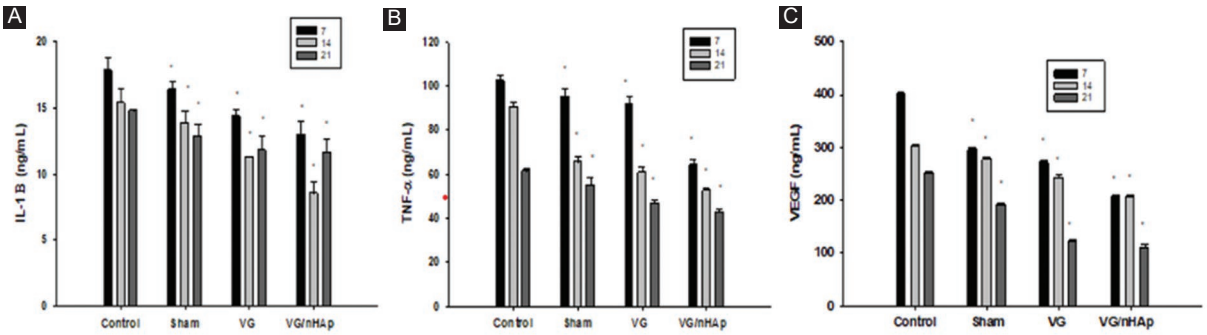

Figure 4. *Interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), VEGF enzyme-linked immunosorbent assay analysis values in blood serum samples obtained from Control, Sham, vaginal gel (VG), VG/nHAp groups on days 7, 14 and 21. According to the* **A:** *Kruskal-Wallis test IL-1β $p < 0.020$,* **B:** *Kruskal-Wallis test TNF-α $p < 0.023$,* **C:** *Kruskal-Wallis VEGF $p < 0.022$ were found when the control and all groups were compared pairwise. $p < 0.05$ was considered significant.*

of the vagina and the typical vaginal mucosa with many transverse folds together with the lamina propria located under it were evident in the control group (Fig. 5A). The basement membrane underlying the epithelium was thick and continuous, and collagen and reticular fibers were regularly arranged in the connective tissue forming the lamina propria (Fig. 5B and C). On the 7th day of the experiment, the number of epithelial layers, keratinization, and mucosal folds decreased, the number of inflammatory cells in the lamina propria increased, the number of collagen and reticular fibers decreased and became irregular in the Sham, VG, VG/nHAp groups compared to the control group. Newly formed blood vessels were significantly increased in the VG/nHAp group on the 7th day of the experiment. In the sham group, the epithelium was irregularly formed in the lamina propria, there were intense inflammatory cells, and collagen and reticular fibers were thin and irregularly arranged. Especially in the VG and VG/nHAp groups, the arrangement of connective tissue fibers in the tunica mucosa increased from the 7th day to the 14th and 21st days and markedly improved (Fig. 5A-C).

On the 7th, 14th, and 21st days of the experiment, the typical multilayered squamous keratinized epithelium of the vagina and the typical vaginal mucosa with many transverse folds with the lamina propria located underneath were evident in the control group (Fig. 5A). The basement membrane in which the epithelium was located was thick and continuous, while collagen and reticular fibers were regularly arranged in the connective tissue forming the lamina propria (Fig. 5B and C). On the 7th day of the experiment, the number of epithelial layers, keratinization, and mucosal folds decreased, the number of inflammatory cells in the lamina propria increased, the number of collagen and reticular fibers decreased and became irregular in the Sham, VG, VG/nHAp groups compared to the control group. Newly formed blood vessels were significantly increased in the VG/nHAp group on the 7th day of the experiment. In the sham group, the epithelium was irregularly formed in the lamina propria, there were intense inflammatory cells, and thin and irregularly arranged collagen and reticular fibers were remarkable. Especially in the VG and VG/nHAp groups, the arrangement of connective tissue fibers in the tunica mucosa increased from the 7th day to the 14th and 21st days and markedly improved (Fig. 5 A-C).

According to table 2, for the score values obtained from the subjects in the control group, no significant difference was found between the 7th, 14th, and 21st day scores in any parameter ($p > 0.05$). For the score values obtained from the subjects in the sham group, no significant difference was found between the 7th, 14th, and 21st day scores of the other parameters, except for the reepithelialisation parameter ($p > 0.05$). There was a significant difference for the re-epithelialization parameter ($p < 0.05$), and significant increases were observed at each follow-up time. Re-epithelialization value reached its highest value on the 21st day. For the score values obtained from the subjects in the VG group, no significant difference was found between the 7th, 14th, and 21st day scores of the other parameters, except for the inflammation parameter ($p > 0.05$). There was a significant difference for the inflammation parameter ($p < 0.05$), and significant decreases were observed at each follow-up time. Re-epithelialization value reached its lowest value at day 21. For the score values obtained from the subjects in the VG/nHAp group, a significant difference was found between the

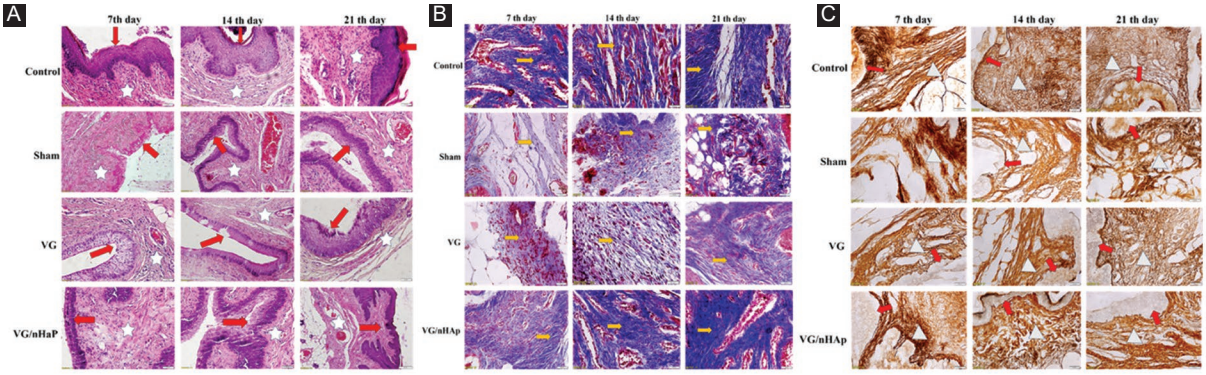

Figure 5. *Microscopic images obtained by staining with hematoxylin eosin (***A***), masson trichrome (***B***) and silver precipitation method (***C***) on paraffin tissue sections on the 7th, 14th, and 21st days in Control, Sham (wound created but no bioadhesive gel applied), VG (vaginal gel applied), and VG/nHAp (vaginal gel with nano hydroxyapatite particles) groups in which vaginal wound healing model was created.* **A:** *Microscopic photograph showing differences in epithelialization (arrow) and inflammation and neovascularization in the lamina propria (star) among Control, Sham, VG, and VG/nHAp groups on days 7, 14, and 21 in an experimental vaginal wound model (x20 Magnification, Hematoxylin and Eosin stain).* **B:** *Microscopic photograph shows differences in collagen fiber distribution (yellow arrows) among Control, Sham, VG, and VG/nHAp groups on days 7, 14, and 21 in an experimental vaginal wound model (x40 Magnification, MT stain).* **C:** *Microscopic photograph shows differences in reticular fiber distribution (white triangles) within the lamina propria (red arrow) beneath the epithelium among Control, Sham, VG, and VG/nHAp groups on days 7, 14, and 21 in an experimental vaginal wound model (x40 Magnification, Silver Imp).*

7th, 14th, and 21st day scores of the other parameters, except for collagen deposit and re-epithelialization parameters ($p < 0.05$). Although there were not enough changes to find a significant difference for re-epithelialization and collagen deposit parameters, increases were observed at follow-up times. For other parameters, significant differences were found between follow-up times and score values ($p < 0.05$). For the parameters of inflammation, granulation tissue amount, and neovascularization, significant decreases were observed at follow-up times, while the lowest values were reached on day 21. Significant increases were observed for the reticular deposit parameter at follow-up times and reached their highest value on the 21st day. The distribution of semi-quantitative score data for vaginal wound healing criteria obtained from the Control, Sham, VG, and n-HAp groups on days 7, 14, and 21 is shown in figure 6A.

According to the Kruskal-Wallis test results, a significant difference was found between the score values obtained on the 7th, 14th, and 21st days in terms of inflammation ($p < 0.05$). The mean ranks of inflammation, granulation, collagen and reticular fiber density, re-epithelialization and neovascularization score values of the subjects in the VG/nHAp group were found to be significantly different in the VG/nHAp group compared to the control group in accordance with the wound healing stages ($p < 0.05$) (Table 3). The distribution of semi-quantitative score data for vaginal wound healing criteria obtained on days 7, 14, and 21 according to the control, sham, VG, and VG/nHAp groups is shown in figure 6B.

Discussion

Surgical incision to the perineum, such as episiotomy, is known to cause various problems on vaginal wound healing. Although different methods have been proposed to reduce the rate of perineal laceration, an effective method has not yet been found due to the complications experienced afterward[27].

In today's biomedical applications, various natural polymer-based gels, hydrogels, and porous scaffolds have significant potential to simultaneously fulfill expected properties[28-30]. Sodium Alginate (SA), an anionic linear polysaccharide composed of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues, holds promise due to its good biocompatibility, biodegradability, non-toxicity, and non-immunogenicity. It has been recognized as a valuable biopolymer[31-33]. Carboxymethyl cellulose (CMC), another natural polymeric biomaterial similar to SA, has been shown to support the stability of cellular environments *in vivo* and promote increased cell viability over time.

Various polymers with bioadhesive properties have been reported in the literature[34,35], including polyacrylates (polycarbophil and carbomer), cellulosic derivatives (sodium carboxymethylcellulose, hydroxypropylmethylcellulose, and hydroxyethylcellulose), and other

Table 2. Friedman test table for semi quantitative score data of vaginal wound healing criteria obtained from Control, Sham, VG, VG/nHAp groups on the 7th, 14th, and 21st days

Groups	Parameters	Days	Mean	Standard deviation	Minimum	Maximum	Mean rank	p
Control	Inflammation	7	0.2	0.447	0	1	2.20	0.368
		14	0	0.000	0	0	1.90	
		21	0	0.000	0	0	1.90	
	Granulation tissue amount	7	0	0.000	0	0	2.00	1.000
		14	0	0.000	0	0	2.00	
		21	0	0.000	0	0	2.00	
	Collagen deposit	7	3	0.000	3	3	2.00	1.000
		14	3	0.000	3	3	2.00	
		21	3	0.000	3	3	2.00	
	Reticular deposit	7	2.8	0.447	2	3	1.80	0.368
		14	3	0.000	3	3	2.10	
		21	3	0.000	3	3	2.10	
	Re-epithelization	7	3	0	3	3	2.00	1.000
		14	3	0	3	3	2.00	
		21	3	0	3	3	2.00	
	Neovascularization	7	2	0	2	2	1.90	0.368
		14	2.2	0.447	2	3	2.20	
		21	2	0	2	2	1.90	
Sham	Inflammation	7	2.8	0.447	2	3	2.30	0.444
		14	2.6	0.548	2	3	2.10	
		21	2.2	0.837	1	3	1.60	
	Granulation tissue amount	7	1.8	0.837	1	3	2.30	0.165
		14	1.8	0.447	1	2	2.30	
		21	1.2	0.447	1	2	1.40	
	Collagen deposit	7	1.2	0.447	1	2	1.50	0.082
		14	1.6	0.548	1	2	2.00	
		21	2	1.000	1	3	2.50	
	Reticular deposit	7	1	0.707	0	2	1.50	0.210
		14	1.4	0.548	1	2	2.00	
		21	2	0.707	1	3	2.50	
	Re-epithelization	7	0.4	0.548	0	1	1.30	0.037*
		14	1	0.707	0	2	2.10	
		21	1.6	0.548	1	2	2.60	
	Neovascularization	7	1.8	0.837	1	3	2.50	0.174
		14	1.2	0.447	1	2	1.90	
		21	1	0.000	1	1	1.60	

(Continues)

Table 2. Friedman test table for semi quantitative score data of vaginal wound healing criteria obtained from Control, Sham, VG, VG/nHAp groups on the 7th, 14th, and 21st days *(continued)*

Groups	Parameters	Days	Mean	Standard deviation	Minimum	Maximum	Mean rank	p
VG	Inflammation	7	2.4	0.548	2	3	2.70	0.039*
		14	1.8	0.837	1	3	1.80	
		21	1.6	0.548	1	2	1.50	
	Granulation tissue amount	7	2.2	0.447	2	3	2.40	0.273
		14	1.8	0.447	1	2	1.90	
		21	1.6	0.548	1	2	1.70	
	Collagen deposit	7	1.6	0.548	1	2	1.60	0.202
		14	2	0.707	1	3	2.00	
		21	2.4	0.548	2	3	2.40	
	Reticular deposit	7	1.2	0.447	1	2	1.50	0.074
		14	1.4	0.548	1	2	1.80	
		21	2.2	0.447	2	3	2.70	
	Re-epithelization	7	1.2	0.837	0	2	1.50	0.109
		14	1.8	0.447	1	2	1.90	
		21	2.4	0.548	2	3	2.60	
	Neovascularization	7	2	0.707	1	3	1.90	0.607
		14	2.2	0.447	2	3	2.20	
		21	2	0.707	1	3	1.90	
VG/nHAp	Inflammation	7	1.6	0.548	1	2	2.70	0.037*
		14	1	0.707	0	2	1.90	
		21	0.6	0.548	0	1	1.40	
	Granulation tissue amount	7	2.8	0.447	2	3	2.80	0.039*
		14	1.8	0.837	1	3	1.80	
		21	1.2	0.837	0	2	1.40	
	Collagen deposit	7	2.2	0.447	2	3	1.50	0.074
		14	2.4	0.548	2	3	1.80	
		21	3	0.000	3	3	2.70	
	Reticuler deposit	7	1.8	0.447	1	2	1.40	0.048*
		14	2.2	0.447	2	3	2.00	
		21	2.6	0.548	2	3	2.60	
	Reepitelization	7	2.2	0.837	1	3	1.50	0.082
		14	2.6	0.548	2	3	2.00	
		21	3	0.000	3	3	2.50	
	Neovascularization	7	3	0.000	3	3	2.90	0.015*
		14	2.2	0.447	2	3	1.80	
		21	1.6	0.548	1	2	1.30	

* indicates a statistically significant difference at $p < 0.05$, VG: vaginal gel.

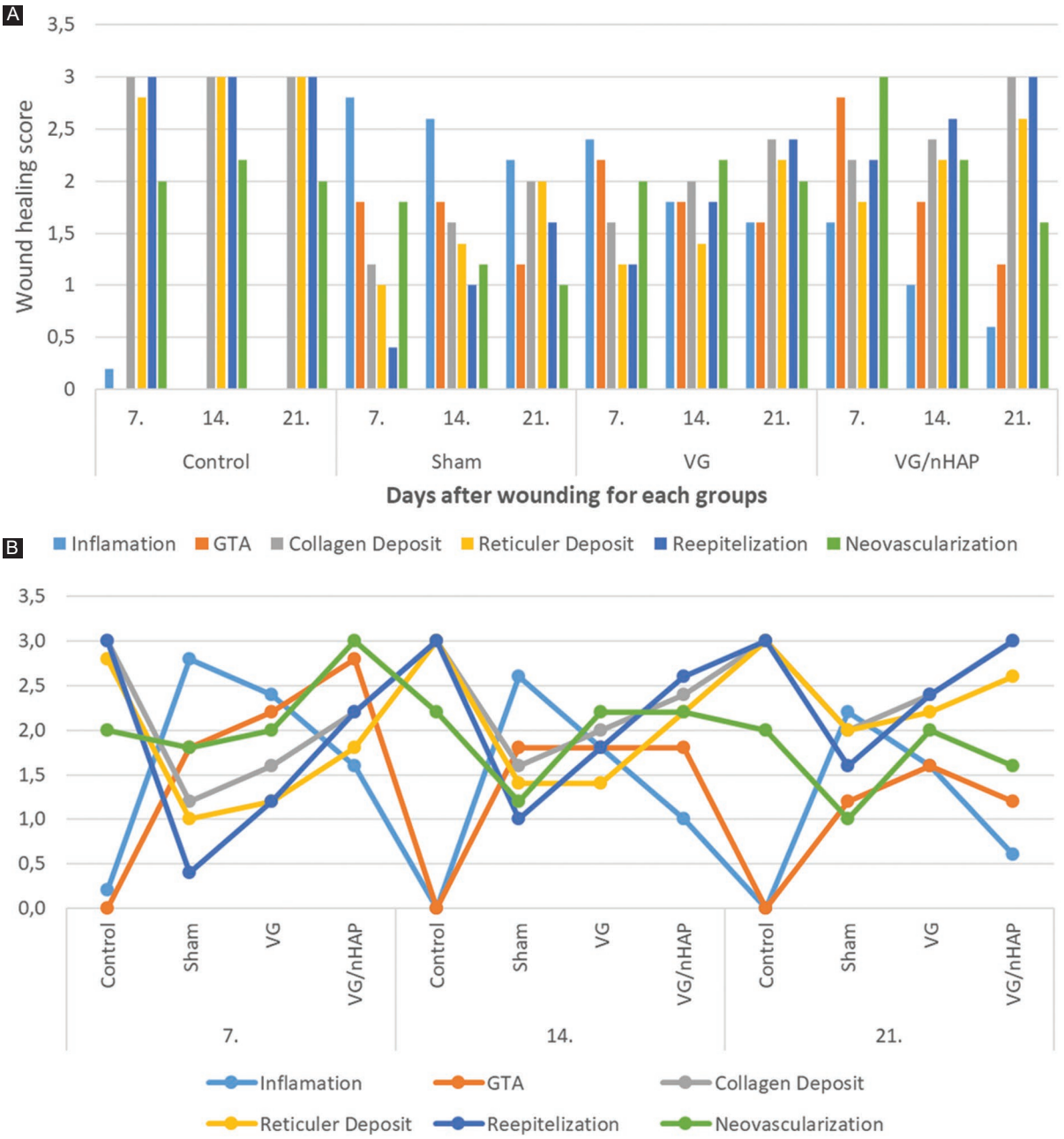

Figure 6. A: *Friedman test graph of semi quantitative score data of vaginal wound healing criteria obtained from Control, Sham, Vaginal gel (VG), n-HAp groups at 7, 14, and 21 days.* **B:** *Kruskal-Wallis test graph of semi quantitative score data of vaginal wound healing criteria obtained from Control, Sham, VG, VG/nHAp groups at 7, 14, and 21 days.*

polysaccharides (chitosan, hyaluronic acid, and gums). Among the limited number of polymer combinations studied for commercial muco-adhesive drug delivery systems, most vaginal semi-solid products are based on cellulosic and polyacrylic polymers[36,37]. Therefore, further research is needed on the application of different combinations of biodegradable biopolymers for bioadhesive VGs. In this study, it was observed that the synthesized polymer combinations increased the biological adhesive properties, and simultaneously, the addition of bioactive n-HAp enhanced the stability of the gels. Polymeric structures designed as biomaterials are among the most suitable options for mimicking the main structure of soft tissues. However, the incorporation of different additives into the polymer matrix can accelerate the development of biological properties and wound healing processes.

Table 3. Kruskal-Wallis analysis table of semi quantitative score data of vaginal wound healing criteria obtained from control, sham, VG, VG/nHAp groups on the 7th, 14th, and 21st days

Inflammation					Granulation tissue amount				
Days	**Groups**	**n**	**Mean rank**	**p**	**Days**	**Groups**	**n**	**Mean rank**	**p**
7	Control	5	3.2	0.002	7	Control	5	3	0.002
	Sham	5	16.2			Sham	5	10.5	
	VG	5	13.6			VG	5	12.3	
	VG/nHAp	5	9			VG/nHAp	5	16.2	
14	Control	5	3.5	0.002	14	Control	5	3	0.006
	Sham	5	16.7			Sham	5	13.1	
	VG	5	12.9			VG	5	13.1	
	VG/nHAp	5	8.9			VG/nHAp	5	12.8	
21	Control	5	4	0.002	21	Control	5	3.5	0.01
	Sham	5	16.3			Sham	5	11.9	
	VG	5	13.8			VG	5	14.7	
	VG/nHAp	5	7.9			VG/nHAp	5	11.9	
Collagen deposit					**Reticular deposit**				
Days	**Groups**	**n**	**Mean rank**	**p**	**Days**	**Groups**	**n**	**Mean rank**	**p**
7	Control	5	17.5	0.002	7	Control	5	17.4	0.005
	Sham	5	4.9			Sham	5	6.1	
	VG	5	7.7			VG	5	7	
	VG/nHAp	5	11.9			n-HAp	5	11.5	
14	Control	5	16.5	0.012	14	Control	5	17.5	0.003
	Sham	5	5.6			Sham	5	6.3	
	VG	5	8.5			VG	5	6.3	
	n-HAp	5	11.4			n-HAp	5	11.9	
21	Control	5	13.5	0.044	21	Control	5	15.5	0.039
	Sham	5	6.9			Sham	5	6.9	
	VG	5	8.1			VG	5	7.9	
	VG/nHAp	5	13.5			VG/nHAp	5	11.7	
Reepitelization					**Neovascularization**				
Days	**Groups**	**n**	**Mean Rank**	**p**	**Days**	**Groups**	**n**	**Mean Rank**	**p**
7	Control	5	17	0.003	7	Control	5	8.5	0.02
	Sham	5	4.3			Sham	5	7.6	
	VG	5	7.9			VG	5	8.9	
	VG/nHAp	5	12.8			VG/nHAp	5	17	

(Continues)

Table 3. Kruskal-Wallis analysis table of semi quantitative score data of vaginal wound healing criteria obtained from control, sham, VG, VG/nHAp groups on the 7th, 14th, and 21st days *(continued)*

Inflammation					Granulation tissue amount				
Days	**Groups**	**n**	**Mean rank**	**p**	**Days**	**Groups**	**n**	**Mean rank**	**p**
14	Control	5	16.5	0.002	14	Control	5	12.6	0.014
	Sham	5	4.1			Sham	5	4.2	
	VG	5	7.9			VG	5	12.6	
	VG/nHAp	5	13.5			VG/nHAp	5	12.6	
21	Control	5	14.5	0.002	21	Control	5	14	0.015
	Sham	5	3.9			Sham	5	4.5	
	VG	5	9.1			VG	5	13.3	
	VG/nHAp	5	14.5			VG/nHAp	5	10.2	

VG: vaginal gel.

Among these additives, bioceramics such as bioglasses, carbon nanostructures, and HAp, which contribute to tissue healing, can be preferred for applications in both hard and soft tissues[38,39]. HAp, one of the bioceramics used in tissue healing, offers fundamental benefits to tissues such as promoting cell growth and proliferation, inducing angiogenesis, and possessing antimicrobial properties[40]. n-HAp also enhances fibroblast activity in injured areas and contributes to wound dressing/burn treatment when incorporated into various soft organic matrices to improve the mechanical properties and thermal stability of the polymer[41]. Therefore, the use of HA microparticles (size < 74 μm) in combination with gels at skin wound sites further enhances fibroblast maturation and re-epithelialization due to the beneficial effects of the ion-charged stored in the gel[42].

The purpose of biocompatibility testing is to determine the suitability of a substance for human use and to see whether it has potentially harmful physiological effects. *In vitro* and in *vivo* studies are performed in biocompatibility tests. Therefore, we performed biocompatibility tests *in vitro* in our study (ISO/EN10993-5). Possible vaginal wound healing material (VG/nHAp) was prepared in four different concentrations and was observed to be non-toxic. In addition, the material did not change the proliferation and morphology of healthy cells. In the study conducted by Malafaya and Reis[43], the toxicity of HAp produced was evaluated in the L929 healthy fibroblast cell line, and it was stated that it was not toxic.

The steps of wound repair in skin and vaginal tissue have common similarities. Compared to skin healing, the proliferation and migration of local vaginal fibroblasts is coordinated with local vaginal epithelial cells at the wound edges to ensure granulation tissue formation and re-epithelialization during the vaginal wound healing process. For example, a scab does not form during vaginal wound healing as in dermal wounds. After injury in dermal tissues, a clot is formed, and inflammatory cells enter the injured tissue, and abundant leukocytes and plasma proteins migrate to the healing area. Initially, neutrophils arrive, sterilize, and debride the wound. Monocytes and tissue macrophages dominate the inflammatory response within 2-3 days. After the initial inflammatory response, inflammation continues with a gradual disappearance of inflammatory cells. Inflammation is intensely observed in the first 7 days of wound healing and gradually decreases[44]. In our study, on the 7th day of vaginal injury, more intense inflammation was observed in the Sham group than in the VG and VG/nHAp groups, while it decreased on the 14th and 21st days. Less inflammation was determined in the VG and VG/nHAp groups compared to the Sham group. The VG/nHAp group had the least number of inflammatory cells.

Cytokines and growth factors, which are protein molecules that provide communication between cells in the body, manage important processes after vaginal injury. Cytokines enable the migration of inflammatory cells and fibroblasts to the wound site during the wound healing phase, and organize the formation and production of extracellular matrix (ECM) by activating cell proliferation and angiogenesis. Both human and animal studies emphasize that high local and circulating TNF-α levels are associated with impaired wound healing. It has been suggested that treating women with TNF-α antagonists following vaginal surgeries may have a therapeutic effect, resulting in an improved wound healing process. However, macrophages are involved in both the inflammatory and proliferation phases of wound healing. TNF-α and IL-1β are proinflammatory cytokines secreted by M1 macrophages during the inflammatory phase. M2 macrophages release polyamines for the induction of VEGF expression and collagen production. If this polarization from M1 macrophages to M2 macrophages is disrupted, wound healing is impaired. This disruption slows the rate of re-epithelialization, granulation tissue formation, and vascularization[45]. Our study supports the literature. A decrease in TNF-α and VEGF values was observed in VG and VG/nHAp groups. There is a decrease in TNF-α and VEGF values in the VG/nHAp group, which shows that the fastest recovery is in this group.

In dermal tissues, fibroblasts have been reported to migrate into acute wounds within 2 days and become the main cell type of granulation tissue on day 4 post-injury. Fibroblasts fill the wound site through migration and increase in number by proliferation. These functions are accompanied by various growth factors. In addition to the behavior of fibroblasts, neovascularization also accompanies the repair process in the vaginal wound. Thus, granulation tissue forms in the vagina on the 4th day[46]. When we look at our study, granulation tissue in the Sham group gradually decreased from day 7 to day 21 because it represents normal vaginal healing. On the other hand, it was determined that the maturation process of granulation tissue in the VG and VG/nHAp groups matured faster than in the control on day 7, and the rate of granulation tissue formation in the VG/nHAp group was higher than the VG group.

Neovascularization is an important component of the wound healing process, involving the branching and expansion of adjacent blood vessels as well as endothelial progenitor cells[47]. In the control group, it was determined that neovascular structures decreased from the 7th day until the 21st day. In the VG and VG/nHAp groups used in the vaginal healing model, neovascular structures increased much more significantly from day 7. New vessel formation was observed more prominently in the VG/nHAp group as of day 7. During wound healing in the skin, fibroblasts have been shown to accumulate increased amounts of fibrillar collagen, which is important for wound healing. This process is regulated by the same growth factors that regulate fibroblast proliferation.

It has been shown that collagen deposition by skin fibroblasts starts within 3-5 days after injury and continues for several weeks, depending on the size of the wound[48,49]. Type 1 and type 3 forms of collagen, which have various subtypes, are commonly found in skin and mucosa. They strongly bind to cells to support epithelial and lamina propria formation and maintain tissue integrity[50]. In our study, we wanted to determine the amount of collagen with masson trichrome stain and reticular fiber (type 3) collagen with the silver precipitation technique to understand whether wound healing occurs properly with the proposed biomaterials. In the sham group, the amount of collagen fiber increased from the 7th to the 21st day after injury.

However, the amount of collagen and reticular fiber in the VG and VG/nHAp groups was higher than in the Sham group. Especially in the VG/nHAP group, there was a significant increase in collagen and reticular fibers compared to the other groups.

The normal vaginal epithelium is moist and thick. The natural shedding of epithelial cells into the vagina leads to the release of glycogen, which is converted into lactic acid by lactobacilli to maintain the balance of the vagina. Epithelial cells are surrounded and supported by an abundant ECM, normally rich in type I and type III collagens. The amount of these two collagen proteins has a significant effect on the symptoms of vaginal injury. It is stated that collagen-based biomaterial application to rats with vaginal atrophy increases vaginal epithelial height and increases cell adhesion molecules and ECM markers[51]. It has been shown that re-epithelialization in dermal tissues occurs within 24-48 h after injury when the spurs of epithelial cells move from the wound edges along the cut edges of the dermis and accumulate basement membrane components supported by type 3 collagen. Eventually, they fused to form a continuous epithelial layer covering the wound. It is stated that wound re-epithelialization in the vagina starts in a short period of time and progresses similarly to that in the skin and is almost completed on days 14-21[2]. In our study, although it was determined that re-epithelialization was completed on the 21st day in the Sham group, epithelial elevation and keratinization did not occur. On the other hand, a significant difference was observed in the VG/nHAp group compared to the other groups as of day 14. In this group, the epithelium was closed, epithelial folds were completed, and mucosal folds became prominent on the 21st day.

Conclusion

The bioadhesive gel prepared with n-HAp was successfully synthesized. In the study, the characterization of the gel was determined by FE-SEM, S-TEM, and XRD. Images of the gel, which exhibited porous structures with optimal mechanical properties similar to bone, were confirmed by what was reported in the literature. After it was observed that the VG was not toxic, it was used in the *in vivo* experiment. In animal experiments, a wound was opened in the vagina of rats and bioadhesive gel was applied. As a result, bioadhesive gel prepared with HAp is seen as promising for the treatment of vaginal wounds. However, its safety and efficacy should be tested in the clinical setting, which is the next step for biocompatibility experiments, by conducting extensive research.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The study does not involve patient personal data nor requires ethical approval. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ORIGINAL ARTICLE

Circulating histone H4 values can relate to disease severity in patients with alcoholic hepatitis and cirrhosis

Los valores circulantes de histona H4 pueden relacionarse con la gravedad de la enfermedad en pacientes con hepatitis alcohólica y cirrosis

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Abstract

Objective: *We aimed to evaluate whether serum histone H4 (sHH4) is associated with alcoholic liver disease (ALD) phenotypes.* **Methods:** *This case–control study included 66 ALD patients and 47 healthy controls (HCs). Patients with ALD were classified into three groups: alcohol-associated steatotic liver, alcoholic hepatitis (AH), and alcoholic cirrhosis (AC). The HIST1H4A kit was used for the enzyme-linked immunosorbent assay of sHH4.* **Results:** *In the AH patients, the median sHH4 value was the highest and the lowest in the HC (3572.32 ng/L vs. 451 ng/L, respectively, p = 0.002). In the AC group, the median sHH4 value was higher in the Child-Pugh classification B (CPC-B) group than in the CPC-A group (p = 0.026). Positive correlations existed between the sHH4 value and the duration of alcohol use and Maddrey's discriminant function scores in patients with AH (rho = 0.886, p = 0.019 for both). In the AC patients, a positive correlation was noted between the sHH4 values and The Model for End-stage Liver Disease Sodium scores (rho = 0.527, p = 0.006).* **Conclusions:** *Increased sHH4 values might be a marker for the severity of AH and AC.*

Keywords: *Alcoholic liver disease. Histone H4. Severity.*

Resumen

Objetivo: *Evaluar si la histona sérica H4 (sHH4) está asociada con fenotipos de enfermedad hepática alcohólica.* **Métodos:** *Estudio de casos y controles que incluyó 66 pacientes con enfermedad hepática alcohólica y 47 controles sanos. Los pacientes con enfermedad hepática alcohólica se clasificaron en tres grupos: hígado esteatósico asociado al alcohol, hepatitis alcohólica (HA) y cirrosis alcohólica (CA). Se utilizó el kit HIST1H4A para el ensayo de inmunoadsorción ligado a enzimas (ELISA) de sHH4.* **Resultados:** *En los pacientes con HA, la mediana del valor de sHH4 fue más alta que en los controles sanos (3572.32 ng/L frente a 451 ng/L; p = 0.002). En el grupo con CA, el valor mediano de sHH4 fue mayor en los pacientes en clase B de Child-Pugh que en aquellos en clase A (p = 0.026). Hubo una correlación positiva entre el valor de sHH4 y la duración del consumo de alcohol y las puntuaciones MDF (Maddrey's Discriminant Function) en los pacientes*

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con HA (rho = 0.886; p = 0.019 para ambos). Se detectó una correlación positiva entre los valores de sHH4 y las puntuaciones MELD-Na (Model for End-stage Liver Disease Sodium) en los pacientes con CA (rho = 0.527; p = 0.006). **Conclusiones:** *Los valores elevados de sHH4 pueden ser un indicador de la gravedad de la HA y la CA.*

Palabras clave: *Enfermedad hepática alcohólica. Histona H4. Gravedad.*

Introduction

Alcohol abuse is a public health care problem. The pathogenesis of alcoholic liver disease (ALD) involves a series of complex interactions, including toxic alcohol metabolites, cytokines, oxidative stress, endotoxins, as well as genetic and immunological factors[1-6].

The accumulation of neutrophils in the liver of ALD patients has been reported to be associated with poor prognosis[6-8]. Disruption of the intestinal barrier due to alcohol leads to bacterial translocation from the gut and increased lipopolysaccharide concentrations in the portal circulation, which in turn triggers the recruitment of neutrophils into the liver[4,6].

In response to bacterial products and chemical agents, neutrophils secrete extracellular traps (NET), including DNA fragments, histones, and bactericidal proteins, a process called NETosis[8]. Histones are basic proteins functioning in the assembly of DNA molecules, participate in the cellular innate immune response, and are responsible for immune-mediated immunothrombosis through toll-like receptor (TLR) activation[8-10]. NET, including histones, revealed diagnostic and prognostic significance in some clinical conditions, including sepsis, trauma, venous thromboembolism, and inflammatory bowel diseases[8]. Neutrophils and histones exert harmful and protective effects on inflammation, called double-edged swords[8].

Exaggerated NETosis can cause tissue damage, and excessive release of the histones from neutrophils into circulation was associated with liver injury in the experimental models of ALD[6,11-14]. In acute liver failure, hepatocyte necrosis triggers the release of damage-associated molecular patterns (DAMPs) that activate immune cells in the liver and circulation, and histones are members of DAMPs[9].

Regarding the associations between the extracellular release of histones in the liver and the pathogenesis of ALD, specifically, we aimed to evaluate whether the serum histone H4 (sHH4) levels of the patients with ALD could serve as diagnostic and prognostic markers correlating to the clinical and laboratory traits of the diseases.

Methods

Study population

Sixty-six patients with ALD and 47 healthy controls (HCs) admitted to our gastroenterology inpatient and outpatient services between October 2021 and July 2023 were enrolled in the study. The Local Ethics Committee approved the study (215/October 27, 2021). The study protocol complies with the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008), as reflected in a priori approval by the Institution's Human Research Committee. Written informed consent was obtained from all participants.

Participants with clinical conditions that can affect sHH4 levels, such as sepsis, inflammatory bowel diseases, or any malignancies, including hepatocellular carcinoma, venous thromboembolism, or trauma, were excluded from the study. Volunteers with severe organ failure, including acute liver failure, acute or chronic infections, autoimmune diseases, diabetes mellitus, and any cause of acute and chronic liver disease, as well as those with acute or chronic hepatitis B (including HBeAg-negative chronic hepatitis B infection) and C infection and illicit drug use, were also excluded from the study.

The patients using excessive alcohol of > 60 g/day for men and > 40 g/day for women for at least 5 years were enrolled in the study[1,15]. The diagnosis of alcohol use disorder was made by a psychiatrist after an interview, according to the Diagnostic and Statistical Manual of Mental Disorders[16]. The HCs were not using alcohol, and no hepatic steatosis or hepatomegaly was reported in ultrasonography (USG).

Data collection

The dose and the duration of alcohol use were noted for each patient with ALD. The body mass index (BMI), comorbidities, and medications were recorded for all participants. Based on the disease history, physical findings, laboratory results, and radiological results, patients with ALD were assigned into three

groups: alcohol-associated steatotic liver (ASL), alcoholic hepatitis (AH), and alcoholic cirrhosis (AC). USG was applied to all participants for the radiologic evaluation.

AH was defined according to clinical and laboratory criteria. In this group, the alcohol intake was continuous for 6 months or more with < 60 days of abstinence before the onset of jaundice, and serum aspartate transaminase (AST) values were higher than alanine aminotransferase (ALT) values (AST/ALT ratio > 1.5), serum total bilirubin > 3.0 mg/dL, and without any other cause[1,15]. Cirrhotic patients were not included in the AH group.

AC was diagnosed according to the stigmata of cirrhosis in the physical examination and to the results of the biochemical tests along with the USG reports. Patients with ALS were non-cirrhotic and had no clinical or laboratory criteria for AH. The ALD patients did not present with the clinical and laboratory findings of acute liver failure and hepatic encephalopathy.

Assessment of the severity of disease in patients with ALD

All participants had blood chemistry tests, including the complete blood count, C-reactive protein (CRP), and D-dimer values, before USG. According to USG, the presence of hepatic steatosis was recorded in all participants and in patients with AH and AC, the presence of ascites was noted[17]. The patients with AH and AC underwent upper gastrointestinal endoscopy, and the presence of esophageal and/or gastric varices was recorded.

The fibrosis-4 (FIB-4) index was used to predict liver fibrosis for patients with ALD[18]. The Maddreys-discriminant function (MDF) tests and the Model for End-stage Liver Disease Sodium (MELD-Na) scores were calculated in patients with AH[19,20]. The patients with AC were classified according to the Child-Pugh classification (CPC A, B, C). The MELD-Na scores were also noted in the cirrhotic patients[21,22].

Measurement of sHH4

The serum for histone H4 (HH4) was separated from venous blood samples. After centrifugation at 5000r *g* for 10 min at 30nut the supernatant serum was stored in Eppendorf tubes at (–) 80be for 12-24 months. For the enzyme-linked immunosorbent assay (ELISA) measurement of sHH4, the Human HH4 (HIST1H4A) Bioassay Technology Laboratory Kit (Cat. No. E5420 Hu, Lot:202305012) was used (Intra-Assay: CV < 8%, Inter-Assay: CV < 10%) with a microplate reader (Biotech Epoch 2 Microplate ELISA Reader, USA).

Statistical analysis

Statistical analyses were performed using jamovi version 2.3.28.0 and easyROC version 1.3.1[23,24]. Descriptive statistics were presented as median and mean with quartiles and standard deviations, respectively, for continuous variables and as frequencies with percentages for categorical ones. Mann-Whitney U and Kruskal-Wallis compared non-parametric continuous variables and analysis of variance for those with parametric features. Furthermore, the χ^2 test was used to test equity with categorical variables. Diagnostic accuracy was studied by receiver operating characteristics (ROCs) curved analysis. The confidence level for statistical significance was defined as 95%.

Results

The study evaluated 66 patients with ALD (62 males, 4 females) and 47 HCs (42 males, 5 females). The demographic, clinical, and histopathological characteristics of the study population are presented in table 1. For gender, males dominated the study population, and there was no statistically significant difference among the groups. The mean age of the patients was the highest in the AC patients (p = 0.001). The mean BMI of the HC group was the lowest, but it was similar among the ALD groups (p < 0.001) (Table 1).

The neutrophil counts were similar in all groups, whereas the median CRP value was the lowest in the HC group (Table 1). While the median Fib-4 index was the highest in the AH group, no difference existed between the patients with AH and AC (p < 0.001) (Table 1). The median D-dimer values were similar between the AH and AC and between the ALS and HC groups (p < 0.001).

In total, sHH4 concentrations were higher in the ALD patients compared to the HCs (878.91 [391.54-1985.92] vs. 451 [359.91-922] ng/L, respectively, p = 0.034). Regarding sHH4 concentrations, there was a statistically significant difference between the subgroups of ALD patients and the HCs (p = 0.002) (Table 1). The median sHH4 value was the highest in the AH patients and the lowest in the HCs (3572.32 ng/L vs. 451 ng/L, respectively, p = 0.002). The median

Table 1. Clinical, demographic, laboratory characteristics, and serum histone H4 values in patients with alcoholic liver diseases and healthy controls

Parameters	Alcohol-associated steatotic liver (n = 34)	Alcoholic hepatitis (n = 6)	Alcoholic cirrhosis (n = 26)	Healthy control group (n = 47)	p
Gender, n (%) Male	33 (97.1)	5 (83.3)	24 (92.3)	42 (89.4)	0.522
Age, median (IQR)	47 (43-53) [c]	45 (41-47) [c]	60 (53-66)[a,b,d]	45 (32-61)[c]	0.001†
Duration of alcohol use (years), median (IQR)	20 (15-30) [c]	21 (15-28)	35 (25-47)[a]	-	< 0.001*
BMI (kg/m^2), mean ± SD	28.29 ± 2.8[d]	29.51 ± 3.75[d]	29.72 ± 3.85[d]	25.22 ± 3.62[a,b,c]	< 0.001*
Presence of hepatic steatosis, n (%)	34 (100)	5 (83.3) [c]	8 (30.7) [b]	-	< 0.001*
Presence of ascites, n (%)	-	3 (50)	11 (42.3)	-	0.732
Esophageal/gastric varices, n (%)	-	3 (50)	16 (61.5)	-	0.604
Fibrosis-4 index, median (IQR)	0.95 (0.74-1.98) [b, c]	4.47 (2.58-7.64) [a]	3.45 (2.52-6.08) [a]	-	< 0.001*
MELD-Na score, median (IQR)	-	20 (9-39)	14 (9-21)	-	0.358
MDF, median (IQR)	-	50.5 (17.5-164)	-	-	-
Child-Pugh class, n (%) A B C	-	-	 10 (38.5) 7 (26.9) 9 (34.6)	-	-
Child-Pugh score, median (IQR)	-	-	7 (5-10)	-	-
Neutrophils (×10^3/μL), median (IQR)	5.26 (3.99-6.55)	4.62 (4-7.2)	3.53 (2.66-6.14)	4.63 (3.79-5.69)	0.056
D-Dimer (ug/mL), median (IQR)	0.45 (0.22-0.82)[c]	0.99 (0.56-4)[d]	1.58 (0.46-4)[a,d]	0.26 (0.1-0.46)[b,c]	< 0.001*
CRP (mg/L), median (IQR)	5.89 (1.63-14.86)[d]	15.6 (8.02-27.5)[d]	7.08 (3-21.1)[d]	2 (0.91-3.36)[a,b,c]	< 0.001*
Serum histone H4 (ng/L), median (IQR)	706.18 (407.04-1376.89)[b]	3572.32 (2387.4-4856)[a,c,d]	789.57 (333.8-2233.8)[b]	451 (359.91-922)[b]	0.002†

*Statistically significant level was lower than 0.001. According to the pairwise comparison, it was different from alcohol-associated steatotic liver.
†Statistically significant level was lower than 0.01.
[a]alcohol-associated steatotic liver.
[b]alcoholic hepatitis.
[c]alcoholic cirrhosis.
[d]healthy control group.
IQR: inter quartile range; SD: standard deviation; BMI: body mass index; MELD-Na: model for end-stage liver disease sodium; MDF: Maddrey's discriminant function; CRP: c-reactive protein.

sHH4 values were similar in the ALS, AC, and HC groups (Table 1).

In the AH and AC groups, the median sHH4 values were higher in the patients with steatosis, but the differences were not statistically significant. Although not significant, the patients with ascites, esophageal, and/or gastric varices also had higher median sHH4 values. According to the CPC in the AC patients, the median sHH4 value was higher in the CPC-B group than in the CPC-A group, but there was no difference between the CPC-B and CPC-C groups (p = 0.026) (Table 2).

There were positive correlations between the sHH4 value and the duration of alcohol use and MDF scores in patients with AH (rho = 0.886, p = 0.019 for both). In the AC group, there was a positive correlation between the sHH4 values and MELD-Na scores (rho = 0.527, p = 0.006). No correlation was reported between the sHH4 values and the Child-Pugh scores, neutrophil counts, and FIB-4 scores in the ALD patients (Table 3).

ROCs curve analyses revealed that the sHH4 concentrations had a predictive value to differentiate AH from the ASL (Area under the curve: 0.931 [95% CI: 0.842-1.00, p < 0.001]; Fig. 1). Serum HH4 levels higher than 0.765 ng/L had a diagnostic accuracy for AH with a sensitivity of 100% (0.541–n/a) and a specificity of 76.5% (95% CI: 0.588-0.893).

Discussion

Harmful alcohol use is a rising global burden and causes morbidity and mortality in the younger ages of life, with over 3 million deaths worldwide every year[2].

Table 2. Serum histone H4 concentrations and clinical and laboratory variables in patients with alcoholic hepatitis and cirrhosis

Group	n	Serum histone H4 (ng/L) Median IQR		p
Alcoholic hepatitis (n = 6)				
Hepatic steatosis				
Absent	2	3572.3	(2050.4-4710)	1
Present	4	3621.7	(2387.4-4856)	
Ascites				
Absent	3	2721.6	(2387.4-4997)	1
Present	3	4423	(1379.2-4856)	
Esophagus and/or gastric varices				
Absent	3	2721.6	(2387.4-4997)	1
Present	3	4423	(1379.2-4856)	
Alcoholic cirrhosis (n = 26)				
Hepatic steatosis				
Absent	24	789.5	(329.8-2109.8)	0.677
Present	2	1409.5	(350.8-2468.2)	
Ascites				
Absent	15	391.5	(325.8-1985.9)	0.443
Present	11	962	(385.3-2377.2)	
Esophagus and/or gastric varices				
Absent	10	371.2	(305.3-1334.8)	0.182
Present	16	1233	(371.2-2287.8)	
Child-Pugh Classification				
A	10	345.4	(305.3-391.5)[b]	0.026*
B	7	2233.8	(447.9-2468)[a]	
C	9	1406	(617.1-2341.8)	

*Statistically significance level was lower than 0.05. According to the pairwise comparison, it was different from Child-Pugh Classification A[a], B[b].

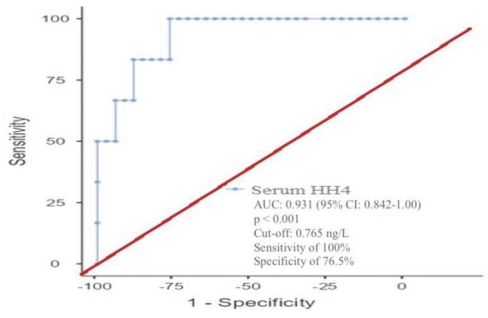

Figure 1. *Receiver operating characteristics curve analyses of the serum histone H4 concentrations, which differentiate alcoholic hepatitis from alcohol-associated steatotic liver (Area under the curve: 0.931 [95% CI: 0.842-1.00, p < 0.001]).*

Alcoholic fatty liver disease, alcohol-related hepatitis, and cirrhosis are consecutive disorders. Liver disease in alcohol use disorder is still a challenging enigma for practitioners[2,6].

Approximately 10-20% of alcoholic patients may subsequently develop hepatitis, and overall, 8-20% of patients with alcoholic steatosis can progress to cirrhosis[25]. Despite the excessive amount of alcohol ingestion, it is not known why some patients are more vulnerable to ethanol, which progresses to advanced liver disease and cirrhosis, while it may be steady in others. Unknown mechanisms may exist in the pathogenesis of ALD[5,6,25].

Neutrophils enhance lipid accumulation in the liver by inducing pro-inflammatory cytokine secretion in ALD[6,26,27]. Histones, classified as H1, H2A, H2B, H3, and H4, are the main members of the NET family, and HH4 is the most investigated molecule[28]. Neutrophils and histones exert harmful and protective effects on inflammation, called double-edged swords[8].

In a model of ethanol-related liver injury, the ethanol-derived acetate was converted to acetyl-CoA, which bound to specific sites on HH3 and HH4 proteins and disrupted the biological function of the cell by altering post-transcriptional protein modification through histone acetylation[11].

In a previous study, mice were treated with alcohol binges and LPS, and endotoxin significantly increased NET formation. In the efferocytosis process, in which apoptotic cells are removed by phagocytic cells, indicators of NET formation, including citrullinated histone-H3, increased in mouse hepatocytes, and citrullinated histone-H3 formation was attributed to the decreased clearance of NET[13].

Li et al. reported that histones are the critical mediators of liver injury in a heat shock (HS) setting through hepatocyte pyroptosis, a novel type of programmed cell death that causes pro-inflammatory cytokine secretion in murine hepatocytes[14]. Serum HH3 (sHH3) levels, which ELISA measured in HS mice, increased in a time- and dose-dependent manner compared to the control group, which was not exposed to HS injury. There were positive correlations between the sHH3 values, serum transaminase levels, and the histological activity scores of mice hepatocytes. Although HH3 was declared a pivotal mediator of hepatic injury, the other histone monomers were not evaluated in that study[14].

The aforementioned body of evidence demonstrates the pathogenic significance of histones in the experimental models of liver disease, and, as noted earlier, over-expressed histones in the tissue might diffuse into circulation[8,11-14]. Thus, we hypothesized that sHH4 concentrations might relate to the clinical traits of ALD patients.

Our results revealed higher sHH4 concentrations in the ALD patients compared to the HCs. Among the ALD groups, the mean sHH4 value was the highest in AH patients and the lowest in the ALS group. In the present study, increased extracellular HH4 release

Table 3. Correlations between the serum histone H4 values and the clinical and laboratory variables of the patients with alcoholic liver diseases

Parameters	Serum histone H4 (ng/L)					
	Alcohol-associated steatotic liver (n = 34)		Alcoholic hepatitis (n = 6)		Alcoholic cirrhosis (n = 26)	
	rho	p	rho	p	rho	p
Duration of alcohol use (years)	–0.255	0.146	0.886	0.019*	–0.005	0.981
Child-Pugh score	-	-	-	-	0.368	0.065
MELD-Na	-	-	0.771	0.072	0.527	0.006**
MDF	-	-	0.886	0.019*	-	-
Neutrophils (x10^3/μL)	–0.157	0.760	–0.371	0.468	0.302	0.134
Fibrozis-4 score	0.071	0.690	–0.143	0.787	0.278	0.169

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.
MELD-Na: model for end-stage liver disease sodium; MDF: Maddrey's discriminant function.

may be due to higher inflammatory activity in AH patients, as noted with high CRP values in that group.

In the AC patients, the mean sHH4 value was the lowest in CPC-A patients, whereas it was the highest in the CPC-B group. In the CPC-A group, as expected, the patients had compensated disease. Although not statistically significant, patients presenting with ascites and varices had higher median sHH4 concentrations. Necrotic hepatocytes that are exposed to ethanol toxicity can act as DAMPs and induce the activation of innate immunity, further aggravating liver injury and subsequent fibrosis, a process that is partly due to histone activation[4,6,9,14]. In our study, increased sHH4 values might indicate susceptibility to hepatic decompensation in AH and AC patients. Nevertheless, the number of patients was low in the CPC groups, and it can be a focus for future research.

Serum D-dimer values are representative of thrombosis, and histones promote thrombosis through TLR activation[8,10,14]. In the present study, the mean D-dimer values of the ALD patients were higher in the AC and AH patients and this result might be a clue for the procoagulant feature of severe ALD.

The Fib-4 index was reported to have a predictive value for hepatocellular carcinoma in alcoholic patients[29]. There was no correlation between the FIB-4 index and sHH4 values in the ALD patients, even in the AC group, who are more prone to developing carcinoma. As a predictive method, it is possible that the Fib-4 index may not exhibit the disease severity in ALD. On the other hand, performing a liver biopsy could be more precise for scoring the fibrosis. However, it is not recommended for ALD patients with significant alcohol use and with no other cause of chronic liver disease because of its complications and ethical considerations[29].

No correlation was reported between the sHH4 values and the MELD-Na values in patients with AH. However, MDF scores, which are a good predictor of mortality, were strongly correlated to sHH4 values[19]. Despite the small sample size of the AH patients, sHH4 values had a predictive value to differentiate AH from ALS. Larger sample-sized cohorts might confirm the diagnostic value of sHH4. In patients with AH, in which time- and dose-dependent continuous ethanol ingestion is the cause, it was noteworthy that the duration of alcohol use was strongly correlated with sHH4 concentrations. The CPC scores did not correlate to sHH4 values in the AC patients, but there were positive correlations between the sHH4 concentrations and MELD-Na values. This may partly be due to the subjective components of the CPC[21]. sHH4 values may be a prognostic marker in patients with AH and AC.

Although the median sHH4 was different among the groups, neutrophil values were similar in the present study. The dichotomous choice of neutrophils is phagocytosis or NETosis, which relates to pro-inflammatory cytokines, chemical stimuli, and antigen size[28]. As noted earlier, histones are known as DAMPs, and cell death in hepatocytes promotes more histone release into circulation from neutrophils[9]. Thus, rather than the counts of neutrophils, the pathogenic significance of neutrophils might exert an influence on liver injury.

Inhibition of NET formation through an anti-citrullinated protein antibody that specifically binds to

citrulline in histones 2A and 4 has been declared to have therapeutic potential in the experimental arthritis model[30]. Li et al. noted that hepatotoxicity and inflammation due to HH3 are reduced after anti-H3 antibody treatment in the HS-induced liver injury model[14]. Abstinence from alcohol is the primary goal in treating alcohol use disorder, and anti-histone treatment might be a candidate modality to abolish liver injury in ALD.

This study is the first clinical trial of the serum HH4 values in patients with ALD and should be considered a preliminary step for future research. The major limitation is the relatively small size of the population, especially in the AH group, as it was a single-centered trial.

Conclusion

In patients with AH and AC, sHH4 concentrations might delineate disease severity. If proven further, larger cohorts may confirm the relationship between the clinical and laboratory traits of disease and sHH4 values in patients with ALD. Diagnostic strategies with the possibility of therapeutic interventions can be developed by identifying new practical and objective biomarkers in ALD.

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Conflicts of interest

The authors declare no conflicts of interest.

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Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ORIGINAL ARTICLE

Using adjuvant radiotherapy for keloid scars: a patient and observer assessment study

Uso de radioterapia adyuvante para cicatrices queloides: estudio de evaluación de paciente y observador

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Abstract

Objective: *The study presented in this article assesses the effectiveness of adjuvant electron beam radiotherapy (RT) in reducing keloid recurrence and improving scar-related outcomes.* **Methods:** *The retrospective study included 32 patients with 36 keloid scars who underwent surgical excision and adjuvant electron beam RT. The patient and observer scar assessment scale patient and observer scar assessment scale was used for all patients after RT.* **Results:** *The results showed no major treatment-related adverse events, and significant correlations were observed between overall score and color, stiffness, thickness, and irregularity of keloid scars. The study highlights that electron beam RT is an effective adjuvant therapy in reducing keloid recurrence and improving scar-related outcomes.* **Conclusion:** *Surgical excision combined with adjuvant RT is an excellent treatment option for keloids, with high patient satisfaction and low recurrence rates.*

Keywords: *Keloid. Radiotherapy. Scar Assessment.*

Resumen

Objetivo: *Evaluar la efectividad de la radioterapia (RT) con electrones en la reducción de la recurrencia de queloides y la mejora de los resultados relacionados con la cicatriz.* **Métodos:** *Estudio retrospectivo con 32 pacientes y 36 queloides que se sometieron a escisión quirúrgica y RT adyuvante. En todos los pacientes se aplicó la Escala de Evaluación de Cicatrices por Paciente y Observador (POSAS, Patient and Observer Scar Assessment Scale) después de la radioterapia.* **Resultados:** *No hubo eventos adversos y se observaron correlaciones significativas entre la puntuación general y el color, la rigidez, el grosor y la irregularidad de las cicatrices queloides. El estudio destaca que la RT es una terapia eficaz para reducir la recurrencia y mejorar los resultados.* **Conclusiones:** *La escisión quirúrgica con RT adyuvante es una excelente opción de tratamiento para queloides, con alta satisfacción del paciente y bajas tasas de recurrencia.*

Palabras clave: *Queloide. Radioterapia. Evaluación de cicatrices.*

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Introduction

Keloid scars are a common benign condition that occurs due to a fibroblast proliferation disease. The primary cause of this disorder is chronic inflammation of the dermis during the wound-healing process, which leads to an abnormal accumulation of collagen[1]. Keloids differ from hypertrophic scars as they typically grow beyond the boundary of the original wound, continuously invading the neighboring healthy skin.

Patients with a higher Fitzpatrick phototype tend to show keloid scars more frequently. The incidence of keloid scars ranges from 4.5% to 16% in individuals with type VI Fitzpatrick skin type compared to only 0.09% in those with type I[2]. Furthermore, genetic conditions, such as Bethlem myopathy and Noonan syndrome can contribute to the development of this condition, as well as a family history of keloid scars[3].

Most studies report a similar distribution of keloid scar incidence between genders. However, Noishiki et al. concluded that before the normal onset age of 15, females have a two-fold incidence compared to men[4]. The pathophysiology of keloids is not yet fully understood. Some studies suggest that hormonal changes during puberty and pregnancy may exacerbate tissue inflammation due to the vasodilatory effect of estrogens on blood vessels. Local risk factors, such as delayed wound healing, depth, and skin tension, also contribute to keloid formation[5]. Inflammation plays a crucial role in the development of keloids by continuously activating fibroblasts. Therefore, any condition that activates these cells in a genetically predisposed individual can increase the probability of a keloid scar[6].

Keloid scars may be asymptomatic or cause itching, pain, erythema, and continuous lump growth, leading to malformations in function and esthetic appearance[7]. Histologically, keloids are characterized by the presence of disarrayed fibrous nodules and hyalinized thick collagen, with a unique appearance in size, pigmentation, and pattern[8].

Although keloid scars are a benign condition, they are often refractory to most treatments and have a high risk of recurrence. Combined treatments are currently used to manage them, aiming to relieve symptoms and improve the appearance of the scar without the risk of recurrence. Several treatments have been described, including the use of medical ointments, compression therapy, silicone gel pads, corticosteroid injections, topical administration of antineoplastic drugs (such as bleomycin, 5-fluorouracyl, and mitomycin), immunotherapy (such as tacrolimus, imiquimod, and interferons), surgical excision, laser treatment, intralesional cryotherapy, and post-operative radiotherapy (PORT)[8]. Radiotherapy (RT) protocol can vary widely, and studies have shown that a dose of 20 Gy in 5 fractions may be associated with a lower recurrence rate of keloid scars[9].

This study aims to assess the effectiveness of PORT in reducing keloid recurrence and improving scar-related outcomes, as evaluated both by patients and by clinicians.

Methods

Our retrospective study included all patients who underwent surgical excision and PORT using electron beams for keloid scars at our institution between May 2016 and September 2022. Demographic and clinical data were collected from the medical charts of the Plastic Surgery and RT Departments.

All patients undertook the patient and observer scar assessment scale (POSAS v 2.0/EN) to evaluate various scar-related parameters, with scores ranging from 1 to 10[10].

The POSAS is composed of two components: the patient scale and the observer scale. Each scale consists of six items that are rated on a numerical scale from 1 to 10, with 1 representing characteristics of normal skin and 10 representing the most severe and imaginable scar. A seventh item in each scale provides an overall assessment of the scar, offering a comprehensive evaluation of the treatment s outcome.

The POSAS observer scale was performed by the same investigator in all patients after RT. A prescription dose of 18 Gy/2 fractions (9 Gy/Fraction) was used, with 6MeV electrons and a 3D conformal technique. PORT treatment started within 24 h after surgery, and the fractions were administered 7 days apart.

Data were analyzed using IBM® SPSS® Statistics, version 28. Quantitative variables were reported either as mean ± standard deviation, when normally distributed, or as median and interquartile range (IQR), when normal distribution was not observed or when considering an ordinal scale variable. For qualitative variables, absolute and relative frequencies were reported for descriptive purposes. Spearman's correlation coefficient was used to determine correlations between POSAS scale parameters, and reliability was assessed through standardized Cronbach's alpha and

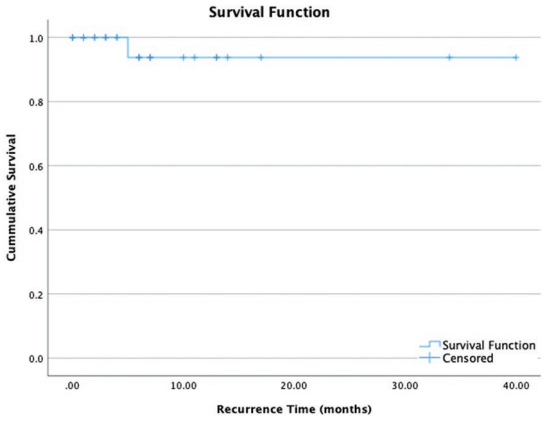

Figure 1. *Recurrence observed, with a 1-year progression-free survival of 93.8%.*

item-by-item sensitivity analysis. Survival was assessed using Kaplan-Meier's method. A type I error of 0.05 was considered for inferential analyses. This study was approved by the Institutional Ethics Board.

Results

Thirty-two patients (13 women and 19 men) with a total of 36 keloid scars were included, with an age at diagnosis of 29.3 ± 13.7 years, ranging from 12 to 69 years. Five patients were African descendants and 27 were Caucasians. The median follow-up period was 3.5 years (IQR: 8.25).

Among the 36 keloid scars, 21 (58.3%) were located in the ear, 5 (13.9%) in the trunk, 2 (7.7%) in the neck, 2 (7.7%) in the breast, and 6 (16.7%) in other locations. No major treatment-related adverse events were reported. Only one recurrence was observed, with a 1-year progression-free survival of 93.8% (Fig. 1). Table 1 summarizes demographic, clinical and treatment related data.

POSAS patient-reported parameters showed a median ranging between 1 and 3, with an overall classification of 2 (IQR of 3). The parameter with the highest score was 'color' (median of 3) and those with the lowest score were 'pain' and 'itching' (median of 1) (Table 2).

Significant correlations were observed between patient overall score and color (ρ = 0.35, p = 0.042), stiffness (ρ = 0.75, p < 0.001), thickness (ρ = 0.74, p < 0.001), and irregularity (ρ = 0.71, p < 0.001).

Reliability for patient-related items was α = 0.796, with stiffness, thickness, and irregularity contributing most on the item-by-item removal sensitivity analysis.

Table 1. Patient, keloids and treatment related characteristics

Variable	p
Age (mean ± SD)	29.3 ± 13.7
Gender, n (%)	
Male	19 (59.4)
Female	13 (40.6)
Race, n (%)	
Black	5 (15.6)
Caucasian	27 (84.4)
Keloid-associated onset event, n (%)	
Iatrogenic/Surgery	21 (58.3)
Piercing	7 (19.4)
Accident	3 (8.3)
Others	5 (13.9)
Locations, n (%)	
Ear	21 (58.3)
Trunk	5 (13.9)
Cervical	2 (7.7)
Breast	2 (7.7)
Others	6 (16.7)
Previous treatments, n (%)	
No	17 (47.2)
Yes	19 (52.8)
POSAS Patient Scale, median (IQR)	
Pain	1 (1)
Itching	1 (1)
Color	3 (2)
Stiffness	2 (3)
Thickness	2 (3)
Irregularity	2 (2)
Overall opinion	2 (3)
POSAS Observer Evaluation Scale, median (IQR)	
Vascularization	1 (1)
Pigmentation	1 (1)
Thickness	1 (2)
Relief	1 (1)
Pliability	1 (1)
Surface Area	1 (1)
Overall opinion	2 (1)
Local recurrence, n (%)	
No	35 (97.2)
Yes	1 (2.8)

IQR: interquartile range; SD: standard deviation.

POSAS observer-reporter parameters showed a median ranging between 1 and 2, with an overall score of 2 (IQR of 1). The parameter with the highest score was color and all other parameters had a score of 1 (Table 2).

All individual observer-assessed parameters showed significant correlations with the observer's overall score (ρ > 0.55, p < 0.001). Reliability for observer-related items was α = 0.898, with thickness, relief, pliability, and area contributing most on the sensitivity analysis. No significant correlation between

Table 2. Patient and observer scar assessment scale value

Parameter	Patient		Observer	
	Median	IQR	Median	IQR
Pain	1	1	1	1
Itching	1	1	1	1
Color	3	2	1	2
Stiffness	2	3	1	1
Thickness	2	3	1	1
Irregularity	2	2	1	1
Opinion	2	3	2	1

patient and observer overall score were observed ($\rho = 0.26$, $p = 0.14$).

Discussion

Keloid scars are a type of benign dermal condition that occurs due to excessive activation of fibroblasts, which leads to the deposition of collagen. This excessive expression of growth factors (such as TGF-ß, PDGF, and VEGF) and cytokines is responsible for the development of keloid scars[11]. Without treatment, the activation cycle progresses over time, leading to an increase in size associated with local symptoms. The definitive treatment for keloid scars involves removing the fibrotic tissue, while breaking the cytokine and collagen cycle.

Numerous treatment options for keloid scars have been proposed, but no treatment is yet defined as gold standard. These options include non-invasive treatments, such as silicone gel pads, medical ointments, and compression therapy, as well as invasive treatments, such as intralesional corticosteroid injections, topical administration of antineoplastic drugs, immunotherapy, cryotherapy, surgical excision, and PORT[8,11].

Surgical excision is a popular option for treating keloids, and it is the first-line treatment for a disabling scar. However, when not combined with other treatments, recurrence rates can range from 45% to 100%[12]. To prevent and treat keloids, present international guidelines recommend intralesional corticosteroid injections as first-line therapy[13]. Although injections can be painful, response rates range from 50% to 100%, and the recurrence rate varies between 9% and 50%[14]. Intralesional injections of 5-fluorouracil have also been found effective in treating hypertrophic, fibrous, and painful scars; however, its use in this setting remains controversial[15].

RT has been used as a treatment option for keloids, and can be an excellent choice when combined with surgical excision. PORT has been found to be the most effective treatment for severe keloid cases, reducing recurrence rates by 55% after 30 months of follow-up[13,15]. However, the mechanism by which RT exerts positive effects in the management of keloids is still uncertain. The possible mechanism is the induction of DNA damage on fibroblasts, preventing collagen synthesis and proliferation, which can inhibit keloid formation. Previous studies have reported good local control rates ranging from 67% to 98% and a recurrence rate of < 10-20% after PORT[16,17].

In our study, we investigated the effectiveness of PORT in the management of keloid scars. The rationale behind utilizing PORT in keloid scars is its potential to achieve better treatment outcomes compared to primary RT alone. This is primarily due to the fact that PORT is administered to a more radiosensitive immature target after surgical excision[18].

The timing of RT following surgical excision is a crucial factor in determining its efficacy, although it remains a topic of debate. Several studies have reported favorable disease control benefits ranging from 10% to 23% when RT is initiated within 24 h after surgery[16,19]. The rationale behind the efficacy of a short time interval is to prevent fibroblast proliferation, which is crucial in keloid formation and recurrence.

In our study, we adhered to the recommended practice of initiating PORT treatment within 24 h after surgery. The radiation fractions were administered at intervals of 7 days apart. Notably, our study observed only one recurrence, resulting in a 1-year progression-free survival rate of 93.8% (Fig. 1). These findings suggest that early initiation of PORT following surgical excision may contribute to improved treatment outcomes and reduced recurrence rates in keloid scars.

Renz et al. reported that lesions treated with 20 Gy had a recurrence rate of 1.6%, compared to 9.6% with < 20 Gy[9]. In this study, a RT protocol of 18 Gy in 2 fractions, 7 days apart was used for all keloids regardless of their location, and the results were considered optimal with only one recurrence observed. The recurrence may have been due to trauma with a ball after the patient's RT sessions, which could explain the final result.

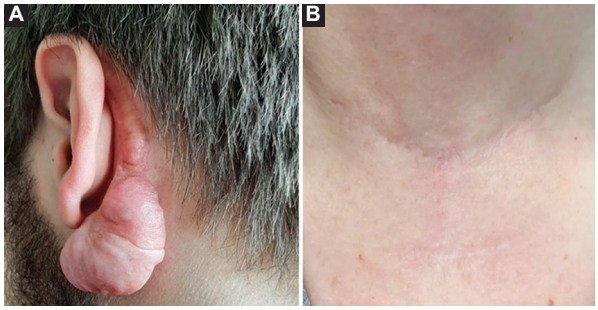

Figure 2. A: *post-auricular keloid before post-operative radiotherapy (PORT).* **B:** *after PORT, 24 months of follow-up.*

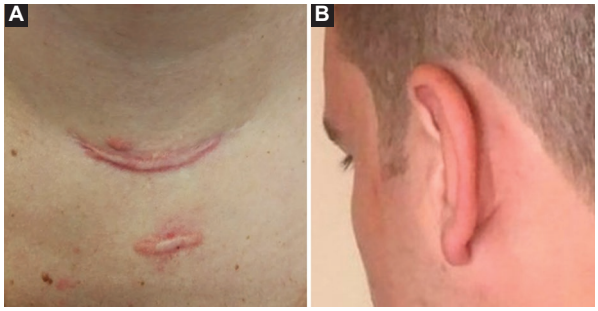

Figure 3. A: *keloid total thyroidectomy, located on the anterior neck keloid, before post-operative radiotherapy (PORT).* **B:** *after PORT, 5 years of follow-up.*

Overall, our study adds to the existing body of evidence supporting the use of PORT in keloid scar management. The favorable results observed (Figs. 2 and 3) in terms of disease control and progression-free survival; further emphasize the importance of timely intervention and adherence to recommended treatment protocols. However, additional research and larger-scale studies are required to confirm these findings and establish optimal guidelines for the timing and administration of PORT in the management of keloid scars. It is also important to consider the potential toxicity associated with PORT. The use of radiation therapy in the treatment of keloid scars can lead to adverse effects, including skin toxicity, such as erythema, desquamation, and hyperpigmentation[20].

Further studies are required to assess the long-term effects of PORT, such as cosmetic outcomes, quality of life, and patient satisfaction. It is also essential to assess the toxicity profile of PORT in keloid treatment and explore strategies to minimize adverse effects while maximizing treatment efficacy by establishing optimal dose schedules and fractionation protocols to balance the potential benefits of treatment with the risk of toxicity.

Conclusion

According to this study, the combination of surgical excision and PORT proves to be a highly effective treatment approach for keloids. It not only demonstrates remarkable patient satisfaction but also significantly reduces the likelihood of recurrence. Nevertheless, it is important to conduct longer-term patient follow-up to comprehensively assess the treatment's effectiveness.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ORIGINAL ARTICLE

Mean arterial pressure difference before and during cardiopulmonary bypass: what should be the ideal mean perfusion pressure?

Diferencia de la presión arterial media antes y durante un bypass cardiopulmonar: ¿cuál debe ser la presión de perfusión media ideal?

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Abstract

Objectives: *The difference between mean arterial pressure (MAP) before cardiopulmonary bypass (CPB) and mean perfusion pressure (MPP) during CPB is thought to be a strong predictor of acute kidney injury (AKI). In this study, we aimed to evaluate whether the difference between MAP and MPP is a good index to predict the development of AKI and what the ideal MPP should be during CPB.* **Methods:** *A total of 296 consecutive patients were included in this retrospective study. MAP-MPP differences of patients who developed AKI and those who did not develop AKI according to standard guidelines and their relation with adverse outcomes were evaluated.* **Results:** *MAP values of patients who did not develop AKI and patients who developed AKI were higher in the group with AKI, 67.60 mmHg versus 64.84 mmHg (p = 0.001). The MAP-MPP difference was 5.07 in the group without AKI and 9.44 in the group with AKI (p = 0.000).* **Conclusion:** *We found that the difference between MAP and MPP is a good index for predicting the development of CPB-related AKI and poor outcomes. We also suggest that patients' preoperative arterial blood pressure should be taken into account for an ideal MPP.*

Keywords: *Cardiopulmonary bypass. Mean arterial pressure. Mean perfusion pressure. Acute kidney injury. Mean arterial pressure-mean perfusion pressure difference.*

Resumen

Objetivo: *La diferencia de la presión arterial media (PAM) antes del bypass cardiopulmonar y la presión de perfusión media (PPM) durante el bypass cardiopulmonar se considera un factor predictivo importante de insuficiencia renal aguda (IRA). En este estudio nos propusimos evaluar si la diferencia entre la PAM y la PPM es un buen índice para predecir el desarrollo de IRA y cuál debe ser la PPM ideal durante el bypass cardiopulmonar.* **Métodos:** *Estudio retrospectivo de 296 pacientes. Se evaluaron las diferencias entre la PAM y la PPM de los pacientes que desarrollaron IRA y los que no la desarrollaron según las directrices estándar y su relación con los resultados adversos.* **Resultados:** *Los valores de PAM de los pacientes que no desarrollaron IRA y de los pacientes que desarrollaron IRA fueron superiores en el grupo con IRA: 67,60 mmHg frente a 64,84 mmHg (p = 0,001). La diferencia PAM-PPM fue de 5.07 en el grupo sin IRA y de 9.44 en el grupo con IRA (p = 0.000).* **Conclusiones:** *Encontramos que la diferencia entre la PAM y la PPM es un buen índice para predecir el desarrollo de IRA*

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relacionada con bypass cardiopulmonar y malos resultados. También sugerimos que debe tenerse en cuenta la presión arterial preoperatoria de los pacientes para obtener una PPM ideal.

Palabras clave: *Bypass cardiopulmonar. Presión arterial media. Presión de perfusión media. Lesión renal aguda. Diferencia PAM-PPM.*

Introduction

A bloodless and immobilized environment is needed in cardiac surgical applications performed with cardiopulmonary bypass (CPB), so a perfusion device (heart–lung machine) is used, which temporarily performs the pump of the heart and the respiratory properties of the lungs. In this process, the patient's heart and lung functions are disabled and perfusion is performed for a certain period of time with the heart–lung machine. Depending on this perfusion process, various changes may occur in metabolism and organs during or after CPB[1-3]. One of these is acute kidney injury (AKI) associated with cardiac surgery[4]. Post-operative renal dysfunction, one of the most common complications of cardiac surgery performed with CPB, is associated with cardiac surgery[5]. AKI is associated with increased morbidity and mortality in the short term. Modifiable and non-modifiable factors may contribute to the development and progression of AKI during cardiac surgery. It is thought that the difference between preoperative mean arterial pressure (MAP) and MAP (mean perfusion pressure [MPP]) during CPB may strongly predict AKI[5-8]. It has also been reported that it may be predictive in many morbidities and mortalities and low MPP is associated with unfavorable outcomes[6,9].

In this study, we aimed to answer two questions. First, we aimed to evaluate whether the difference between MAP and MPP is a good index for predicting the development of AKI. Second, we aimed to find the answer to the question of what should be the ideal MAP during CPB, that is, the ideal perfusion pressure.

Methods

Type of research

This study is a retrospective clinical study.

Ethical dimension of the research

In this study, approval was obtained from the institution and the local ethics committee (Harran University Clinical Research Ethics Committee) (Date: March 18, 2024-Approval no: HRÜ/February 24, 2025). Since the study was retrospective, consent was not required from the participants. The study was conducted in accordance with the principles of the Helsinki Declaration.

Study population

In this retrospective study, patient data of the last 1 year before ethics committee approval were included (March 1, 2023-March 1, 2024). The study included data of 296 consecutive patients who underwent CPB-guided cardiac surgery (coronary artery bypass graft [CABG], aortic or mitral valve replacement) in the Cardiovascular Surgery Clinic of Mehmet Akif Inan Training and Research Hospital after applying the exclusion criteria.

Exclusion and inclusion criteria

Patients with chronic hypertension or hypotension, patients undergoing emergency bypass, patients scheduled for additional cardiac surgery such as aortic aneurysm or dissection, reoperations, chronic renal disease, chronic diabetes, chronic autoimmune disease, systemic inflammatory disease, chronic liver disease, hematological disease, and history of atrial fibrillation were excluded. After applying the exclusion criteria, adult patients aged between 20 and 85 years who underwent CPB-guided cardiac surgery in the last consecutive year were included in the study.

Formation of groups

In this study, patients were grouped and compared in two different ways to analyze the results. First, patients were divided into two groups as those who developed AKI and those who did not develop AKI to evaluate the relationship between the MAP-MPP difference and AKI. Second, the MAP-MPP difference was compared with the presence or absence of other post-operative adverse events and variables.

Data collection method

Data of the patients were obtained from computer, operating theater records, perfusion follow- up records, intensive care unit follow-up cards, and file records.

Procedures and variables used in the research

The data of the patients obtained in this study were recorded and entered into the computer. Descriptive data of the patients (age, gender, height, weight, body surface area [BSA], flow, ejection fraction percentage [EF%], aortic cross clamp time, total perfusion time, and type of surgery performed [CABG numbers, aortic valve, mitral valve, etc.]); MAP value (last arterial pressure measured just before induction of anesthesia) and MPP value (MPP recorded every 20 min while connected to the heart-lung machine), post-operative adverse outcomes; and acute liver failure (ALF), major bleeding, need for ultrafiltration during CPB, need for pacemaker, need for intra-aortic balloon pump (IABP), need for CPB weaning defibrillation, need for CPB weaning inotropes, intensive care unit (ICU) inotrope requirement, intubation time (hours), ICU length of stay (days), hospital stay (days), neurological complications (paralysis or delirium), and mortality rate data were recorded.

CPB (perfusion) method

Standard coronary and valvular heart surgery techniques were performed in all patients. After midline sternotomy in coronary heart surgery patients, arterial cannulation was performed from the ascending aorta and venous cannulation was performed from the right atrium with a single venous cannula (two-stage venous conduit). Left mammary artery graft was used in all cases. Saphenous vein graft was applied to other coronary grafts. Complete revascularization was performed in all patients. In valvular heart surgery patients, in addition to standard surgical techniques, in mitral valve replacements after midline sternotomy, arterial cannulation was performed from the ascending aorta and venous cannulation was performed with two venous cannulae from the vena cava superior and vena cava inferior (bicaval cannulation). In aortic valve replacements, arterial cannulation was performed from the ascending aorta and venous cannulation was performed from the right atrium with a single venous cannula (two-stage cannulation).

Blood flow rates (Flow) of the patients included in the study during extracorporeal circulation were determined non-pulsatile according to their BSAs (2.4 L/min/m^2). Oxygenators and tubing sets suitable for the patient's weight and cannula diameters suitable for BSAs were used. Membrane oxygenator/tubing sets with integrated arterial filter were used. Tubing set venous line diameter was 1/2 and arterial line diameter was 3/8. All patients were subjected to 32°C hypothermia during extracorporeal circulation. Preoperative MAP was adjusted to prevent severe hypotension > 50 mmHg. The overall target MAP was 60 mmHg. Arterial line pressures were maintained between 150 and 180 mmHg on average during CPB. MPP was kept around 60 mmHg. Active clotting time was kept at 480 s and above by providing adequate anticoagulation. As prime solution, 1,200 mL of balanced solution (isolate), 150 mL of 20% mannitol, 5 thousand units of heparin, and 2 g of cefazolin were used. Blood cardioplegia solution was used in all patients. In patients in whom isothermic blood cardioplegia solution (32°C) was used, the initial amount of cardioplegia solution was administered as kg × 15 mL (full dose) and the maintenance dose was administered as half dose (1/2) every 20 min.

AKI Identification

AKI was defined using RIFLE criteria in which three damage layers were defined[10]:

1. Risk-serum creatinine increase × 1.5 or glomerular filtration rate (GFR) > 25%; basal or urine output < 0.5 mL/kg/h × 6 h[10],
2. Damage-serum creatinine increase × 2.0 or GFR > 50% or urine output < 0.5 mL/kg/h × 12 h and[10]
3. Insufficiency-serum creatinine × 3.0, GFR > 75% or serum creatinine level ≥ 4 mg/dL; or urine output < 0.3 mL/kg/h × 24 h or anuria for 12 h[10].

The first 24 h after cardiac surgery was considered in the assessment of AKI. The rationale for this "early" definition was to capture AKI that was most likely attributable to intraoperative factors, such as CPB, rather than factors in the post-operative period. Other adverse outcome events were defined according to standardized guidelines.

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS)® 17.0

Table 1. Demographic and operative characteristics of the study population

Variables	No AKI (mean ± SD or n,%) (n = 206) (%)	AKI (mean ± SD or n,%) (n = 90) (%)	p
Age (year) (mean ± SD)	62.83 ± 9.23	63.98 ± 9.41	0.292
Gender, n (%)			
Male	124, 60.2	55, 61.1	0.882
Female	82, 39.8	35, 38.9	
Surgical type, n (%)			
CABGX1	11, 5.3	4, 4.4	0.207
CABGX2	30, 14.6	16, 4.4	
CABGX3	43, 20.9	22, 24.4	
CABGX4	48, 23.3	22, 24.4	
CABGX5	39, 18.9	18, 20	
MVR	19, 9.2	4, 4.4	
AVR	16, 7.8	4, 4.4	
Height (cm) (mean ± SD)	163.11 ± 9.93	164.48 ± 8.11	0.368
Weight (kg) (mean ± SD)	73.50 ± 15.59	75.20 ± 10.41	0.666
BSA m^2 (mean ± SD)	1.81 ± 0.20	1.84 ± 0.14	0.534
Flow (L) (mean ± SD)	4.33 ± 0.51	4.42 ± 0.34	0.539
Pre-operative EF % (mean ± SD)	46.48 ± 11.67	45.50 ± 10.87	0.902
Cross clamp time (minutes) (mean ± SD)	76.41 ± 36.85	71.93 ± 19.57	0.634
Total perfusion time (minutes) (mean ± SD)	110.46 ± 44.06	107.90 ± 31.07	0.806
Intubation time (hours) (mean ± SD)	7.17 ± 4.82	6.68 ± 2.06	0.200
ICU duration (days) (mean ± SD)	1.98 ± 1.48	2.22 ± 0.95	0.000
Duration of hospital stay (days) (mean ± SD)	5.32 ± 2.99	8.57 ± 3.47	0.000

AKI: acute kidney injury; Mean ± SD: mean ± standard deviation; N: frequency; %: percent; CABG: coronary artery bypass graft; MVR: mitral heart valve; AVR: aortic heart valve; BSA: body surface area; EF: ejection fraction; ICU: intensive care unit.

computer program (version 17.0, SPSS, Chicago, IL, USA). Means and standard deviations were calculated for continuous and ordinal data. Kolmogorov-Smirnov test and Shapiro-Wilk test were used to assess normality distribution. Compera Means (Independent-Samples T Test [Student T Test]) and Nonparametric Test (2 Independent Samples [Mann-Whitney U]) tests were used to evaluate normal and non-normally distributed data, respectively. Receiver-operating characteristic (ROC) curve analysis was performed to test the ability of MAP and MAP-MPP difference to predict the association with AKI outcomes. The areas under the ROC curves (area under the curve) were expressed using the mean 95% confidence interval. Frequency and percentage analyses were performed for nominal data. A $p < 0.05$ was considered statistically significant.

Results

In this retrospective study, data from 296 patients fulfilling the criteria were included. AKI developed in 90 (30.4%) of these patients in the first 24 h postoperatively. As shown in table 1, demographic data including age, gender, surgical procedure, height, weight, BSA, flow, pre-operative EF%, aortic cross clamp time, and total perfusion time were similar in the patient groups who did not develop AKI and those who developed AKI ($p = 0.292$; $p = 0.882$; $p = 0.207$; $p = 0.368$; $p = 0.666$; $p = 0.534$; $p = 0.539$; $p = 0.902$; $p = 0.634$; $p = 0.806$, respectively).

While the extubation times of patients who did not develop AKI and those who developed AKI were similar with a mean of 7.17 h versus 6.68 h ($p = 0.200$), the ICU length of stay was higher in the group who developed AKI with a mean of 1.98 days versus 2.22 days ($p = 0.000$). In addition, the duration of hospital stay was also higher in the group that developed AKI with 8.57 days versus 5.32 days ($p = 0.000$) (Table 1).

MAP values of patients who did not develop AKI and patients who developed AKI were higher in the group with AKI, 67.60 mmHg versus 64.84 mmHg ($p = 0.001$). However, MPP values were similar in both groups ($p = 0.104$). The MAP-MPP difference was 5.07 in the group without AKI and 9.44 in the group with AKI ($p = 0.000$) (Table 2).

ROC curve analysis was performed to predict AKI outcomes in patients undergoing CPB- guided cardiac surgery. According to these results, the test power of

MAP-MPP difference was determined to be 72.8%. At the same time, Roc analysis related to MAP revealed that the test power was 61.6%. In the analysis, the cut-off value for MAP-MPP difference was found to be 2.50 (87.8% sensitivity and 73.8% specificity) ($p < 0.001$). The cut-off value for MAP was 47 (98.9% sensitivity and 99.0% specificity) ($p = 0.002$) (Table 3 and Fig. 1).

The MAP-MPP difference values of patients who did not develop ALF were 6.28, while those who developed ALF were 8.85 and the difference was higher ($p = 0.047$). While the MAP-MPP difference was 6.27 in patients who did not need ultrafiltration during CPB, it was 9.23 in patients who needed ultrafiltration and the difference was higher ($p = 0.027$). While the MAP-MPP difference was 6.26 in patients who did not need pacemaker, it was 9.46 in those who needed pacemaker and the difference was higher ($p = 0.031$). While the MAP-MPP difference was 6.15 in patients who did not need CPB weaning defibrillation, it was 7.84 in patients who needed CPB weaning defibrillation and the difference was higher ($p = 0.045$). While the MAP-MPP difference was 4.87 in patients who did not need CPB weaning inotropes, it was 6.65 in those who needed CPB weaning inotropes and the difference was higher ($p = 0.026$). While the MAP-MPP difference was 5.06 in patients without ICU inotrope requirement, it was 6.76 in patients with ICU Inoptop Requirement, and the difference was higher ($p = 0.030$). There was no significant difference between the rates of major bleeding, need for IABP, neurological complications (stroke or delirium), and mortality and the MAP-MPP difference ($p = 0.959$; $p = 0.822$; $p = 0.577$; $p = 0.467$, respectively) (Table 4).

Table 2. Comparison of MAP, MPP, and MAP-MPP differences of the groups and relationship with AKI

Variables	No AKI (Mean ± SD) (n = 206)	AKI (Mean ± SD) (n = 90)	p
MAP (mmHg) (mean ± SD)	64.84 ± 6.05	67.60 ± 5.88	0.001
MPP (mmHg) (mean ± SD)	60.09 ± 5.95	59.48 ± 4.61	0.104
MAP-MPP difference	5.07 ± 3.36	9.44 ± 5.89	0.000

AKI: acute kidney injury; Mean ± SD: mean ± standard deviation; MAP: mean arterial pressure; MPP: mean perfusion pressure; MAP-MPP difference: difference between mean arterial pressure and mean perfusion pressure.

Table 3. Receiver-operating characteristic curve analysis

Risk factors	AUC (95)	Cut off	p	Sensitivity	Specificity
MAP-MPP difference	0.728 (0.660-0.797)	2.50	0.000	87.8	73.8
MAP	0.616 (0.545-0.686)	47.00	0.002	98.9	99.0

AUC: area under the curve; MAP-MPP difference: difference between mean arterial pressure and mean perfusion pressure; MAP: mean arterial pressure.

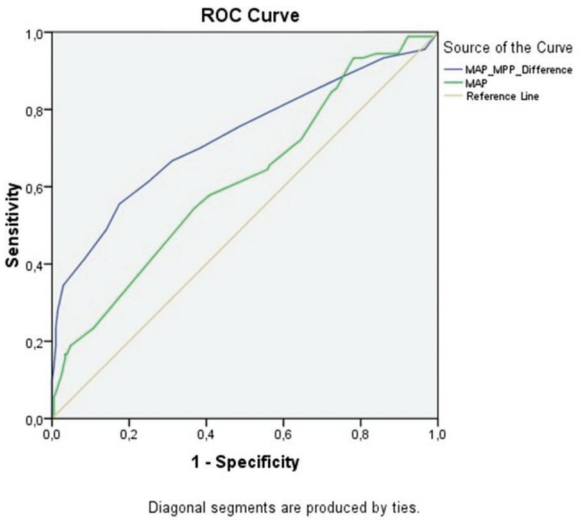

Figure 1. *Receiver-operating characteristic curve analysis of mean arterial pressure (MAP), mean arterial pressure, and mean perfusion pressure difference (MAP-mean perfusion pressure difference) in predicting the outcome of acute kidney injury.*

Discussion

In CPB-guided cardiac surgery, prediction of adverse post-operative outcomes and predetermination of standards during CPB are of great importance. In this study, we determined that the MAP-MPP difference is a strong predictive marker for the development of AKI after CPB and that MPP during CPB indexed to BSA should be evaluated together with pre-operative MAP. We found that indexing MPP only to BSA was associated with unfavorable results. With the data we obtained, we found that it is not sufficient to consider only the BSA in the calculation of the ideal MPP, and the pre-operative MAP of the patient should also be taken into consideration. We also found that high MAP-MPP difference was associated with ALF, need for ultrafiltration during CPB, need for pacemaker, need for CPB weaning defibrillation, need for CPB weaning inotropes, and need for ICU inoptop.

Table 4. The relationship between MAP-MPP difference and other post-operative adverse events

Variables	Negative situation existence	n, %	MAP-MPP difference (mean ± SD)	p
ALF	No	282, 95.3	6.28 ± 4.67	0.047
	Yes	14, 4.7	8.85 ± 5.34	
Major hemorrhage	No	280, 94.6	6.41 ± 4.75	0.959
	Yes	16, 5.4	6.18 ± 4.43	
Ultrafiltration requirement during CPB	No	283, 95.6	6.27 ± 4.68	0.027
	Yes	13, 4.4	9.23 ± 5.05	
Need for pacemaker	No	283, 95.6	6.26 ± 4.63	0.031
	Yes	13, 4.4	9.46 ± 5.83	
Need for IABP	No	291, 98.3	6.40 ± 4.69	0.822
	Yes	5, 1.7	6.20 ± 7.29	
CPB weaning the defibrillation requirement	No	252, 85.1	6.15 ± 4.56	0.045
	Yes	44, 14.9	7.84 ± 5.40	
CPB weaning inotrope requirement	No	41, 13.9	4.87 ± 4.03	0.026
	Yes	255, 86.1	6.65 ± 4.79	
ICU inoptop requirement	No	63, 21.3	5.06 ± 3.37	0.030
	Yes	233, 78.7	6.76 ± 4.97	
Neurological complications	No	294, 99.3	6.40 ± 4.74	0.577
	Yes	2, 0.7	7.00 ± 1.41	
Mortality	No	291, 98.3	6.41 ± 4.70	0.467
	Yes	5, 1.7	6.12 ± 6.15	

Mean ± SD: mean ± standard deviation; N: frequency; %: percent; MAP-MPP difference: difference between mean arterial pressure and mean perfusion pressure; ALF: acute liver failure; CPB: cardiopulmonary bypass; IABP: intra-aortic balloon pump; ICU: intensive care unit.

These findings are among the advantages of this study.

Some studies have also reported that the difference between arterial pressure before CPB and perfusion pressure during CPB is a strong determinant of AKI[7,9]. Kanji et al. performed a prospective observational study on 157 consecutive high-risk patients who underwent cardiac surgery with CPB. In their study, they reported that the difference between MAP and MPP was independently associated with early post-operative AKI in high-risk patients. They also stated that these factors were potentially identifiable and modifiable[7]. Rajagopalan et al. also reported that low MPP indexed to BSA is a strong predictor of poor outcomes after heart transplantation in patients with high pre-transplant venous pressure. They retrospectively studied 250 heart transplant recipients who underwent isolated heart transplantation in a single center between October 2012 and March 2020. They stated that in patients with high right atrial pressure, the acceptable blood pressure during vasodilator therapy should be higher, especially in patients with high BSA[9]. The results of our study are compatible with the literature and the results of this study support our study. Dhanyee et al. investigated the relationship between pre-operative and CPB MAP difference and AKI in cardiac surgery patients undergoing valve surgery. They included 112 patients who underwent valve and valve plus CABG with CPB. In their study, they reported that MAP and CPB flows were not associated with early post-operative AKI. However, they reported that the pressure difference in the early post-operative period independently predicted the development of AKI[11]. In our study, we found that MAP alone predicted AKI. Although other results were

similar, we think that this difference may be due to the patient population.

Molina-Andujar et al. investigated the association of the MAP-MPP difference with a high incidence of AKI in cardiac surgery patients in a randomized controlled trial. At the end of their study, they reported that individualized hemodynamic management did not reduce the incidence of AKI compared with standard treatment. However, they also stated that personalized perfusion pressure management was safe. They also stated that there was no difference between the groups in terms of extrarenal complications. They also reported that there was no difference in terms of mean ICU length of stay and post-operative hospital stay, transfusion requirement, cardiovascular complications, or mortality[12]. In our study, we found that the MAP-MPP difference was related to AKI. We think that the fact that we evaluated AKI in the first 24 h after surgery in our study and they evaluated AKI in the 1st week after cardiac surgery may have an effect on the emergence of different results from the study of Molina-Andujar et al.

It has been reported that higher perfusion pressure and pump flow during CPB are also beneficial for renal function[13]. However, in our study, we think that MPP alone does not mean anything and should be evaluated together with MAP. We think that this difference may be due to the fact that pre-operative MAP was not evaluated in the related study. In a meta-analysis study, it was reported that high blood pressure or low blood pressure target during CPB may cause little or no difference in patient outcomes, including AKI and mortality[14]. In another study in the literature, it was reported that they could not detect systematic differences in terms of pump flow or MPP between patients who developed AKI after CPB and those who did not. They stated that they obtained little information from their observational studies regarding the effect of changes in pump flow and MPP on the risk of AKI. However, they stated that a clinical study to evaluate the effects of more target pump flow and MPP on the risk of AKI is needed[15]. Although these data in the literature emphasize the justification of the results of our study, they suggest that MPP during CPB should be evaluated in a patient-based manner and pre-operative MAP should also be evaluated. In addition, when our study results are compared with the data in the literature, we think that the MAP-MPP difference may be a predictor for the detection of CPB-related AKI and may also suggest a solution for ideal perfusion pressure.

De la Hoz et al. reported that intraoperative hypotension during cardiac surgery was associated with increased risk of AKI, mortality, or stroke[6]. In contrast to this study, Turner et al. reported that low perfusion pressure and low perfusion flow during CPB were not predictors of post-operative AKI, stroke, or death[16]. In our study, we found that lower perfusion pressure compared to pre-operative blood pressure, i.e., MAP-MPP difference, was associated with AKI but not with neurological complications (stroke or delirium) and mortality, and we think that these differences may be due to the patient population and other different surgical dynamics.

In the literature, it is reported that CPB may cause well-defined hemostatic activation and acquired coagulopathy, which may be complicated by life-threatening bleeding. It has been reported that major bleeding may occur in 10% of patients in cardiac surgery, and major bleeding may occur in 20-40% of patients, especially in reoperations or complex surgeries and may require blood transfusion[17]. In our study, we think that the MAP-MPP difference, i.e., perfusion pressures between pre-operative and during CPB, is not related with major bleeding or has no significant effect on major bleeding.

It has been reported that the use of ultrafiltration is beneficial and safe in CPB-related AKI[18]. However, it has also been reported that excessive ultrafiltration volume contributes to the risk of development of AKI[19]. Considering this benefit-harm debate, the presence of the need for ultrafiltration during CPB, which we found to be associated with the MAP-MPP Difference, emphasizes the importance of the MAP-MPP difference and supports our opinion that patient-based perfusion pressure assessment is required.

According to flow rate clinical guidelines, the target blood flow during CPB depends on BSA and temperature and is usually 2.2-2.8 L/min/m^2 under conditions of shallow hypothermia[20]. However, there has always been controversy regarding the optimal setting of flow rates and perfusion pressures. However, in our study, we think that pre-operative blood pressure should be taken into consideration in addition to this approach. There is also a correlation between oxygen delivery during CPB and AKI. Since oxygen delivery can be adjusted with the pump flow rate, it is a modifiable factor. In this case, the targeted perfusion method comes to the fore. Oxygen delivery provided with a higher pump flow rate has also been reported to contribute to a lower incidence of AKI[21]. In this case, individualized blood pressure management should be

introduced to ensure a higher flow rate. It has also been reported that blood pressure management may be associated with end-organ dysfunction after cardiac surgery[22]. Based on this, the ideal perfusion pressure comes to the forefront once again. At this point, the importance of individualized perfusion pressure emerges. Although these data are promising, specialized and skilled perfusionists will always be needed for the management of extracorporeal circulation equipment.

Limitations of the study

The limitations of this study include the retrospective and single-center nature of the study. Although the results of the study are at a generalizable statistical level, we think that multicenter and more patient populations will give more comprehensive results.

Conclusion

The MPP during CPB is managed as standard BSA × 2.4 L. However, in our study, we found that the patient's pre-operative arterial blood pressure should also be taken into account because we found that the difference between MAP and MPP is a good index for predicting the development of CPB-related AKI. Therefore, we think that it is important to prioritize patient-based physiology for ideal MPP. In conclusion, MPP indexed only to BSA, excluding pre-operative arterial pressure, is a strong predictor of poor outcomes after cardiac surgery.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The authors have obtained approval from the Ethics Committee for the analysis of routinely obtained and anonymized clinical data, so informed consent was not necessary. In this study, approval was obtained from the institution and the local ethics committee (Harran University Clinical Research Ethics Committee) (Date: March 18, 2024-Approval no: HRÜ/February 24, 25).

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ORIGINAL ARTICLE

Respiratory monitoring during endoscopic procedures: efficacy and clinical significance of integrated pulmonary index, randomized controlled trial

Monitoreo respiratorio durante procedimientos endoscópicos: eficacia y significado clínico del índice pulmonar integrado, ensayo controlado aleatorizado

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Abstract

Objective: *In sedation practices, respiratory monitoring, particularly for endoscopic procedures, remains crucial due to the risk of respiratory complications. Despite standard monitoring recommendations, significant hypoventilation may occur, leading to adverse events. Integrated pulmonary index® (IPI) offers comprehensive respiratory status assessment, yet its utility in endoscopic sedation remains unclear.* **Methods:** *A prospective, double-blind, randomized controlled trial was conducted at Kartal City Hospital between July and September 2022. Patients aged 18-80 undergoing endoscopic procedures were randomized into standard monitoring (Group 1) or capnography with IPI monitoring (Group 2). Both groups received standard monitoring, whereas Group 2 additionally had capnography monitoring.* **Results:** *Of the 200 patients included, no significant differences were observed in demographics or procedure types between groups. Apnea duration was significantly lower in Group 2 (IPI group). Group 2 showed higher peripheral oxygen saturation (SpO_2) and IPI values at specific intervals compared to Group 1. However, the occurrence of apnea did not significantly differ between groups.* **Conclusion**: *While capnography with IPI monitoring showed advantages in reducing apnea duration and maintaining higher SpO_2 levels, these differences were not clinically significant. Capnography's role as an adjunct to standard monitoring in preventing respiratory complications during endoscopic procedures needs further evaluation, considering its cost implications.*

Keywords: *Sedation. Respiratory monitoring. Endoscopic procedures. Capnography. Integrated pulmonary index. Hypoxemia.*

Resumen

Objetivo: *En las prácticas de sedación, el monitoreo respiratorio, en especial para procedimientos endoscópicos, sigue siendo crucial debido al riesgo de complicaciones respiratorias. A pesar de seguir las recomendaciones de monitoreo estándar, puede ocurrir una hipoventilación significativa, lo que conlleva eventos adversos. El índice pulmonar integrado (IPI) ofrece una evaluación completa del estado respiratorio; sin embargo, su utilidad en la sedación endoscópica sigue siendo incierta.* **Métodos:** *Los pacientes de 18 a 80 años sometidos a procedimientos endoscópicos fueron asignados al azar para recibir monitoreo estándar (grupo 1) o monitoreo de capnografía con IPI (grupo 2). Ambos grupos recibieron monitoreo estándar, pero el grupo 2 tuvo además monitoreo de capnografía.* **Resultados:** *De los 200 pacientes incluidos, la duración de la apnea fue significativamente menor en el grupo 2 (IPI). El grupo 2 mostró valores más altos de SpO_2 y del IPI en intervalos específicos en comparación con el grupo 1. Sin embargo, la ocurrencia de apnea no difirió significativamente entre los grupos.*

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Conclusiones: *Aunque la capnografía con monitoreo IPI mostró ventajas en la reducción de la duración de la apnea y el mantenimiento de niveles más altos de SpO_2, las diferencias no fueron clínicamente significativas. El papel de la capnografía como complemento al monitoreo estándar en la prevención de complicaciones respiratorias durante los procedimientos endoscópicos requiere una evaluación adicional, considerando sus implicaciones económicas.*

Palabras clave: *Sedación. Monitoreo respiratorio. Procedimientos endoscópicos. Capnografía. Índice pulmonar integrado. Hipoxemia.*

Introduction

In sedation practices, monitoring, particularly respiratory monitoring, is crucial for anesthesiologists. Even with moderate sedation targeted for endoscopic procedures, patients remain susceptible to drug-induced airway obstruction, aspiration, respiratory depression, and progression to deep sedation. Current guidelines recommend standard monitoring of heart rate (HR), non-invasive blood pressure (NBP), and peripheral oxygen saturation (SpO_2) during procedures requiring sedation, such as endoscopy. However, significant alveolar hypoventilation can occur even with normal SpO_2 values measured by pulse oximetry. Neither clinical observation nor pulse oximetry can detect hypoventilation, apnea, hypercarbia, and consequent early signs of acidosis, arrhythmias, and myocardial depression[1]. In addition, it has been demonstrated that apnea and respiratory changes often occur well before significant hypoxemia[2,3]. Therefore, early detection of desaturations accelerates interventions to prevent prolonged hypoxemia. Integrated pulmonary index® (IPI), designed for respiratory monitoring and early detection of desaturation, automatically calculates using four components (1: End-tidal carbon dioxide [$EtCO_2$], 2: Respiratory rate [RR], 3: SpO_2, and 4: HR), providing a summary of the patient's ventilation and oxygenation status based on a single parameter. IPI values are scored between 1 and 10. Values between 7 and 10 reflect stable respiratory parameters, whereas values below 7 indicate the need for respiratory intervention[4].

The primary hypothesis of this study is to investigate the necessity of measuring IPI for monitoring respiratory status in patients undergoing endoscopic procedures under deep sedation and to evaluate whether IPI monitoring helps reduce hypoxemic events compared to standard monitoring. The secondary hypothesis involves measures to address complications such as hypotension, bradycardia, and restoring normoventilation.

Methods

This study was planned as a prospective, double-blind, randomized controlled trial and was approved by the local ethics committee (Kartal Dr. Lütfi Kirdar City Hospital Clinical Research Ethics Committee; decision no. 2022/514/228/17). It was designed according to the principles outlined in the Helsinki Declaration and was conducted at Kartal City Hospital between July and September 2022. Written informed consent was obtained from all patients for participation in the study.

The study aimed to include patients aged 18-80 undergoing diagnostic sedation for endoscopic procedures (endoscopy and colonoscopy), of both genders, classified as American Society of Anesthesiologists (ASA) class I-III. Patients with significant cardiopulmonary comorbidities (uncontrolled hypertension, chest pain, advanced heart failure, etc.), ASA 4 and above, propofol allergy, and those aged under 18 or over 80 were excluded from the study. Patients with procedure abandonment, refusal of sedation, or device malfunction were also excluded. The Consolidated Standards of Reporting Trials flow diagram was used for patient enrollment (Fig. 1).

Intervention and anesthesia regimen

After pre-operative evaluation, patients were randomly assigned to either the standard monitoring group (Group 1) or the capnography monitoring group with IPI (Capnostream®/Covidien) (Group 2) according to a randomization table generated by a researcher who did not participate in the study. Each group was assigned a random code of 0 or 1, and these codes were placed in sealed envelopes. Patient monitoring commenced based on the code selected from the envelope. Both groups received standard monitoring, including clinical observation, pulse oximetry, NBP, and HR monitoring. In the IPI group, capnographic data were recorded for the evaluation of patients'

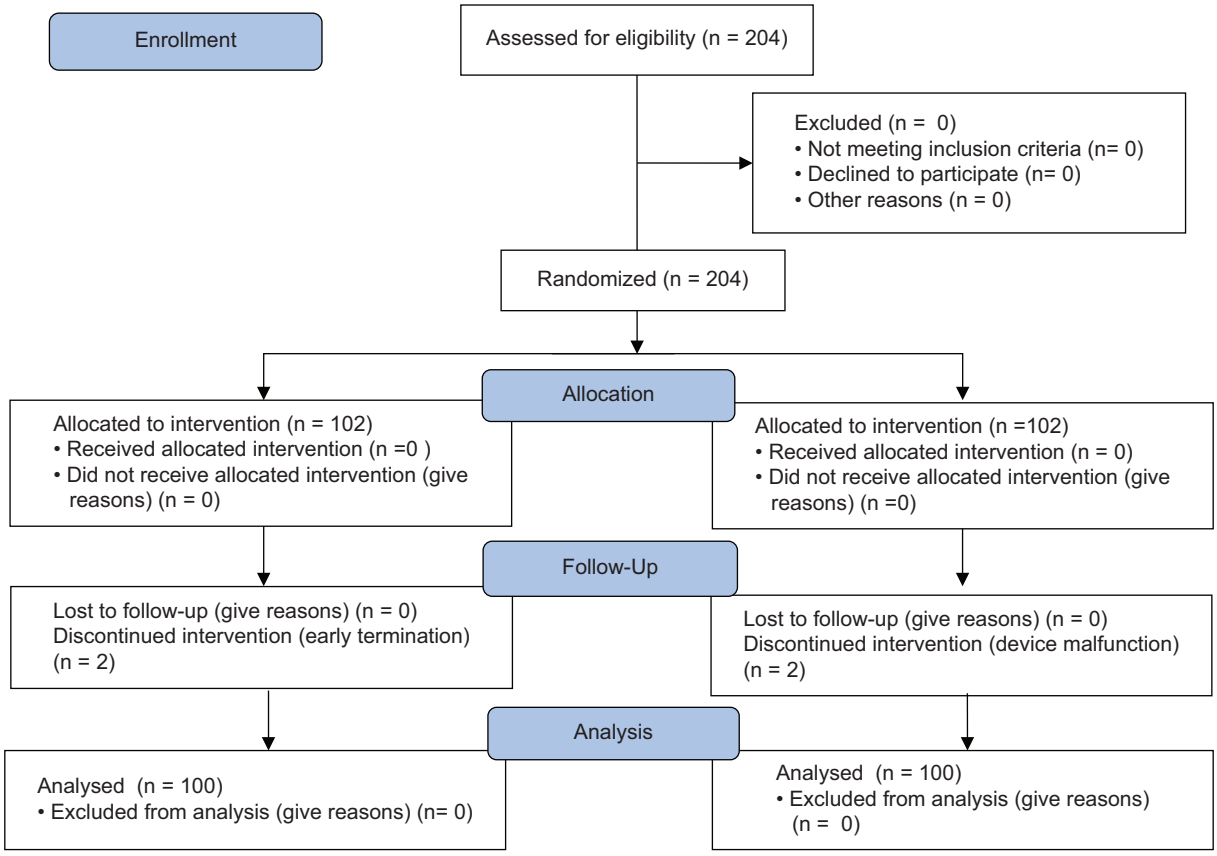

Figure 1. *Consort flow diagram.*

respiratory status. In the standard group, capnographic data with IPI were continuously recorded, but an opaque black cover was placed over the capnography screen. All data were recorded by an independent observer not involved in endoscopic and sedation procedures.

In both groups, a special apparatus with a nasal cannula was used to continuously sample CO_2 content during both inhalation and exhalation (Smart CapnoLine Plus O_2 Microstream; Oridion Medical, Needham, MA, United States) (Fig. 2). The sampling line was connected to the capnography monitor, and sedation was administered with standard doses of anesthetic drugs before starting the endoscopic procedure, with an oxygen flow of 4 L/min. Sedation was achieved using standard doses of anesthetic drugs (2 mg midazolam, 1 mg/kg propofol, and 50 mcg fentanyl). Lower initial doses of propofol were administered to elderly patients or those with pre-existing serious comorbidities. After the initial dose of sedatives, additional doses of propofol (0.5 mg/kg) were administered as needed to achieve an adequate sedation level. The depth of sedation was assessed using the Observer Assessment of Alertness/Sedation Scale (OAA/S), and a target OAA/S score of 3 was maintained. Anesthesia depth was also measured using bispectral index (BIS), and titration of anesthetic drugs was performed to maintain BIS values between 60 and 80.

Patient's vital signs, including HR, NBP, SpO_2, IPI, RR, $EtCO_2$, BIS, and OAA/S scores, were recorded at the start of the procedure, 1 min after induction, and then at 3, 5 min intervals during the procedure, and post-procedure. A capnographic criterion for apnea was defined as the absence of $EtCO_2$ for 10 s. After the completion of the study, a retrospective analysis was performed in Group 1 to determine the number of apneic episodes (> 10 s) detected by capnographic monitoring (including IPI).

In Group 1, interventions for hypoxic events (defined as a decrease in SpO_2 > 5% or hypoventilation < 5 breaths/min) were based on clinical observation and SpO_2 evaluation. In Group 2, interventions were initiated when $EtCO_2$ remained at 0 mmHg for more than 10 s or when IPI was < 7, and the capnography

IPI	Patient Status
10	Normal
8-9	Within normal range
7	Close to normal range; requires attention
5-6	Requires attention and may require intervention
3-4	Requires intervention
1-2	Requires immediate intervention

Figure 2. *Apnea condition, patient status, and integrated pulmonary index values.*

monitor provided both auditory and visual alarms for apnea. Interventions to improve ventilation and/or oxygenation included cessation or reduction of sedatives, increasing oxygen support, stimulating the patient, chin lift, or jaw thrust maneuvers.

At the end of the procedure, in addition to respiratory complications, other complications such as hypotension, bradycardia, bleeding, aspiration, etc., were recorded.

After completion of the endoscopic procedure, monitor connections were discontinued provided that patients could provide a meaningful verbal response and hemodynamic parameters were stable. The Modified Aldrete Score (MAS) was used for post-procedure recovery assessment. Patients with MAS > 9 were transferred to the post-anesthesia care unit. Subsequently, patients were transferred to the recovery room and discharged to the inpatient ward from the endoscopy unit.

Study outcomes and measurement

The primary outcome of the study was defined as a decrease of at least 5% in SpO_2 or apnea lasting ≥ 10 s. The secondary outcome included hypotension (systolic blood pressure < 90 mmHg and/or a decrease of ≥ 25% from baseline), bradycardia (HR ≤ 60), and interventions to restore normoventilation.

The device manufacturer had no role in the design, data collection, data analysis, or manuscript preparation of this study.

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences version 25. The data of the study were summarized using descriptive statistical methods (mean, frequency, and percentage). The Shapiro-Wilk test was used to determine whether continuous variables followed a normal distribution. Fisher's exact test was applied to evaluate independence between categorical variables in 2 × 2 contingency tables. Mann-Whitney test was employed to compare non-normally distributed continuous variable data between the two groups to investigate differences. The Wilcoxon signed-rank test was used for the difference test between dependent groups. A significance level of 0.05 was considered for all tests.

G*Power Version 3.1.9.7 was used for sample size calculation. The mean decrease in oxygen saturation during interventional endoscopy under sedation was calculated as 6 ± 4.5% based on a previous study[5]. To detect a 50% reduction in this parameter with additional IPI assessment, 81 patients were required for each group (using the Mann-Whitney test with a two-sided significance level of $p < 0.01$ and 95% power). A total of 195 patients were planned to be enrolled, considering a 20% dropout rate.

Results

A total of 204 patients were planned to be enrolled and randomized into groups. However, due to premature termination of endoscopic procedures in Group 1 and device malfunction in two patients in Group 2, a total of 200 patients were included in the study, with 100 patients in each group. Of these patients, 109 (54.5%) were female, and 91 (45.5%) were male. The mean age of all patients was 54.98 years. A total of 62 patients (31%) underwent gastroscopy, 76 patients (38%) underwent colonoscopy, and 62 patients (31%) underwent both gastroscopy and colonoscopy.

Statistical analysis between groups showed no significant differences in age, gender, body mass index, chronic disease, ASA score, or type of procedure ($p > 0.05$). There were also no significant differences between groups in the need for additional propofol dose and intervention requirement ($p > 0.05$) (Table 1).

Since there were 100 patients in each group, the frequency given in table 1 is also the percentage. Among the variables, only the duration of apnea was statistically significant, with a $p < 0.05$. The duration

of apnea (seconds) in Group 2 was significantly lower than in Group 1 (p: 0.000).

The mean SpO_2 values at 3 and 5 min in Group 2 were significantly higher than those in Group 1 (p: 0.017, p: 0.050). Similarly, the mean IPI value at 3 min in Group 2 was significantly higher than that in Group 1 (p: 0.00) (Table 2).

There was no statistically significant difference in HR and NBP values between groups ($p > 0.05$) (Table 2).

Although the percentage of patients with apnea duration of 10 s or more was higher in Group 1 (52.3%) compared to Group 2 (47.7%), this difference was not statistically significant (p: 0.229) (Table 3).

There is no difference between groups in terms of the average decreased percentage of SpO_2 after induction compared to baseline. However, regarding the percentage decreases after baseline at 3 and 5 min, the average SpO_2 decrease percentage in Group 1 is significantly higher than that in Group 2 ($p < 0.05$) (Table 4).

There is a significant difference between the groups in terms of the number of patients with decreases in SpO_2 between baseline and 3 min, categorized as < 5% or 5% or more. There have been higher rates of 5% or higher SpO_2 decreases in Group 1 (p:0.000) (Table 5).

Spearman Rho correlation coefficients between SpO_2 and IPI differences at baseline, 3 min, and 5 min are provided. It is understood that the decrease in IPI compared to SpO_2 is considerably higher from baseline to induction, the difference closes after 3 min, and at 5 min, the decrease in SpO_2 exceeds that in IPI (Fig. 3).

Primer outcome measures

Hypoxemia (SpO_2 decrease percentage) occurred more frequently in Group 1 compared to Group 2. Apnea was detected in 62 patients in Group 2. However, hypoxemia developed in only 20 of these patients.

Secondary outcome measures

There was no significant difference between the two groups in terms of intervention requirement (p: 0.752), hypotension, and bradycardia. No serious adverse effects, such as orotracheal intubation, cardiopulmonary resuscitation, or death, were observed in either group.

Table 1. Demographic data and test results for differences between variables

	Group		
Variables	**IPI (Group 2)**	**SM (Group 1)**	**p**
Age, median (SD)	55.54 ± 1.23	54.43 ± 1.19	0.463[1]
Gender			
Female	55	54	
Male	45	46	
BMI (SD)	23.86 ± 3.5	23.76 ± 3.32	0.823[1]
Chronic disease			
+	16	14	0.575[2]
–	84	86	
ASA score			
1	18	17	
2	64	63	
3	18	20	
Type of procedure			
G	30	28	0.938[3]
C	38	40	
G + C	32	32	
Additional propofol			
–	40	39	0.280[2]
+	60	61	
Intervention requirement			
–	40	40	0.752[3]
+	60	60	
Duration of apnea, sec, mean (SD)	18.26 ± 1.15	28.16 ± 1.59	0.000[3]

[1]Mann–Whitney.
[2]Fisher's exact test.
[3]χ^2 test.
IPI: integrated pulmonary index; SM: standard monitoring; BMI: body mass index; SD: standard deviation; G: gastroscopy; C: colonoscopy.

Discussion

The use of capnography is a standard part of monitoring in patients undergoing general anesthesia. However, its role is being investigated in procedures requiring sedation. In this study, we aimed to demonstrate whether early interventions with capnography monitoring could be beneficial in preventing sedation-related hypoxemia. We found that there was no significant difference between the two groups in terms of the occurrence of apnea and interventions. However, capnography was superior to standard pulse oximetry in shortening the apnea duration.

The similarity in the average reduction of oxygen saturation between the IPI group and the control group led to the conclusion that there is no discernible

Table 2. Comparison of hemodynamic parameters between groups

Group	Basal SpO_2	1st min SpO_2	3rd min SpO_2	5th min SpO_2	Post-proc SpO_2	Basal IPI	1st min IPI	3rd min IPI	5th min IPI	Post-proc IPI
IPI (Group 2)										
Mean	98.74	96.39	96.63	96.71	97.04	9.59	6.21	7.27	7.64	8.88
SD	0.16	0.26	0.23	0.23	0.19	0.05	0.19	0.15	0.14	0.05
SM (Group 1)										
Mean	98.84	96.14	95.60	95.94	97.00	9.59	6.02	6.43	7.57	8.87
SD	0.17	0.26	0.28	0.25	0.19	0.05	0.19	0.17	0.14	0.06
p	0.891	0.507	**0.017**	**0.050**	0.874	1.000	0.425	**0.000**	0.711	0.986
Group	**Basal HR**	**1st min HR**	**3rd min HR**	**5th min HR**	**Post-proc HR**	**Basal NBP**	**1st min NBP**	**3rd min NBP**	**5th min NBP**	**Post-proc NBP**
IPI (Group 2)										
Mean	79.46	79.05	78.96	78.16	93.96	93.07	91.97	91.34	90.45	90.01
SD	1.40	1.31	1.37	1.31	1.69	1.66	1.62	1.42	1.27	0.22
SM (Group 1)										
Mean	79.48	79.03	78.94	78.13	93.65	92.42	91.62	90.93	90.25	90.01
SD	1.39	1.31	1.37	1.31	1.66	1.58	1.59	1.37	1.24	0.22
p	0.798	0.984	0.982	0.973	0.972	0.886	0.811	0.873	0.906	0.960

IPI: integrated pulmonary index; SM: standard monitoring; SD: standard deviation; post-proc: post-procedure; HR: heart rate; NBP: non-invasive blood pressure; Mann–Whitney U test.

Table 3. Apnea status between groups

	Group		
Apnea status	**IPI (Group 2)**	**SM (Group 1)**	**p**
No apnea observed or apnea duration < 10 s			
n	38	32	0.229
Percentage	54.3	45.7	
10 s or more apnea duration			
n	62	68	
Percentage	47.7	52.3	

IPI: integrated pulmonary index; SM: standard monitoring. Fisher's exact test.

Table 4. Difference in mean SpO_2 decrease percentages between groups compared to baseline

	Group		
SpO2 decrease interval	**IPI (Group 2)**	**SM (Group 1)**	**p**
Mean percentage decrease in SpO_2 from baseline to 1 min	2.38	2.63	0.408
Mean percentage decrease in SpO_2 from baseline to 3 min	2.14	3.18	0.017
Mean percentage decrease in SpO_2 from baseline to 5 min	2.06	2.84	0.050

IPI: integrated pulmonary index; SM: standard monitoring.

advantage in utilizing the IPI index for interventional endoscopic procedures. However, the use of the IPI did not demonstrate any additional clinical benefit. Our study did not detect significant complications such as the need for mechanical ventilation, cardiopulmonary resuscitation, or death. Still, it was evaluated based on the results of larger studies utilizing capnography[6,7].

European guidelines recommend additional capnography monitoring for prolonged procedures or deep sedation[8,9]. Various studies have shown that supplemental capnography monitoring in addition to standard monitoring aids in preventing desaturation during standard endoscopic procedures such as esophagogastroduodenoscopy and colonoscopy[6,10,11]. Garah et al. found in a study conducted on 109 pediatric patients undergoing upper endoscopy under sedation that the IPI alerted to all apneic episodes and hypoxic events, whereas pulse oximetry only detected hypoxic episodes[4]. However, these results contradict our findings. No difference in the occurrence of apnea was found between the two groups.

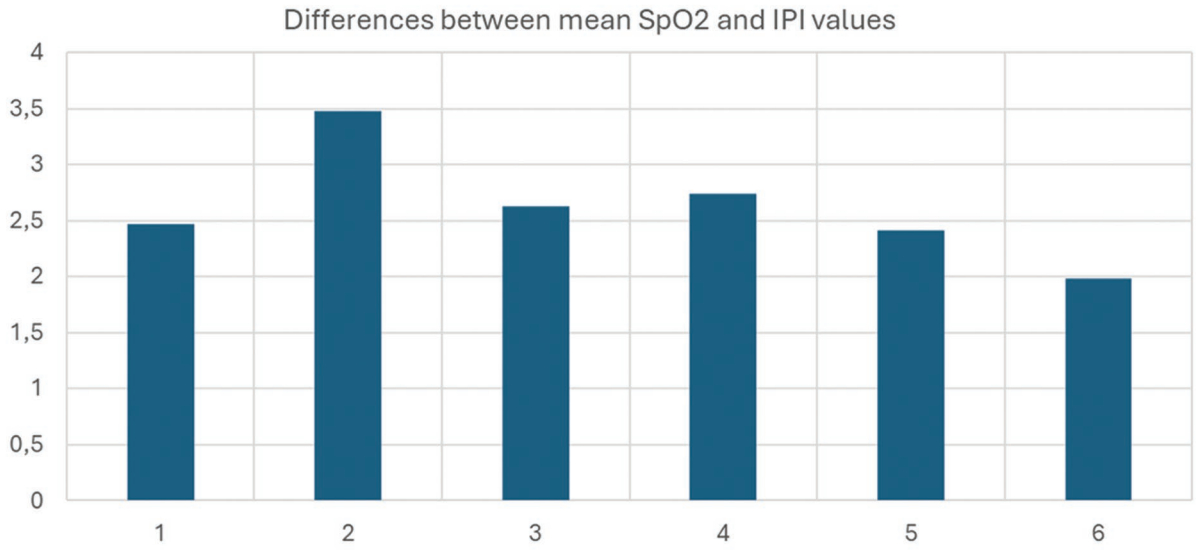

Figure 3. *Spearman rho correlation coefficients between SpO_2 and integrated pulmonary index values. (1) IPI baseline to 5th min, (2) SpO_2 baseline to 5th min, (3) IPI baseline to 3rd min, (4) SpO_2 baseline to 3rd min, (5) IPI baseline to 1st min, and (6) SpO_2 baseline to 1st min. IPI: integrated pulmonary index.*

Table 5. Differences in SpO_2 decrease percentages between groups according to period

Group	Rate of decrease in SpO_2 from baseline to induction		p
	< 5%	5% or more	
IPI (n)	89	11	0.414
SM (n)	87	13	
Group	**Rate of decrease in SpO_2 from baseline to 3 min**		
	< 5%	**5% or more**	
IPI (n)	98	2	0.000
SM (n)	83	17	
Group	**Rate of decrease in SpO_2 from baseline to 5 min**		
	< 5%	**5% or more**	
IPI (n)	93	7	0.083
SM (n)	86	14	

IPI: integrated pulmonary index; SM: standard monitoring.

Berkenstadt et al. observed 113 events requiring attention in patients undergoing moderate sedation during colonoscopy, but they noted only limited concordance between irregular respiratory parameters and the IPI[12]. Riphaus et al. compared the control group with the IPI group in a study involving 170 endoscopy patients undergoing deep sedation. They noted that IPI did not provide a significant clinical advantage. They noted no significant difference in the average maximum decrease in SpO_2 and hypoxic events between the groups but found that the IPI was effective in reducing the frequency of apneic episodes[8]. In our study, apnea was detected in 62 patients in the IPI group, but hypoxemia developed in only 20 of these patients. In addition, a significant correlation was found only between SpO_2 decrease at 3 min and the IPI, with no differences observed at other time intervals.

Mehta et al. and Qadeer et al. defined respiratory disturbances as a reduction of > 75% in the amplitude of respiratory waves lasting ≥ 5 s in their studies involving patients undergoing endoscopic retrograde cholangiopancreatography and endoscopic ultrasound[13,14]. In both studies, there was no significant difference in terms of respiratory disturbances between the control group and the capnography group. Furthermore, no association was observed between respiratory disorders and hypoxic events, which our study also supports.

In our study, contrary to a study by Vargo et al. that showed the inadequacy of clinical observation for assessing ventilation, abnormal ventilation and apnea observed clinically in the standard monitoring group were similar to those in the IPI group[2]. Only the duration of apnea differed between the two groups (IPI: 18.26 s;

SI: 28.16 s). However, this difference did not yield a clinically significant result. There were also no significant differences in interventions between the two groups.

In addition, while apnea was detected in 62% of patients in the IPI group, oxygen desaturation occurred in only 20% of these patients. In the literature, the main mechanism of hypoxic events is described as a series of events starting with apnea and leading to hypoxemia[15,16]. However, when additional oxygen is present and consequently an increase in basal oxygen partial pressure, only some impaired ventilation episodes result in hypoxemia. While these episodes are detected by the IPI, pulse oximetry measures hemoglobin oxygenation and may not adequately reflect the patient's ventilation status. Furthermore, inadequate respiratory activity or complete absence of chest movement, signs of apnea, are often not detected during standard monitoring. However, measures can be taken when pulse oximetry warns of a subsequent decrease in SpO_2 due to hypoxemia. Peveling-Oberhag et al. found that blind capnographic measurements in the standard monitoring group had an average delay of more than a minute in detecting apnea and hypoxemia, and early application of interventions (including jaw lift/chin thrust maneuvers) in the capnography group led to a decrease in hypoxemic events[17]. However, neither our study nor any previous study reports that additional capnography monitoring during endoscopic sedation prevents side effects such as death or serious morbidity[18]. In our study, neither serious side effects nor deaths occurred. In addition, there is a significant increase in costs associated with increased capnographic monitoring. However, no controlled trial has demonstrated a mortality benefit, even with the widespread use of pulse oximetry. In our study, no life-threatening conditions requiring intervention emerged, and there was no notable difference in the need for intervention between the groups.

Berkenstadt et al. found no difference in RR, SpO_2, and HR between groups when they grouped patients according to IPI values. However, high $ETCO_2$ values were obtained in the high IPI group[12]. In our study, there was no difference in hemodynamic parameters between the two groups.

Our study has various limitations. Hypoxemic events were observed before clinically significant results emerged, and we did not have any patients with severe hypoxemia ($SpO_2 < 85\%$). However, it is unclear whether severe hypoxemia can serve as a sufficient cause for sedation-related complications. In addition, routine oxygen supplementation may lead to high SpO_2 measurements, masking hypoventilation, but its use is recommended during endoscopy due to guidelines advocating its routine use.

Conclusion

Although capnography appears to reduce the number and duration of hypoxic events during endoscopic procedures, the results are not clinically significant. Capnography can significantly contribute to the prevention of respiratory complications; however, it should be noted that the presence of a normal capnogram does not guarantee adequate oxygenation. Therefore, while capnography cannot replace standard patient monitoring and clinical observation, it can be accepted as a useful adjunct. However, due to limited clinical benefits and increased costs associated with the use of capnography, we do not consider capnography necessary during endoscopic procedures.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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ARTÍCULO DE REVISIÓN

Tratamiento quirúrgico del linfedema secundario al cáncer de mama: revisión actualizada

Surgical treatment of breast cancer-related lymphedema: an updated review

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Resumen

El linfedema secundario al cáncer de mama es una complicación frecuente tras la disección ganglionar y la radioterapia, con un impacto negativo en la calidad de vida de las pacientes. El objetivo de esta revisión es presentar los avances en el tratamiento quirúrgico del linfedema relacionado con cáncer de mama, destacando las técnicas más recientes y su papel en la prevención y el manejo en distintas etapas de la enfermedad. Se llevó a cabo una revisión exhaustiva de la literatura disponible en bases de datos internacionales (PubMed, Scopus, Web of Science), enfocada en estudios originales, revisiones sistemáticas y metaanálisis que abordaran las técnicas quirúrgicas para el manejo del linfedema secundario al cáncer de mama. En la revisión se incluyeron artículos publicados en los últimos 10 años. Se describen distintas estrategias quirúrgicas, como la anastomosis linfático-venosa, la transferencia de ganglios linfáticos vascularizados, la reconstrucción linfática inmediata y la liposucción. La elección de la técnica depende de la etapa clínica del linfedema, de la disponibilidad de equipos y de la experiencia en microcirugía. La evidencia actual indica que el tratamiento quirúrgico temprano puede disminuir la progresión del linfedema, mejorar la calidad de vida y reducir el número de episodios infecciosos. La cirugía linfática ofrece resultados prometedores en la prevención y el tratamiento del linfedema secundario al cáncer de mama. Aunque se han logrado avances significativos —incluida la supermicrocirugía y los procedimientos de prevención inmediata— persiste la necesidad de ensayos clínicos aleatorizados que aporten mayor solidez a las recomendaciones. Un abordaje interdisciplinario y la selección adecuada de las pacientes son cruciales para optimizar los resultados.

Palabras clave: *Linfedema. Cáncer de mama. Reconstrucción linfática. Anastomosis linfático-venosa. Supermicrocirugía.*

Abstract

Breast cancer-related lymphedema is a common complication after axillary lymph node dissection and radiotherapy, significantly affecting patients' quality of life. This review aims to outline the latest surgical advances for his management, highlighting current techniques and their role in prevention and treatment at various stages of the disease. An extensive literature review was performed in major databases (PubMed, Scopus, Web of Science), focusing on original studies, systematic reviews, and meta-analyses addressing surgical techniques for the management of breast cancer-related lymphedema. Articles published in the last 10 years were included. Several surgical approaches are described, including lymphaticovenous anastomosis, vascularized

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lymph node transfer, immediate lymphatic reconstruction, and liposuction. The choice of procedure depends on the clinical stage of lymphedema, the availability of expertise and micro-surgical equipment. Current evidence shows that early surgical management can reduce disease progression, improve quality of life, and lower the incidence of infectious episodes. Lymphatic surgery offers promising outcomes in both prevention and treatment of breast cancer-related lymphedema. Although significant advances —such as supermicrosurgery and immediate prophylactic procedures— have been made, there is still a need for randomized clinical trials to strengthen clinical recommendations. An interdisciplinary approach and careful patient selection are critical to optimizing outcomes.

Keywords: *Lymphedema. Breast cancer. Lymphatic reconstruction. Lymphaticovenous anastomosis. Supermicrosurgery.*

Introducción

El linfedema es una condición clínica compleja caracterizada por una insuficiencia en el sistema linfático, que se manifiesta como una acumulación anormal de líquido rico en proteínas en el espacio intersticial de las extremidades superiores e inferiores.

El linfedema asociado al cáncer de mama ocurre después de la disrupción del sistema linfático por disección axilar ganglionar, radiación o terapia con taxanos. Se estima que afecta a una de cada cinco pacientes con cáncer de mama, lo que provoca un impacto significativo en las esferas psicosocial, emocional y económica[1,2]. Esta disfunción en el transporte linfático generalmente provoca inflamación crónica de la extremidad afectada posterior a una fibrosis progresiva, hipertrofia del tejido graso, daño estructural de los vasos linfáticos y una afectación significativa en la calidad de vida de las pacientes[3-5]. El manejo del linfedema requiere un enfoque interdisciplinario que involucra la colaboración de diversas especialidades, como cirugía plástica, oncología quirúrgica, cirugía de mama, radiología y fisioterapia especializada en linfedema, entre otras[6]. Cada disciplina aporta su experiencia para optimizar los resultados en las pacientes.

Entre los factores de riesgo, la obesidad ha sido identificada como un determinante significativo en el desarrollo de linfedema posoperatorio en pacientes con cáncer de mama, lo que resalta la importancia de implementar estrategias dirigidas al control y la pérdida de peso en aquellas con riesgo de desarrollar linfedema secundario[6,7].

Aunque el linfedema no tiene cura, su manejo conservador, basado en la terapia descongestiva completa o con drenaje linfático manual, además de compresión, ejercicio y cuidado de la piel, representa el método de referencia para prevenirlo y mejorar la condición local en etapas tempranas.

El tratamiento quirúrgico se reserva para casos específicos, principalmente cuando el tratamiento conservador fracasa. Sus objetivos incluyen prevenir la progresión de la condición, reducir la frecuencia de episodios infecciosos o inflamatorios, y mejorar tanto la estética como la función de las áreas afectadas.

La cirugía linfática desempeña un papel cada vez más relevante en pacientes con cáncer de mama, interviniendo tanto en la prevención como en el tratamiento en etapas tempranas y avanzadas. A pesar de los avances significativos en esta área, incluyendo el desarrollo de técnicas de supermicrocirugía y un enfoque creciente en la prevención del linfedema en los últimos años, la evidencia de alta calidad sigue siendo limitada debido a la escasez de estudios clínicos aleatorizados[8,9].

Estadificación del linfedema

El sistema de estadificación de la International Society of Lymphology se basa en la exploración física, las manifestaciones clínicas, las mediciones y los estudios de imagen[8,9] (Tabla 1). En algunos centros se emplean sistemas modificados que combinan la gravedad de los síntomas, las diferencias circunferenciales y los hallazgos de estudios de imagen, categorizando el linfedema en grados de leve a muy grave[10].

La bioimpedancia es una técnica no invasiva que se utiliza para medir la resistencia eléctrica del cuerpo al paso de una corriente eléctrica de baja intensidad. En el contexto del diagnóstico de linfedema, la bioimpedancia se emplea para evaluar y monitorear la acumulación de líquido en los tejidos y los cambios en la composición corporal. La corriente eléctrica pasa a través del cuerpo, y su resistencia varía según el contenido de agua y electrolitos en los tejidos. El líquido linfático acumulado en el linfedema afecta esta resistencia, ya que los tejidos con mayor contenido de agua conducen mejor la electricidad. La técnica puede detectar diferencias entre extremidades afectadas y no afectadas, y monitorear los cambios en una extremidad a lo largo del tiempo. Tiene la ventaja de poder identificar el linfedema en etapas iniciales, antes de que los datos visibles

Tabla 1. Estadificación clínica del linfedema de la International Society of Lymphology

Etapa	Descripción
Etapa 0 (latente)	Linfedema subclínico, sin edema, pero con evidencia de función linfática afectada. Puede existir meses o años antes de que el edema ocurra
Etapa 1	Edema con fóvea, reversible. No hay fibrosis palpable
Etapa 2	Edema con fóvea que no responde a elevación
Etapa 2b	Edema sin fóvea secundario a fibrosis grave
Etapa 3 (elefantiasis linfostática)	Hinchazón grave, fibrosis y engrosamiento de tejidos. Acantosis (hiperpigmentación), hiperqueratosis y papilomatosis (crecimiento de verrugas)

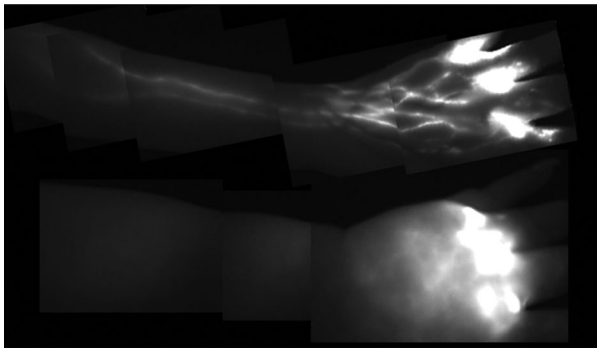

Figura 1. *Linfografía con verde de indocianina. En la imagen superior se ve una extremidad normal con canales linfáticos lineales. En la imagen inferior se ve una extremidad con linfedema, con reflujo dérmico y estancamiento del contraste.*

aparezcan; además, no es invasiva, los resultados se obtienen en pocos minutos y permite medir cambios precisos en el volumen de líquidos[11-13].

Actualmente, el uso de la linfografía con verde de indocianina ha permitido ampliar el panorama de la fisiología linfática, permitiendo visualizar los canales linfáticos permeables *in vivo* (Fig. 1).

Los estudios sugieren el uso del sistema de clasificación del MD Anderson Cancer Center (MDACC) (Tabla 2), que permite orientar la toma de decisiones sobre el tratamiento, el cual básicamente consiste en realizar anastomosis linfático-venosa cuando hay canales visibles[14-16].

Además de la clasificación MDACC, en diversas instituciones se han implementado algoritmos de tratamiento que integran un enfoque multidisciplinario para la prevención y el manejo del linfedema asociado a cáncer de mama. Cho et al.[17] han propuesto un enfoque algorítmico basado en la estadificación del MDACC y la gravedad del linfedema, priorizando el uso de anastomosis linfático-venosa en estadios tempranos y la transferencia de ganglios linfáticos vascularizados en estadios avanzados. Este algoritmo enfatiza la vigilancia temprana, la colaboración interdisciplinaria y el uso de reconstrucción linfática inmediata (abordaje microquirúrgico preventivo para la restauración linfática [LYMPHA, *lymphatic microsurgical preventive healing approach*], *bypass* linfático-venoso profiláctico y reconstrucción linfática inmediata inguinal) en pacientes sometidas a disección de ganglios linfáticos axilares, con seguimiento posoperatorio estricto a 24 meses[17].

Existen otras técnicas adicionales, como la linforresonancia y la linfangiografía por resonancia magnética, descrita por Pons et al.[18], que consiste en la inyección intradérmica de gadolinio y la obtención de una imagen por resonancia que permite identificar canales profundos, una visión e imagen integral de la anatomía linfática y la posibilidad de localizar canales para su próxima derivación[18,19] (Fig. 2).

Técnicas quirúrgicas en el tratamiento actual del linfedema

El tratamiento quirúrgico del linfedema se divide en dos enfoques principales:

- Terapia fisiológica: se centra en la restauración o reconstrucción del drenaje linfático.
- Terapia escisional: incluye procedimientos como la liposucción y técnicas resectivas con injerto de piel, como la técnica de Charles.

Reconstrucción linfática inmediata

Se atribuye a Boccardo la técnica LYMPHA, publicada en 2011, la cual marcó el inicio del concepto de reconstrucción linfática inmediata. Su objetivo es realizar una derivación linfático-venosa en la misma cirugía en la que el flujo linfático es interrumpido por una disección de ganglios linfáticos axilares[20-22].

Inicialmente, la técnica consistía en la introducción de varios vasos linfáticos «en bloque» dentro de una vena afluente axilar, pero con el tiempo han surgido variantes que han refinado el procedimiento, incluyendo la eliminación de grasa en la anastomosis, ya que su presencia se ha asociado con una mayor tasa de falla. También se han incorporado mejoras como la técnica «en pulpo»[23] y refinamientos como el uso de un injerto venoso interposicional para evitar tensión en la anastomosis[24] (Fig. 3).

Tabla 2. Sistema de clasificación del linfedema del MD Anderson Cancer Center

Estadio	Recomendación de manejo
Estadio 0 Sin reflujo dérmico	Vigilancia activa con monitoreo regular mediante bioimpedancia y linfografía con verde de indocianina Medidas preventivas, como fisioterapia y seguimiento por especialistas en linfedema Consideración de reconstrucción linfática inmediata (reconstrucción linfática inmediata inguinal, LYMPHA, *bypass* linfático-venoso profiláctico) en pacientes sometidas a disección ganglionar axilar
Estadio 1 Vasos linfáticos permeables y reflujo dérmico mínimo	Terapia descongestiva completa que incluye drenaje linfático manual, compresión, ejercicios y cuidado de la piel Anastomosis linfático-venosa recomendada, ya que aún existen vasos linfáticos funcionales
Estadio 2 Número moderado de vasos linfáticos permeables y reflujo dérmico segmentario	Anastomosis linfático-venosa viable si los vasos linfáticos funcionales son identificados mediante verde de indocianina Terapia descongestiva completa
Estadio 3 Pocos vasos linfáticos permeables con reflujo dérmico extenso	Combinación de anastomosis linfático-venosa y transferencia de ganglios linfáticos vascularizados en casos seleccionados Terapia de compresión y fisioterapia como soporte posoperatorio
Estadio 4 Reflujo dérmico que afecta la mano	La transferencia de ganglios linfáticos vascularizados es la principal opción quirúrgica, ya que la fibrosis impide la realización de anastomosis linfático-venosa Terapia descongestiva completa intensificada con medidas de compresión avanzada y drenaje linfático manual
Estadio 5 El verde de indocianina no se desplaza proximalmente desde el sitio de inyección	Transferencia de ganglios linfáticos vascularizados con resección de tejido fibrótico si es necesario Liposucción en casos de linfedema avanzado con hipertrofia grasa significativa Terapia descongestiva completa como tratamiento complementario para mantenimiento de la reducción del volumen

LYMPHA: *lymphatic microsurgical preventive healing approach* (abordaje microquirúrgico preventivo para la restauración linfática).

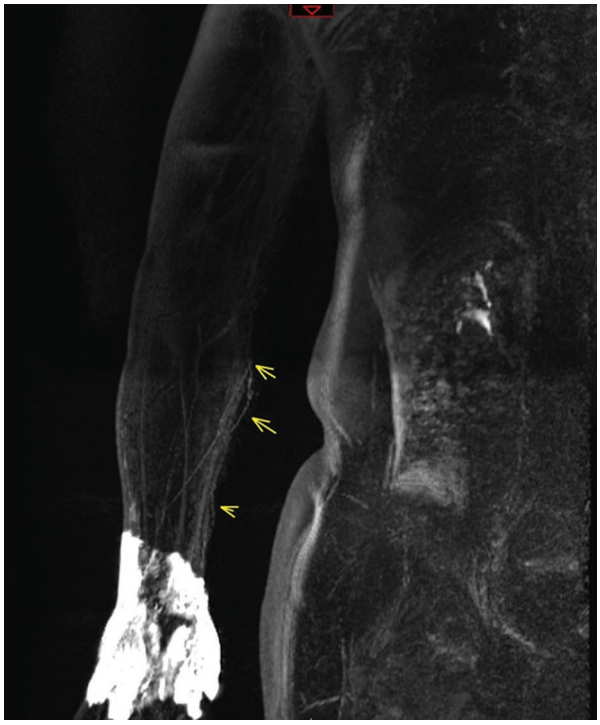

Figura 2. *Linforresonancia magnética con administración de gadolinio intradérmico. Se aprecian canales linfáticos lineales en el antebrazo.*

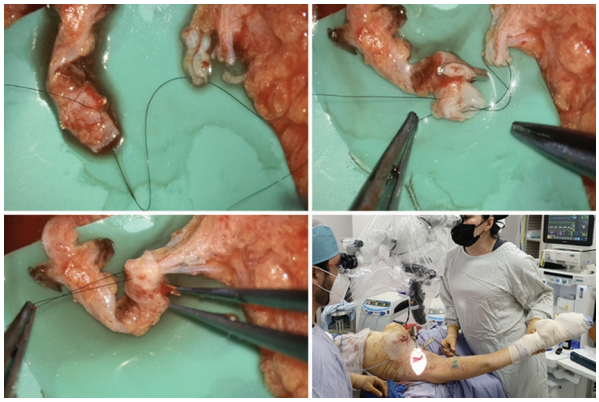

Figura 3. *Reconstrucción linfática inmediata. Técnica de «pulpo», anastomosis múltiple con sutura invaginante. Brazo en abducción para corroborar la ausencia de tensión.*

En la actualidad, esta técnica es conocida como reconstrucción inmediata o *bypass* linfático-venoso profiláctico. Su objetivo es prevenir el desarrollo del linfedema relacionado con cáncer de mama al restaurar el drenaje linfático interrumpido tras una disección de ganglios linfáticos axilares.

Los estudios publicados reportan que el uso de esta técnica reduce el riesgo de linfedema del 40% al 8%.

Además, los metaanálisis realizados han demostrado su efectividad y costo-efectividad[25-27], y varios grupos internacionales están trabajando en la generación de evidencia de alto nivel para establecer recomendaciones clínicas[3,28,29].

La reconstrucción linfática inmediata no solo ha sido utilizada en cáncer de mama, sino también en melanoma y cánceres ginecológicos. Morotti et al.[30] describieron la reconstrucción linfática inmediata inguinal en 12 extremidades inferiores tras una disección de ganglios linfáticos inguinales, reportando solo un caso de linfedema leve (8.3%).

Consideraciones quirúrgicas

Aunque la derivación linfático-venosa es un procedimiento conceptualmente sencillo, su ejecución en quirófano representa un reto. Por ejemplo, en casos de linfadenectomía estándar en bloque puede ser difícil encontrar una vena receptora de longitud y calibre adecuados.

La curva de aprendizaje varía según la especialidad del cirujano:

- Para el cirujano resectivo, el desafío radica en identificar y preservar las venas receptoras adecuadas durante la disección ganglionar. En México, este procedimiento generalmente es realizado por un cirujano oncólogo con experiencia en cirugía de mama, incluyendo la disección linfática axilar de los niveles I y II.
- Para el cirujano reconstructivo, la dificultad se centra en el conocimiento profundo de la anatomía venosa y linfática, la identificación confiable de los canales linfáticos diana y la selección adecuada de las venas receptoras; además de dominar la técnica de anastomosis microvasculares.

Tratamiento fisiológico

Anastomosis linfático-venosa y derivación linfático-venosa

La primera anastomosis linfático-venosa experimental fue reportada por Jacobson en un modelo canino en 1962. Posteriormente, Yamada realizó la primera aplicación clínica en un paciente con linfedema obstructivo en 1967. Durante la década de 1970, O'Brien y su equipo implementaron la técnica en humanos, mientras que varios otros intentaron perfeccionarla sin obtener resultados satisfactorios.

En el año 2000, Koshima introdujo un enfoque basado en supermicrocirugía, en el que los linfáticos subdérmicos se conectan a vénulas subdérmicas adyacentes de menos de 0.8 mm de diámetro[31]. Este procedimiento, conocido como anastomosis linfático-venosa, permite la derivación del flujo linfático hacia el sistema venoso, su destino fisiológico natural en la unión del conducto torácico con la circulación venosa a nivel de la vena subclavia. Al redirigir el flujo linfático hacia pequeñas vénulas, se evita la obstrucción del sistema linfático.

Los estudios han demostrado que la anastomosis linfático-venosa tiene una alta efectividad en etapas tempranas del linfedema, y actualmente se investiga su aplicación incluso en casos avanzados (estadio III). Esta técnica es particularmente eficaz cuando aún existen linfáticos funcionales y el tejido circundante no ha desarrollado fibrosis grave. Un porcentaje significativo de pacientes sometidas a este procedimiento han logrado suspender el uso de prendas de compresión, incluso en seguimientos a largo plazo[32].

Procedimiento quirúrgico

En la evaluación preoperatoria se realiza un mapeo linfático mediante linfografía con verde de indocianina, lo que permite identificar vasos linfáticos funcionales y vénulas adyacentes.

El procedimiento puede realizarse bajo anestesia general o local con sedación. Se practican incisiones de 1 a 2 cm en los sitios previamente marcados, tras lo cual los vasos linfáticos y las vénulas son identificados y disecados cuidadosamente con la ayuda de un microscopio quirúrgico.

La conexión entre el linfático y la vénula se lleva a cabo mediante técnicas de supermicrocirugía, utilizando suturas ultrafinas de calibre 11-0 o 12-0. Las configuraciones de anastomosis pueden ser término-terminal, término-lateral o combinaciones de ambas.

Para verificar la permeabilidad de la anastomosis, se observa el paso de un colorante, como verde de indocianina o azul patente.

Dado que es posible integrar la visión infrarroja al microscopio quirúrgico, se puede evaluar en tiempo real la permeabilidad de la anastomosis linfático-venosa durante la cirugía. Sin embargo, una de las principales limitantes de esta técnica es la baja penetración de la luz utilizada para estimular la fluorescencia del pigmento, la cual alcanza solo hasta 2 cm bajo la piel (Fig. 4).

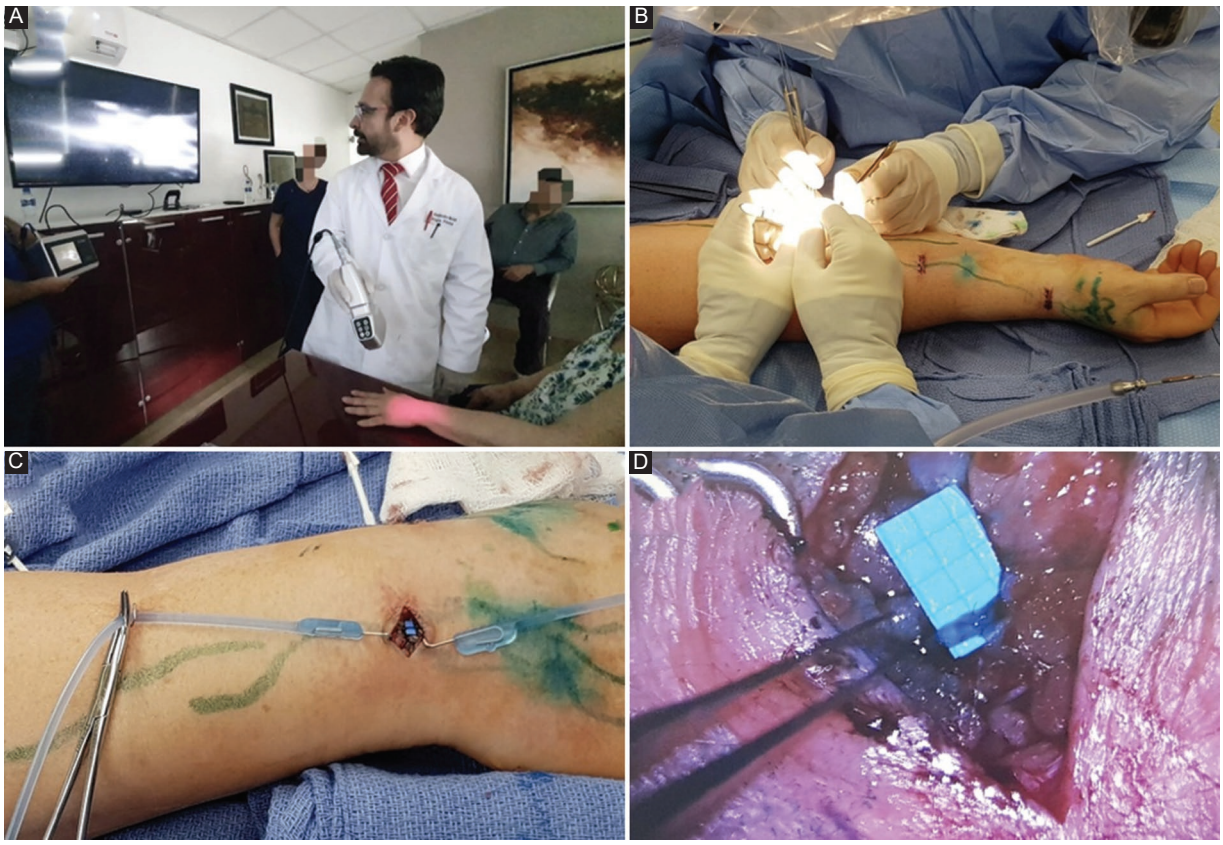

Figura 4. *Procedimiento de trasplante de ganglios linfáticos gastroepiploicos al brazo.* **A:** *trabajo de equipos simultáneos, toma de ganglios gastroepiploicos por laparoscopia y preparación de la zona receptora.* **B:** *colgajo de ganglios gastroepiploicos dividido en dos colgajos.* **C***: colgajos in situ.* **D***: posoperatorio inmediato.*

Cuidados posoperatorios y complicaciones

En el posoperatorio se requiere el uso continuo de prendas de compresión para mantener un flujo linfático adecuado y prevenir la acumulación de linfa. Se realizan controles clínicos regulares para monitorear posibles complicaciones, como infecciones, persistencia del edema o fallo de la anastomosis.

La mayoría de las series publicadas sobre la cirugía de *bypass* linfático reportan tasas bajas de complicaciones. Entre los eventos adversos más frecuentes se encuentran problemas menores de cicatrización, episodios de celulitis y fístulas linfáticas[33,34].

Transferencia de ganglios linfáticos vascularizados

La transferencia de ganglios linfáticos vascularizados es una técnica quirúrgica moderna y prometedora para el tratamiento del linfedema, especialmente en pacientes en estadios avanzados, en quienes la anastomosis linfático-venosa no es viable debido a fibrosis grave o daño irreversible de los vasos linfáticos. También está indicada en casos refractarios a tratamientos conservadores y en pacientes con episodios recurrentes de celulitis. En algunos casos, la transferencia de ganglios linfáticos vascularizados puede combinarse con anastomosis linfático-venosa para mejorar los resultados[35].

Inicialmente descrita y popularizada por Becker et al.[36], esta técnica ha llevado a la búsqueda de sitios donadores de ganglios linfáticos que minimicen el riesgo de morbilidad en la zona donante, evitando así la aparición de linfedema en dicha región. Se ha demostrado que preservar el suministro vascular durante la transferencia mejora significativamente el grado de linfedema y la función linfática[37].

Mecanismos de acción

Existen dos mecanismos principales por los cuales la transferencia de ganglios linfáticos sanos contribuye a restablecer el flujo linfático:

– Función de «esponja»: el tejido vascularizado en el área afectada, generalmente dañada por fibrosis y radioterapia, absorbe líquido intersticial y lo drena a través de la anastomosis venosa del colgajo.

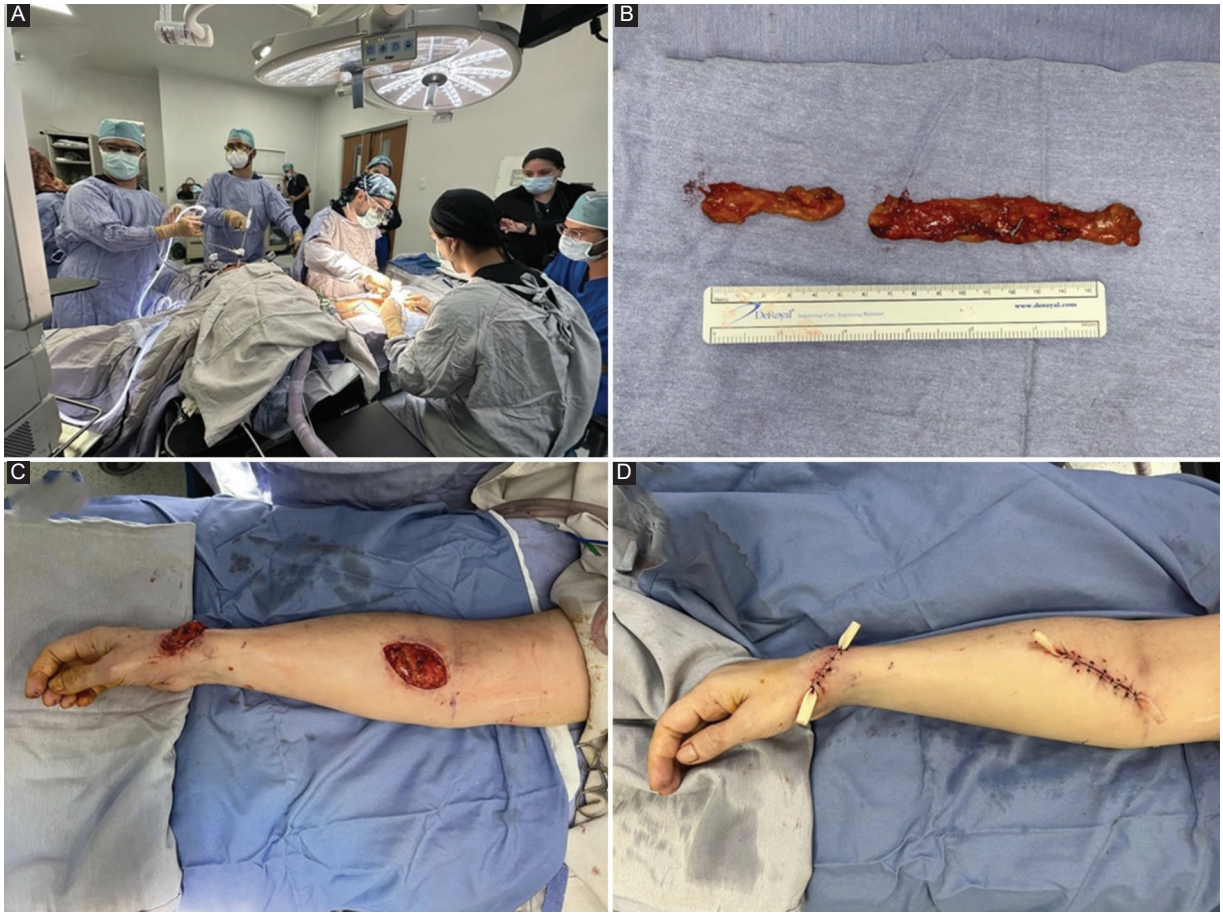

Figura 5. *Anastomosis linfático-venosa.* **A:** *localización de canales linfáticos con linfografía con verde de indocianina y cámara infrarroja.* **B:** *vista general de la disposición en la mesa de quirófano bajo el microscopio.* **C:** *acercamiento, incisiones hasta el tejido celular subcutáneo, de aproximadamente 2 cm de longitud.* **D:** *anastomosis linfático-venosa in situ. Cuadrícula de contraste de 1 mm por cuadro.*

– Función de «bomba»: los ganglios linfáticos funcionales trasplantados facilitan la circulación linfática en la zona receptora.

Además, los ganglios linfáticos trasplantados favorecen la liberación de factores de crecimiento linfático, promoviendo la linfangiogénesis, es decir, la formación de nuevas conexiones linfáticas entre el colgajo y los vasos linfáticos receptores[38].

Sitios donantes y técnica quirúrgica

Los sitios donantes preferidos en la actualidad son los siguientes: submental, supraclavicular, toracodorsal, inguinal y gastroepiploico (Fig. 5).

El colgajo inguinal es una evolución del colgajo perforante de la arteria circunfleja ilíaca superficial, el cual se obtiene de la porción lateral del área inguinal para disminuir el riesgo de linfedema en la zona donante. Para optimizar la seguridad del procedimiento se emplea mapeo linfático inverso, utilizando una doble técnica para identificar los ganglios que drenan la extremidad del donante (Fig. 6).

En caso de que el sitio receptor (axila, tobillo o ingle) haya sido sometido a cirugía o radioterapia, se realiza una escisión amplia del tejido cicatricial con el fin de preparar un lecho adecuado para el colgajo. Aparentemente no existe diferencia en el resultado si el colgajo se posiciona en una zona distal (muñeca) o en una zona proximal (axilar)[39].

Las mujeres que requieren reconstrucción mamaria simultánea y tratamiento del linfedema pueden ser candidatas ideales para la reconstrucción mamaria con colgajos abdominales inferiores, incluyendo ganglios linfáticos inguinales. En estos casos, los vasos toracodorsales suelen seleccionarse como vasos receptores, permitiendo así la colocación del tejido linfático en el espacio axilar[40] (Fig. 7).

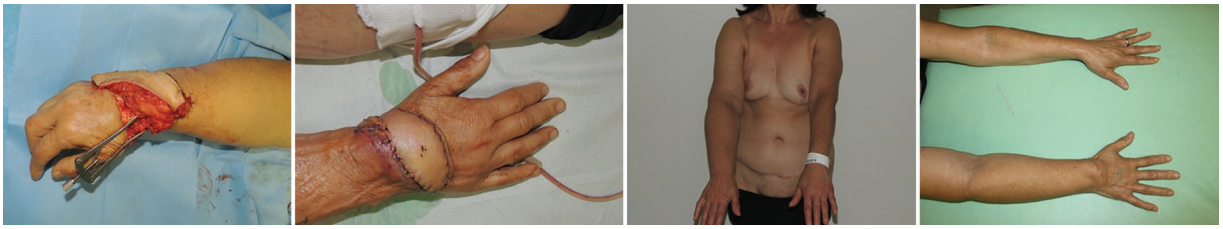

Figura 6. *Colgajo de ganglios linfáticos inguinales.* **A:** *inset de colgajo en la muñeca.* **B:** *posoperatorio inmediato del colgajo.* **C:** *linfedema preoperatorio* **D:** *seguimiento posoperatorio con mejoría del linfedema.*

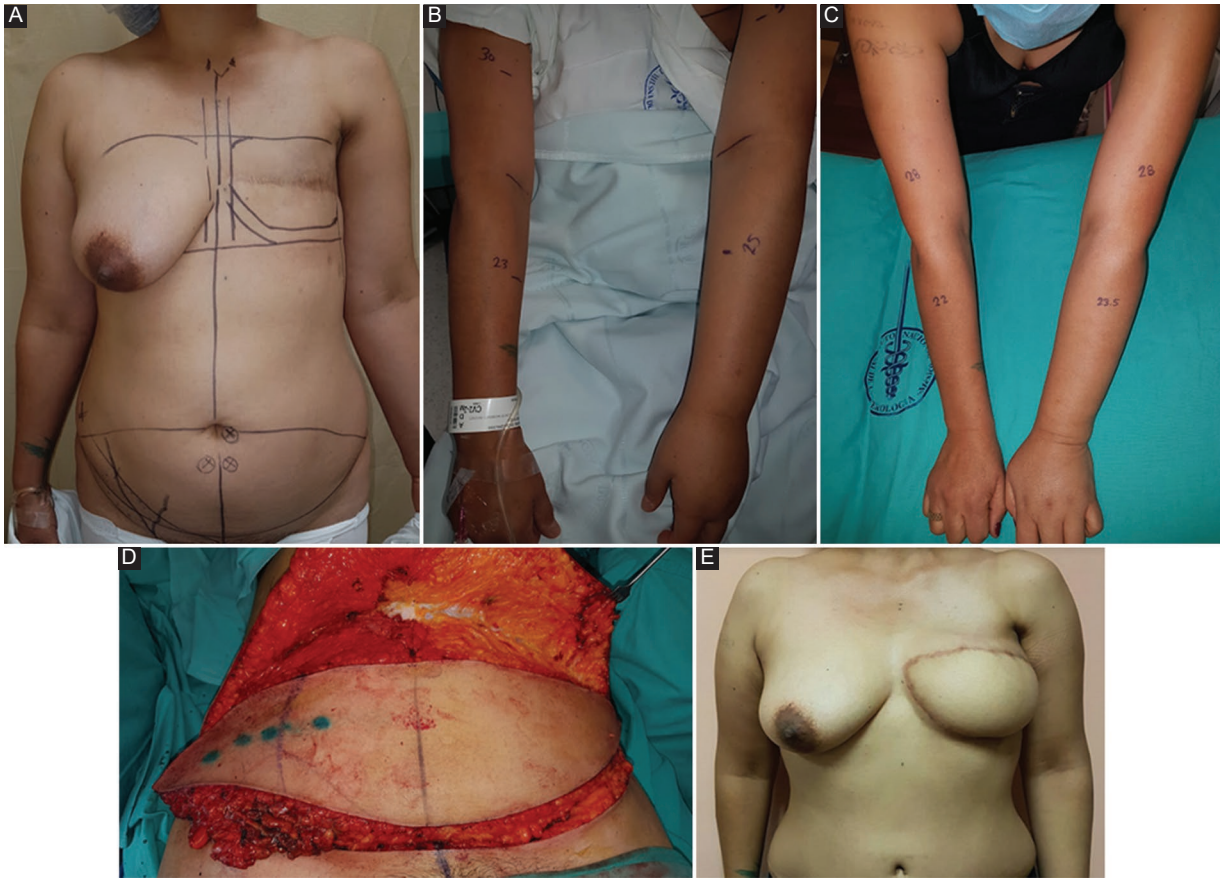

Figura 7. *Procedimiento de colgajo de vasos perforantes de la arteria epigástrica inferior profunda combinado con ganglios linfáticos inguinales.* **A:** *fotografía preoperatoria con ausencia de mama.* **B:** *preoperatorio con linfedema de brazo izquierdo.* **C:** *posoperatorio con mejoría del linfedema del brazo izquierdo y reducción de la circunferencia.* **D:** *colgajo de vasos perforantes de la arteria epigástrica inferior profunda incluyendo ganglios linfáticos inguinales.* **E:** *posoperatorio con seno reconstruido y linfedema tratado.*

Complicaciones

Si bien la transferencia de ganglios linfáticos vascularizados ha demostrado ser un procedimiento generalmente seguro, pueden presentarse complicaciones, como pérdida del colgajo, linfedema en el sitio donante, seroma, linfocele, infección y complicaciones en la cicatrización de la herida[41].

Interposición de colgajos de canales linfáticos funcionales

El uso de técnicas de visualización linfática, como el verde de indocianina, ha permitido el traslado de colgajos de canales linfáticos con una orientación anatómica precisa, funcionando como un «puente» en áreas dañadas, como la axila. Este procedimiento

Tabla 3. Visión integral de las aplicaciones y los resultados de las principales técnicas quirúrgicas en el tratamiento del linfedema

Técnica	Estadio del linfedema más indicado	Indicaciones	Limitaciones	Complicaciones	Porcentaje de reducción del volumen
LYMPHA	Prevención de linfedema	Pacientes con disección ganglionar axilar	Aún hay debate sobre su eficacia; se encuentran en proceso estudios aleatorizados	No se han reportado	Disminuye el riesgo de linfedema del 40% al 9% de los casos
Anastomosis linfático-venosa	Estadios tempranos con vasos linfáticos funcionales	Pacientes con linfedema inicial, transporte linfático parcial	Requiere equipo de fluorescencia y habilidades microquirúrgicas	Fístulas linfáticas, infecciones menores	Hasta 44-55% y mejora en 73% de los casos
Transferencia de ganglios linfáticos vascularizados	Estadios avanzados con fibrosis o daño irreversible de vasos linfáticos	Pacientes con linfedema avanzado o no aptas para anastomosis linfático-venosa	Riesgo de linfedema en el sitio donador, técnica microquirúrgica compleja	Linfedema del sitio donador, seroma, infección, pérdida del colgajo	Hasta 47%; mejor cuando se combina con anastomosis linfático-venosa
LIFT	Etapas II y III	Zonas identificadas de interrupción del flujo, como heridas o cicatrices	Requiere equipo de fluorescencia y habilidades microquirúrgicas	Linfedema del sitio donador, seroma, infección, pérdida del colgajo	
Liposucción	Estadios avanzados con fibrosis e hipertrofia grasa significativa	Reducción del volumen en casos refractarios a terapia de compresión	Uso continuo de prendas de compresión, riesgo de daño a vasos linfáticos	Celulitis, daño linfático, parestesias menores	20-23% (hasta 106% combinado con terapia de compresión)
Exéresis directa con injertos de piel	Casos graves y refractarios con importante discapacidad funcional	Casos extremos en los que las actividades diarias están muy afectadas	Alta morbilidad, pobre resultado estético, técnica invasiva	Cicatrices, infecciones crónicas, irregularidades del contorno	Variable, depende del caso; solo se usa en casos extremos

LIFT: *lymphatic interpositional flap transfer* (transferencia de colgajo linfático interpuesto); LYMPHA: *lymphatic microsurgical preventive healing approach* (abordaje microquirúrgico preventivo para la restauración linfática).

facilita la reconexión de canales linfáticos sanos y el restablecimiento del flujo linfático en la zona afectada.

En la técnica LIFT *(lymphatic interposicional flap transfer),* un colgajo perforante que incluye vasos linfáticos es transferido a un sitio receptor con el objetivo de «puentear» la interrupción causada por la ablación de un tumor y conectar los cabos linfáticos proximales y distales[42].

Tratamiento escisional

Liposucción

La acumulación de líquido linfático en las extremidades provoca un aumento en la deposición y la hipertrofia del tejido adiposo. La liposucción, un procedimiento que utiliza una cánula metálica fenestrada conectada a un sistema de succión al vacío, permite aspirar la grasa subcutánea y reducir el volumen de las extremidades afectadas. Sin embargo, uno de los posibles riesgos de esta técnica es el daño adicional a los vasos linfáticos restantes[43]. Para minimizar este riesgo se prefiere la liposucción longitudinal, ya que preserva en mayor medida la integridad de los vasos linfáticos, razón por la cual ha sido descrita como «preservadora de vasos linfáticos». Por otro lado, la liposucción circunferencial puede ser una opción adecuada cuando se busca un resultado estético más uniforme, siempre que se realice con precaución.

Brorson[44] describió la técnica de liposucción bajo isquemia, la cual permite la extracción de tejido adiposo con una disminución significativa en la pérdida de sangre. Posterior al retiro de grasa, se puede realizar la resección de la piel sobrante y redundante en el mismo procedimiento.

La principal desventaja de la liposucción es la necesidad de utilizar prendas de compresión de forma continua (24 horas al día) después de la cirugía para mantener el equilibrio logrado. La interrupción de este

Tabla 4. Ventajas y desafíos de la cirugía robótica aplicada en reconstrucción linfática

Ventajas	
Precisión extrema	Mayor precisión gracias a los instrumentos robóticos. Estos pueden moverse en rangos que superan las capacidades de las manos humanas
Minimización de trauma	Los movimientos controlados del robot reducen el riesgo de daño a los tejidos circundantes, lo cual es crucial en estructuras linfáticas pequeñas y delicadas
Mejor visualización	Los sistemas ofrecen una visión tridimensional ampliada, facilitando la identificación de vasos linfáticos y venas minúsculas
Ergonomía para el cirujano	La plataforma robótica reduce la fatiga del cirujano durante procedimientos prolongados, mejorando la calidad de la intervención
Resultados potencialmente mejores	Una mayor precisión podría traducirse en un mejor drenaje linfático, disminución de los síntomas y una recuperación más rápida de la paciente
Desafíos y consideraciones	
Costo elevado	Los sistemas son costosos, tanto en términos de adquisición como de mantenimiento, lo que puede limitar su accesibilidad
Curva de aprendizaje	Requiere una capacitación extensa para los cirujanos
Indicaciones específicas	No todas las pacientes con linfedema son candidatas ideales para cirugía robótica. Su uso debe considerarse caso por caso, basado en la gravedad del linfedema, la viabilidad de las estructuras linfáticas y otros factores
Duración del procedimiento	Si bien la precisión es mayor, los procedimientos robóticos pueden ser más largos, especialmente durante las fases iniciales de la adopción
Evidencia clínica	Aunque los resultados preliminares son prometedores, se necesitan estudios a mayor escala y a largo plazo para establecer la superioridad definitiva de la cirugía robótica sobre las técnicas tradicionales

régimen puede provocar la reaparición del linfedema debido a la acumulación recurrente de líquido linfático. Por lo tanto, la liposucción es un procedimiento reductivo que no mejora la función linfática.

Escisión directa

La escisión directa está reservada para casos graves de linfedema debido a su alta morbilidad, ya que en algunos casos la escisión radical puede ser la única opción para recuperar la función.

Entre las técnicas descritas se encuentra el procedimiento atribuido a Charles, el cual se caracteriza por la resección completa de la piel y el tejido subcutáneo, con preservación de la fascia profunda y de la planta del pie, cubriendo el defecto con un injerto de piel obtenido del tejido resecado[45].

Además, se han desarrollado otras técnicas dirigidas a restablecer la conexión entre el sistema linfático profundo y superficial. Estas incluyen resección de piel y tejido blando a través de una incisión elíptica a lo largo de la cara medial del brazo combinada con una amplia escisión de la fascia profunda, y uso de colgajos de piel desepitelizada que se posicionan a lo largo del paquete neurovascular en toda la longitud de la incisión, con el objetivo de favorecer la comunicación linfática entre ambos sistemas[46] (Tabla 3).

Cirugía robótica

El uso de la cirugía robótica en el tratamiento del linfedema representa una evolución significativa en la supermicrocirugía reconstructiva. Esta tecnología ha ampliado las posibilidades quirúrgicas al mejorar la precisión del cirujano, proporcionar visión ampliada y disminución del temblor, lo que resulta especialmente beneficioso en las técnicas de anastomosis linfático-venosas. Sus ventajas y desventajas se exponen en la tabla 4.

Conclusiones

La cirugía linfática ofrece resultados prometedores en la prevención y el tratamiento del linfedema asociado al cáncer de mama. Aunque se han logrado avances significativos, como la supermicrocirugía y los

procedimientos de prevención inmediata, persiste la necesidad de ensayos clínicos aleatorizados que aporten mayor solidez a las recomendaciones. Un abordaje interdisciplinario y la selección adecuada de las pacientes son cruciales para optimizar los resultados.

La cirugía robótica tiene un gran potencial en el tratamiento quirúrgico del linfedema, especialmente en procedimientos de microcirugía como la anastomosis linfático-venosa y la transferencia de ganglios linfáticos vascularizados. Sin embargo, su implementación debe considerarse en función de los recursos disponibles, la experiencia del equipo quirúrgico y la selección adecuada de las pacientes. Con el avance continuo de la tecnología y la capacitación especializada, es probable que esta modalidad se convierta en un estándar en el manejo quirúrgico del linfedema en centros especializados.

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Conflicto de intereses

Los autores declaran no tener conflicto de intereses.

Consideraciones éticas

Protección de personas y animales. Los autores declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

Confidencialidad, consentimiento informado y aprobación ética. Los autores han seguido los protocolos de confidencialidad de su institución, han obtenido el consentimiento informado de los pacientes, y cuentan con la aprobación del Comité de Ética. Se han seguido las recomendaciones de las guías SAGER, según la naturaleza del estudio.

Declaración sobre el uso de inteligencia artificial. Los autores declaran que no utilizaron ningún tipo de inteligencia artificial generativa para la redacción de este manuscrito.

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LETTER TO THE EDITOR

Comments on "sigmoid volvulus and descending colon adenocarcinoma, a double cause of intestinal obstruction"

Comentarios a "Vólvulo del sigmoides y adenocarcinoma de colon descendente, una doble causa de obstrucción intestinal"

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To the Editor,

We read with interest the paper written by Senejoa et al[1]. on colon malignancy complicating sigmoid volvulus (SV). Although both colon malignancy and SV are relatively common causes of colonic obstruction, their co-occurrence is a very rare clinical entity with seven cases reported to date[1-4]. My colleagues and I have a 57.5-year (from June 1966 to January 2024) experience including 1,076 cases with SV, which is the most comprehensive single-center SV series over the world[5]. In the light of this experience, our comments relate to the comorbidity of SV and colon malignancy.

First, similar to its low worldwide incidence, there is only one case (0.1%) with sigmoid colon carcinoma complicating SV in our 1,076 case series. Most likely, the rarity of this comorbidity prevents the detailed definition of its pathophysiology[1-4]. In our opinion, sigmoid colon masses may trigger SV using their driving force, while malignancies localized in the other segments may increase bowel peristalsis, resulting in SV. In addition, in our experience, both SV and colon malignancy may mimic each other due to their similar clinical presentations.

Second, most likely due to the rarity of this comorbidity, the treatment algorithm is not identified clearly[1-4]. In our opinion, in cases with sigmoid colon malignancy, resection followed by primary anastomosis or stoma is the preferred surgical option. However, the masses localized in the other segments muck up the treatment plan. In such cases, if the sigmoid is gangrenous, primary anastomosis or stoma following double-segment resection is required. Conversely, in addition to the resection of the tumoral segment, sigmoid detorsion with or without resection, or a recurrence-reducing procedure such as sigmoidopexy, mesoplasty, or extraperitonealization, are the main options in patients with viable sigmoid colon.

We congratulate the authors and we look forward to their reply.

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Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The

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Confidentiality, informed consent, and ethical approval. The authors have obtained approval from the Ethics Committee for the analysis of routinely obtained and anonymized clinical data, so informed consent was not necessary. Relevant guidelines were followed.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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CARTA AL EDITOR

El olfato, una ventana a la mente

Smell, a widow to the mind

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Introducción

En la novela *El perfume: historia de un asesino,* el escritor alemán Patrick Süskind nos presenta el peculiar caso de Jean Baptiste Grenoulle, dotado de un olfato excepcional, pero incapaz de emitir un olor propio. El autor describe la historia de Grenoulle desde su nacimiento y su infancia, momentos en los que padece múltiples adversidades, hasta su vida adulta, cuando se convierte en un gran perfumista cuya pasión por los olores y las fragancias femeninas es tal que recurre al homicidio de mujeres para obtenerlas y preservarlas. Así, en esta novela se resalta el papel tan importante que puede llegar a tener el olfato en la vida de los seres humanos. Por ello, las alteraciones olfativas, ya sea por un proceso infeccioso o no infeccioso, pueden por un lado predecir el desarrollo de una patología o ser el resultado en sí mismas de la patología. Cualquiera que sea el origen, esta afección sin duda afecta la vida personal, conductual y social de los individuos que la padecen.

Entre los sentidos, el olfato es el menos explorado y, al menos en el ser humano, suele ser considerado como un sentido más intuitivo que informativo. Quizás por esta razón es que existe poca consciencia de la importancia de su funcionamiento y su carácter de indispensable para el bienestar. Los problemas relacionados con la vista o el oído son causa de exámenes sistemáticos, incluso desde edades tempranas, mientras que los padecimientos del olfato suelen pasar inadvertidos.

La biología evolutiva ha mostrado que se requiere el sentido del olfato para la supervivencia de los animales, incluido el hombre, en quien el desdén cultural lo ha considerado solo un vestigio de «animalidad» (ya que olfatear es una acción característica del comportamiento de muchos animales). El olfato nos permite identificar nutrimentos, nos brinda seguridad (para evitar alimentos potencialmente contaminados), mejora nuestra calidad de vida (autoestima y ánimo) e influye directamente en nuestra conducta (higiene personal, vida sexual, etc.). Este sentido trabaja al compás de cada ciclo respiratorio, permitiendo en cada inhalación la entrada de moléculas odoríferas a la nariz y al sistema respiratorio, para su procesamiento final en áreas cerebrales primarias y secundarias. Entre los vertebrados, los tipos de receptores olfatorios son comunes y muy similares en sus aspectos estructural y funcional (percepción). Sin embargo, a diferencia de otros vertebrados, en el ser humano la información percibida es integrada en amplias áreas cerebrales, como la región arquicortical (corteza entorrinal) y neocortical (cortezas orbitofrontal e insular), y muestran importantes interconexiones con áreas cerebrales encargadas de la memoria (lo que explica por qué recordamos un olor y por qué podemos asociar un olor a un evento pasado), el lenguaje y funciones neurovegetativas (particularmente el sueño), por lo que participa e influye en la conducta y las emociones[1,2].

Anatomía del olfato

En los humanos, la cavidad nasal tiene unos 2.5 cm^2 de superficie donde se concentran cerca de 50 millones de células receptoras con 8-20 cilios cada una, inmersas

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en una capa de moco producida por las glándulas de Bowmann[3]. La unión de una partícula odorífera a su receptor olfativo genera cambios en el potencial de la membrana de la de la neurona cuyos axones se proyectan desde el epitelio nasal a través de la lámina cribosa hasta el bulbo olfatorio, y de esta estructura se emiten conexiones a estructuras olfativas centrales primarias, como la corteza piriforme (que incluye los sistemas límbico y paralímpico, estructuras reconocidas por controlar la respuesta instintiva y emocional), el núcleo olfativo anterior, el tubérculo olfativo, la amígdala y la corteza entorrinal, así como estructuras secundarias como el hipocampo, el tálamo, la corteza orbitofrontal y el cerebelo[4]. En la figura 1 se ilustran estas estructuras.

Un rasgo importante de la vía olfatoria en los humanos es la ausencia del órgano vomeronasal (estructura olfatoria auxiliar especializada en la percepción de feromonas), del que solo queda una estructura remanente, y también la disminución del tamaño de los cornetes en comparación con otros vertebrados, como los perros, entre otros, que tienen mayor capacidad olfativa que nosotros. El mecanismo molecular de la detección de partículas odoríferas por sus receptores conduce a la apertura de canales iónicos y la consecuente activación de las neuronas olfatorias.

El sistema olfativo clásico está evolutivamente muy conservado en los insectos, los reptiles y los mamíferos. En los vertebrados, particularmente está representado por una gran familia de genes que codifican para proteínas receptoras que se expresan en los cilios de los receptores olfatorios: 350 genes en el humano, 1948 en el elefante, 1767 en la rata, 1391 en el ratón, 2129 en la vaca y 1100 en el perro[5,6]. Esta diversidad señala que el número de genes implicados en el proceso de oler no está directamente relacionado con el tamaño de las especies ni con su grado de evolución.

En los humanos existe un marcado dimorfismo sexual en el sentido del olfato: las mujeres tienen una mejor capacidad de percepción y discriminación de los olores que los hombres[7], lo cual probablemente se correlaciona con un mayor desarrollo del área prefrontal orbital y pudiera asociarse a su vez con su sistema hormonal y reproductivo. La capacidad olfativa también es diferente entre grupos raciales; por ejemplo, los afroamericanos y los hispanos experimentan una pérdida funcional más temprana con el envejecimiento en comparación con las poblaciones caucásicas[7], aunque se desconoce la razón de esa diferencia.

Clasificación de los trastornos olfativos

Estos trastornos se clasifican en cuantitativos, caracterizados por una afección de la intensidad más que de la calidad del aroma y que van desde hiposmia hasta anosmia, y cualitativos caracterizados por una percepción olfativa alterada (disosmia), como la distorsión en la calidad de un aroma (parosmia) o la percepción de un aroma sin un estímulo olfativo (alucinaciones olfativas o fantosmia)[8].

¿Por qué es tan importante el olfato en los humanos?

Cuando percibimos un olor, no solo se activa la corteza entorrinal, sino también conjuntamente la corteza orbitofrontal. De hecho, tenemos representaciones diferentes para olores agradables (región rostromedial, corteza orbitofrontal) y desagradables (región lateral izquierda, corteza orbitofrontal), lo cual implica la activación directa de áreas del sistema límbico que tienen la capacidad de interaccionar con el sistema endocrino (sin mediar estructuras corticales) y con el sistema nervioso periférico. El sistema límbico es una región cerebral que incluye el hipotálamo, el hipocampo y la amígdala, y que se encarga del procesamiento de las emociones y el afecto. Dentro de los factores de riesgo para trastornos olfativos se encuentran el tabaquismo[9-11], el uso de drogas por inhalación y trabajar en fábricas[12,13], entre otros[14]. Al igual que las alteraciones visuales y auditivas, los trastornos olfativos incrementan con la edad[7,15]. Recientemente, en una cohorte de 3503 pacientes adultos americanos se investigó la asociación de disfunción olfativa con mortalidad por todas las causas a 5 años[16]. Los autores observaron que la disfunción olfativa en adultos mayores de 65 años se correlacionó con mayor riesgo de muerte, pero las causas detrás de esta correlación son desconocidas y se requiere mayor investigación.

¿Cómo impactan los trastornos olfativos en la calidad de vida e incluso en la mortalidad?

Mas allá de la función sensorial pura (percepción y discriminación de moléculas olfativas), que nos permite regular la conducta alimentaria[17] o social[15,18], el sentido del olfato participa en el procesamiento de las emociones, a través del sistema por conexiones con la amígdala y la corteza entorrinal; de igual modo, debido a su

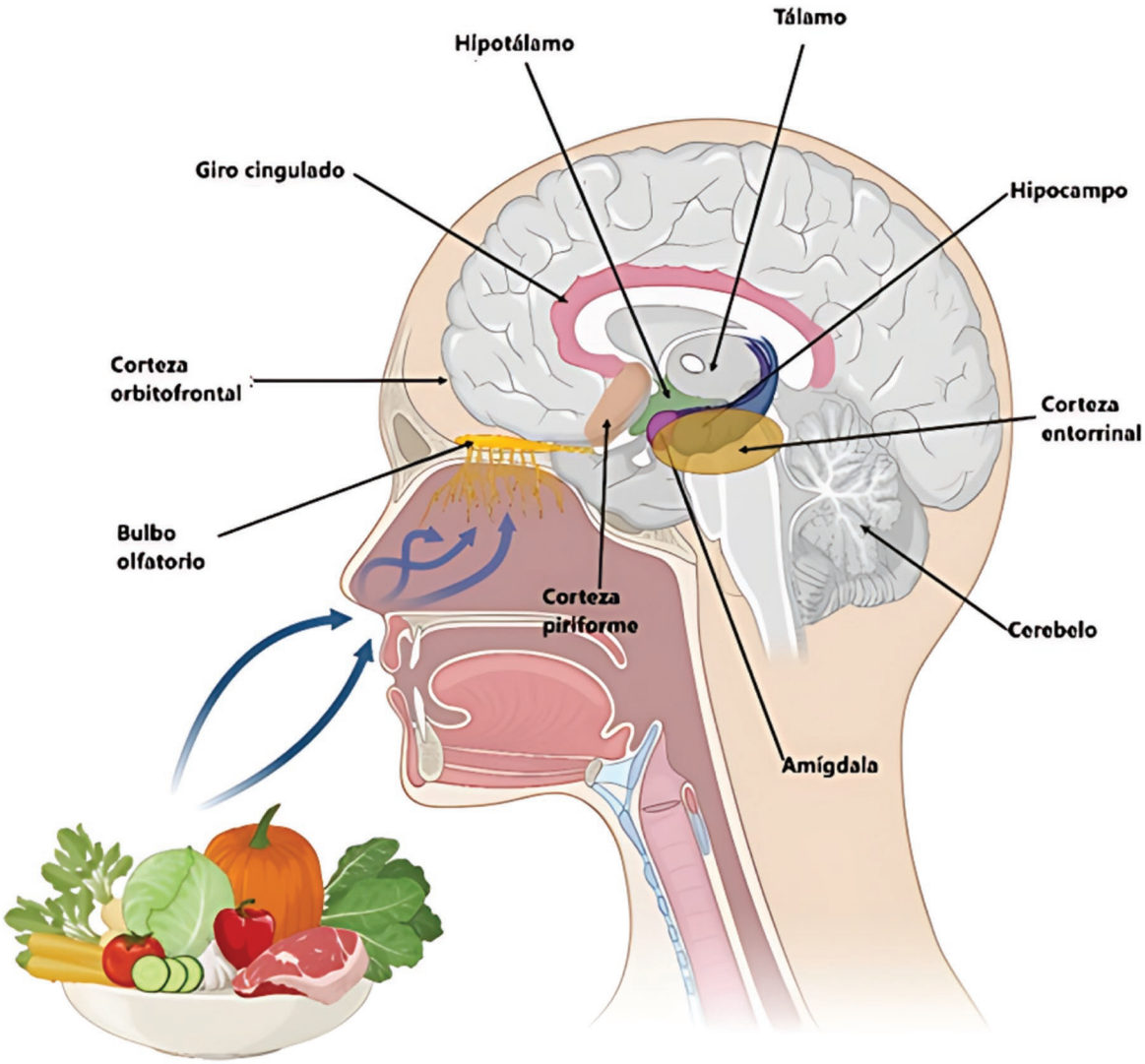

Figura 1. *Estructuras cerebrales primarias y secundarias relacionadas con el olfato. La unión de una partícula odorífera a su receptor olfativo genera cambios en la membrana de la neurona. Esta información es llevada al bulbo olfatorio y, de este, a estructuras primarias (corteza piriforme, asiento de estructuras del sistema límbico [cíngulo, amígdala] que participan en la respuesta instintiva y emocional) y secundarias (corteza orbitofrontal, cerebelo, etc.) que participan también en la integración del estímulo.*

relación con la corteza orbitofrontal, tiene un papel en el aprendizaje y la memoria. El olfato es considerado también un sensorio «emocional» *per se,* capaz de dar un valor moral positivo (agradable) o negativo (desagradable) a un tipo de olor. Ciertos olores pueden inducir un estado emocional. En la novela *En busca del tiempo perdido,* Marcel Proust resalta la importancia de los olores sobre sus recuerdos y los vínculos que crea, por ejemplo, cuando un día, al encontrarse abrumado por la tristeza, huele y prueba una magdalena mojada en té, y de manera repentina y vívida se transporta a recuerdos de su infancia. En efecto, los estímulos olfativos modulan las emociones, y viceversa[15].

Los pacientes con trastornos olfativos (como la anosmia, que es la pérdida total del olfato) y gustativos presentan problemas de autoestima y del ánimo[19]. Estos individuos tienen dificultades en la ingesta, la preparación de alimentos, la autoseguridad, la higiene personal y la vida sexual. En efecto, casi la mitad de los afectados refieren sensación de aislamiento, insatisfacción con la vida y ansiedad o depresión[20]. Aunque los mecanismos involucrados en el desarrollo de los trastornos del ánimo no están bien dilucidados, se considera que influye no solo la limitación en las actividades cotidianas (por afección olfativa), sino también la desregulación del sistema límbico y hormonal[21,22].

Se han reportado diferencias significativas en una escala de depresión (evaluada por el puntaje del Inventario de Depresión de Beck) entre pacientes con función olfativa normal (normosmia) y con algún grado

Tabla 1. Causas infecciosas de trastornos del olfato

Agentes infecciosos que condicionan disfunción olfativa	Agentes infecciosos que utilizan la vía olfativa para invadir el sistema nervioso
Virus (influenza, parainfluenza, rinovirus, coronavirus) Bacterias (*Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis*)	Bacterias (*Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae*) Virus (coronavirus, influenza) Protozoos (*Naegleria fowleri, Balamuthia mandrillaris, Acanthamoeba* spp.)

de disfunción (cuantitativa: hiposmia vs. anosmia). En ese estudio, los autores concluyeron que los pacientes con depresión presentan una reducción del desempeño de la función olfatoria y que los pacientes con disfunción olfativa tienen síntomas depresivos que se relacionan con la gravedad de la pérdida olfativa[23]. Estas manifestaciones parecen asociadas con la limitación de las actividades cotidianas, como por disminución en las aferencias al sistema límbico[9].

En diversos padecimientos neurodegenerativos (por ejemplo, las enfermedades de Alzheimer y de Parkinson), así como en trastornos neuropsiquiátricos (depresión, ansiedad y autismo), se reportan manifestaciones olfativas entre los síntomas prodrómicos (síntomas iniciales que preceden al desarrollo de una enfermedad)[24,25]. También algunos procesos infecciosos pueden inducir trastornos olfativos. El ejemplo más reciente, y tal vez de los más reconocidos por el impacto que ha tenido en todo el mundo, es la infección por el SARS-CoV-2. Sin duda, una de las primeras manifestaciones reportadas en esta infección fue el compromiso cuantitativo de la función olfativa (hiposmia o anosmia), en particular con la cepa silvestre, con un 73% de incidencia en comparación con las variantes alfa (41%) y delta (48%)[26]. Aunque aún no se conocen los alcances ni la gravedad a largo plazo de las secuelas de la COVID-19, el desarrollo de efectos cognitivos graves y la demencia son una preocupación. En este contexto, los estudios de seguimiento en convalecientes han demostrado que los pacientes que presentaron COVID-19, a 6 meses tras la infección presentaban cefaleas y deterioro cognitivo que se correlacionaron con la gravedad de la afección olfativa[27-30] Así mismo, existe la posibilidad de que estos trastornos olfativos puedan incrementar el riesgo de secuelas neurológicas o enfermedades neurodegenerativas en el largo plazo[27,31]. En la tabla 1 se muestran los principales agentes infecciosos que utilizan la vía olfatoria para acceder al sistema nervioso central, o que comprometen su función.

Conclusiones

A pesar de que existe una gran variedad de causas infecciosas y no infecciosas de disfunción olfativa, que hacen compleja su comprensión y tratamiento, es necesario establecer programas educativos y clínicos enfocados en la prevención de las alteraciones olfativas, así como identificar a las poblaciones de alto riesgo para brindar asesoría y reducir las consecuencias negativas en su salud y calidad de vida.

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Consideraciones éticas

Protección de personas y animales. Las autoras declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

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LETTER TO THE EDITOR

Comment on: "Does perivascular fibrin glue application have a preventive effect for the endothelial damage on saphenous vein graft? An experimental model"

Comentario a: "¿La aplicación de pegamento de fibrina perivascular tiene efecto preventivo del daño endotelial en el injerto de vena safena? Un modelo experimental"

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Dear Editor,

We read with interest the paper by Badem et al.[1] entitled "Does perivascular fibrin glue application have a preventive effect for the endothelial damage on saphenous vein graft? An experimental model." We congratulate the authors. We would like to make some comments.

In this *ex vivo* experimental study, 40 pieces of saphenous vein graft (SVG) were obtained as "excess" from a total of 20 patients. In coronary bypass surgery, sometimes the prepared grafts cannot be used due to some unexpected reasons, such as the target vessel diameter not being bypassable. However, this does not happen very often. How many total cases were performed during the time examined to reach the number of 20 patients, with excess SVG, included in this study? In a study designed in this way, were the saphenous parts included in the study of standard length? Or was the standard length of the saphenous vein obtained in all patients? Was it possible to obtain the excess saphenous vein in every case?

Another issue that interests us is how the saphenous vein harvesting technique is performed. We think that "no-touch harvesting" should be preferred in such a study rather than methods that may cause trauma to the saphenous vein. It is known that excessive vascular distension with classical vascular cannulation will both damage the intima and affect graft patency[2]. Which of the endoscopic/no-touch/conventional techniques were used for SVG harvesting in these patients? In the arterial system model designed for the study, and working successfully, SVGs were exposed to blood flow under 120 mmHg pressure for 60 min. In the experimental model created under the same conditions by Stooker et al.,[3] which is cited as a reference in your study, the pressure was designed as 60 mmHg, and the blood flow was 100 mL/min. In your study design, how many mL/min was the "intrasaphenous blood flow" set under 120 mmHg pressure? Were coronary blood flow rate and coronary perfusion pressure taken into consideration when making these calculations?

Finally, SVG remains the most preferred graft in multi-vessel coronary surgery in the world[4]. In your study, it was observed that significant intimal damage occurred in the grafts that were presented as the control group and in which fibrin glue was not applied. How do you interpret the fact that such significant intimal damage occurs in SVGs exposed to blood flow under 120 mmHg pressure for 60 min? If the experimental environment created meets physiological conditions, we think that the obtained data should be interpreted carefully.

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Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the Institutional Ethics Committee.

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Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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LETTER TO THE EDITOR

Coinfections and comorbidities observed in COVID-19 during the influenza season in the pediatric patients: correspondence

Coinfecciones y comorbilidad observadas en pacientes pediátricos con COVID-19 durante la temporada de influenza: correspondencia

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Dear Editor,

We would like to share ideas on "Coinfections and comorbidities observed in COVID-19 during the influenza season in the pediatric patient[1]." In this study, data from 163 patients diagnosed with COVID-19 or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during the 2020-2021 peak influenza season were evaluated. According to the study, children and preschoolers were the next most vulnerable age groups to illness, with teenagers having the highest risk. In addition, incidences of bacterial and viral coinfection with parvovirus B-19 and herpes type I were found in the study. There were four occurrences of fatality (2.4%), with the primary comorbidities being obesity, arterial hypertension, and acute lymphoblastic leukemia.

The very small sample size of 163 patients in this study is a possible drawback. More reliable findings on the risk factors and outcomes linked to SARS-CoV-2 infection in various age groups and with different comorbidities might be made with a larger sample size. Furthermore, the study only examined patients who were admitted to one hospital, which would restrict how broadly the results can be applied to other demographics or environments.

Future research on the effect of COVID-19 immunization on the prognosis of patients infected with SARS-CoV-2 might be beneficial. This could enhance patient treatment and provide information for public health initiatives. In addition, future studies may examine the possibility of re-infection or a decline in immunity over time, as well as the long-term consequences of SARS-CoV-2 infection in pediatric patients. Finally, studying the efficacy of various SARS-CoV-2 infection treatment approaches, such as immunomodulatory treatments or antiviral drugs, may aid in improving COVID-19 patient outcomes.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The study does not involve patient personal data nor requires ethical approval. The SAGER guidelines do not apply.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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