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EDITORIAL
Modernización del uso de la clozapina en Venezuela.

La clozapina (CLZ), es un fármaco antipsicótico (AP), atípico, sintetizado en 1958, y relacionado químicamente con algunos antidepresivos tricíclicos y con la clorpromazina. Se considera atípico, por su baja propensión a inducir efectos adversos (EAs) motores, y fue introducido para su uso en Europa a comienzos de 1970. Sin embargo, en 1975 el Sistema Finlandés de fármacovigilancia, atribuyó la muerte de 8 sujetos a agranulocitosis asociada a la CLZ, por lo cual su uso fue restringido. En 1988 Kane y col.[1], demostraron la superioridad de la CLZ en comparación con otros APs, en un trascendente ensayo clínico aleatorizado en pacientes con esquizofrenia resistente al tratamiento [1].

A partir de los años 90s, se autorizó el uso de la CLZ solo en la esquizofrenia, en la ideación suicida asociada a la esquizofrenia y al trastorno esquizoafectivo. Sin embargo, continuó restringida debido a la intensa monitorización hematológica requerida, y a los numerosos EAs que se fueron identificando progresivamente (ver adelante). En conjunto, la CLZ, fue considerada un fármaco de uso ocasional y relativamente relegada por las instituciones psiquiátricas en todo el mundo[1].

A mediados de los años 90, comenzó entonces una nueva etapa en la historia de la CLZ. Por una parte, la comprensión de su farmacocinética ha permitido entender mejor sus complejos EAs, y por otro lado, su uso aunque no aprobado oficialmente, en diversos trastornos psiquiátricos y neurológicos, ha demostrado tal efectividad tal, que ha llevado a que se acuñe la expresión “La Modernización de la Clozapina” [1]. En la actualidad, la CLZ también se utiliza de manera no aprobada, en casos severos de trastorno bipolar, ansiedad generalizada, trastornos del neurodesarrollo, trastornos de conducta asociados con demencia, personalidad límite, en psicosis asociadas con el abuso de sustancias y a la enfermedad de Parkinson, y en la discinesia tardía [1,2].

Los expertos en el uso de la CLZ, recomiendan enfáticamente, que se establezcan “Clínicas de CLZ”, como una sección de los departamentos de psiquiatría clínica, donde un personal especialmente entrenado, atienda pacientes tratados con el fármaco, y donde se cuantifiquen sus niveles séricos y los de su metabolito principal. Recientemente comprobamos que, de los países de la América Latina, sólo Chile cuenta con tal servicio.[2] Tenemos entonces, a nuestra disposición, un medicamento altamente efectivo, pero con un rango de seguridad muy estrecho, pues sus EAs están directamente relacionados con su concentración sanguínea. El llamado Índice Terapéutico de un fármaco, resulta de dividir la concentración plasmática por encima de la cual hay riesgo de toxicidad, entre la concentración efectiva mínima. La CLZ tiene el Índice Terapéutico más bajo entre todos los APs de uso en la actualidad, (600/350 ng/mL = 1.7) [3].

Los EAs asociados a la CLZ pueden organizarse cronológicamente. A corto plazo (1-4 meses de su inicio), pueden ocurrir neumonía, miocarditis, neutropenia, fiebre inexplicable, hipotensión, síncope, eosinofilia con síntomas sistémicos, hepatitis y pancreatitis. A mediano y largo plazo (> 4 meses) pueden presentarse hipomotilidad gastrointestinal (HMGI), síndrome metabólico, priapismo y cáncer hematológico y no hematológico. En cualquier momento del tratamiento, pueden ocurrir HMGI, neutropenia y disminución del

umbral convulsivo. Actualmente, se discute en cuáles condiciones se puede reintroducir la CLZ una vez resueltos los EAs [1,3,4].

La CLZ es metabolizada principalmente por el citocromo P450 1A2 (CYP1A2).

Un importante hallazgo, de nuestro grupo de investigación liderizado por el Dr. José de León, en la Universidad de Kentucky, fue que los sujetos de origen asiático, tienden a presentar un metabolismo lento del fármaco, en contraste con sujetos de grupos étnicos afro-americanos o caucásicos, quienes presentan un metabolismo promedio o rápido.[3,4] En la práctica, esto se traduce en que los pacientes con ancestros asiáticos, remotos o cercanos, que comprenden un porcentaje significativo de la población andina venezolana y de otros países de la América Latina , deben ser tratados con dosis relativamente bajas de CLZ. En conjunto, hemos identificado 6 grupos étnicos en la población mundial que requieren dosis distintas de CLZ. A todo esto, se suma que numerosos fármacos inducen o inhiben el metabolismo de la CLZ, mediante el citocromo P450 1A2, y que la obesidad y la inflamación lo inhiben.[3,4]

En Venezuela, los centros asistenciales públicos o privados, en Venezuela carecen de recursos para monitorear los niveles sanguíneos de la CLZ, por lo que los psiquiatras venezolanos, debemos optimizar la evaluación clínica y la tecnología disponible, para prevenir, diagnosticar y tratar precozmente los EAs que pueda causar este medicamento. Los expertos han recomendado disminuir la frecuencia de la monitorización hematológica durante el tratamiento.

La CLZ es subutilizada en psiquiatría y neurología en Venezuela, hecho que contrasta con que hasta el año 2024, en América del Sur, nuestro país poseía el mayor número de publicaciones científicas rigurosas sobre los EAs del fármaco en América del Sur[5]. En consecuencia, nuestro grupo de investigación en Mérida, Venezuela, y en Querétaro, México, está realizando un programa de optimización en la educación científica, por parte del personal dedicado al cuidado de la Salud Mental en ambos países.

Con el presente Editorial, deseamos estimular el interés científico de psiquiatras y neurólogos venezolanos sobre el tema. Por ejemplo, recientemente, solicitamos por vía electrónica a 401 psiquiatras del país, su opinión sobre el uso de la CLZ, sus EAs, y la necesidad de crear una guía de uso, acorde a las condiciones locales. La participación fue muy baja, puesto que solo 78 (19,45%) doctores respondieron a la convocatoria. Una mayor participación de nuestros médicos en este y otro tipo de estudios, redundará en beneficio de pacientes con enfermedades mentales y neurológicas severas.

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Modernizing the use of clozapine in Venezuela.

Abstract. Until few years ago, clozapine (CLZ) was scarcely used in psychiatric patients in Venezuela, even when it was an effective antipsychotic drug in severe mental disorders. This was mainly due to the severe adverse effects (AEs) sometimes occurring during CLZ treatment, and unpredictable drug availability. Even though Venezuela leads South American countries in the number of high-quality published studies about the drug's AEs, local clinicians are still reluctant to its use. Recent knowledge in CLZ pharmacokinetics allows a safer administration. Our research group has promoted an education program about CLZ use in Venezuela, but few colleagues' have answered our request. We hope this Editorial will improve this attitude, which may lead to improving life quality in psychiatric and neurological patients with severe disorders.

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Phillygenin reduced neuropathic pain by inhibiting the rats' TLR4/MyD88/NF-κB pathway.

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Keywords: neuropathic pain; phillygenin; proinflammatory cytokines; TLR4, MyD88.

Abstract. To elucidate the effects of phillygenin (PHI) and the potential mechanism on TLR4 and MyD88/NF-κB signalling in neuropathic pain in animal studies, chronic constriction injury (CCI) models were constructed for neuropathic pain induction using male Sprague-Dawley rats. PHI (20 mg/kg) was delivered through intragastric administration. Von Frey and Hargreaves tests were implemented to determine the 50% paw-withdrawal threshold (PWT) and paw-withdrawal latency (PWL). A nitric oxide (NO) assay was used for NO level detection, and an ELISA assay was employed to measure the expression of proinflammatory cytokines. Western blotting and RT-qPCR were conducted for protein and mRNA level detection. Treatment with PHI significantly enhanced 50% of PWT and PWL. PHI significantly decreased the levels of NO and reduced the levels of TNF-α, IL-1β, and IL-6. PHI also downregulated TLR4 and MyD88 expressions and inhibited the phosphorylation of NF-κB. PHI ameliorated inflammatory status and alleviated neuropathic pain in CCI rats, targeting TLR4 and suppressing MyD88/NF-κB signalling.

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Filigenina reduce el dolor neuropático en ratas inhibiendo la vía del TLR4/MyD88/NF-κB.

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Palabras clave: dolor neuropático, filigenina, citocinas proinflamatorias, TLR4, MyD88.

Resumen. Para dilucidar los efectos de la filigenina (PHI) y el mecanismo potencial en la señalización de TLR4 y MyD88/NF-κB en el dolor neuropático en estudios con animales, se construyeron modelos de lesión por constricción crónica (CCI) para la inducción del dolor neuropático utilizando ratas Sprague-Dawley macho. La PHI (20 mg/kg) se administró por vía intragástrica. Se implementaron las pruebas de von Frey y Hargreaves para determinar el 50% del umbral de retracción de la pata (PWT) y la latencia de retracción de la pata (PWL). Se utilizó un ensayo de óxido nítrico (NO) para la detección del nivel de NO, y se empleó un ensayo ELISA para medir la expresión de citocinas proinflamatorias. Se realizaron transferencia Western y RT-qPCR para la detección del nivel de proteína y ARNm. El tratamiento con PHI mejoró significativamente el 50% del PWT y la PWL. La PHI disminuyó significativamente los niveles de NO y redujo los niveles de TNF-α, IL-1β e IL-6. PHI también disminuyó la expresión de TLR4 y MyD88 e inhibió la fosforilación de NF-κB. PHI mejoró el estado inflamatorio y alivió el dolor neuropático en ratas con CCI, actuando sobre TLR4 y suprimiendo la señalización MyD88/NF-κB.

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INTRODUCTION

Neuropathic pain usually has a chronic progression and affects a significant number of individuals globally, leading to physical, emotional, and economic burdens [1]. It arises due to a dysfunction in the nervous system, causing abnormal signalling and processing of pain signals [2]. Patients with neuropathic pain experience various symptoms such as shooting or burning pain, numbness, tingling, and hypersensitivity to stimuli [3]. Various factors can cause neuropathic pain, including injury, infection, diabetes, or chemotherapy. This condition causes a substantial burden to the patients, and the current treatments for neuropathic pain are often limited in efficacy and associated with adverse effects [4]. Hence, the development of alternative treatment agents for the clinical therapy of neuropathic pain is urgently needed.

Toll-like receptor 4 (TLR4) belongs to the TLR family that initiates the innate immune response [5]. TLR4 has been found in various cells, including immune cells and neurons. It was reported that TLR4 could be activated by lipopolysaccharides (LPS) and damage-associated molecular patterns (DAMPs) [6]. Activation of TLR4 leads to the upregulation of myeloid differentiation factor 88 (MyD88). Moreover, the activated TLR4 could promote the nuclear factor-κB (NF-κB) pathway, resulting in the upregulation of various inflammatory factors and chemokine production [7]. TLR4 participates in the occurrence and progression of neuropathic pain. Publications have shown that

TLR4 increases in the spinal cord in animal models of neuropathic pain [8], and TLR4-deficient mice exhibit reduced pain behavior in various neuropathic pain models [9]. Therefore, implementing TLR4 antagonists or inhibitors might have analgesic effects in neuropathic pain treatment.

Phillygenin (PHI) is a bioactive compound found in *Forsythia suspensa*, traditionally used for treating inflammatory and infectious diseases [10]. PHI exhibits a wide range of bioactive capabilities, including improving the inflammatory status, preventing cancer progression, and protecting against neuron injury [11]. The modulation of different cellular processes, such as NF-κB-related inflammation and PI3K/Akt-mediated tumor cell proliferation, accounts for the diverse properties of the substance [12]. Recent research indicates that PHI could suppress TLR4 activation, further inhibiting the NF-κB signalling pathway downstream, suggesting its potential as a treatment target for the therapy of inflammatory and neuropathic conditions [13]. However, the mechanism of action underlying the potential use of PHI in treating neuropathic pain remains unclear.

This study explored the potential effects of PHI in neuropathic pain progression and its underlying mechanism. We implemented rat chronic constriction injury (CCI) models for in vivo studies on neuropathic pain. We hypothesized that treatment with PHI could significantly inhibit TLR4 expression and suppress MyD88/NF-κB signalling, reducing the neuropathic behavior in CCI rats. Our study aimed to provide new insights into the potential use of PHI for the clinical therapy of neuropathic pain.

METHODS

Animal models

Male Sprague-Dawley rats (210-250 g) were purchased from the Hunan Sileck Jingda Experimental Animal Co., Ltd. All rats were kept in individual cages with a 12 h light/dark cycle, constant temperature (22 ± 2°C) and humidity (50 ± 10%), and food and water were freely available. Both male and female rats were included in the sample. The final allocation of 40 rats comprised an equal representation of both sexes to ensure a comprehensive assessment of the experimental outcomes. The acclimatization of animals (at least seven days) was performed before they underwent various treatments and experiments. The Animal Care and Use Committee of the Wuhan Fourth Hospital reviewed and authorized all animal experiments.

The CCI model was implemented as per a previous study [14]. During surgery, the rats received continuous anesthesia with 2% isoflurane in oxygen, and the exposition of the left sciatic nerve was made at the mid-thigh level. Subsequently, 4-0 chromic gut was used as loose ligatures for making ties around the nerve with a 1-mm interval. The tightened ligatures were further made to produce a mild nerve constriction, which induced the CCI condition. Layers of 4-0 silk sutures were used to close the wound, and all rats were then kept in a warm environment for recovery. Rats that exhibited motor dysfunction or showed signs of infection were excluded from the study. A total of 40 rats were then randomly allocated into four groups (10 rats in each, according to previous studies)[15, 16]: (1) Sham group: rats underwent the same surgical process as the CCI group, but without performing the nerve ligation; (2) CCI group, where CCI surgery was implemented; (3) CCI+PHI group, where CCI surgery was implemented and rats received daily intragastric administration of PHI (20 mg/kg, using DMSO as vehicle, accordingly to previous studies) [11, 17] for 14 consecutive days. PHI was obtained from a commercial supplier, specifically the Shanghai Yuanye Biotechnology Co., Ltd., which provided a high-purity grade of PHI suitable for our experimental needs; (4) CCI+NC group, where CCI surgery was implemented and rats were treated with the same volume of normal saline. Once the CCI model was established and the rats had re-

covered from the anesthesia, they received daily intragastric administration of PHI (20 mg/kg) or the same volume of normal saline in the CCI+NC group for 14 consecutive days. Researchers performing data analysis and histological assessments were blinded to the treatment groups. After model establishment, the rats were euthanized, and their spinal cords were collected for subsequent experiments. Researchers performing data analysis and histological assessments were blinded to the treatment groups.

Von Frey Test

Von Frey assay (Ugo Basile, Italy) was used to assess mechanical allodynia before surgery and on postoperative days 0, 2, 4, 6, 8 10, 12 and 14. All rats were maintained in boxes that contained a wire mesh bottom and acclimatized for 30 minutes. After acclimation, the von Frey test was conducted on the mid-plantar surface of the ipsilateral and contralateral hind paw. The cutoff was set at 26 g, and the data were collected automatically. Pain-like responses, such as an abrupt withdrawal of the paw, licking, or vigorously shaking, were noted. The 50% paw withdrawal threshold (PWT) was determined through the up-down approach as described in a previous study [18].

Hargreaves Test

The Hargreaves test was performed on rats before surgery and on postoperative days 0, 2, 4, 6, 8, 10, 12, and 14 to assess thermal hyperalgesia. The Plantar Test Apparatus (Ugo Basile, Italy) was obtained for this purpose. A total of 30 minutes of acclimatization were first made. A radiant heat source treated the mid-plantar surface of the right hind paw, and the paw withdrawal latency (PWL) was recorded. A 20-second cutoff threshold was set for the heat stimulation test to prevent injury. A decrease in PWL was considered a sign of heat hyperalgesia. The behavioral tests were accomplished by experimenters blinded to the group assignments [18].

Nitric oxide (NO) assay

The expression of NO was detected using a 2,3-diaminonaphthalene (DAN) assay kit. First, the tissue samples were first isolated and homogenized in PBS at 4°C and then centrifuged at 4°C for 15 minutes (12,000 rpm). Next, the supernatant was obtained, and 50 μL of the sample and 50 μL of DAN solution (5 mM in 0.62 M HCl) were added and maintained for 15 minutes at room temperature. Afterward, NaOH (100 μL, 2 M) was used to terminate the reaction. The intensity was measured using a fluorescence spectrophotometer (excitation wavelength: 365 nm, emission wavelength: 450 nm).

ELISA assay

Following the manufacturer's protocol, TNF-α, IL-1β, and IL-6 expressions were measured using a commercial ELISA kit (R&D Systems, USA). Briefly, the spinal cord tissue samples were obtained as described above. Then, 200 μL of detection reagent was added and maintained for 2 h, after which 200 μL of substrate solution was added and maintained for 1 h in the dark. A 50 μL stop solution was added to terminate the reaction, and the absorbance was monitored through a microplate reader (450 nm).

RT-qPCR

TRIzol reagent (Invitrogen, USA) was obtained to isolate total RNA from the spinal cord tissues, following the manufacturer´s protocol. Then, total RNA was reverse transcribed into cDNA using a PrimeScript RT Reagent Kit (Takara Bio, Japan). The expression levels of mRNAs were quantified using an SYBR Premix kit (Takara Bio, Japan) and a StepOnePlus Real-Time PCR system (Applied Biosystems, USA). The mRNA levels were normalized to that of GAPDH, and the calculation was performed as per the $2^{-\Delta\Delta Ct}$ method. The primers used are listed as follows:

TLR4-F: 5'- GAATGCTAAGGTTGGCAC TCTC -3'

TLR4-R: 5'- CTCAGGCAGGAAAGGAA-CAATG -3'

MyD88-F: 5'- GCTGAGAGGAAGAGTTC-TAC -3'

MyD88-R: 5'- CAGTGATAACCCTGGAC-TAC -3'

NF-κB: 5'- AGACCTGGAGCAAGCCATT AG -3'

NF-κB: 5'- CGGACCGCATTCAAGTCAT AG -3'

GAPDH: 5'- TTCAACGGCACAGTCAAG G -3'

GAPDH: 5'- GTCTTCTGAGTGGCAGT-GATG -3'

Western blotting

For Western blot analysis, spinal cord tissue samples underwent homogenization in RIPA buffer (Roche, Switzerland), which was added to the protease inhibitor cocktail. Then, quantification was performed via BCA Protein Assay kit (Thermo Scientific, USA). After separation on 10% SDS-PAGE, proteins were transferred to PVDF membranes (Millipore, USA). Then, 5% non-fat milk was obtained for blocking. Subsequently, the membranes were incubated with primary antibodies against TLR4 (ab22048, Abcam, 1:1000), MyD88 (ab219413, Abcam, 1:1000), p-NF-κB (#3039, CST, 1:1000), NF-κB (#6956, CST, 1:1000), and β-actin (ab8226, Abcam, 1:1000) overnight at 4°C. The membranes were further incubated with secondary antibodies (1:1000) for 2 hours at room temperature. The protein levels of target genes were quantified by visualizing protein bands using enhanced chemiluminescence reagents (Millipore, USA) and analyzed through ImageJ (National Institutes of Health, USA). Normalization to β-actin expression levels was performed.

Statistical analysis

All data were presented as means ± standard deviation (SD) and analyzed using GraphPad Prism 8. One-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test was used to determine the statistical significance of differences among multiple groups. $P<0.05$ was considered statistically significant.

RESULTS

The data illustrated in the behavioral tests revealed that rats subjected to the CCI model had significantly lower 50% PWT and PWL (Figs 1A and B) compared to the Sham group. However, when compared with CCI rats, the additional administration of PHI dramatically enhanced 50% of PWT and PWL. Moreover, the implementation of NO and ELISA assays revealed, respectively, that the levels of NO and the inflammatory cytokines TNF-α, IL-1β, and IL-6 significantly increased after establishing the CCI model (Fig 2). In contrast, administration of PHI significantly reduced these expression levels. Western blotting and RT-qPCR (Figs. 3A and 3B) results showed that the levels of TLR4, MyD88, and p-NF-κB were significantly increased by CCI induction, while treatment with PHI significantly reversed the change in these expression levels. And the results will be presented as "relative quantification (RQ) using the 2^(-ΔΔCt) method," indicating the fold change in expression levels compared to the control group.

DISCUSSION

In our study, treatment with Phillygenin (PHI) significantly enhanced the 50% paw withdrawal threshold (PWT) and paw withdrawal latency (PWL), indicating an effective analgesic effect. Notably, PHI treatment resulted in a marked decrease in nitric oxide (NO) levels, alongside a reduction in proinflammatory cytokines, including TNF-α, IL-1β, and IL-6. Furthermore, our findings demonstrated that PHI downregulated the expression of TLR4 and MyD88, key components in the inflammatory pathway, and inhibited the phosphorylation of NF-κB, a critical factor in inflammation and pain signalling.

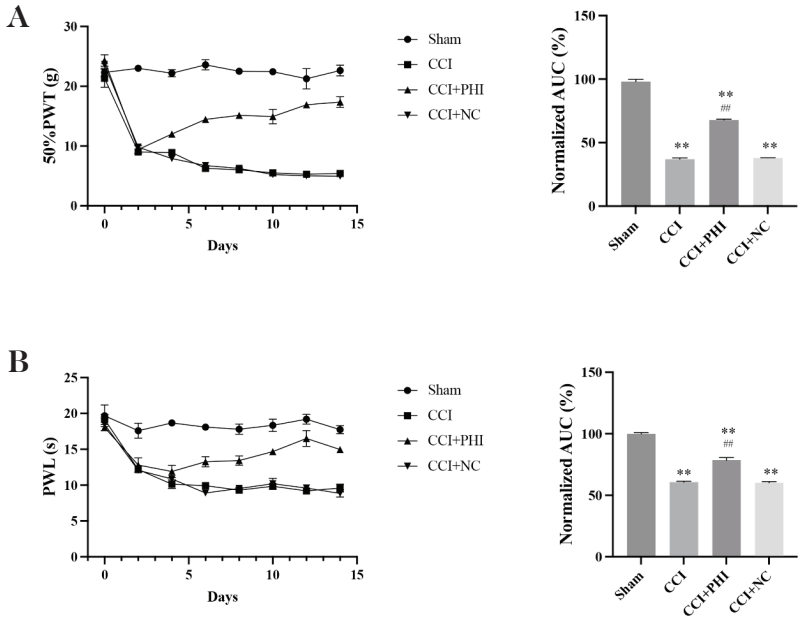

Fig. 1. PHI treatment significantly attenuated mechanical allodynia and thermal hyperalgesia. (A) The time course effect of PHI treatment on mechanical allodynia was assessed using the 50% paw withdrawal threshold (PWT). (B) The time course effect of PHI treatment on thermal hyperalgesia was determined by measuring paw withdrawal latency (PWL). Data are presented as means ± SD. Statistical analyses were performed using a One-way variance analysis (ANOVA). Significant differences are indicated as **P<0.01 vs Sham group and ##P<0.01 vs CCI group (n=10). Abbreviations: CCI, chronic constriction injury; PWT, paw withdrawal threshold; PWL, paw withdrawal latency.

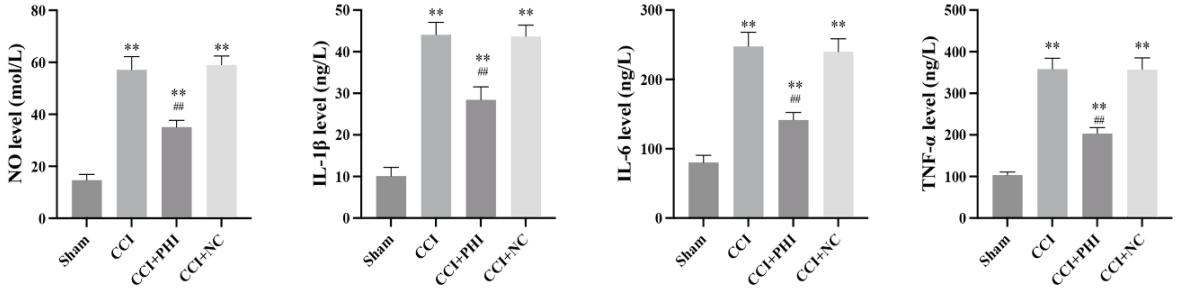

Fig 2. PHI treatment significantly reduced the inflammatory status in CCI rats, as indicated by the levels of nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) measured after treatment. Data are presented as means ± SD. Statistical analyses were conducted using a One-way analysis of variance (ANOVA). Significant differences are indicated as **P<0.01 vs Sham group and ##P<0.01 vs CCI group (n=10). Abbreviations: CCI, chronic constriction injury; NO, nitric oxide; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6. The Y-axis of the graphs denotes the "Levels" of these inflammatory markers.

These results suggest that PHI may alleviate pain by reducing inflammation and modulating key signalling pathways, highlighting its potential as a therapeutic agent in pain management.

In our study, we observed that treatment with Phillygenin (PHI) significantly enhanced the 50% paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) in CCI rats, indicating its potential efficacy in alleviating neu-

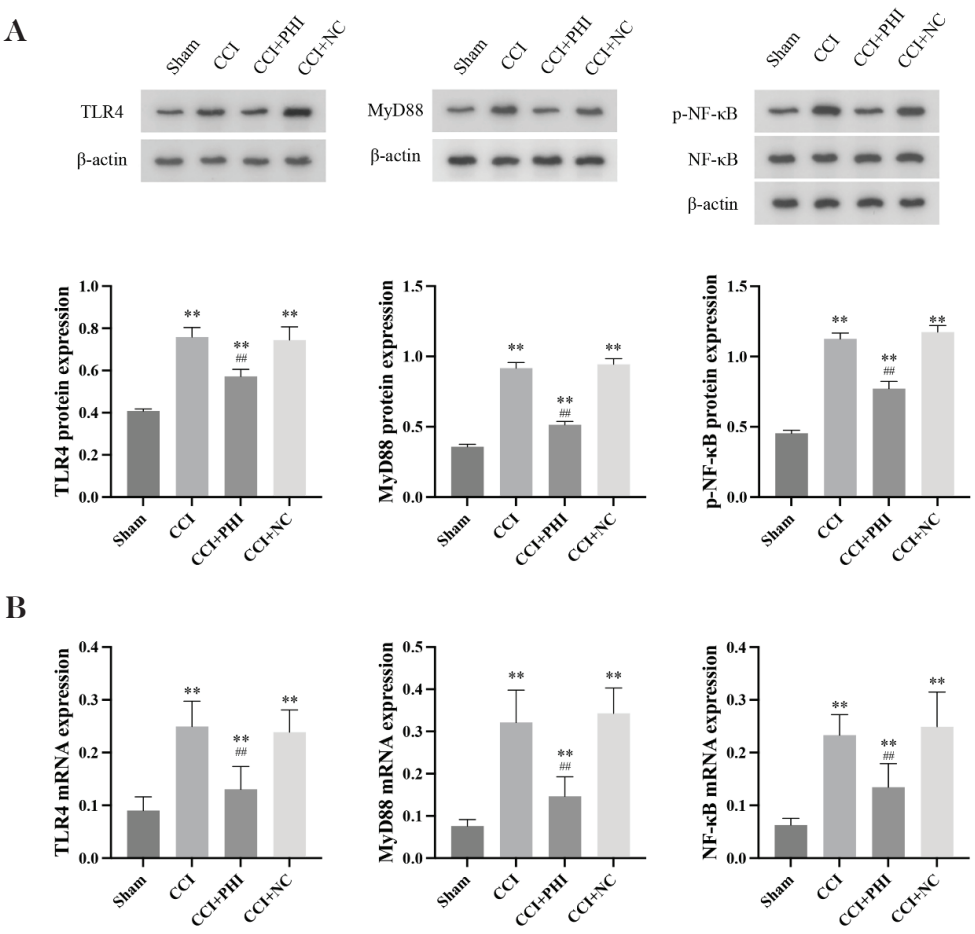

Fig. 3. PHI treatment inhibited CCI rats' TLR4/MyD88/NF-κB signalling pathway. (A) Protein expression levels of TLR4, MyD88, and phosphorylated NF-κB (p-NF-κB) relative to total NF-κB were determined by western blotting. (B) mRNA expression levels of TLR4 and MyD88 were assessed using RT-qPCR. Data are presented as means ± SD. And the results will be presented as "relative quantification (RQ) using the 2^(-ΔΔCt) method," indicating the fold change in expression levels compared to the control group. Statistical analyses were performed using a One-way analysis of variance (ANOVA). Significant differences are indicated as **P<0.01 vs Sham group and ##P<0.01 vs CCI group (n=10). Abbreviations: CCI, chronic constriction injury; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; p-NF-κB, phosphorylated NF-κB.

ropathic pain. This finding aligns with recent literature suggesting that neuropathic pain is closely associated with elevated inflammatory factors and increased TLR4 expression [18]. Specifically, TLR4 signalling has been implicated in mediating neuropathic pain and exacerbating inflammatory responses, as demonstrated in studies indicating that partial sciatic nerve ligation leads to heightened TLR4 levels and subsequent pain sensations [19]. Another study suggested that the increased TLR4 level was linked to partial sciatic nerve ligation-mediated neuropathic pain [20]. Moreover, our findings support Zhang *et al.*'s observations that opioid receptor agonists can non-selectively activate TLR4 and increase proinflammatory cytokines like TNF-α, IL-1β, and IL-6, contributing to neuroinflammation.

In contrast, our results demonstrate that PHI significantly decreased the levels of these inflammatory cytokines, suggesting a potential mechanism for its analgesic effects [21]. Li *et al.* reported enhanced TLR4 and MyD88 levels in the dorsal root ganglion following paclitaxel treatment, which correlated with diminished PWT and PWL [18]. Our study extends these findings by showing that PHI not only modulates TLR4 expression but also reduces inflammation, thereby improving pain thresholds in CCI rats. Overall, our results suggest that targeting TLR4 could be a viable strategy for alleviating neuropathic pain. The integration of our findings with existing literature underscores the importance of inflammatory pathways in neuropathic pain and positions PHI as a promising candidate for further investigation in pain management strategies.

PHI has been found to participate in various aspects of inflammatory status relief and in reducing TLR4 expression. For instance, in our study, we noted that administration of PHI inhibited LPS-induced inflammatory responses and apoptosis in BEAS-2B cells. This inhibition was linked to the subsequent activation of PPAR-γ signalling due to the downregulation of MMP-8. By reducing MMP-8 levels, PHI effectively alleviated acute lung injury [22]. Additionally, a previous study found that dietary PHI supplementation reduced malondialdehyde and inflammatory mediator production, increased antioxidant enzyme contents and Bcl-2 levels, and ameliorated aflatoxin B1-induced liver damage [23]. Xue *et al*. found that PHI reversed the expression of SOD and MDA and downregulated the levels of TNF-α, IL-1β, IL-6, and IL-10 in a model of colitis in mice, and reduced the proportion of tyrosine kinase Src activated by TLR4, suggesting that PHI might be a potential drug candidate that could effectively safeguard against colitis [13]. Our study found that PHI administration might ameliorate neuropathic pain in CCI rats, potentially through mechanisms such as the downregulation of TLR4, inhibition of MyD88/NF-κB signalling, and reduction of proinflammatory cytokines. Similarly, our findings demonstrated that PHI played a beneficial role in reducing inflammation in CCI rats, suggesting it may serve as an alternative therapeutic strategy for neuropathic pain. In our study, we observed that treatment with Phillygenin (PHI) significantly enhanced the 50% paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) in CCI rats, indicating its potential as an analgesic agent.

Our results suggest that PHI may alleviate neuropathic pain by targeting the TLR4 and MyD88/NF-κB signalling pathways. While these findings are promising, a more detailed mechanistic explanation is warranted to elucidate how PHI exerts its effects. One potential mechanism involves the downregulation of TLR4 expression by PHI, which could reduce the activation of downstream inflammatory pathways. By inhibiting TLR4, PHI may prevent the subsequent activation of MyD88 and the phosphorylation of NF-κB, leading to a decrease in proinflammatory cytokines such as TNF-α, IL-1β, and IL-6. This reduction in inflammatory mediators may alleviate pain and contribute to restoring normal neuronal function in the context of neuropathic pain. Additionally, PHI may modulate oxidative stress pathways. By decreasing nitric oxide (NO) levels, PHI could help mitigate oxidative damage that often accompanies neuropathic conditions, further supporting neuronal health and function. This dual action-reducing inflammation while protecting neurons–could provide a comprehensive approach to pain management. While our findings align with existing literature, further investigation is needed to explore these proposed mechanisms. Future studies should aim to clarify the specific interactions of PHI with TLR4 and MyD88/NF-κB, potentially utilizing molecular and cellular assays to provide insight into the therapeutic implications of targeting these pathways in neuropathic pain. This enhanced mechanistic understanding would strengthen the rationale for PHI as a viable therapeu-

tic strategy in clinical settings. We utilized a nitric oxide (NO) assay to assess NO levels, a key signalling molecule involved in various physiological and pathological processes, including inflammation and pain. Our results indicated that Phillygenin (PHI) treatment significantly reduced NO levels in CCI rats. This reduction is particularly noteworthy, as elevated NO levels are often associated with increased pain sensitivity and inflammatory responses in neuropathic pain models. The decrease in NO levels suggests that PHI may exert an analgesic effect by modulating the nitric oxide signalling pathway. Elevated NO contributes to pain by enhancing nociceptive signalling and promoting neuroinflammation. By lowering NO levels, PHI may help restore the balance of neuroinflammatory mediators, thereby alleviating pain sensations. Furthermore, the reduction of NO could also imply potential protective effects on neuronal health, as excessive NO can lead to oxidative stress and neuronal damage. This aspect is crucial, as it highlights PHI's dual role in alleviating pain through anti-inflammatory effects and promoting neuronal survival in a neuropathic context.

Previous publications have investigated the role of the TLR4 and its downstream MyD88/NF-κB signalling in inflammation and neuropathic pain. Wang et al. indicated that the upregulation of the TLR4 and MyD88/NF-κB signalling participated in the development of inflammation and contributed to vascular dementia [24]. MicroRNA-27a modulated the TLR4 and MyD88/NF-κB signalling, further decreasing proinflammatory cytokine levels and ameliorating acute lung injury [25]. We referenced the findings of Liu et al., who suggested that activating the GABA receptor, specifically the GABAA receptor, might inhibit TLR4 and MyD88/NF-κB signalling pathways, thereby ameliorating the progression of diabetic neuropathic pain [26]. Our results indicate that PHI influences the TLR4 expression and inhibits MyD88/NF-κB signalling, highlighting its crucial role in neuropathic pain.

In our study, we conducted behavioral tests to assess mechanical allodynia and thermal hyperalgesia in CCI rats following PHI treatment. A one-way ANOVA was initially applied to establish differences between the groups, and this analysis is represented in the bar graphs of Fig. 1. These graphs illustrate each group's total magnitude of mechanical allodynia and thermal hyperalgesia. Additionally, the line graphs in the same figure display intriguing patterns of changes in nociceptive variables over time. Given the temporal nature of these data, we agree that a time-oriented analysis would enhance the depth of our results. Thus, we will perform a two-way ANOVA to analyze the time-dependent effects of PHI on these behavioral measures in our future studies. This approach will allow us to examine both the treatment and time factors, providing a more comprehensive understanding of the dynamics of pain response. The initial analysis using one-way ANOVA provided valuable insights into the overall effects of PHI on mechanical allodynia and thermal hyperalgesia. However, the rich temporal data displayed in the line graphs suggested that a more nuanced approach could be beneficial. A two-way ANOVA is more appropriate for analyzing changes across time points and treatment conditions.

By incorporating this analysis, we can better interpret the interaction effects of treatment and time on pain responses, enhancing our understanding of the pharmacodynamics of PHI. In acknowledging the potential limitations of our study, several factors warrant consideration. Although we used 40 rats divided into four groups, a larger sample size may have provided more robust data and enhanced the statistical power of our findings. Smaller sample sizes can lead to variability and may not fully capture the effects observed. Other factors, such as environmental stressors, the time of day, and individual animal differences, could influence pain responses. While we attempted

to control for these variables, they may still introduce bias or variability in our results.

Our study demonstrated that PHI treatment could reduce TLR4 levels and attenuate the inflammatory status in CCI rats, indicating that targeting the TLR4 and its downstream MyD88/NF-κB signalling might be a viable therapeutic strategy for neuropathic pain. However, our study needed further investigation on other inflammatory factors and molecular pathways related to PHI and neuropathic pain to explore its underlying mechanism further.

Conflicts of interest

All authors declare no conflict of interest.

Ethics Approval

The ethics approval was obtained from the Ethics Committee of Wuhan Fourth Hospital.

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Author´s contributions

LH conceived and designed experiments; performed experiments and data analysis; provided technical support, data collection and analysis; and wrote the manuscript. WPZ conceived and designed experiments; provided technical support, data collection and analysis; and wrote the manuscript. MJL conceived and designed experiments; provided technical support, data collection and analysis; and wrote the manuscript. RCL conceived and designed experiments; provided technical support, data collection and analysis; and wrote the manuscript.

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Values of hemodynamic changes of fetal vessels evaluated by color Doppler ultrasound for fetuses with growth restriction.

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Key words: color; Doppler ultrasound; fetal growth; growth restriction; restriction.

Abstract. We aimed to explore the values of hemodynamic changes of fetal vessels evaluated by color Doppler ultrasound (CDUS) for fetal growth restriction (FGR). A retrospective analysis was performed on clinical data of 75 pregnant women who received prenatal examination from January 2021 to August 2023 and whose fetuses were diagnosed with FGR (FGR group) and 75 pregnant women whose fetuses were healthy in the same period and were considered as the healthy group. CDUS was performed on the fetuses. The values of indicators of umbilical artery, middle cerebral artery and aortic arch isthmus for assessing pregnancy outcomes were investigated. The FGR group had significantly lowered arterial resistance index (RI), blood flow pulsatility index (PI), and systolic and diastolic velocity (S/D) levels of the middle cerebral artery and peak systolic velocity (PSV)/end-systolic reflux velocity (ESRV) level of aortic arch isthmus but significantly elevated RI, PI, and S/D levels of umbilical artery in comparison with those of the healthy group ($p<0.05$). The areas under the receiver operating characteristic curves (AUCs) of RI, PI, and S/D of the umbilical artery in diagnosing FGR were 0.893, 0.893 and 0.900, respectively, AUCs of RI, PI, and S/D of the middle cerebral artery were 0.812, 0.874 and 0.910, respectively, and AUC of PSV/ESRV was 0.857 ($p<0.05$). The incidence rate of severe hypoxia was significantly higher in the fetuses with a more significant RI value of the middle cerebral artery and a larger PSV/ESRV value than those with a smaller RI value of the middle cerebral artery and a smaller PSV/ESRV value ($p<0.05$). The changes in umbilical artery RI, middle cerebral artery RI, and PSV/ESRV were unrelated to fetal survival rate ($p>0.05$). Fetal umbilical artery, middle cerebral artery and aortic arch isthmus parameters detected through CDUS are all sensitive indices for assessing FGR.

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Valores de los cambios hemodinámicos de los vasos fetales evaluados mediante ecografía Doppler a color en fetos con restricción del crecimiento.

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Palabras clave: color; ecografía Doppler; crecimiento fetal; parámetro; restricción de crecimiento, flujo sanguíneo arterial.

Resumen. Nuestro objetivo fue explorar los valores de los cambios hemodinámicos de los vasos fetales evaluados mediante ecografía Doppler a color (CDUS) en la restricción del crecimiento fetal (FGR). Se realizó un análisis retrospectivo de los datos clínicos de 75 mujeres embarazadas que recibieron un examen prenatal desde enero de 2021 hasta agosto de 2023 y cuyos fetos fueron diagnosticados con FGR (grupo FGR) y 75 mujeres embarazadas cuyos fetos estaban sanos en el mismo período y se consideraron como el grupo sano. Se realizó CDUS en los fetos. Se investigaron los valores de los indicadores de la arteria umbilical, la arteria cerebral media y el istmo del arco aórtico para evaluar los resultados del embarazo. El grupo FGR tuvo un índice de resistencia arterial (IR), un índice de pulsatilidad del flujo sanguíneo (IP) y niveles de velocidad sistólica y diastólica (S/D) significativamente reducidos de la arteria cerebral media y un nivel de velocidad sistólica máxima (PSV)/velocidad de reflujo telesistólico (ESRV) del istmo del arco aórtico, pero niveles significativamente elevados de IR, IP y S/D de la arteria umbilical en comparación con los del grupo sano ($p<0,05$). Las áreas bajo las curvas de características operativas del receptor (AUC) de RI, PI y S/D de la arteria umbilical en el diagnóstico de FGR fueron 0,893, 0,893 y 0,900, respectivamente, las AUC de RI, PI y S/D de la arteria cerebral media fueron 0,812, 0,874 y 0,910, respectivamente, y el AUC de PSV/ESRV fue 0,857 ($p<0,05$). La tasa de incidencia de hipoxia grave fue significativamente mayor en los fetos con un valor de RI más significativo de la arteria cerebral media y un valor de PSV/ESRV mayor que aquellos con un valor de RI menor de la arteria cerebral media y un valor de PSV/ESRV menor ($p<0,05$). Los cambios en el RI de la arteria umbilical, el RI de la arteria cerebral media y el PSV/ESRV no se relacionaron con la tasa de supervivencia fetal ($p>0,05$). Los parámetros de la arteria umbilical fetal, la arteria cerebral media y el istmo del arco aórtico detectados mediante ecografía endoscópica son todos índices sensibles para evaluar la RCF.

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INTRODUCTION

Fetal growth restriction (FGR), a pregnancy complication, refers to the inability of fetuses to achieve their genetic growth potential, which has such characteristics as pathological lag of intrauterine growth rate in an intrauterine growth curve. FGR is also one of the common perinatal complications, accounting for about 30% of perinatal deaths, and is detected in 50% of perinatal infants with intrauterine hypoxia during de-

livery, which is the second leading cause of perinatal deaths [1,2]. FGR has an association with various adverse perinatal outcomes, such as stillbirth, neonatal death, and neonatal diseases (intraventricular hemorrhage, neonatal hyperbilirubinemia, and hypoglycemia, among others.), probably having negative impacts on the neurobehavioral development of affected children in the long term and increasing the risk of such diseases as obesity, diabetes, and cardiovascular and cerebrovascular diseases in such children. The pathogenesis of FGR remains unclear, but previous studies have manifested that maternal nutrition, placental transfer, fetal inheritance and other relevant factors are implicated in the development of FGR. However, given that it is hard to diagnose FGR in the first trimester of pregnancy, FGR is usually diagnosed after delivery or in late gestation, which further highlights the importance of accurate ultrasound examination during the first trimester of pregnancy to assess fetal growth indicators dynamically. CDUS is a common imaging examination approach in clinical practice, characterized by good safety, non-invasion, simple operation and free-radiation. At 18-22 weeks of pregnancy, most morphological and structural abnormalities of fetuses can be screened out through ultrasound. As one of the crucial parameters in human physiological evaluation, arterial blood flow can illustrate the benefits of fetal metabolism, and blood flow velocity distribution is of great significance in clinical measurement [3]. A study also denoted a close relationship between the pathological change of FGR and abnormal changes in uterine-placental-fetal blood circulation.[4] For this reason, early assessment of blood flow changes in the umbilical artery, middle cerebral artery and fetal heart is conducive to early diagnosis and early intervention of FGR, which is significant for improving fetal prognosis.

In this study, 75 pregnant women who received prenatal examination and whose fetuses were diagnosed with FGR in our hospital from January 2021 to August 2023 were enrolled as subjects to analyze the value of relevant intraabdominal fetal parameters detected by CDUS in assessing FGR.

PATIENTS AND METHODS

General data

Seventy-five pregnant women with FGR fetuses receiving prenatal examinations in our hospital from January 2021 to August 2023 were selected as observation subjects (FGR group). The inclusion criteria were set as follows: 1) Pregnant women whose fetuses met the diagnostic criteria for FGR,[5] 2) those who were singleton, naturally conceived and in the third trimester of pregnancy, 3) those whose fetuses had no response to fetal heart monitoring, 4) those with decreased fetal movement, 5) those who were healthy in the past, without history of genetic diseases, and 6) those with complete clinical data and no data loss. The exclusion criteria involved 1) pregnant women whose fetuses had structural malformations and chromosome abnormalities before delivery based on ultrasound examination, 2) those whose fetuses were complicated by severe congenital diseases, 3) those whose fetuses suffered from endogenous homologous FGR induced by fetal chromosomal abnormalities, 4) those with abnormities or spiral edema in umbilical cord insertion point, or single umbilical artery, 5) those complicated by prenatal complications or comorbidities, 6) those with placental morphological changes, including choriocarcinoma or other lesions, or 7) those with nervous system or mental disease. Meanwhile, 75 pregnant women with healthy fetuses undergoing prenatal examination in our hospital in the same period were selected as the healthy group. Pregnant women in the FGR group (n=75) were aged 22-35 years old, with an average of (28.85±5.85) years old, and had a gestational age of 33-43 weeks, with a mean of (37.45±4.15) weeks. In terms of parity, there were 46 primiparas and 29 multipa-

ras. In the healthy group (n=75), pregnant women were aged 23-35 years old, with an average of 28.45±5.85 years old, and had a gestational age of 35-42 weeks, with a mean of 37.85±6.18 weeks. As to parity, there were 42 primiparas and 33 multiparas. No statistically significant differences between the two groups were found in age, parity and gestational age ($p>0.05$). This study was conducted with approval from the hospital's Ethics Committee.

Examination methods

Routine prenatal ultrasound was measured using a real-time ultrasound imager with a linear or convex array probe (abdominal probe frequency of 3.0-3.5 MHz, with 5.0 MHz for thin pregnant women) as follows. After exposing the abdomen of pregnant women, couplant was smeared on the examination area, and the probe was placed on the area to observe whether there was any abnormality in fetal position, placental position and uterine appendages. Besides, a series of inspections were carried out on the fetuses from head to toe, and biological measurements were made on various standard cross sections, including biparietal diameter, head circumference, abdominal circumference, and femur length. In addition, the gestational age was predicted, fetal weight was estimated, and fetal position and fetal number were judged. Additionally, amniotic fluid and the position and maturity of the placenta were observed, and the depth of amniotic fluid and the thickness and area of the placenta were measured. Moreover, observations were made on physiological phenomena such as the fetal heart, fetal movement, and fetal swallowing. In our hospital, suspected FGR would be determined if the fetal weight assessed by ultrasound was less than the 10^{th} percentile of the average weight of normal fetuses of the same gestational age or two standard deviations below the average weight.

CDUS was conducted with the DC-35Pro diagnostic system (Mindray Medical Equipment Co., Ltd., China). In brief, pregnant women were guided to lie in the supine position, and the fetal weight and head circumference were routinely measured. Then, the probe was placed vertically in the lower abdomen and tilted to one side to find and display the external iliac artery by the parasagittal section. Next, CDUS was employed to identify the uterine artery crossing the external iliac artery. Thereafter, considering that the uterine artery extends along one side of the uterus to the fundus of the uterus, the scanning direction of the probe was adjusted so that the main uterine artery was parallel to the sound beam as much as possible. Next, the spectrum Doppler sampling gate was placed on the main uterine artery 1 cm below the intersection point for measurement. After that, the contralateral uterine artery was measured in the same way. Thereafter, the middle cerebral artery was examined as follows. At the standard biparietal diameter section (the brain midline was perpendicular to the sound beam as far as possible), the probe was moved in parallel to the fetal skull base until a pair of great wings of sphenoid bone appeared between the anterior cranial fossa and the middle cranial fossa. The middle cerebral artery starts from the left and right sides of the middle part of the arterial ring, goes to both sides of the brain and slightly deviates to the forehead. The sampling volume was set to 2-3 mm, the probe was placed at the point 3-5 mm away from the starting point of the Willis arterial ring, and the Doppler angle was adjusted as close as possible to 0° (not more than 30°), and more than three continuous and stable pulse Doppler waveforms were obtained. Finally, the arterial resistance index (RI), blood flow pulsatility index (PI), and systolic/diastolic velocity (S/D) levels of the middle cerebral artery were measured.

Afterwards, the measurement of the umbilical artery was performed. In brief, the middle part of the umbilical cord floating in amniotic fluid was selected for examination, and the angle between the Doppler sound

beam and umbilical blood vessel should be less than 30° to obtain the Doppler spectrum of the umbilical artery. After at least three continuous and stable waveforms appeared, the image was frozen to measure the RI, PI, and S/D levels. Afterwards, the three-vessel and trachea section of the fetal heart was found to measure the peak systolic velocity (PSV) and end-systolic reflux velocity (ESRV) levels of aortic arch isthmus, followed by calculation of PSV/ESRV.

Observation of indicators

The changes in RI, PI, and S/D levels of the fetal middle cerebral artery and umbilical artery and the PSV/ESRV level of fetal aortic arch isthmus were compared between the two groups.

Pregnancy outcomes were assessed. In brief, pregnancy outcomes were observed, and abnormal pregnancy outcomes, namely severe hypoxia (Apgar score ≤3 points after birth, stillbirth, neonatal death, and presence of hypoxic complications including cerebral palsy) and mild hypoxia (3 points < Apgar score ≤7 points, small for gestational age, relieving of hypoxia symptoms after birth, and absence of complications), were recorded.

Statistical analysis

The SPSS 20.0 software was employed for statistical analysis. Measurement data were expressed by ($\bar{x} \pm SD$) and subjected to the *t*-test. Count data were expressed by % and subjected to the χ^2 test. The receiver operating characteristics (ROC) curves were plotted to assess the diagnostic value of ultrasound parameters in FGR, $p<0.05$ suggested that the difference was statistically significant.

RESULTS

Middle cerebral artery parameters

The RI, PI, and S/D levels of the middle cerebral artery were obviously lower in the FGR group than those in the healthy group ($p<0.05$) (Table 1).

Table 1
Middle cerebral artery parameters.

Group	n	Middle cerebral artery		
		RI	PI	S/D
Healthy	75	0.72±0.16	1.60±0.37	4.56±0.59
FGR	75	0.51±0.12	1.33±0.41	3.26±0.57
t		9.093	4.234	13.724
p		<0.001	<0.001	<0.001

Measurement data were expressed by ($\bar{x} \pm SD$) and subjected to the *t*-test. FGR: Fetal growth restriction; PI: pulsatility index; RI: resistance index; S/D: systolic/diastolic velocity.

Umbilical artery indicators

The RI, PI, and S/D levels of umbilical artery were significantly higher in the FGR group than those in the healthy group ($p<0.05$) (Table 2).

Table 2
Umbilical artery parameters.

Group	n	Umbilical artery		
		RI	PI	S/D
Health	75	0.56±0.12	0.75±0.16	2.01±0.34
FGR	75	0.87±0.24	1.19±0.48	2.89±0.36
t		10.005	7.531	15.391
p		<0.001	<0.001	<0.001

Measurement data were expressed by ($\bar{x} \pm SD$) and subjected to the *t*-test. FGR: Fetal growth restriction; PI: pulsatility index; RI: resistance index; S/D: systolic/diastolic velocity.

Aortic arch isthmus indicators

The PSV/ESRV level of the aortic arch isthmus was markedly lower in the FGR group than in the healthy group [(2.86±0.62) *vs.* (3.85±0.78)] ($t=8.605$, $p<0.05$).

Diagnostic value of umbilical artery, middle cerebral artery and aortic arch isthmus indicators in FGR

As shown in ROC curves, the area under the ROC curves (AUCs) of RI, PI, and S/D of the umbilical artery in diagnosing FGR were 0.893, 0.893 and 0.900, respectively ($p<0.05$). The AUCs of RI, PI, and S/D of

middle cerebral artery in diagnosing FGR were 0.812, 0.874 and 0.910, respectively ($p<0.05$). The AUC of PSV/ESRV of aortic arch isthmus in diagnosing FGR was 0.857 ($p<0.05$) (Table 3 and Fig. 1).

Evaluation of pregnancy outcomes based on umbilical artery, middle cerebral artery and aortic arch isthmus indicators

Combined with the ability to assess pregnancy outcomes by simply comparing blood flow parameters in clinical practice, ROC curves were adopted for the analysis of pregnancy outcomes based on the RI values of the umbilical artery and middle cerebral artery and the cutoff value of PSV/ESRV. It was found that the incidence rate of severe hypoxia was higher in fetuses with a more significant RI value of the middle cerebral artery and a larger PSV/ESRV value than those with a smaller RI value of the middle cerebral artery and a smaller PSV/ESRV value ($p<0.05$) (Table 4).

Table 3
Diagnostic value of umbilical artery, middle cerebral artery and aortic arch isthmus indicators in FGR.

Indicator		Area under the curve	95% confidence interval	p	Sensitivity	Specificity	Cut-off	Youden index
Umbilical artery	RI	0.893	0.839~0.946	<0.001	76.00	86.00	0.66	0.720
	PI	0.893	0.843~0.942	<0.001	93.33	70.67	1.02	0.640
	S/D	0.900	0.850~0.950	<0.001	86.67	81.33	2.76	0.680
Middle cerebral artery	RI	0.812	0.735~0.890	<0.001	76.00	86.67	0.69	0.627
	PI	0.874	0.815~0.933	<0.001	74.67	88.00	1.38	0.627
	S/D	0.910	0.857~0.963	<0.001	84.00	89.33	3.38	0.733
Aortic arch isthmus	PSV/ESRV	0.857	0.797~0.917	<0.001	84.00	73.33	3.36	0.573

ESRV: End systolic reflux velocity; PI: pulsatility index; PSV: peak systolic velocity; RI: resistance index; S/D: systolic/diastolic velocity.

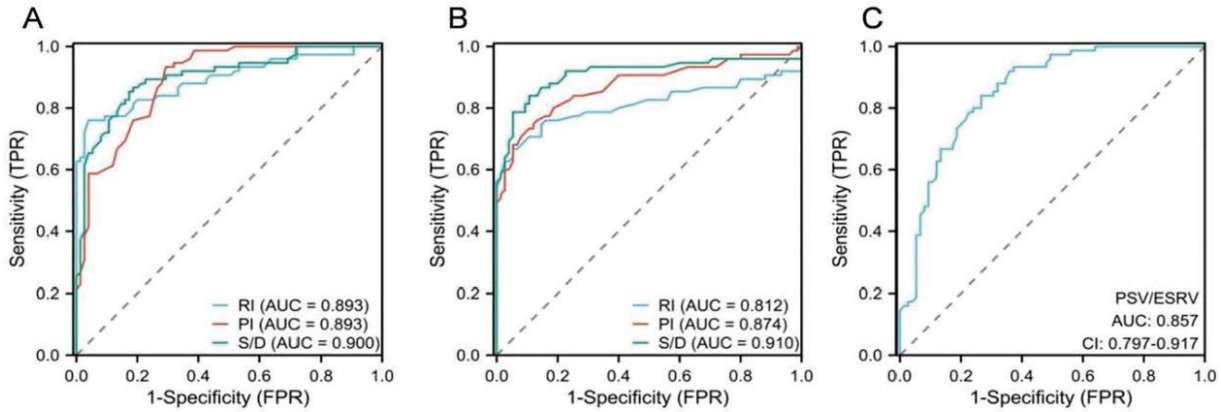

Fig. 1. ROC curves of the umbilical artery, middle cerebral artery and aortic arch isthmus are indicators of FGR. A: Diagnostic value of umbilical artery in FGR. B: Diagnostic value of middle cerebral artery in FGR. C: Diagnostic value of aortic arch isthmus in FGR. AUC: Area under the curve; FGR: fetal growth restriction; PI: pulsatility index; ROC: receiver operator characteristic; RI: resistance index; S/D: systolic/diastolic velocity.

Table 4
Evaluation of pregnancy outcomes based on various parameters.

Indicator	n	Abnormal pregnancy outcome		χ^2	p
		Mild hypoxia	Severe hypoxia		
RI of umbilical artery >0.66	60	23 (38.33)	37 (61.67)	1.114	0.291
RI of umbilical artery ≤0.66	15	8 (53.33)	7 (46.67)		
RI of middle cerebral artery >0.69	55	15 (27.27)	40 (72.73)	16.816	<0.001
RI of middle cerebral artery ≤0.69	20	16 (80.00)	4 (20.00)		
PSV/ESRV >3.36	53	17 (32.08)	36 (67.92)	6.386	0.012
PSV/ESRV ≤3.36	22	14 (63.64)	8 (36.36)		

Count data were expressed by % and subjected to the χ^2 test. ESRV: End systolic reflux velocity; PSV: peak systolic velocity; RI: resistance index.

Table 5
Evaluation of fetal survival rate based on various parameters.

Indicator	n	Fetal outcome		χ^2	p
		Survival	Death		
RI of umbilical artery >0.66	60	53 (88.33)	7 (11.67)	1.930	0.165
RI of umbilical artery ≤0.66	15	15 (100.00)	0 (0.00)		
RI of middle cerebral artery >0.69	55	48 (87.27)	7 (12.73)	2.808	0.094
RI of middle cerebral artery ≤0.69	20	20 (100.00)	0 (0.00)		
PSV/ESRV >3.36	53	46 (86.79)	7 (13.21)	3.205	0.073
PSV/ESRV ≤3.36	22	22 (100.00)	0 (0.00)		

Count data were expressed by % and subjected to the χ^2 test. ESRV: End systolic reflux velocity; PSV: peak systolic velocity; RI: resistance index.

Evaluation of fetal survival rate based on umbilical artery, middle cerebral artery and aortic arch isthmus indicators

The fetal survival rates were further evaluated based on the umbilical artery, middle cerebral artery, and aortic arch isthmus indicators. The changes in umbilical artery RI, middle cerebral artery RI, and PSV/ESRV were not related to fetal survival rate ($p>0.05$) (Table 5).

DISCUSSION

As one of the common perinatal complications, FGR is closely related to placental dysfunction and decreased fetal reserve capacity, leading to high perinatal fetal mortality and a high incidence rate of long-term complications [6]. Therefore, early screening of FGR and early intervention are significant for improving the prognosis of fetuses with FGR.

CDUS has no significant effect on fetal growth and development due to noninvasive and radiation-free operation, and it can be used to evaluate the blood perfusion of the fetal-placental circulation by observing fetal vascular hemodynamic changes [7,8]. Normally, with increasing gestational week, the diastolic blood flow of the umbilical artery increases, and S/D, PI, and RI levels decrease[9,10]. However, FGR may occur when there is a decrease in villous vascular branches, an increase in circulatory resistance, and a decrease in the total cross-sectional area of the vascularized lumen in the placenta, and

an elevation in S/D, PI, and RI levels of the umbilical artery. In severe cases, the risk of adverse events, such as intrauterine distress, asphyxia, and even death of the fetus, may increase [11,12]. In this study, the RI, PI, and S/D levels of umbilical artery in the FGR group were significantly higher than those of the healthy group ($p<0.05$), indicating that the blood flow in the umbilical artery of fetuses with FGR was in a high resistance state.

According to the hemodynamic principle of fetal placental circulation, villous vascular bed increases in the second and third trimesters of pregnancy, the resistance of fetal placental circulation and the S/D decrease, and the placental blood flow increases accordingly, which is conducive to the growth and development of fetuses.[13] The results of this study revealed that the S/D value of FGR fetuses significantly increased, probably due to prolonged hypoxia and nutritional deficiencies. Moreover, fetal development is closely related to placental blood flow. As a result, the S/D value indirectly reflects the fetal-placental circulation state and intrauterine conditions. Also, the ROC curve analysis results showed that the AUCs of RI, PI, and S/D of the umbilical artery in the diagnosis of FGR were 0.893, 0.893 and 0.900 ($p<0.05$), respectively, indicating that abnormal umbilical artery hemodynamics can affect the supply of nutrients to fetuses. Regular monitoring of umbilical artery hemodynamic changes is favorable for the early diagnosis and clinical management of FGR.

As an important branch of the internal carotid artery, the middle cerebral artery markedly affects the changes in fetal cerebral circulation, and its hemodynamic alterations are closely related to fetal cranial blood circulation and hypoxia [14,15]. In the case of insufficient cerebral blood supply and oxygenation, the hypoxia and ischemia of the fetus are aggravated, causing damage to other organs and affecting the prognosis [16,17]. In this study, the RI, PI, and S/D levels of the middle cerebral artery in the FGR group were significantly lower than those in the healthy group ($p<0.05$), probably because FGR activated the cerebral protective effect to self-regulate and contract peripheral vasculature to increase the blood supply to the heart, brain, and other vital organs. As a result, monitoring the changes in the resistance parameters of the middle cerebral artery can evaluate the effect of fetal hypoxia on FGR. The results of ROC curve analysis herein revealed that the AUCs of RI, PI, and S/D of the middle cerebral artery in the diagnosis of FGR were 0.812, 0.874, and 0.910 ($p<0.05$), with high specificity and Youden index. Moreover, the analysis of pregnancy outcomes based on the cut-off value of RI showed that a high proportion of fetuses with severe hypoxia had an RI >0.69. Therefore, the blood flow parameters of the middle cerebral artery can be used as indicators for the prenatal ultrasound diagnosis of FGR.

PSV/ESRV can reflect the blood flow of the aortic arch isthmus. When a fetus has a reduced blood supply, the body activates the compensatory mechanism to protect important organs such as the heart and brain and increase the perfusion of such organs, increasing ESRV level and decreasing PSV/ESRV [18,19]. In this study, the PSV/ESRV level of the aortic arch isthmus in the FGR group was significantly lower than that in the healthy group ($p<0.05$), suggesting that FGR can also be evaluated based on hemodynamic changes in the aortic arch isthmus. Probably, the decreased blood oxygen level during the increase in the resistance to fetal peripheral blood flow cannot meet the needs of fetal growth and development, so the body initiates a compensatory mechanism to promote dilatation to increase the perfusion of blood flow. Also, local anaerobic glycolysis increases in a state of hypoxia, producing metabolites such as lactate and adenosine, which can dilate blood vessels and reduce cardiac output [20]. Additionally, the results of ROC curve analysis revealed that the AUC of PSV/ESRV of aortic arch isthmus in diagnosing FGR was 0.857 ($p<0.05$), with the sensi-

tivity and specificity of 84.00% and 73.33%, respectively. The infants with diagnostic value >3.36 accounted for a significantly high proportion. This indicates that the blood flow changes in the aortic arch isthmus are valuable for diagnosing FGR. The early monitoring of the PSV/ESRV level changes is conducive to diagnosing FGR at an early stage and can help guide the treatment.

In conclusion, fetal umbilical artery, middle cerebral artery, and aortic arch isthmus parameters detected by CDUS are all sensitive indicators for evaluating FGR, and the determination of optimal diagnostic value for each flow parameter is valuable for the clinical determination of FGR and intrauterine hypoxia, and for improving the prognosis. However, due to the short duration of this study, the values of fetal parameters detected by CDUS in evaluating the severity and prognosis of FGR have not yet been analyzed. In the future, the research duration will be increased, and the source of subjects will be expanded for in-depth investigation.

Conflicts of interest

The authors declare they have no conflicts of interest.

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Pesquisa y diagnóstico de trastorno depresivo en una comunidad rural de Venezuela.

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Palabras clave: trastorno depresivo; PHQ-9; desempleo; Venezuela; salud rural.

Resumen. La pandemia de la COVID-19 ha desencadenado un incremento mundial en la incidencia de consultas psicológicas con relación a ansiedad y trastorno depresivo, que, en conjunto con la crisis humanitaria en Venezuela, produjo un aumento en los casos reportados de estas patologías a nivel nacional. Actualmente, no se encuentran disponibles trabajos que documenten la prevalencia de trastorno depresivo en comunidades rurales de Venezuela. El objetivo de este trabajo fue determinar la prevalencia de trastorno depresivo en La Marroquina, comunidad rural situada en el Estado Yaracuy, Venezuela, mediante un estudio de tipo transversal, realizado en julio del 2022, con una población estimada de 611 pacientes y un muestreo de 160. El estudio comprendió dos fases: una entrevista breve, donde se realizó el Cuestionario de Salud del Paciente 9 (PHQ-9) y en una segunda fase, entrevista con un especialista en Psiquiatría, quien utilizó la escala de Depresión de Hamilton (HAM-D) para el diagnóstico de depresión. En el triaje psiquiátrico de depresión, 67 pacientes obtuvieron puntajes de PHQ-9>10 puntos, de los cuales sólo 39 asistieron a consulta. Al realizar la evaluación psiquiátrica de los mismos, se diagnosticó trastorno depresivo en 30 pacientes, para una prevalencia en la comunidad de La Marroquina de 18,75%. Se encontró una asociación estadísticamente significativa entre riesgo de depresión según PHQ-9 y desempleo. El hecho de que las escalas no permiten diferenciar entre diversos tipos de trastorno depresivo, así como el estigma social de acudir a una consulta psiquiátrica pueden causar un subregistro. La Marroquina tiene una alta prevalencia de trastorno depresivo, que se relaciona con el desempleo como factor de riesgo más importante.

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Screening and diagnosis of depressive disorder in a rural community in Venezuela.

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Keywords: depressive disorder; PHQ-9; unemployment; Venezuela; rural health.

Abstract. The COVID-19 pandemic has triggered a global increase in the incidence of psychological consultations related to anxiety and depressive disorder, which, together with the humanitarian crisis in Venezuela, produced a surge in reported cases of these pathologies nationwide. Currently, there are no works available that document the prevalence of depressive disorder in rural communities in Venezuela. This work aimed to determine the prevalence of depressive disorder in La Marroquina, a rural community in Yaracuy State, Venezuela, through a cross-sectional study conducted in July 2022, with an estimated population of 611 patients and a sample of 160. The study included two phases: a brief interview, where the Patient Health Questionnaire 9 (PHQ-9) was conducted and in a second phase, an interview with a Psychiatry specialist who used the Hamilton Depression Scale (HAM-D) for the diagnosis of depression. In the psychiatric triage for depression, 67 patients obtained PHQ-9 scores >10 points, of which only 39 attended a consultation. When performing the psychiatric evaluation of these patients, the depressive disorder was diagnosed in 30 patients, with a prevalence in the community of La Marroquina of 18.75%. A statistically significant association was found between the risk of depression according to the PHQ-9 and unemployment. The fact that the scales do not allow differentiating between different types of depressive disorder, as well as the social stigma of attending a psychiatric consultation, may cause underreporting in our study. La Marroquina has a high prevalence of depressive disorder, which is related to unemployment as the most important risk factor.

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INTRODUCCIÓN

La Organización Mundial de la Salud (OMS) estimó que para el 2020, 264 millones de personas aproximadamente, sufrirían de depresión, con 850.000 muertes atribuibles cada año [1], por lo que la organización adoptó un plan de acción integral sobre la salud mental (2013-2030), con el objetivo de alcanzar una meta mundial, sin embargo se hace referencia a que los sistemas de salud aún no han dado un abordaje eficiente para el tratamiento y atención de trastornos mentales, especialmente en países de ingresos bajos y medios [2]. Asimismo, en un contexto de pandemia COVID-19 (2020-2021) las consultas psicológicas más frecuentes tuvieron relación con depresión, ansiedad y estrés crónico [3, 4].

Para 2015, la depresión ya era la cuarta causa de discapacidad, según la relación entre años vividos con discapacidad observados y esperados, según el Estudio de Carga Global de la Enfermedad de 2015 (Global Burden of Disease Study 2015) [5], dentro este informe explican que, analizar datos de enfermedades crónicas y mentales se torna más difícil en países de bajos ingresos y en conflicto, entre los cuales figura Venezuela,

debido a la escasez o pobre calidad de datos epidemiológicos.

En Venezuela, existe un registro limitado de estudios que describan la prevalencia, factores de riesgo, tratamiento y pronóstico del trastorno depresivo en la población, sin embargo, se encontró que la prevalencia de síntomas de depresión en la población urbana de la región Guayana de Venezuela, fue de 2,7% según EVESCAM [6], resultados similares a lo descrito por otros autores en comunidades rurales en el Estado Trujillo[7]. Múltiples estudios en países desarrollados han caracterizado diferencias entre prevalencias de depresión en poblaciones rurales y urbanas [8-12], aunque un reciente metaanálisis evidenció que la urbanidad se asocia a una mayor prevalencia de trastorno depresivo solo en países desarrollados [13].

Se ha descrito que en comunidades rurales debido a mayores índices de pobreza y estigma social asociado a patologías mentales, aunados al difícil acceso a la salud, y particular ausencia de servicios de salud mental, producen una tendencia negativa de los pacientes a ir a consultas especializadas de psicología y/o psiquiatría [14]; fenómeno que se puede inferir está presente en Venezuela, debido a su alta proporción de población rural (45%) y al usar el criterio actualizado de Chomitz y col. para la diferenciación de ruralidad [15, 16], ya que el criterio oficial solo incluye la variable "tamaño de población", por lo que existe la posibilidad de sesgo.

La Marroquina es una comunidad rural del estado Yaracuy, que tiene acceso a consultas especializadas durante jornadas médicas-odontológicas, realizadas cada 8 meses por parte de Fundación Proyecto Mayū [Organización no gubernamental sin fines de lucro, perteneciente a la Coordinación de Grupos de Extensión de la Universidad Central de Venezuela (UCV)] [17], por lo que se planteó estimar la prevalencia de los trastornos depresivos en la comunidad e identificar factores de riesgo asociados, la primera vez que se llevó una consulta de psiquiatría a la comunidad.

PACIENTES Y MÉTODOS

Diseño del estudio y población

Se trató de un estudio de campo, de corte transversal, realizado en la comunidad de La Marroquina, estado Yaracuy, ubicada en el centro occidente de Venezuela, con latitud: 10°19'14.02", longitud: -68°40'55.2" [18]. Es una comunidad rural cerca de múltiples haciendas, con caminos de tierra, casas de bloque y de cemento, techos de zinc, pisos de cemento. Cuenta con un Consejo Comunal, una Escuela Integral Bolivariana "Mercedes Flores de Ramírez", y un Centro de atención médica tipo I de la Misión "Barrio Adentro", "Dr. Vicente Pérez Dávila", sin embargo, la asistencia médica es intermitente y no cuentan con farmacia.

La recolección de datos se llevó a cabo por los integrantes del Proyecto Mayū, durante la jornada de salud realizada por el mismo, entre el 30 de junio y el 2 de julio de 2022. Un mes antes, el equipo de investigación en conjunto con especialistas de la Fundación Proyecto Mayū, se trasladaron a la comunidad de La Marroquina acompañados de líderes comunitarios y personal de salud de la zona, invitando a todos los habitantes a participar. A través de juntas bisemanales los líderes comunitarios difundieron información sobre el estudio a los líderes de familia; por otra parte, el personal de salud invitó a los habitantes durante consultas médicas domiciliarias realizadas semanalmente. El total de habitantes de la comunidad La Marroquina, mayores de 18 años, registrados en el Centro Electoral "Escuela Integral Bolivariana La Marroquina" para el censo electoral de febrero 2012, fue de 611 personas. Se realizó un muestreo probabilístico según los asistentes a la jornada realizada entre el 30 de junio y 2 de julio de 2022, fue constituido por todos aquellos pacientes de la comunidad de La Marroquina, que cumplieron con los criterios de inclusión: asistencia a la jornada de salud, adulto igual o mayor a 18 años, residencia en la comunidad de La Marroquina y aceptación del consentimiento

informado, indistintamente de sexo, nivel de instrucción, ocupación y/o comorbilidades; con criterios de exclusión: menores de 18 años, no asistir a la jornada de salud. Para un nivel de confianza de 95%, alfa de 5% (0.05), un error de muestreo de 6,5% (0,065), tamaño poblacional de 611 habitantes, y proporción esperada 50% (0,5); se entrevistaron 160 personas.

Encuesta

El estudio se realizó en 2 fases (Anexo 1): una **primera fase** de despistaje, realizada por estudiantes de pregrado de Medicina a través de una entrevista breve, haciendo uso del Cuestionario de Salud del Paciente 9 (Patient Health Questionnaire-9 o PHQ-9), en el que se hace referencia a síntomas sugestivos de trastorno depresivo mayor, durante las dos semanas previas a la entrevista [19] (Anexo 2); y una **segunda fase** realizada por un médico especialista en psiquiatría, que luego de una entrevista clínica, aplicó la Escala de Depresión de Hamilton (HAM-D), la cual se usa en pacientes con diagnóstico de trastorno depresivo, para clasificar la severidad del mismo [20, 21] (Anexo 3). Se planificó el estudio incluyendo dos fases, debido a que se ha descrito que HAM-D no es una herramienta efectiva para el despistaje de trastorno depresivo, sino para su clasificación posterior al diagnóstico, en contraparte con PHQ-9 que cumple con esta función.

El PHQ-9 fue aplicado a todos los pacientes que acudieron al triaje de la jornada de salud, este instrumento consta de 9 preguntas, dispuestas en forma de escala de tipo adjetival que evalúa la presencia de síntomas en las dos últimas semanas ("nada en absoluto", "varios días", "más de la mitad de los días" y "casi todos los días", que se puntúa de 0 a 3 y el puntaje puede ser 0 a 27. Puntajes mayores o iguales a 10 tienen sensibilidad de 88% y especificidad de 88% para trastorno depresivo mayor [19],. Los pacientes que tuvieron puntajes mayores o iguales a 10 fueron referidos a la consulta de psiquiatría para la segunda fase.

La segunda fase fue realizada durante la consulta con el especialista en Psiquiatría, quien luego de la entrevista clínica, aplicó la encuesta HAM-D. Este instrumento contiene 21 variables, medidas con escalas de 3 ó 5 puntos, y un puntaje total entre 0 y 7 es considerado normal (o en remisión) mientras que puntajes entre 8 y 13 son considerados consistentes con depresión menor, puntajes comprendidos entre 14 y 18 con depresión moderada, entre 19 y 22 con depresión grave, y mayores de 23 con depresión muy grave [21, 23]. A los pacientes diagnosticados con depresión, en la consulta del especialista, les fue indicado tratamiento farmacológico oportuno e individualizado.

Utilizando tablas dinámicas de Excel y EpiInfo 7®. Se realizaron tablas de distribución de frecuencias con sus respectivos intervalos de confianza y tablas de contingencia detalladas con las que se calcularon Odds Ratio y Chi^2 para establecer la asociación de variables, con un punto de corte de $p<0.05$.

Aspectos éticos. El presente trabajo incluyó la recolección de información de seres humanos, cumpliendo con los principios básicos de la ética de investigación enmarcados dentro de la 8va Revisión de la Declaración de Helsinki [24]. Para ello, se garantizó la autonomía, confidencialidad y privacidad de los datos obtenidos, los instrumentos solo fueron aplicados a participantes luego de otorgar al equipo su consentimiento informado, voluntario y previa explicación de los objetivos, riesgos y metodología a usar. Este proyecto fue avalado por la comisión de bioética de la Escuela de medicina "José María Vargas" (29-06-2021). Los pacientes diagnosticados con depresión tuvieron seguimiento por el psiquiatra vía online y presencial 8 meses después, en la siguiente jornada. Asimismo, les fue suministrado tratamiento médico en los casos que el psiquiatra consideró pertinente.

RESULTADOS

De los 456 pacientes que asistieron a la jornada de salud, 160 cumplieron con los criterios de inclusión, por lo que fueron eva-

luados en el triaje psiquiátrico y se les realizó la prueba PHQ-9, con el resultado de 67 pacientes con una alta probabilidad de depresión y 93 pacientes con bajo riesgo de depresión (Tabla 1). Los pacientes con puntajes elevados fueron referidos a la consulta especializada en Psiquiatría para su evaluación, y posteriormente se les aplicó la escala Hamilton-D; se evidenció que de los 39 pacientes que asistieron, 76,92% fueron diagnosticados con trastorno depresivo.

Tabla 1
Distribución de pacientes con base a resultados de PHQ-9.

	n	%
Depresión		
Improbable	93	58,13
Probable	67	41,88

Al analizar las variables demográficas se encontró que la mediana de la edad fue de 38 años (DE = 16.26), mayormente entre los 30 y 49 años (40,63%), sexo femenino (73,75%), con presencia de comorbilidades, de etiología cardiovascular, neurológica, psiquiátrica, respiratoria, gastrointestinales, entre otras misceláneas (69,4%); desempleados (61,88%), en una relación estable, siendo concubinato o casados (61,88%). Entre los datos obtenidos (Tabla 2), se encontró una asociación estadísticamente significativa entre el estado laboral y la alta probabilidad de depresión (p = 0,031).

Mediante la evaluación del riesgo de depresión asociado a las variables demográficas, se encontró que el ser catalogado como "empleado" disminuyó las probabilidades de presentar alta probabilidad de depresión en 81.3% [OR 0,187 (I.C.: 0,034-0,966)], de manera significativa (p = 0,033). Las demás variables no obtuvieron una asociación estadísticamente significativa (Tabla 3).

Tabla 2
Variables demográficas de 160 pacientes encuestados mediante PHQ-9 en La Marroquina, Edo. Yaracuy, Venezuela.

	n	%	p*
Edad			0,836
18-29	39	24,37	
30-49	65	40,63	
50-64	35	21,88	
≥65	21	13,13	
Sexo			0.095
Femenino	118	73,75	
Masculino	42	26,25	
Laboral			0.031
Desempleado	99	61,88	
Empleado	61	38,13	
Estado civil			0.611
Soltero	61	38,13	
Unido	99	61,88	
Comorbilidades			0.607
Ausentes	49	30,63	
Presentes	111	69,38	

*Los valores de p fueron obtenidos mediante la prueba de Chi².

Tabla 3
Factores asociados a pacientes con puntajes elevados en PHQ-9.

	Odds ratio (Intervalo de confianza de 95%)	p*
Sexo	0,24 (0,034-1,472)	0,104
Comorbilidades	0,665 (0,123-3,631)	0,607
Laboral	0,187 (0,034-0,966)	0,033
Estado civil	1,472 (0,14-5,559)	0,625

*Los valores de p fueron obtenidos mediante la prueba exacta de Fisher.

DISCUSIÓN

Se determinó que, para julio del 2022, 41,8% de la población encuestada en una zona rural de Venezuela (La Marroquina, Estado Yaracuy), a la que se le aplicó el test PHQ-9, tenía una alta probabilidad de de-

presión. Dentro de dicho grupo, solo 55% de los pacientes acudió de forma voluntaria a la consulta psiquiátrica, en la cual se les aplicó el test HAM-D, de ellos el 76,92% (n= 30) obtuvieron un resultado positivo con ambas escalas, diagnosticándose depresión para una prevalencia de 18% en dicha comunidad.

La escasa asistencia de los pacientes referidos a la consulta con el especialista en Psiquiatría, probablemente se deba al estigma social asociado a la salud mental [25-30], lo que limita frecuentemente la solicitud de atención y el tratamiento psiquiátrico, lo que podría apuntar a que hubo un subregistro de depresión en la comunidad de La Marroquina. Se ha descrito que las personas con patologías psiquiátricas presentan mayores desigualdades con respecto al acceso a servicios médicos, por ende, mayor morbimortalidad [31, 32].

Resulta importante señalar que al ser comparadas ambas escalas (HAM-D y PHQ-9) en 15 centros hospitalarios en China [33], se encontró que el test PHQ-9 es una alternativa eficiente y fiable para detectar depresión en pacientes, lo que concuerda con lo descrito por autores en Colombia [23], siendo propuesta como una herramienta confiable [34].

Dentro del grupo de pacientes evaluados clínicamente y en los que se realizó el test HAM-D, se corroboró el diagnóstico de trastorno depresivo en 30 pacientes, observando un total de 18% de pacientes deprimidos con respecto a toda la muestra, superando la prevalencia esperada en comparación con la prevalencia a nivel mundial [2], y a nivel nacional [6, 7]. Las enfermedades mentales como la depresión tienen una estrecha relación de influencia del país a donde pertenezca quién las padece; es decir, aquellas personas que viven en un país de bajos ingresos o que han pasado por un cambio económico desfavorable, tienen de 1,5 hasta 3 veces más probabilidades de sufrir depresión o ansiedad [35].

Un estudio realizado en 51 centros de 21 países diferentes en los cinco continentes, y en situaciones económicamente diversas, señaló que la prevalencia de depresión fue mayor en áreas urbanas (13% vs. 9% en zonas rurales), y concluyó que haber padecido depresión aumenta el riesgo de cualquier causa de mortalidad en un 17% [32]. En países desarrollados también se ha demostrado mayor prevalencia de depresión en áreas urbanas [36].

Se encontró una relación estadísticamente significativa entre el estatus laboral y depresión, ya que más del 70% de los pacientes con probable depresión, se encontraba desempleado, resultados que difieren de lo observado por otros autores en poblaciones urbanas a nivel nacional [37]. En un estudio realizado en España [38] señalaron que el desempleo no solo afecta de forma negativa la economía individual, también desestabiliza aspectos como el desarrollo social de la persona, organización temporal, sentimiento de culpa e improductividad, por lo que el desempleo constituye un factor de riesgo para la salud mental. Las personas desempleadas presentan mayor prevalencia de depresión, la depresión en pacientes desempleados suele implicar mayores gastos económicos debido a su incapacidad para trabajar, y se ha asociado a menores tasas de re-empleo. Se ha descrito que entre mayor tiempo la persona se encuentre desempleada, habrá mayor deterioro de la salud mental [39].

El presente estudio tiene varias limitaciones: la metodología no permite diferenciar entre diversos tipos de depresión, ya que las escalas son utilizadas como herramientas de tamizaje y solo fueron diagnosticados con trastorno depresivo los pacientes que voluntariamente decidieron acudir a consulta con especialista. Otra limitación es el estigma social hacia la búsqueda de ayuda psicológica/psiquiátrica, y de igual manera hacia la depresión, que hace que muchos pacientes dejen de ir a dicha consulta.

En conclusión, **la** población de La Marroquina tiene una alta prevalencia de depresión, aun tomando en cuenta las limitaciones de este trabajo. El estigma social asociado a enfermedades mentales en la comunidad, ocasiona que los pacientes no acudan a la consulta psiquiátrica para confirmar el diagnóstico y

estadiaje del trastorno depresivo. Sin embargo, utilizar instrumentos de tamizaje es valioso en la atención primaria a una comunidad. En esta oportunidad permitió asociar variables con la probable depresión y se demostró que el factor de riesgo más importante para depresión en la comunidad de La Marroquina es el desempleo, seguido de las comorbilidades. Se recomienda realizar estudios comparativos en Venezuela entre poblaciones rurales y urbanas; y proveer a la comunidad La Marroquina de atención tanto primaria como especializada para patologías que socaven la salud mental de sus habitantes.

Conflicto de interés

Los autores declaran que no hay conflicto de interés.

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Contribución de los Autores

JCM realizó el diseño del estudio y llevó a cabo la consulta psiquiátrica (recolección de datos. JLL, KJL-L MIG, VR-R, MA, YC-M y RTC participaron en la recolección de datos y redacción del anteproyecto. MAPF, JLL y ODO-A supervisaron y redactaron la versión final.

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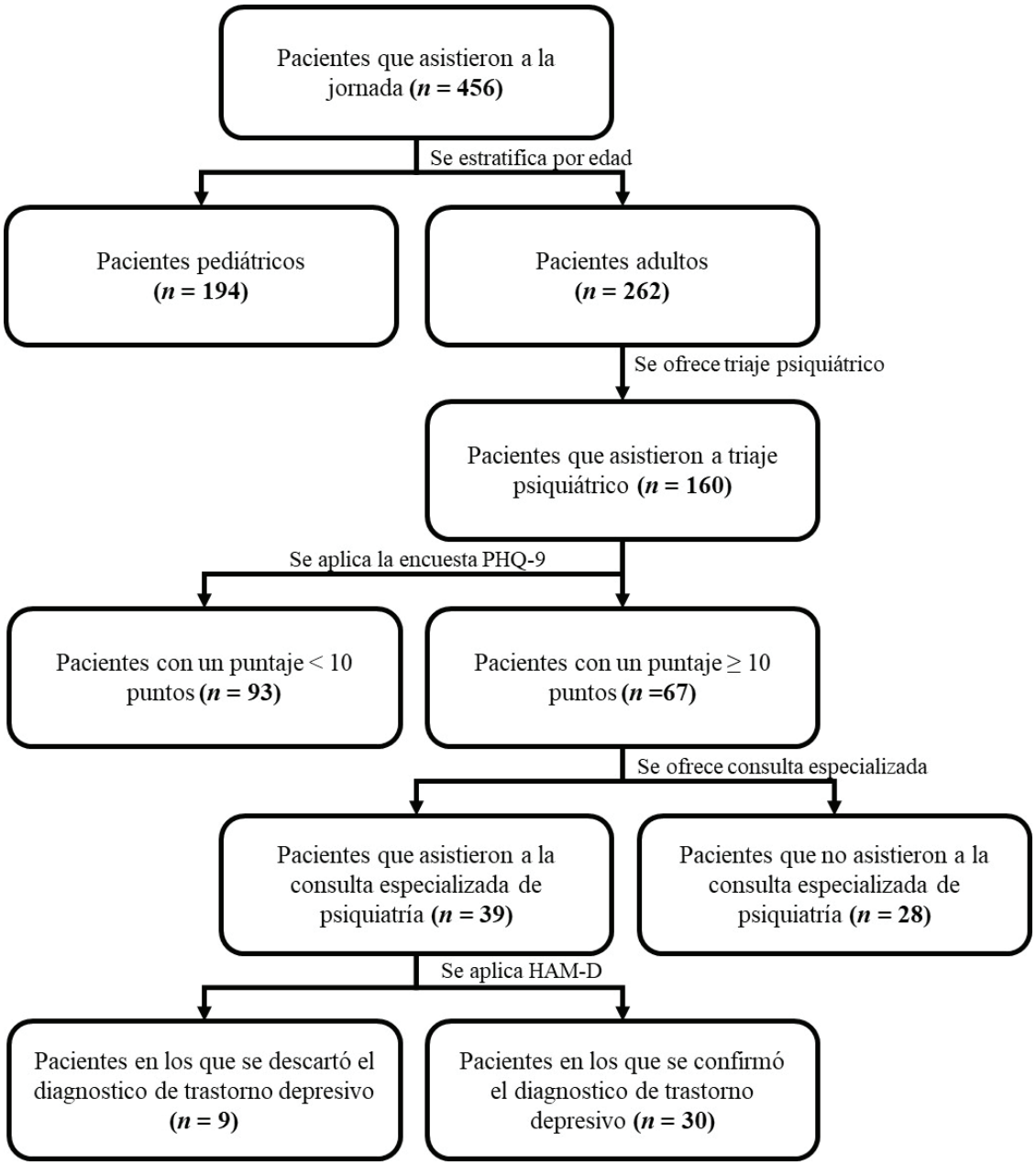
Anexo 1. Flujograma.



Anexo 2.
Cuestionario PHQ-9

Nombre y Apellido: _____ CI:_____ Edad: _____ Sexo:_____

Estado: _____ Municipio: _____ Parroquia: _____

Comorbilidades (Cardiovasculares / Metabólicas / Obesidad / Insomnio / Mentales): _____

Situación laboral actual: _____

Situación sentimental: _____

Durante las últimas 2 semanas, ¿qué tan seguido le han afectado cualquiera de los siguientes problemas? *(Marque con una "✔" para indicar su respuesta)*	**Para nada**	**Varios días**	**Más de la mitad de los días**	**Casi todos los días**
1. Poco interés o placer en hacer las cosas	0	1	2	3
2. Se ha sentido decaído(a), deprimido(a), o sin esperanzas	0	1	2	3
3. Dificultad para dormir o permanecer dormido(a), o ha dormido demasiado	0	1	2	3
4. Se ha sentido cansado(a) o con poca energía	0	1	2	3
5. Con poco apetito o ha comido en exceso	0	1	2	3
6. Se ha sentido mal con usted mismo(a) – o que es un fracaso o que ha quedado mal con usted mismo(a) o con su familia	0	1	2	3
7. Ha tenido dificultad para concentrarse en cosas tales como leer el periódico o ver televisión	0	1	2	3
8. ¿Se ha estado moviendo o hablando tan lento que otras personas podrían notarlo?, o por el contrario – ha estado tan inquieto(a) o agitado(a), que se ha estado moviendo mucho más de lo normal	0	1	2	3
9. Ha pensado que estaría mejor muerto(a) o se le ha ocurrido lastimarse de alguna manera	0	1	2	3

FOR OFFICE CODING 0 + _____ + _____ + _____
=Total Score: _____

Si usted marcó cualquiera de estos problemas, ¿qué tan difícil fue hacer su trabajo, las tareas del hogar o llevarse bien con otras personas debido a tales problemas?

Para nada difícil	**Un poco difícil**	**Muy difícil**	**Extremadamente difícil**
☐	☐	☐	☐

Anexo 3.

Cuestionario HAM-D

Nombre y Apellido: _____ CI: _____ Edad: _____ Sexo: _____

Estado: _____ Municipio: _____ Parroquia: _____

Comorbilidades (Cardiovasculares / Metabólicas / Obesidad / Insomnio / Mentales): _____

Situación laboral actual: _____Situación sentimental: _____

ESCALA DE HAMILTON PARA LA DEPRESIÓN

Versión de JA Ramos-Brieva y A Cordero-Villafáfila

1- ESTADO DE ÁNIMO DEPRIMIDO_____[ ]
0 *Ausente*
1 *Ligero*: actitud melancólica; el paciente no verbaliza necesariamente el descenso del ánimo
2 *Moderado*: llanto ocasional, apatía, pesimismo, desmotivación....
3 *Intenso*: llanto frecuente (o ganas); introversión; rumiaciones depresivas; pérdida del gusto por las cosas
4 *Extremo*: llanto frecuente (o ganas); frecuente tendencia al aislamiento; contenidos depresivos exclusivos en el pensamiento o la comunicación verbal; pérdida de la capacidad de reacción a estímulos placenteros

2- SENTIMIENTOS DE CULPA_____[ ]
0 *Ausente*
1 *Ligero*: autorreproches, teme haber decepcionado a la gente
2 *Moderado*: ideas de culpabilidad; sentimiento de ser una mala persona, de no merecer atención
3 *Intenso*: la enfermedad actual es un castigo; meditación sobre errores, malas acciones o pecados del pasado; merece lo que padece
4 *Extremo*: ideas delirantes de culpa con o sin alucinaciones acusatorias

3- SUICIDIO_____[ ]
0 *Ausente*
1 *Ligero*: la vida no vale la pena vivirla
2 *Moderado*: desearía estar muerto o piensa en la posibilidad de morirse
3 *Intenso*: ideas o amenazas suicidas
4 *Extremo*: serio intento de suicidio

4- INSOMNIO INICIAL (si toma hipnóticos y no puede evaluar, puntúe 1)_____ [ ]
0 *Ausente*
1 *Ocasional*: tarda en dormir entre media y una hora (<3 noches/semana)
2 *Frecuente*: tarda en dormir más de una hora (3 ó más noches /semana)

5- INSOMNIO MEDIO (si toma hipnóticos y no puede evaluar, puntúe 1)_____ [ ]
0 *Ausente*
1 *Ocasional*: está inquieto durante la noche; si se despierta tarda casi una hora en dormirse de nuevo (<3 noches/semana)
2 *Frecuente*: está despierto durante la noche, con dificultades para volver a conciliar el sueño; cualquier ocasión de levantarse de la cama (excepto para evacuar), o necesidad de fumar o leer tras despertarse debe puntuar 2 (3 ó más noches seguidas por semana)

6- INSOMNIO TARDÍO (si toma hipnóticos y no puede evaluar, puntúe 1)_____ [ ]
0 *Ausente*
1 *Ocasional*: se despierta antes de lo habitual (<2 horas antes; <3 días por semana)
2 *Frecuente*: se despierta dos o más horas antes de lo habitual 3 ó más días por semana)

7- TRABAJO Y ACTIVIDADES_____ [ ]
0 *Ausente*
1 *Ligero*: ideas o sentimientos de incapacidad o desinterés. Distíngalo de la fatiga o pérdida de energía que se puntúan en otra parte.
2 *Moderado*: falta de impulso para desarrollar las actividades habituales, las aficiones o el trabajo (si el paciente no lo manifiesta directamente, puede deducirse por su desatención, indecisión o vacilación ante el trabajo y otras actividades).
3 *Intenso*: evidente descenso del tiempo dedicado a sus actividades; descenso de su eficacia y/o productividad. En el hospital se puntúa 3 si el paciente no se compromete al menos durante tres horas/día a actividades (Trabajo hospitalario o distracciones) ajenas a las propias de la sala. Notable desatención del aseo personal.
4 *Extremo*: dejó de trabajar por la presente enfermedad. No se asea o precisa de gran estímulo para ello. En el hospital se puntúa 4 si el paciente no se compromete en otras actividades más que a las pequeñas tareas de la sala o si precisa de gran estímulo para que las realice.

8- INHIBICIÓN_____ [ ]
0 *Ausente*
1 *Ligera*: ligera inhibición durante la entrevista; sentimientos ligeramente embotados; facies inexpresiva.
2 *Moderada*: evidente inhibición durante la entrevista (voz monótona, tarda en contestar las preguntas).
3 *Intensa*: entrevista difícil y prolongada; lentitud de movimientos al caminar.
4 *Extrema*: estupor depresivo completo; entrevista imposible.

9- AGITACIÓN_____ [ ]
0 *Ausente*
1 *Ligera*: mueve los pies; juega con las manos o con los cabellos
2 *Moderada*: se mueve durante la entrevista, se agarra a la silla; se retuerce las manos; se muerde los labios; se tira de los cabellos; mueve ampliamente los brazos, se muerde las uñas, las manos...
3 *Intensa*: no puede estarse quieto durante la entrevista; se levanta de la silla.
4 *Extrema*: la entrevista se desarrolla "corriendo", con el paciente de un lado para otro, o quitándose la ropa, o arrancándose los cabellos; el paciente parece desconcertado y "desatado".

10- ANSIEDAD PSÍQUICA_____ [ ]
0 *Ausente*
1 *Ligera*: tensión subjetiva e irritabilidad.
2 *Moderada*: tensión objetiva, evidente; preocupación por trivialidades.
3 *Intensa*: actitud aprensiva evidente en la cara y el lenguaje.
4 *Extrema*: crisis de ansiedad observadas, la ansiedad forma la mayor parte del contenido de su comunicación espontánea, verbal o no verbal.

11- ANSIEDAD SOMÁTICA_____ [ ]
0 *Ausente*
1 *Ligera*: un solo síntoma o síntoma dudoso o varios síntomas de un mismo sistema.
2 *Moderada*: varios síntomas de distintos sistemas.
3 *Intensa*: múltiples síntomas de varios sistemas simultáneamente.
4 *Extrema*: numerosos síntomas persistentes e incapacitantes la mayor parte de las veces.

12- SÍNTOMAS SOMÁTICOS GASTROINTESTINALES_____ [ ]
0 *Ausentes*:
1 *Ligeros*: pérdida de apetito, pero come sin necesidad de estímulo; sensación de pesadez en el abdomen.
2 *Intensos*: pérdida de apetito, no come aunque se le estimule, o precisa de gran estímulo para comer; precisa o solicita laxantes o medicación para sus síntomas gastrointestinales.

13- SÍNTOMAS SOMÁTICOS GENERALES_____ [ ]
0 *Ausentes*:
1 *Ligeros*: fatigabilidad, pérdida de energía, pesadez en extremidades, espalda, cabeza; algias en el dorso, cabeza, músculos.
2 *Intensos*: fatigabilidad y pérdida de energía la mayor parte del tiempo; cualquier síntoma somático bien definido o expresado espontáneamente.

14- SÍNTOMAS GENITALES (preguntar siempre)_____ [ ]
0 *Ausentes*: o información inadecuada o sin información (emplear lo menos posible estas dos últimas).
1 *Ligeros*: descenso de la líbido; actividad sexual alterada (inconstante, poco intensa).
2 *Intensos*: pérdida completa de apetito sexual; impotencia o frigidez funcionales.

15- HIPOCONDRÍA_____ [ ]
0 *Ausente*:
1 *Ligera*: preocupado de sí mismo (corporalmente).
2 *Moderada*: preocupado por su salud.
3 *Intensa*: se lamenta constantemente. Solicita ayuda, etc.
4 *Extrema*: ideas hipocondríacas delirantes.

16- PÉRDIDA DE INTROSPECCIÓN_____ [ ]
0 *Ausente*: se da cuenta de que está enfermo, deprimido.
1 *Ligera*: reconoce su enfermedad, pero la atribuye a la mala alimentación, al clima, al exceso de trabajo, a una infección viral, a la necesidad de descanso, etc.
2 *Moderada*: niega estar enfermo o el origen nervioso de su enfermedad.

17- PÉRDIDA DE PESO_____ [ ]
0 *Ausente*:
1 *Ligera*: probable pérdida de peso asociada a la enfermedad actual; pérdida superior a 500 gr/semana ó 2,5 kg/año (sin dieta).
2 *Intensa*: pérdida de peso definida según el enfermo; pérdida superior a 1 kg/semana ó 4,5 kg/año (sin dieta).

PUNTUACIÓN TOTAL_____ [ ]

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Clinical impact of early enteral nutrition on postoperative pain, gastrointestinal function and nutritional status in colorectal cancer patients.

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Keywords: colorectal cancer; early enteral nutrition; postoperative pain; analgesic pump.

Abstract. This research aimed to clarify the clinical impact of early enteral nutrition (EN) on postoperative pain, gastrointestinal function and nutritional status of colorectal cancer (CRC) patients. Eighty rectal cancer patients undergoing surgery in our hospital from October 2021 to October 2023 were selected as research subjects and divided into an experimental group (EG) and a control group (CG) using a random number table method, with 40 cases each. Both groups received conventional nursing, including preoperative, intraoperative, and postoperative nursing. The CG received a traditional preoperative routine diet and postoperative EN support. The EG received five-day preoperative EN support and postoperative parenteral nutrition support based on a traditional preoperative routine diet. The analgesic effect indicators, pain scores, gastrointestinal function recovery indicators, adverse reactions and nutritional indicators in both groups received measurement and comparison. The Average additional amount of flurbiprofen axetil in the EG decreased relative to those in the CG ($p<0.05$). At six h and 12 h after surgery, VAS scores in the EG were lower than those in the CG during the same period; at 24 h and 48 h after surgery, no statistical significance in VAS scores was shown between both groups ($p>0.05$). The bowel sound recovery time, first defecating time, first exhaust time, and first getting-out-of-bed time in EG were inferior relative to those in the CG ($p<0.05$). The incidence of adverse reactions in the EG was reduced relative to that in the CG ($p<0.05$). Before surgery and one day after surgery, no statistically significant differences in total protein (TP) and serum albumin (ALB) levels were shown between both groups ($p>0.05$); three days and seven days after surgery, TP and ALB levels in the EG exhibited an elevation relative to those in CG during the same period ($p<0.05$). In conclusion, early EN can improve not only postoperative gastrointestinal function and nutritional status of patients but also mitigate postoperative pain and facilitate postoperative recovery with high safety, which is worthy of further clinical promotion.

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Impacto clínico de la nutrición enteral temprana sobre el dolor post-quirúrgico, función gastrointestinal y estado nutricional de pacientes con cáncer colorrectal.

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Palabras clave: cáncer colorrectal; nutrición enteral temprana; dolor postoperatorio; bomba analgésica.

Resumen. Esta investigación tuvo como objetivo aclarar el impacto clínico de la nutrición enteral temprana (EN) en el dolor posoperatorio, la función gastrointestinal y el estado nutricional de los pacientes con cáncer colorrectal (CCR). Se seleccionaron ochenta pacientes con cáncer de recto sometidos a cirugía en nuestro hospital desde octubre de 2021 hasta octubre de 2023 como sujetos de investigación y se dividieron en un grupo experimental (GE) y un grupo de control (GC) utilizando un método de tabla de números aleatorios, con 40 casos cada uno. Ambos grupos recibieron enfermería convencional, incluida enfermería preoperatoria, intraoperatoria y posoperatoria. El GC recibió una dieta de rutina preoperatoria tradicional y apoyo de EN posoperatorio. El GE recibió apoyo de EN preoperatoria durante cinco días y apoyo de nutrición parenteral posoperatoria basado en una dieta de rutina preoperatoria tradicional. Los indicadores de efecto analgésico, las puntuaciones de dolor, los indicadores de recuperación de la función gastrointestinal, las reacciones adversas y los indicadores nutricionales en ambos grupos recibieron medición y comparación. La cantidad adicional promedio de flurbiprofeno axetilo en el GE mostró un descenso en relación con los del GC ($p<0,05$). A las seis y 12 h después de la cirugía, las puntuaciones VAS en el GE fueron inferiores a las del GC durante el mismo período; a las 24 y 48 h después de la cirugía, no hubo significación estadística en las puntuaciones VAS entre ambos grupos ($p>0,05$). El tiempo de recuperación del sonido intestinal, el tiempo de la primera defecación, el tiempo del primer escape y el tiempo del primer levantamiento de la cama en el GE fueron inferiores en relación con los del GC ($p<0,05$). La incidencia de reacciones adversas en el GE se redujo en relación con la del GC ($p<0,05$). Antes de la cirugía y un día después de la cirugía, no se mostraron diferencias estadísticamente significativas en los niveles de proteína total (TP) y albúmina sérica (ALB) entre ambos grupos ($p>0,05$); tres y siete días después de la cirugía, los niveles de TP y ALB en el GE exhibieron una elevación en relación con los del GC durante el mismo período ($p<0,05$). En conclusión, la EN temprana puede mejorar no solo la función gastrointestinal posoperatoria y el estado nutricional de los pacientes, sino también mitigar el dolor posoperatorio y facilitar la recuperación posoperatoria con alta seguridad, lo que merece una mayor promoción clínica.

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INTRODUCTION

Colorectal cancer (CRC) is a prevalent malignant gastrointestinal tumor in clinical practice, and CRC patients all suffer from varying degrees of malnutrition [1]. Tumor cachexia, the primary reason for malnutrition in tumor patients, often results from metabolic abnormalities and reduced food intake, characterized by a negative balance between energy and protein metabolism[2,3]. Malnutrition can attenuate patients' immune systems and quality of life, elevate surgery-related complications, and even enhance the mortality rate [4]. The nutritional metabolism of CRC patients has common characteristics in most malignancies, such as insulin resistance, lipid peroxidation, accelerated protein conversion, and elevated acute phase protein synthesis, among others[5]. On the other hand, gastrointestinal dysfunction is a unique cause of malnutrition in CRC patients [6]. Thus, CRC patients are more likely to suffer from malnutrition and weakened immune function. Furthermore, high metabolic status, prolonged fasting, and impaired intestinal mucosal barrier and immune function due to surgical treatment further deteriorate the nutritional status of CRC patients, thereby affecting postoperative recovery and reducing their quality of life [7,8]. Thus, adequate and reasonable nutritional interventions during the perioperative period have become a crucial component of comprehensive treatment for CRC.

For patients who plan to undergo therapeutic surgery but have preoperative malnutrition or nutritional risks, enteral nutrition (EN) support or combined enteral and parenteral nutrition (PN) support treatment is preferred [9,10]. The therapeutic effect of nutrition support should be directly reflected in improving postoperative gastrointestinal function and elevation of the nutritional status. In clinical practice, open surgery is usually applied to treat CRC patients, whereas surgery takes quite a long time and can result in remarkable trauma to patients; patients often have to endure pain, especially during bowel movements, which often makes them hesitant to undergo surgery [11,12]. Thus, effective nutritional intervention remains necessary to control postoperative pain.

This research aimed to clarify the clinical impact of early EN on postoperative pain, gastrointestinal function and nutritional status of CRC patients, which may guide postoperative nursing work for gastrointestinal tumors.

MATERIALS AND METHODS

General data

Eighty rectal cancer patients undergoing surgery in our hospital from October 2021 to October 2023 were selected as research subjects and divided into an experimental group (EG) and a control group (CG) using a random number table method, with 40 cases each. **Inclusion criteria:** 1) Age ranging from 50-70 years old; 2) major clinical manifestations including changes in bowel habits and stool characteristics, abdominal discomfort, abdominal masses, intestinal obstruction, anemia, etc.; 3) diagnosed as rectal cancer through colonoscopy and histopathological examination, and all underwent anterior resection of the rectum as clinical therapy; 4) the patient's condition was stable, conscious, and had good communication and expression abilities; 5) all were informed of this research and signed an informed consent. **Exclusion criteria:** 1) Those with advanced rectal cancer, severe liver and kidney dysfunction, and intestinal inflammation; 2) emergency surgery, conversion to laparotomy, and inability to establish pneumoperitoneum during surgery; 3) patients with blurred consciousness, cognitive and communication barriers, mental illness; and 4) female patients during pregnancy and childbirth. This research received approval from our hospital's ethics committee.

METHODS

Both groups received conventional nursing, including preoperative, intraoperative, and postoperative nursing.

Preoperative nursing: (1) Preoperative education: After patients were admitted, nursing staff informed the patients of the approximate stage and time of treatment and explained the importance of early postoperative activities. They were provided timely guidance for different psychological problems, patiently answered patients' doubts about treatment, and helped patients smoothly pass through the perioperative period. (2) Preoperative intestinal preparation: A semi-liquid or low-residue diet was administered one day before surgery. Then, 750 mL of glucose water was administered to patients ten and two hours before surgery. Fasting and water deprivation occurred six and two hours before surgery, and no gastrointestinal decompression tube was placed.

Intraoperative nursing: The Dixon surgery procedure (transabdominal radical resection of the rectum) was used as a surgical method performed under general anesthesia. An intraoperative insulation blanket was used during surgery to prevent hypothermia. Infusion and flushing liquids received appropriate warming.

Postoperative nursing: (1) Analgesia: Postoperative patients received patient-controlled intravenous analgesia (PCA) intervention. The analgesic pump formula was: Flurbiprofen axetil (150 mg) + Dezocine (50 mg) + Tropisetron (8 mg) + Dexmedetomidine (60 ug) + normal saline (100 mL). The analgesic pump speed was 1.2-1.5 mL/h, with patient-controlled speed at around 1.5 mL/h, with a locking time of 15 min. When the patient's pain index was relatively high, and PCA was inadequate to mitigate it, flurbiprofen axetil could be added each time additionally.

Nursing staff created an analgesic pump usage card, and after patients returned to the ward, provided a detailed introduction to the working principle, usage method, and adverse reactions of the analgesic pump to patients and their family members, improving patients and their family members' predictability of adverse reactions and preventing severe complications that might endanger patients' life safety. Nursing staff improved acute pain work mode. All nursing staff regularly inquired about and evaluated patients' postoperative pain, responded promptly to patients' pain, provided timely feedback to ensure that doctors administered expedient treatment and analgesic intervention, and summarized clinical medication effects to improve medication plans continuously. (2) Tube management: antibiotics were administered 2-3 days after surgery to shorten the retention time of drainage tubes and related catheters. According to wound healing, urinary catheters and nasogastric tubes were removed within 24-48 hours after surgery, and drainage tubes were removed on the fifth day after surgery. (3) Postoperative activities: 12 hours after surgery, patients engaged in bed activities such as turning over and sitting under a doctor's or nurse's guidance. The next day after surgery, nursing staff encouraged and guided patients to engage in getting-out-of-bed activities.

Nutrition intervention: The Experimental Group (EG) received postoperative early enteral nutrition (EN) based on a standard preoperative routine diet. In contrast, the Control Group (CG) received standard total parenteral nutrition (TPN) support for seven days following surgery. The total liquid intake for both groups was 50 $ml \cdot kg^{-1} \cdot d^{-1}$, with energy provided at a rate of 105 $kJ \cdot kg^{-1} \cdot d^{-1}$ and nitrogen intake at 0.2 $g \cdot kg^{-1} \cdot d^{-1}$. The nitrogen-to-calorie ratio was 1:552 kJ. Nutritional support was provided as a "fully-integrated" solution administered via peripheral veins. The EG received the same caloric and nitrogen intake as the control group. 500 mL of Nutrison Fibre (NUTRICIA) was administered on the first day after surgery. On the second day, the volume increased to 1000 mL; from the third to the

seventh day, 1500 mL of Nutrison Fibre was given daily. Nutrison Fibre supplied 4180 kJ of calories, 40 g of protein, and 6.4 g of nitrogen per 1000 mL, and it also contained vitamins, dietary fiber, and microelements. After recovery of gastrointestinal function, the diet should gradually transition from liquid or semi-liquid to general.

Observation indicators

1. Analgesic effect indicators: The average additional amount of Flurbiprofen axetil in both groups was recorded.
2. Pain scores: The pain degree in both groups at six, 12, 24, and 48 hours after surgery received evaluation with the Visual Analog Pain Scale (VAS) [13]. Patients' pain scores during rest and activity (coughing, turning over, deep breathing, etc.) were recorded, with a score range of 0-10 points. A score of 0-3 points indicated mild pain, 4-6 points indicated moderate pain, and 7-10 points indicated severe pain.
3. Gastrointestinal function recovery indicators: The bowel sound recovery time, first defecating time, first exhaust time, and first getting-out-of-bed time in both groups were recorded.
4. Adverse reactions: Both groups' adverse reactions (majorly vomiting and nausea) received recording.
5. Nutritional indicators: The total protein (TP) and serum albumin (ALB) levels in both groups before surgery and 1, 3, and 7 days after surgery were measured with a colorimetric method.

STATISTICAL ANALYSIS

Statistical analysis of data of this research was performed with the SPSS 27.0® software. Counting data were expressed as %, followed by the χ^2 test for intergroup comparisons. Measurement data conforming to a normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm SD$), followed by t-tests for intergroup comparisons. The difference was statistically significant when $p < 0.05$.

RESULTS

Comparison of general data between both groups

The CG included 25 (62.50%) males and 15 (37.50%) females with a mean age of 58.84 ± 4.47 years. Based on TNM staging, 17 (42.50%) cases were in stage I, 13 (32.50%) were in stage II, and 10 (25.00%) were in stage III. The examination of comorbidities in CG patients showed that seven (17.50%) patients had hypertension and nine (22.50%) patients had diabetes. EG included 22 (55.00%) males and 18 (45.00%) females with a mean age of 59.24±4.40 years. Based on TNM staging, 18 (45.00%) cases were in stage I, 14 (35.00%) were in stage II, and eight (20.00%) were in stage III, and the examination of co-morbidities in EG patients showed that five (12.50%) patients had hypertension and 10 (25.00%) patients had diabetes. No statistical significance in gender, age, TNM staging, and comorbidities was found in the two groups (Table 1).

Table 1
General data in both groups.

Groups	N	Gender [n (%)]		Age (years)	TNM staging [n (%)]			Comorbidities [n (%)]	
		Male	Female		I	II	III	Hypertension	Diabetes
CG	40	25 (62.50)	15 (37.50)	58.84±4.47	17 (42.50)	13 (32.50)	10 (25.00)	7 (17.50)	9 (22.50)
EG	40	22 (55.00)	18 (45.00)	59.24±4.40	18 (45.00)	14 (35.00)	8 (20.00)	5 (12.50)	10 (25.00)
$\chi 2/t$		0.464		0.896	0.288			0.406	
p		0.496		0.373	0.866			0.816	

Comparison of analgesic effect indicators between both groups

The average additional amount of Flurbiprofen axetil in EG was lower than in the CG, and there was a significant statistical difference between the two groups ($p < 0.0001$) (Fig.1).

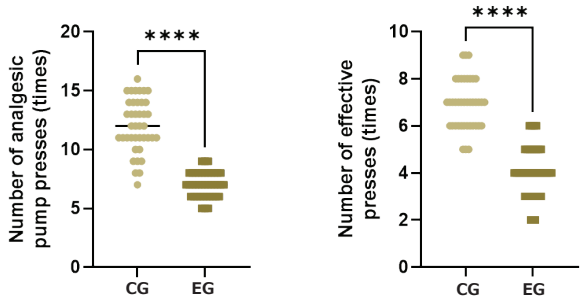

Fig. 1. Analgesic effect indicators in both groups.
Note: EG versus CG, ****P<0.0001.

Comparison of pain scores between both groups

At six hours and 12 hours after surgery, VAS scores in the EG were inferior to those in CG during the same period, indicating a statistical significance difference ($p < 0.001$). At 24 h and 48 h after surgery, neither group exhibited statistically different significance in VAS scores (Fig. 2).

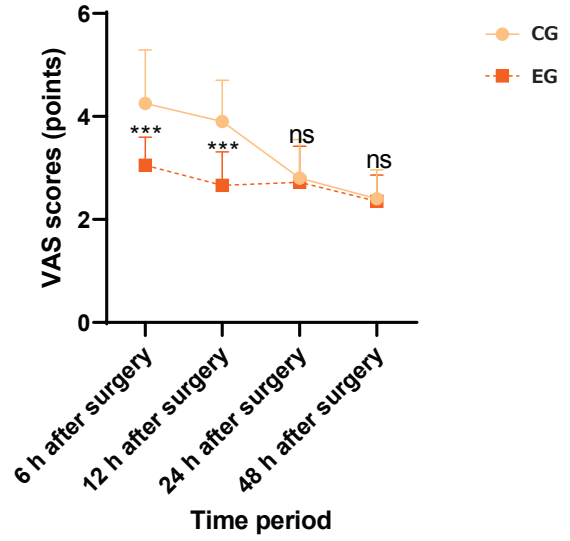

Fig. 2. Pain scores in both groups of patients.
Note: EG versus CG, ns=no significance. ***p<0.001.

Comparison of gastrointestinal function recovery indicators between both groups

The bowel sound recovery time, first defecating time, first exhaust time, and first getting-out-of-bed time in the EG were reduced relative to those in CG, indicating a statistical significance difference ($p < 0.0001$) (Fig. 3).

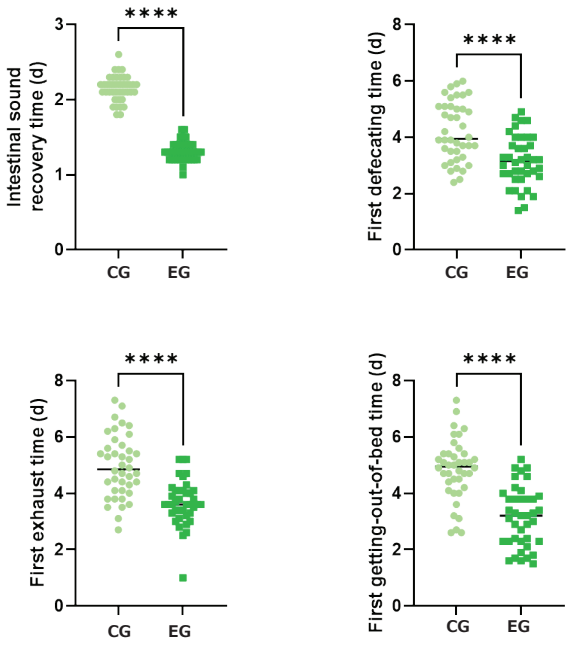

Fig. 3. Gastrointestinal function recovery indicators in both groups.
Note: EG versus CG .****p<0.0001.

Comparison of incidence of adverse reactions between both groups

The incidence of adverse reactions in EG was lower than in the CG, indicating a statistical significance difference ($p < 0.043$) (Table 2).

Comparison of nutritional indicators between both groups

Before surgery and one day after surgery, no statistically significant difference in TP and ALB (borrowed protein) levels was exhibited between both groups. At three days and seven days after surgery, TP and ALB levels in EG were elevated relative to those in the CG during the same period, indicating statistical significance ($p<0.001$) (Fig. 4).

Table 2. Incidence of adverse reactions in both groups.

Groups	N	Nausea	Vomiting	Others	Total incidence of adverse reactions [n (%)]
CG	40	5	3	0	8 (20.00)
EG	40	1	1	0	2 (5.00)
χ^2		/	/	/	4.114
p		/	/	/	0.043

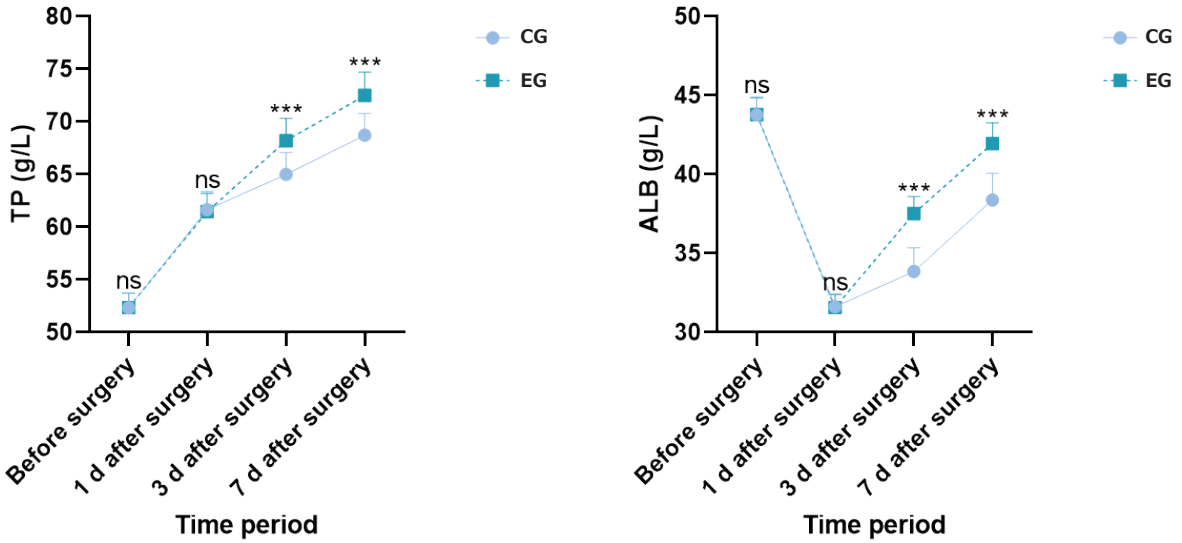

Fig. 4. Nutritional indicators in both groups.
Note: EG versus CG, ns=no significance, ***p<0.001.

DISCUSSION

CRC patients often have varying degrees of malnutrition or risk of malnutrition before surgery. Currently, tumor cachexia, the primary reason for malnutrition in tumor patients, often results from metabolic abnormalities and reduced food intake; its primary feature is the negative balance between energy and protein metabolism. Research has depicted that symptoms and signs such as anorexia, emaciation, and weight loss due to imbalanced nutritional needs and intake by metabolic abnormalities and reduced food intake in patients often cannot be wholly reversed through individual nutritional interventions [14].

CRC patients often experience abnormal intestinal function [15]. Before providing nutrition support to patients, appropriate nutrition support pathways should be selected based on their intestinal function. Nutrition support therapy majorly includes EN and PN. PN is the major nutrition support pathway, which can keep the intestine in a relatively static state of function and provide timely supplement nutrients needed by the body, which is beneficial for the recovery of damaged intestines [16,17]. Nevertheless, long-term total PN support can lead to intestinal mucosal atrophy, weakened mucosal barrier function, and translocation of intestinal microbiota, inducing complications such as enterogenous infections [18]. Thus, EN is preferred for patients who require nutrition support for therapy. EN support is inexpensive and more in line with physiology, which can stimulate secretion of digestive

fluids and gastrointestinal hormones, facilitate gastrointestinal peristalsis and gallbladder contraction, elevate intestinal blood flow, maintain normal growth of mucosal cells and gut microbiota, help maintain the integrity of chemical, mechanical, and immune barriers of the intestinal mucosa, and reduce complications, which is conducive to improving the overall state of patients [19,20]. Herein, the CG only received TPN after surgery, while the EG received early EN before and after surgery. The results depicted that bowel sound recovery time, first defecating time, first exhaust time, and first getting-out-of-bed time in the EG were diminished relative to those in the CG, indicating that early EN probably can facilitate the recovery of gastrointestinal function of patients and further ameliorate their nutritional status, which better meets the nutritional and gastrointestinal needs of CRC patients.

Surgery is a major treatment for non-terminal stage CRC, and comprehensive treatments such as surgery and psychological elements can lead to a high metabolic stress state in the body after surgery, which can further worsen malnutrition. Research has demonstrated that tumor patients with preoperative malnutrition have remarkably higher incidence and mortality rates of postoperative complications relative to those with good nutritional status [21,22]. Serum protein levels are the most commonly applied indicators reflecting the nutritional status of patients, including TP and ALB. In this regard, no statistically significant differences in TP and ALB levels were observed between both groups before and one day after surgery. Three and seven days after surgery, TP and ALB levels in EG were elevated relative to those in the CG during the same period, indicating that early EN probably can facilitate visceral protein synthesis and enhance the overall nutritional status of patients.

Postoperative pain, as a complication of surgery, has always been a hot research topic that needs urgently to be solved in clinical, surgical patients after surgery, and also a vital factor affecting the degree of postoperative recovery and psychological stress state of patients [23]. Common Western medicine pain relief methods after surgery include epidural analgesia, patient-controlled analgesia, and oral opioid analgesics, among others. Herein, both groups received PCA intervention, and in addition, Nutrison Fibre was chosen for the EG as an alternative diet for early nutritional intervention. The results showed that the average additional amount of flurbiprofen axetil in the EG was lower relative to those in CG. Within 24 hours after surgery, VAS scores in the EG were lower than those in the CG during the same period. No statistically significant difference in VAS scores was shown between both groups 24 hours later, indicating that early EN probably reduces the additional amounts of analgesics and mitigates patients' pain levels. Moreover, the incidence of adverse reactions in the EG was reduced relative to that in the CG, indicating that early EN probably can attenuate adverse gastrointestinal events in patients, improve the safety of postoperative use of analgesic pumps, and thereby enhance the prognosis of patients.

In conclusion, early EN can improve patients' postoperative gastrointestinal function and nutritional status, mitigate postoperative pain, and facilitate postoperative recovery with high safety, which is worthy of further clinical promotion.

Limitation

The subjective method using the nutritional screens (GLIM, MUST, NSR) was impossible due to the time limit.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

None.

Contribution of authors

All authors have been contributed equally to this research article.

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Health profile of patients subjected to gastric bypass at Clinics Hospital of Acre, Brazil.

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Keywords: obesity; bariatric surgery; weight recovery; weight loss; public health

Abstract. The study aimed to analyze the health profile of women undergoing Roux-en-Y gastric bypass according to the time elapsed since surgery. Ninety-three women who underwent this procedure at the Clinics Hospital in Acre, Brazil, from 2008-2017, were divided into three groups according to the post-surgery period: G1 (n = 37) up to two years; G2 (n = 20) from two to four years; G3 over four years (n = 36) after surgery. Pre-surgery and post-surgery clinical, anthropometric, and dietary variables were analyzed through a 24-hour recall. The postoperative time was 16.9±7.9 months (G1); 33.9±9.2 months (G2) and 75.3±19.1 months (G3). In the postoperative period, there was a decrease in the number of patients who practiced physical activity (35.5-33.3%). The mean percentage of excess weight loss was 66.1±15.4%. Satisfactory results were achieved by 88.17% (% PEP ≥50%). 67% of the patients presented weight reacquisition, proportional to the postoperative time ($p<0.001$). The dietary survey indicated a daily energy consumption of 1262.75±424.11 kcal. The macronutrient distribution showed 59.25±8.33% for carbohydrates, 24.26±6, 90% for lipids and 17.12±6.68% for proteins. The mean protein intake was lower in group G1 (16.09±6.23), and lipid intake slightly increased over time. Bariatric surgery had a significant impact on the reduction of comorbidities, medication use, and the loss of excess weight. However, the nutrient adequacy and the increasing incidence of weight regain in the post-surgery period demonstrated that bariatric surgery does not end the obesity treatment, but it is only a step that requires periodic monitoring.

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Perfil de salud de los pacientes sometidos a bypass gástrico en el Hospital de Clínicas de Acre, Brasil.

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Palabras clave: obesidad; cirugía bariátrica; recuperación de peso; pérdida de peso; salud pública.

Resumen. El objetivo del estudio fue analizar el perfil de salud de mujeres sometidas a bypass gástrico Roux-en-Y según el tiempo transcurrido desde la cirugía. Noventa y tres mujeres que se sometieron a este procedimiento en el Hospital de Clínicas en Acre, Brasil, de 2008 a 2017, se dividieron en tres grupos según el período posoperatorio: G1 (n = 37) hasta dos años; G2 (n = 20) de dos a cuatro años; G3 más de cuatro años (n = 36) desde la cirugía. Se analizaron variables clínicas, antropométricas y dietéticas preoperatorias y posoperatorias a través de un recordatorio de 24 horas. El tiempo posoperatorio fue de 16,9 ± 7,9 meses (G1); 33,9 ± 9,2 meses (G2) y 75,3 ± 19,1 meses (G3). En el posoperatorio, hubo una disminución en el número de pacientes que practicaban actividad física (35,5-33,3%). El porcentaje medio de pérdida de exceso de peso fue de 66,1±15,4%. Se obtuvieron resultados satisfactorios en el 88,17% (% PEP ≥50%). El 67% de los pacientes presentó readquisición de peso, proporcional al tiempo postoperatorio (p<0,001). La encuesta dietética indicó un consumo energético diario de 1262,75±424,11 kcal. La distribución de macronutrientes mostró 59,25±8,33% para carbohidratos, 24,26±6,90% para lípidos y 17,12±6,68% para proteínas. La ingesta media de proteínas fue menor en el grupo G1 (16,09±6,23%), y la ingesta de lípidos aumentó ligeramente con el tiempo. La cirugía bariátrica tuvo un impacto significativo en la reducción de comorbilidades, uso de medicamentos y pérdida de exceso de peso. Sin embargo, la adecuación de nutrientes y la creciente incidencia de recuperación de peso en el período postoperatorio demostraron que la cirugía bariátrica no pone fin al tratamiento de la obesidad, sino que es sólo un paso que requiere seguimiento periódico.

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INTRODUCTION

Obesity is currently one of the biggest public health problems in the world. Its projection, according to the World Health Organization, is that in 2025, 2.3 billion adults will be overweight, and more than 700 million will be obese. Being a global epidemic of chronic conditions, a multifactorial etiology whose treatment involves different approaches, the main ones are dietary guidelines, physical activity practice, and, in cases where these measures are not enough, the use of medications and surgical intervention [1].

Conventional treatment for severe obesity still produces unsatisfactory results since 95% of patients regain their initial weight in up to 2 years. Due to the need for a more effective intervention in the clinical management of severely obese individuals, an indication of surgery is the most effective treatment for obesity control [2].

In Brazil, bariatric surgery is among the procedures of high complexity offered by the Unified Health System (SUS), according to the regulation established on April 21, 2001. Its indication must be according to CFM Resolution No. 1,766/05, which designates the following requirements for its accomplishment: being over 18 years of age, presenting a Body Mass Index (BMI) of 40 kg/m^2 or more or equal to or greater than 35 kg/m^2 and comorbidities such as diabetes, sleep apnea, hypertension, dyslipidemia, coronary disease, osteoarthritis, and others. In addition, the subject must have undergone conventional methods and have psychological conditions to follow the indications suggested after surgery [2].

According to the Brazilian Society of Bariatric and Metabolic Surgery (SBCBM), throughout 2018, 63,969 bariatric surgeries were performed, 49,521 of which for supplementary health (health plans), according to data from the National Supplementary Health Agency (ANS), 11,402 surgeries by the Unified Health System (SUS) and 3,046 private surgeries. The total number of procedures performed in 2018 was 4.38% higher than in 2017, when approximately 61.283 thousand surgeries were performed by SUS and ANS [3].

Although bariatric surgery is an effective clinical strategy for promoting weight loss, reducing comorbidities, and improving quality of life, it is essential to emphasize that it does not promote the cure of obesity but rather its control. Therefore, although the surgical treatment demonstrates satisfactory results, some individuals present postoperative complications related to organic and behavioral problems that damage clinical and nutritional aspects [4,5].

The Brazilian population has extensive demographic, social, and cultural diversity, which is why population studies that assess the health conditions of the Amazonian peoples and their main conditions allow us to broaden the understanding of the magnitude of diseases, analyze their historical trends, and observe risks of morbidity and mortality in these population groups.

From this perspective, the objective of this study was to analyze the clinical, nutritional, and weight requirements of women undergoing Roux-en-Y gastric bypass according to the time elapsed post-surgery.

PATIENTS AND METHODS

Study design and data collection

The research, carried out at the Clinics Hospital of the State of Acre from August to December 2017, was quantitative, qualitative, and retrospectively observational. The project was submitted to the Research Ethics Committee of the Clinics Hospital /HC of the State of Acre and approved through opinion nº 1,979,084.

Patient inclusion criteria were age 18 or older, having a BMI ≥ 35kg/m^2 associated with a disease or BMI ≥ 40kg/m^2, and having undergone bariatric surgery from 2008 to 2017. The collection took place through an outpatient appointment, which was scheduled by the hospital's Obesity and Quality of Life Group and held every Wednesday and Thursday afternoon in the outpatient clinic of the Rio Branco Clinic Hospital—Acre, Brazil. Telephone contact with the patients and during the support group meetings were made. The researcher and two nutritionally trained academics collected the data. At the time of collection, the participants were informed about the study's relevance, and they voluntarily participated by signing the Informed Consent Form (TCLE).

The evaluation consisted of a questionnaire to identify the patient with the following information: personal data, socioeconomic data, schooling, previous clinical history, current clinical history, anthropometric evaluation, and dietary assessment. It was also evaluated whether or not the patient did nutritional monitoring during the postoperative period. All items were self-reported by the patients, except for the anthropometric data of the preoperative

period, which were obtained from the medical records and the current anthropometric data collected during the outpatient care performed by the researchers.

The study included 114 patients who underwent Roux-en-Y gastric bypass from 2008 to 2017 at the Acre State Clinic Hospital. Among these patients, ninety-three (81.57%) were women and twenty-one (18.42%) were men. Among the patients selected, seven refused to participate, and two died before being evaluated.

The patients were divided into three groups, according to the postoperative period, to compare the variables of interest in the study: group (G1) for up to twenty-four months, group (G2) from twenty-four months to forty-eight months, and group 03 (G3) more than forty-eight months.

After analysis, it was decided to exclude males since there was a statistically significant difference between surgery time and sex. 47.6% of males were in the 25 to 48-month group. Thus, the analyses were based only on female patients.

Clinical Evaluation

The clinical evaluation was performed in two parts: clinical history before surgery and current clinical history. The clinical history in the pre-surgical period was based on the presence of associated diseases, medication use, and physical activity practice. The current clinical history was based on the same information, adding only the use of nutritional supplements and the presence or absence of gastrointestinal disorders [4,5] in the postoperative period.

Obesity is a clinical condition with a high risk for developing other chronic diseases [6], and the following comorbidities were analyzed during the clinical evaluation of the patients: arterial hypertension, dyslipidemia, arthritis, hormonal changes, diabetes mellitus type II, apnea obstructive sleep, and edema [7]. For the evaluation of drug use [6], they were categorized into antihypertensive, antidiabetic, anti-lipemic, antidepressants, appetite suppressants, and others [8]. Patients were asked whether or not they performed regular activities to evaluate physical activity, what their activities were, and how often they practiced them: once, twice, three to four times a week, and five or more times a week.

Regarding the presence or absence of gastrointestinal disorders in the postoperative period, patients were questioned if, at any time, they had at least one of these complications: vomiting, nausea, diarrhea, constipation, and abdominal distension. Regarding supplementation in the postoperative period, the consumption of the following supplements was evaluated: polyvinyl alcohol and minerals, vitamin B12, calcium, and ferrous sulphate.

Nutritional and Dietetic Assessment

For the anthropometric evaluation, the following data were collected: weight (kg) on the day of surgery; height (m); maximum weight achieved in the preoperative period; current weight; minimum weight achieved in the postoperative period; waist circumference (cm); body composition through electrical bioimpedance; reacquisition of weight and percentage of the loss of excess weight (PEP).

The current body weight was measured on a pre-calibrated digital weighing scale with a Welkin 300 kg capacity, and the patient was instructed [8] to remove the shoes, climb backwards to the equipment and remain still with the feet in the center of the platform.

The vertical stadiometer (coupled to the scale) was used for stature measurement, with a scale of 0.5cm. The patient was instructed to keep the arms extended along the body, the head erect, and the stare fixed in a horizontal plane. The body mass index (BMI) was calculated by the weight ratio (kg) divided by the height (m) squared. This index was calculated using preoperative and current weights to evaluate its classification in both moments. The values found were classified according to the WHO [9].

The patients' body composition was evaluated through electrical bioimpedance (BIA) using a Maltron BioScan [10] 915/916 Analyzer.

The following variables were analyzed: lean mass (kg), fat mass (kg), total body water (L), and basal metabolic rate (Kcal).

The loss of excess weight (PEP) was calculated according to the equation indicated by Deitel[11], and weight reacquisition was evaluated by comparing the current weight with the minimum weight reached in the postoperative period.

The quantitative feeding evaluation was performed through a 24-hour recall (R24h) using the Avanutri 2.0 Nutrition Software based on the food composition table (TACO)[12].

Nutrient intake adequacy was evaluated according to the cut-off points of the DRIs19 (Dietary Reference Intakes), considering adequate intake as 50 to 60% carbohydrates, 25 to 30% lipids, and 10 to 15% protein [12].

The weight reacquisition was evaluated by comparing the current weight collected with the minimum weight reached in the postoperative period and the value in kilograms (kg) when the weight was regained.

Data Analysis

Statistical Package for Social Sciences (SPSS) 10.0 and SigmaPlot 14.5 were used to analyze the data. For the qualitative variables, absolute (n) and relative (%) frequencies were described, and the Pearson chi-square test was used to evaluate the difference in the proportion of the outcomes according to the independent variables. The mean (μ) and standard deviation (SD) describe the quantitative variables. The T/Mann-Whitney test was used to evaluate the difference in means of the outcomes according to the independent variables. ANOVA/Kruskal-Wallis was used for the evaluation between two means and three or more means, ANOVA/Kruskal-Wallis was used, according to the Shapiro-Wilk Normality test (p-value).

RESULTS

Ninety-three women with a mean age of 41.8 ± 7.6 years, divided into three groups were evaluated according to the postoperative time: group one (G1) up to twenty-four months, group two (G2) of twenty-four months to forty-eight months and group three (G3) over forty-eight months. In the first group, the mean postoperative time was 16.9 ± 7.9 months. In the second group, 33.9 ± 9.2 months, and in the third group, a mean of 75.3 ± 19.1 months.

As described in Table 1, 29% of the patients evaluated reported having a complete secondary level, 24.7% an incomplete upper level, and only 22.6% a complete upper level.

The income classification showed that 17.2% up to one minimum wage 58% had a family income of up to four minimum wages, 18.3% up to seven and 6.5% more than seven minimum wages.

Regarding whether or not to perform nutritional follow-up after surgery, 52.7% said to follow up, and 47.3% did not do nutritional monitoring. Among the groups, the G1 group (25 to 48 months) attended most nutritional consultations, with 42.9% of the patients.

When the groups were compared, the lowest percentage of patients (23.3%) using medication was found in group 2 (25 to 48 months). 98.9% of the patients reported using medications in the preoperative period, with antihypertensives (32.3%) and anxiolytics (24.7%) being the most commonly used medications. In the postoperative period, this index decreased to 46.2%.

Only 35.5% of the patients reported doing some physical activity before surgery, which was the most performed: walking (22.6%) and bodybuilding (8.6%). The frequency of physical activity reported by the majority (21.5%) was 3 to 4 times per week. The number of patients who practiced physical activity decreased to 33.3% in the postoperative period. The types of exercise most performed by them were walking (20.4%) and bodybuilding (8.6%). Most patients (19.4%) reported having physical activity 3 to 4 times a week.

Regarding alcohol consumption, 61.3% reported not consuming. However, 27.9% reported consuming eventually, and 10.8% weekly.

Table 1
General characterization of the study population.

Variable	Groups			Total	p
	Group 1	Group 2	Group 3		
	n = 37	n = 20	n = 36		
	n (%)	n (%)	n (%)	n (%)	
Schooling					
Incomplete Elementary School	4 (66.7)	0 (0.0)	2 (33.3)	6 (6.5)	0.11
Complete Elementary School	1 (20.0)	0 (0.0)	4 (80.0)	5 (5.4)	
Incomplete High School	1 (9.10)	5 (45.5)	5 (45.5)	11 (11.8)	
Complete High School	12 (44.4)	4 (14.8)	11 (40.7)	27 (29.0)	
Incomplete Higher Education	5 (21.7)	8 (34.8)	10 (43.5)	23 (24.7)	
Complete Higher Education	14 (66.7)	3 (14.3)	4 (19.0)	21 (22.6)	
Family income					
Up to 1 minimum wage	8 (50,0)	2 (12.5)	6 (37.5)	16 (17.2)	0.495
From 2 to 4 minimum wages	22 (40.7)	10 (18.5)	22 (40.7)	54 (58.0)	
From 5 to 7 minimum wages	5 (29.4)	5 (29.4)	7 (41.2)	17 (18.3)	
More than 7 minimum wages	2 (33.3)	3 (50.0)	1 (16.7)	6 (6.5)	
Nutritional monitoring					
Yes	21 (42.9)	12 (24.5)	16 (32.7)	49 (52.7)	0.437
No	16 (36.4)	8 (18.2)	20 (45.5)	44 (47.3)	
Use of medicines					
Yes	19(42.2)	10(23.3)	14(32.6)	43 (46.2)	0.526
No	18(36.0)	10(20.0)	22(44.0)	50 (53.8)	
Physical activity practice					
Yes	13(41.9)	5(16.1)	13(41.9)	31 (33.3)	0.669
No	24(38.7)	15(24.2)	23(37.1)	62 (66.7)	
Alcohol use					
Never	24 (42.1)	12 (21.1)	21 (36.8)	57 (61.3)	0.229
Eventually	12 (46.2)	6 (23.1)	8 (30.8)	26 (27.9)	
Weekly	1 (10.0)	2 (20.0)	7 (70.0)	10 (10.8)	
Smoking					
Non-smoking	31 (38.3)	17 (21.0)	33 (40,7)	81 (87.0)	0.574
Smoker	6 (50.0)	3 (25.0)	3 (25.0)	12 (13.0)	

* p values (Pearson chi-square test).

Eighty-seven percent of the patients said they were not smokers, and 13% said they were former smokers.

Regarding using nutritional supplements in the postoperative period, 59.13% of the patients reported taking supplementation, and 40.86% reported not using any supplement. Comparing the groups, the G1 group (25 to 48 months) used the most supplementation (52.7%).

Table 2 shows the participants' anthropometric variables (weight, height, percentage of fat mass, fat mass, and lean mass).

Table 2
Anthropometric data of the study population.

Variable	Groups			Total	p
	Group 1	Group 2	Group 3		
	n = 37	n = 20	n = 36		
	μ ± SD	μ ± SD	μ ± SD	μ ± SD	
Pre-surgery					
Weight surgery (kg)	117.71 ± 12.08	125.81* ± 15.24	124.94* ± 14.33	122.25 ± 14.04	0.037
BMI surgery (kg/m²)	46.10 ± 4.40	47.60 ± 6.12	48.17 ± 4.62	47.22 ± 4.93	0.188
Maximum weight (kg)	124.42 ± 12.76	129.86 ± 14.55	130.54 ± 13.14	128.00 ± 13.48	0.122
Overweight (kg)	20.85 ± 10.83	22.58 ± 7.55	21.68 ± 8.15	21.54 ± 9.13	0.792
Post-surgery					
Current weight (kg)	78.47 ± 11.24	81.83 ± 8.27	80.40 ± 7.81	79.92 ± 9.42	0.421
Current BMI (kg/m²)	31.09 ± 4.87	31.07 ± 3.13	31.17 ± 3.62	31.12 ± 4.03	0.995
Minimum weight (kg)	76.15 ± 11.97	76.64 ± 8.89	72.66 ± 9.71	74.88 ± 10.58	0.269
Fat mass (%)	30.60 ± 5.49	31.52 ± 5.15	32.17 ± 3.64	31.40 ± 4.77	0.373
PEP**(%)	65.64 ± 18.18	67.22 ± 11.64	66.19 ± 14.61	66.19 ± 15.46	0.936

PEP** Percentage of excess weight loss. μ ± SD: average ± Standard Deviation.

The p values are determined by the ANOVA/Kruskal-Wallis test between groups according to the Shapiro-Wilk Normality test. *Different from group 1.

Regarding the anthropometric evaluation, the mean preoperative BMI was 47.2±4.9 kg/m². Ninety-eight percent of the patients were classified as grade III (IMC>40kg/m²), and 2% were classified as grade II obesity. In the postoperative period, the mean BMI was 31.1±4.0 kg/m², the mean of group one being 31.10±4.8 kg/m², group two 31.1±3.1 kg/m², and group three of 31.1±3.6 kg/m².

The mean percentage of excess weight loss was 66.1±15.4%, and the following means were found in the groups: G1 65.64±18.1%, G2 67.22±11.6 and G3 66,19±14.6%. Satisfactory results were achieved by 88.1% of the patients who presented PEP≥50%. There was no significant difference between groups (p=0.05).

Table 3 contains the results obtained in the 24-hour recall for mean daily caloric intake, macronutrients, and micronutrients.

Concerning nutrient intake, the dietary survey (R24hs) pointed to the average daily energy consumption of 1262.75±424.11 kcal. There was no difference in energy consumption between the groups evaluated (p>0.05).

The distribution of the macronutrient percentage indicated a mean intake of 59.25±8.33% for carbohydrates, 24.26±6.90% for lipids, and 17.12±6.68% for proteins. There was no significant difference between the groups regarding the intake of macro and micronutrients (p>0.05).

Although the macronutrient intake was not significant between the groups, the mean protein intake was lower in group 01 (16.09±6.23), and that of lipid showed a slight increase over time.

Regarding the regularity of meal times, 69.89% of the patients reported not eating regularly. Only 30.10% stated that they had regular meals. Regarding the number of meals performed daily, most participants had a proper fractionation of their meals; 63.4% stated they had 3 to 4 meals/day, while only 18.3% reported doing 1 to 2 meals/day.

Table 3
Daily nutrient intake.

Variable	Groups			Total	p
	Group 1	Group 2	Group 3		
	n = 37	n = 20	n = 36		
	$\mu \pm$ SD	$\mu \pm$ SD	$\mu \pm$ SD	$\mu \pm$ SD	
Heat Transfer (kcal)	1201.5 ± 460.81	1395.4 ± 523.93	1252.0 ± 302.06	1262.7 ± 424.11	0.255
Carbohydrates (%)	58.68 ± 9.69	59.41 ± 8.58	59.74 ± 6.74	59.25 ± 8.33	0.860
Protein (%)	16.09 ± 6.23	17.30 ± 6.53	18.03 ± 7.19	17.12 ± 6.68	0.466
Lipids (%)	23.91 ± 7.42	23.78 ± 6.88	24.88 ± 6.49	24.26 ± 6.90	0.788
Calcium (mg)	480.70 ± 369.71	488.91 ± 360.42	522.51 ± 413.07	498.65 ± 381.57	0.891
Iron (mg)	9.86 ± 8.62	14.82 ± 10.10	12.98 ± 8.14	12.14 ± 8.90	0.102
Thiamine (mg)	1.43 ± 2.59	2.25 ± 5.64	1.10 ± 0.78	1.48 ± 3.10	0.414
Vitamin B_{12} (mcg)	2.22 ± 3.84	2.64 ± 6.59	0.72 ± 0.77	1.73 ± 3.96	0.135
Folate (mcg)	182.30 ± 311.47	203.37 ± 290.38	229.65 ± 282.99	205.16 ± 293.75	0.792
Zinc (mg)	5.48 ± 4.42	6.91 ± 5,17	8.59 ± 17.42	6.99 ± 11.43	0.512

$\mu \pm$ SD: average ± Standard Deviation. The p values were determined by the ANOVA/Kruskal-Wallis test between groups according to the Shapiro-Wilk Normality test.

The evaluation of water consumption showed that 74.2% of the patients consumed 1 to 2 liters of water/per day, 19.4% above 2 liters, and 6.5% up to 1 liter of water/per day.

73.3% of the patients reported food intolerances. The most mentioned foods were tapioca (59.1%), açaí (49.5%), rice (43%), fried foods (37.6%), and milk. Food intolerances were not reduced according to the postoperative time ($p>0.05$).

Sixty-seven percent of the patients presented weight reacquisition, with a mean reacquisition of 14.6±10.8 kg. Among the patients who presented weight reacquisition, 27.4% were from G1 (2 to 24 months), 21% from G2 (25 to 48 months), and 51.6% from G3 (above 48 months). It was observed that the reacquisition of weight was proportional to the postoperative time ($p<0.001$).

Of the several factors analyzed that could influence weight reactivity (age, preoperative BMI, percentage of excess weight loss, basal metabolic rate, and caloric intake), none had a significant influence on postoperative weight reactivity ($p>0.05$) (Table 4).

Ninety-nine percent of the patients presented at least one obesity-related disease in the preoperative period. After the surgical procedure, this index decreased to 36.7%, showing a significant reduction in the presence of all comorbidities ($p=0.01$), as shown in Fig. 1.

DISCUSSION

Currently, bariatric surgery is considered the most effective strategy for managing and treating severe obesity. However, several studies show that the surgical procedure does not end treatment, necessitating auxiliary therapies associated with continuously monitoring risk factors by a multiprofessional team [13].

In Brazil, the highest prevalence of severe obesity is concentrated in women. The higher prevalence of women undergoing bariatric surgery may be justified by a social issue that involves the beauty pattern,

Table 4
Analysis of factors associated with weight re-acquisition.

Variable	Total (n = 93)	weight re-acquisition		p
		Yes	No	
		n = 62	n = 31	
	$\mu \pm SD$	$\mu \pm SD$	$\mu \pm SD$	
Surgery time	43.19 ± 29.68	52.32 ± 29.07	24.94 ± 21.60	0.001
Age	41.86 ± 7.68	42.84 ± 7.40	39.90 ± 7.98	0.82
Preoperative BMI	47.22 ± 4.93	47.33 ± 4.75	47.00 ± 5.36	0.759
Percentage of Excessive Weight Loss	4.17 ± 0.65	4.21 ± 0.58	4.10 ± 0.79	0.435
Basal Metabolic Rate	1463.56 ± 166.27	1456.45 ± 148.60	1477.76 ± 198.92	0.563
Fat Mass (Percentage)	31.40 ± 4.77	31.55 ± 4.10	30.50 ± 5.86	0.199
Caloric intake	1262.75 ± 424.12	1302.46 ± 410.52	1183.33 ± 446.28	0.203

$\mu \pm SD$: average ± Standard Deviation. The p values are determined by the T/Mann-Whitney test between groups according to the Shapiro-Wilk Normality test.

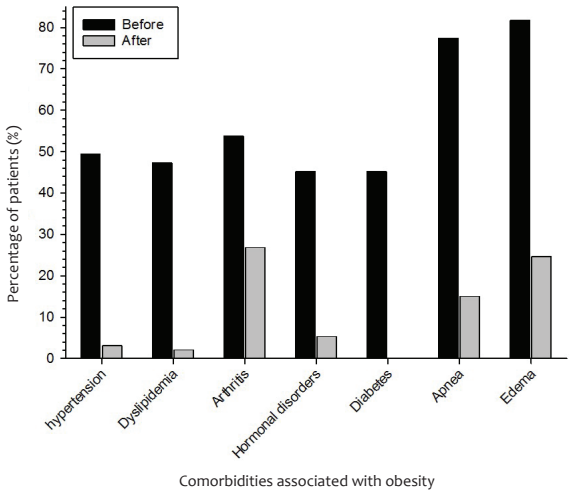

Fig. 1. Comorbidities associated with obesity before and after operation.

the appreciation of leanness, and the social pressure for weight loss to be greater in this public. In addition, women seek more health services than men [14-16].

According to information published by the Brazil Agency website, 70% of bariatric surgeries are performed by women[17]. Research carried out in different sociocultural contexts has shown that women are the majority of patients who seek a surgical procedure to treat obesity[18,19].

Comparing data from the pre-and postoperative periods of the population evaluated in this study showed improvement in all obesity-related comorbidities. Several studies [20-22] described the reduction of the presence of comorbidities.

In this study, most patients reported the use of drugs in the preoperative period, and in the postoperative period, there was a significant reduction. Possibly, this result is directly related to the reduction of comorbidities diagnosed before surgery. According to Ceneviva *et al.* [23], the reduction of comorbidities and the use of medications is proportional to weight reduction in the postoperative period of bariatric surgery. A meta-analysis investigated the impact of surgery on weight and reduction of significant comorbidities in more than 136 studies, totalling 22,094 patients (72.6% women) with a mean age of 39. The results found that the reduction of comorbidities was proportional to the loss of excess weight, similar to that found in this research [24].

A study of 342 patients (261 women and 81 men) who underwent Roux-en-Y gastric bypass showed that, as the mean BMI decreased in a postoperative period of 1,2,5 and 10 years, the associated comorbidities

were also reduced in the short, medium, and long term [25].

Regarding the practice of physical activity, we observed in this study that there was a reduction in the postoperative period, which may contribute to the reacquisition of weight. Bariatric surgery, combined with guided physical exercise, potentiates the reduction of comorbidities and contributes to a better quality of life for the patient [26]. Although no significant result was found between the association of physical activity practice and weight reactivity in this study, it is known that those who become more active present better weight control postoperatively compared to people who do not exercise physically [27]. Weich *et al.* identified that 30% of the patients who adhered to regular physical activity had better weight control in the postoperative period of bariatric surgery [28].

Regarding using nutritional supplements in the postoperative period, most patients evaluated in this study did not use any supplementation. When comparing the groups, it was observed that group 02 was the least medication user. This result may be related to the non-attendance of these patients in the health unit for nutritional follow-up since it was the group that less frequently attended consultations with nutritionists. Regarding whether or not to attend the nutritional consultation, 49% of the patients stated that they did not attend the consultations. Similar results were described by Magro et al. and Souza JMB [27,29], evidencing that periodic nutritional monitoring greatly influences dietary habits and adherence to supplementation.

Regarding the anthropometric evaluation, this study did not observe a significant difference in BMI values between the groups evaluated in the postoperative period. This study confirmed that satisfactory results were achieved by 88.17% of the patients (% PEP ≥50%), with similar results [24].

The dietary survey (R24h) indicated an average daily energy consumption of 1262.7 ± 424.1 kcal. There was no significant difference between groups in terms of nutrient intake. A similar result was described by Brolin RE *et al.* [30].

Carbohydrate intake was 57±6.4%, the only macronutrient that presented intakes compatible with current nutritional recommendations. In this study, we found similar results since the percentage of carbohydrate intake was also the only one that showed adequacy of the current daily recommendations [31].

Although the macronutrient intake was not significant between the groups, the mean protein intake was lower in group 01 (16.09 ± 6.23), and that of lipids showed a discrete increase over time. The average protein intake in grams was shown to be inadequate since the minimum recommendation is 60 to 70 grams per day. A study showed similar results when citing the average protein intake performed by most patients in the postoperative period [13].

Compared to the DRIs-recommended Daily Intake Means, inadequacy was observed in all micronutrients analyzed: calcium, iron, thiamine, vitamin B_{12}, folate, and zinc. Other studies [30-32] also reported low vitamin B12, iron, zinc, iron, calcium, and folate intake.

In patients over 18 years of both genders submitted to Roux-en-Y gastric bypass gastroplasty, it was evidenced that 30.83% of subjects had vitamin B_{12} deficiency, 29.1% had iron deficiency, and 14.1% had calcium deficiency [30-32].

The majority of patients adequately fractionated their meals. Thirty-five reported similar results, identifying that 62.1% of the individuals evaluated consumed four or five meals daily.

Food intolerances were reported by 73.3% of the patients, similar to that found by other authors [30-35,] who also identified that the foods that caused the most discomfort were rice, sweets, and meat. In this study, the most mentioned foods were tapioca (59.1%), açaí (49.5%), rice (43%), fried foods (37.6%), milk (32.3%), and sweet (31,2%).

Foods such as tapioca and açaí have not yet been cited in other studies because they are regional foods.

Regarding weight reacquisition, 67% of the patients regained weight, with a mean reacquisition of 14.6 ± 10.8 kg. [27], finding that 46% of the patients regained weight in two years postoperatively and 63.6% in four years.

In this study, most patients who presented reacquisition were in G3 (above 48 months). Thus, reacquisition was significantly proportional to postoperative time, although no significant relationship was found with income, daily caloric intake, basal metabolic rate, body composition, physical exercise, and nutritional monitoring.

As observed in this study, the literature [30-35] points out a greater incidence of weight reacquisition after two years of surgery, which is attributed to the longer time elapsed after surgery.

The occurrence of weight relapse, especially in patients with a more extended postoperative period, is associated with worsening comorbidities. There is little data on patients with more than 10 years of postoperative, which increases the concern and the need for more research in the area [31].

In the last five years, the scientific literature has pointed out that so far, bariatric surgery is the most effective method to treat obesity and can play an essential role in reducing the direct and indirect costs of obesity treatment [36]. The procedure increases fertility rates and improves breastfeeding, providing benefits to infant and maternal health[37] However, it pointed out that issues associated with bone mineralization [38], digestive motility [39], and nutritional deficiencies should be carefully observed [40].

The results found in this study evidenced that the public that most demanded bariatric surgery in the Western Amazon is the female population.

Among the individuals evaluated, there was a significant reduction in comorbidities associated with obesity and, consequently, a decrease in medication use. The loss of excess weight was satisfactory concerning the surgical procedure adopted (Roux-en-Y gastric bypass), and daily caloric intake, as well as protein, vitamin, and mineral intake, especially in the first two postoperative years, presented inadequacies compared to the current recommended nutrient recommendations.

Most patients did not perform periodic nutritional monitoring, which can demonstrate non-attendance to consultations with other health professionals since the answering service is integrated. In addition, most of the patients did not adequately use nutritional supplementation. The foods with the most significant potential for food intolerances in the region were tapioca, açaí, sweets, and milk.

Regarding weight reacquisition, the higher the postoperative period of the patients, the greater the weight reacquisition was found. This fact did not present significant relevance when compared to variables such as income, physical activity practice, nutritional monitoring, basal metabolic rate and daily energy intake. This result is worrisome and must be investigated in other research, seeking to describe the determining factors for the reality found.

Bariatric surgery significantly reduced comorbidities, medication use, and excess weight loss. However, the adequacy of nutrients and the increasing incidence of weight reactivity in the postoperative period demonstrated that bariatric surgery does not end the treatment of obesity; on the contrary, it is only a step that requires periodic monitoring by health professionals.

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Conflicts of interest

Authors declare that they have no conflicts of interest.

Ethical approval

The project was submitted to the Research Ethics Committee of the Clinics Hospital /HC of the State of Acre and approved through opinion nº 1,979,084.

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Phyllanthin from *Phyllanthus amarus* protects the myocardium during pressure overload-induced cardiac hypertrophy by inhibiting the angiotensin-converting enzyme.

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Keywords: angiotensin-converting enzyme; aortic stenosis; cardiac hypertrophy; collagen-I; Phyllanthin; *Phyllanthus amarus*; pressure overload.

Abstract. Ischemic heart disease results from obstruction of blood flow and leads to myocardial infarction. Various lignans of herbal origin have been shown to protect against cardiotoxicity. The present study aimed to assess the potential of phyllanthin, identified from a standardized methanolic extract of *Phyllanthus amarus* (PAME), against pressure overload-induced cardiac hypertrophy in experimental rats. Lignan was identified in PAME using HPLC. Ligating the abdominal aorta induced cardiac hypertrophy in Wistar rats (220-240g). Then they were treated with (n=15, each) either distilled water (10 mL/kg, aortic stenosis control), lisinopril (15 mg/kg), or PAME (50, 100 and 200 mg/kg) for 28 days. Lignan compounds were identified using UV spectra in PAME, and HPLC analysis showed the presence of phyllanthin at 25.30 retention time with an area of 70.22%. Treatment with PAME (100 and 200 mg/kg) significantly and dose-dependently (p<0.01 and p<0.001) ameliorated AS-induced elevation in absolute and relative heart weights, increased serum biomarker levels, and alterations in electrocardiographic and hemodynamic functions. PAME effectively inhibited AS-induced oxide-nitrosative stress dose-dependently (p<0.01 and p<0.001). Up-regulated mRNA expression of cardiac angiotensin-converting enzyme (ACE) and Collagen-I were also markedly inhibited (p<0.01 and p<0.001) by PAME. Furthermore, PAME significantly reduced (p<0.01 and p<0.001) pressure overload-induced alterations in cardiac histopathology. In conclusion, phyllanthin identified from *P. amarus* ameliorated pressure overload-induced cardiac hypertrophy by inhibiting ACE and collagen-I formation pathways to alleviate hypertension and fibrosis. These findings collectively suggest that *P. amarus* represents promising therapy for managing ischemic heart diseases.

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La filantina de la *Phyllanthus amarus* protegió el miocardio durante la hipertrofia cardíaca inducida por sobrecarga de presión mediante la inhibición de la enzima convertidora de angiotensina.

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Palabras clave: Enzima convertidora de angiotensina; estenosis aórtica; hipertrofia cardíaca; colágeno-I; Filantina; *Phyllanthus amarus*; sobrecarga de presión.

Resumen. La cardiopatía isquémica es el resultado de la obstrucción del flujo sanguíneo del corazón y conduce al infarto de miocardio. Se ha demostrado que varios lignanos de origen herbario protegen contra la cardiotoxicidad. El presente estudio tuvo como objetivo evaluar el potencial de la filantina, identificada a partir de un extracto metanólico estandarizado de *Phyllanthus amarus* (PAME), contra la hipertrofia cardíaca inducida por sobrecarga de presión en ratas experimentales. El lignano se identificó en PAME mediante HPLC. La ligadura de la aorta abdominal indujo hipertrofia cardíaca en ratas Wistar (220-240 g). Luego se las trató con (n = 15, cada una) agua destilada (10 ml/kg, control de estenosis aórtica), lisinopril (15 mg/kg) o PAME (50, 100 y 200 mg/kg) durante 28 días. Los compuestos de lignano se identificaron utilizando espectros UV en PAME, y el análisis de HPLC mostró la presencia de filantina en un tiempo de retención de 25,30 con un área de 70,22%. El tratamiento con PAME (100 y 200 mg/kg) mejoró significativamente y de manera dosis-dependiente ($p<0,01$ y $p<0,001$) la elevación inducida por AS en los pesos cardíacos absolutos y relativos, aumentó los niveles de biomarcadores séricos y las alteraciones en las funciones electrocardiográficas y hemodinámicas. PAME inhibió eficazmente el estrés óxido-nitrosativo inducido por AS de manera dosis-dependiente ($p<0,01$ y $p<0,001$). La expresión de ARNm regulada al alza de la enzima convertidora de angiotensina cardíaca (ECA) y el colágeno-I también fueron inhibidos notablemente ($p<0,01$ y $p<0,001$) por PAME. PAME redujo significativamente ($p<0,01$ y $p<0,001$) las alteraciones inducidas por sobrecarga de presión en la histopatología cardíaca. En conclusión, la filantina identificada en *P. amarus* mejoró la hipertrofia cardíaca inducida por sobrecarga de presión al inhibir las vías de formación de la ECA y del colágeno-I para aliviar la hipertensión y la fibrosis. Estos hallazgos en conjunto sugieren que *P. amarus* ofrece una terapia prometedora para el manejo de las enfermedades cardíacas isquémicas.

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INTRODUCTION

Ischemic heart disease (IHD) is the most prevalent cardiovascular disease (CVD) and is characterized by the accumulation of plaques within the walls of the coronary arteries, resulting in a decreased flow of blood to the cardiac tissue [1]. This obstruction of blood flow leads to acute coronary syndromes such as unstable angina or myocardial infarction [2]. IHD significantly impacts long-term global development and, as per

the World Health Organization reports, an annual toll of 17.7 million lives lost to IHD, imposing a pervasive social and economic burden globally [3].

Treatment interventions to manage IHD include antiplatelet agents (such as aspirin or clopidogrel) to reduce the risk of thrombosis, statins (such as lovastatin, atorvastatin, simvastatin, and rosuvastatin) to reduce the risk of atherosclerosis, beta-blockers (such as timolol, metoprolol, atenolol, and propranolol) to decrease heart rate and blood pressure, calcium channel blockers (such as amlodipine, nicardipine, diltiazem, verapamil) to enhance blood flow to the cardiac tissue, angiotensin-converting enzyme (ACE) inhibitors (lisinopril), or angiotensin receptor blockers to lower blood pressure and improve heart function [4]. Despite extensive exploration of these treatment regimens, their side effects, such as toxicity to various organs, limit their availability as definitive therapeutic or prophylactic interventions for managing IHD [5]. However, the cost of treatment and its efficacy in a fraction of patients limit its implications for optimizing patient outcomes and improving quality of life. Thus, for the effective management of IHD, a multidisciplinary approach is needed.

Among the diverse species of medicinal plants, the *Phyllanthus* spp. (*Euphorbiaceae*) has been used in traditional medicine for thousands of years and in Thai folk medicine for treating various ailments, including diabetes, diarrhea, hepatitis, abdominal pain, and various kidney diseases [6]. Among the *Phyllanthus* spp., *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae) is a valuable medicinal plant, extensively distributed across tropical and subtropical zones, including Asia, Africa, the West Indies, and South America [7]. *P. amarus* has been extensively studied for its hepatoprotective, antiviral, antiulcer, antiepileptic, anti-asthmatic, antidiabetic, anti-inflammatory, anticancer, and antioxidant properties [8-12]. Pharmacological studies have reported that various bioactive compounds, including alkaloids, polyphenols, tannins, flavonoids, sterols, volatile oils, lignans, and triterpenes are responsible for this array of pharmacological activities. Phyllanthin and hypophyllanthin from *P. amarus* have been reported as inflammatory markers, including tumor necrosis factor-α (TNF-α), which exerts their antiulcer potential [12]. Furthermore, the inhibitory effect of phyllanthin on TNF-α, interleukins, heme oxygenase-1, and transforming growth factor-beta supports its anti-asthmatic properties [11]. However, its potential against IHD is yet to be evaluated. This study aimed to assess the potential of phyllanthin identified from a standardized methanolic extract of *Phyllanthus amarus* aerial parts against pressure overload-induced cardiac hypertrophy in experimental rats.

MATERIALS AND METHODS

Animals

Ninety adult Wistar rats (male, 220-240 g, 7-8 weeks, were purchased from the animal house of Qingdao Central Hospital), with the following housing considerations: temperature: 24°C±1°C, relative humidity: 45-55%, normal dark/light cycle with free access to standard pellet chow and water. The Qingdao Central Hospital approved the experimental protocol (Protocol Number: 559974002). All surgeries were performed under sodium thiopental anesthesia, and efforts were made to minimize suffering.

Preparation and identification of a standardized methanolic extract of *P. amarus*

This procedure was performed according to a previously reported method [12]. Briefly, the quantity (500 g) of air-dried powder (Mesh size-16) of the aerial parts of *P. amarus* was macerated with distilled methanol at room temperature by soaking and eventually stirring for seven days and then filtered. The filtrate was dried in a tray dryer and maintained at 40°C. A semi-solid methanolic extract of *P. amarus* (PAME) was obtained, and

colloidal silicon dioxide was added and dried in a vacuum tube dryer. Phytochemical analysis of PAME was performed to identify phyllanthin content using high-performance liquid chromatography (HPLC). Analyses were conducted using an HPLC system (Camag, Muttenz, Switzerland) with an RP C18, 5μ, 250 X 4.6 mm, and 1.5 mL/min flow rate. Acetonitrile: Buffer (40:60 v/v) was used as the mobile phase for isolation and detection. The buffer consisted of 0.136 g of potassium hydrogen phosphate and 0.5 mL of o-phosphoric acid. The optimum injection volume was 20 μL, and the detection wavelength of the detector was set to 230 nm. The autosampler temperature was maintained at 10°C, and the system pressure was 1000 psi.

Induction of pressure overload-induced cardiac hypertrophy and treatment schedule

Wistar rats were anesthetized using sodium thiopental (35 mg/kg, intraperitoneally) and the abdominal aorta above the left renal artery was exposed by cutting the mid-abdomen. Then, it was constricted using a 40 mm cannula (0.9 sizes) ligation and withdrawn after 10 min [13]. After a week of recovery, the rats were randomly assigned to various groups. The rats received the following treatments (15 rats per group): aortic stenosis control (AS, received distilled water [DW], 10 mL/kg), lisinopril (15 mg/kg), and PAME (50, 100, and 200 mg/kg). The dosages of lisinopril (15 mg/kg) and PAME (50, 100, and 200 mg/kg) were selected based on previous studies [9,12]. Other groups of age- and body-weight-matched sham rats were maintained without aortic ligation and treated with DW (10 mL/kg). Rats were treated orally with DW, lisinopril, or PAME for 28 days.

Behavioral and biochemical determination

On the 29th day, blood was collected using the retro-orbital puncture method from anesthetized rats (urethane, 1.25 g/kg, intraperitoneally), and serum (six rats per group) was separated to evaluate the parameters including creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) using various reagent kits (Accurex Biomedical Pvt. Ltd., Mumbai, India).

Electrocardiographic (ECG) and hemodynamic functions (including heart rate and blood pressure viz, systolic blood pressure [SBP], diastolic blood pressure [DBP], and mean arterial blood pressure [MABP]) were estimated (six rats per group) after blood collection using an AD Instrument data-acquisition system (LabChart 7.3; AD Instrument Pvt. Ltd., Australia).

Animals were sacrificed by cervical dislocation. Cardiac tissue was isolated and perfused with cold phosphate-buffered saline to flush blood from the tissue and stored at -70°C. A previously reported method was used to determine the levels of total protein, superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA), and nitric oxide (NO) in cardiac tissue homogenates (six rats per group) [14].

Reverse transcription polymerase chain reaction (RT-PCR) analysis was used to determine the messenger ribonucleic acid (mRNA) expression of angiotensin-converting enzyme (ACE; forward primer: CCTGATCAACCAGGAGTTTGCAGAG, reverse primer: GCCAGCCTTCCCAGGCAAACAGCAC, base pair: 303) and collagen-I (forward primer: GAGCGGAGAGTACTGGATCG, reverse primer: GGTTCGGGCTGATGTACCAG, base pair: 218) in cardiac tissue (6 rats per group) [15,16]. β-actin was used as a reference standard (forward primer: GCCATGTACGTAGCCATC, reverse primer: GAACCGCTCATTGCCGAT, base pair: 375).

Finally, cardiac tissue from each group (three rats per group) was isolated and fixed in 10% formalin for histopathological evaluation. Briefly, cardiac tissues were cut in sections of 3-5 μm thickness by microtome and stained by hematoxylin-eosin. The samples were mount-

ed by disterene phthalate xylene (DPX). For myocardial fibers staining, Yuccafine™ Masson's trichrome staining kit (Yucca Diagnostics, India) was used. Each tissue section's photomicrographs were observed using Cell Imaging software for Life Science microscopy (Olympus Soft Imaging Solution GmbH, Munster, Germany). Microscopic scoring (0-4) of histological observations (myocardial degeneration, interstitial inflammation, and hemorrhage) was performed by an experienced histologist, unaware of the treatment groups, as described previously [15].

Statistical analysis

Data for all parameters (except histopathological findings) are expressed as mean ± standard error of the mean (SEM), and data for histopathological findings are expressed as medians (Q1, Q3). Data analysis was performed using the GraphPad Prism software (version 5.0; GraphPad, San Diego, CA, USA). Data were analyzed using one-way analysis of variance (ANOVA), and Tukey's multiple range test was used for *post hoc* analysis. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Isolation and identification of phyllanthin from PAME

PAME had a 52.78% w/w yield with glycosides, lignans, steroids, tannins, and phenolic compounds. The lignan compounds were identified by ultraviolet (UV) spectroscopy (Fig. 1A). The total run time for the HPLC column was 40 min, and phyllanthin was identified at a retention time (RT) of 25.30 min with an area of 70.22% (Fig. 1B).

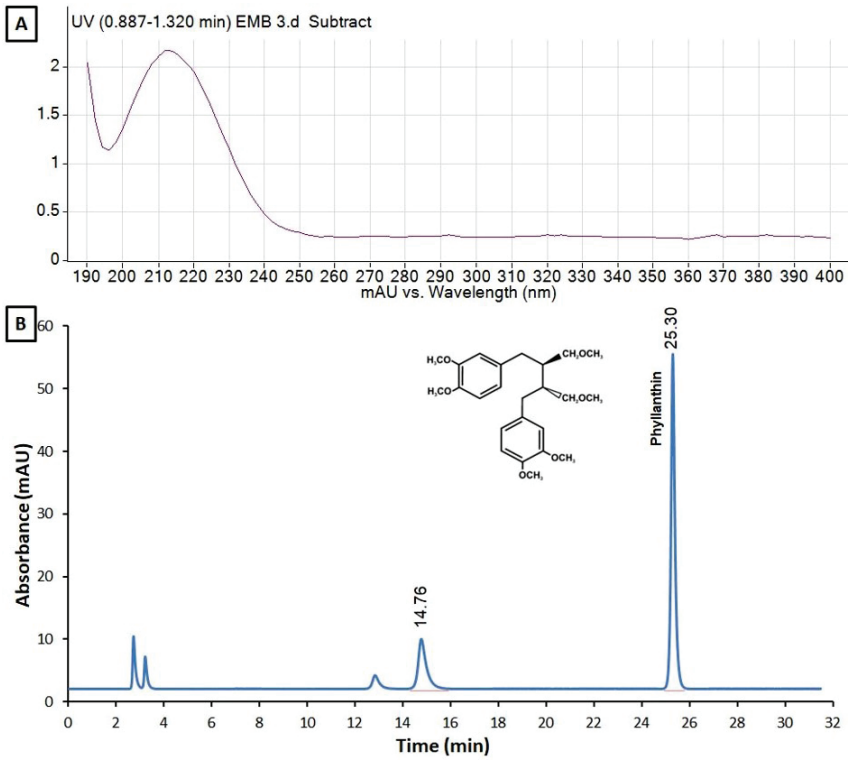


Fig. 1. A – UV spectra of standardized *P. amarus* extract; and **B** – HPLC chromatogram showing the peak of phyllanthin (RT: 25.30 min).

HPLC: High-performance liquid chromatography; mAU: milli-absorbance unit; min: minute; nm: nanomole; RT: retention time; UV: ultraviolet.

Effect of PAME on body weight and relative heart Weight

The body weights of sham rats and rats in the AS control and treatment groups did not differ significantly. Ligation of the abdominal aorta also did not cause any significant change in the body weight of AS control rats compared to sham rats. Compared to sham rats, ligation of the abdominal aorta caused a significant increase ($p<0.001$) in heart weight (absolute) and heart weight to body weight ratio (relative heart weight) in AS control rats. In contrast, treatment with lisinopril (15 mg/kg) resulted in significant attenuation ($p<0.001$) in absolute and relative heart weights compared to the AS control rats. Compared with AS control rats, PAME (100 and 200 mg/kg)-treated rats also showed a significant and dose-dependent decrease ($p<0.01$ and $p<0.001$) in absolute and relative heart weights. Administration of PAME (50 mg/kg) did not protect against AS-induced increase in cardiac weight (Table 1).

Effect of PAME on electrocardiographic and hemodynamic functions

Fig. 2 depicts AS-induced alterations in electrocardiographic recordings and their amelioration by PAME. The heart rate of AS control rats was significantly ($p<0.001$) lower than that of sham rats. In contrast, treatment with lisinopril (15 mg/kg) resulted in a significant increase ($p<0.001$) in heart rate when compared to the AS control rats. Treatment with PAME (100 and 200 mg/kg) resulted in a significant and dose-dependent increase ($p<0.01$ and $p<0.001$) in heart rate compared to that in the AS control rats (Fig. 2 and Table 2).

There was a significant ($p<0.001$) prolongation in the QRS, QT, QTc, PR, RR, and ST intervals in AS control rats compared with sham rats. However, treatment with lisinopril (15 mg/kg) significantly ($p<0.001$) inhibited the prolongation of QRS, QT, QTc, PR, RR, and ST intervals compared with AS control rats. Treatment with PAME (100 and 200 mg/kg) also resulted in a significant ($p<0.001$) decrease in QRS, QT, QTc, PR, RR, and ST intervals compared to AS control rats (Fig. 2 and Table 2).

SBP, DBP, and MABP in AS control rats were significantly ($p<0.001$) lower than those in sham rats. In contrast, treatment with lisinopril (15 mg/kg) resulted in a significant ($p<0.001$) increase in SBP, DBP, and MABP in the AS control rats. Treatment with PAME (100 and 200 mg/kg) also significantly and dose-dependently ($p<0.01$ and $p<0.001$) increased SBP, DBP, and MABP compared with the AS control rats (Table 2).

Effect of PAME on serum biochemistry

CK-MB, LDH, and ALP levels were significantly ($p<0.001$) higher in the AS control rats than in the sham rats. Treatment with lisinopril (15 mg/kg) significantly ($p<0.001$) decreased CK-MB, LDH, and ALP levels compared to AS control rats. Treatment with PAME (100 and 200 mg/kg) reduced the CK-MB, LDH and ALP significantly and dose-dependently ($p<0.001$ and $p<0.001$) compared to AS control rats. However, there was no significant decrease in CK-MB, LDH, and ALP levels in PAME (50 mg/kg)-treated rats compared to those in AS control rats (Table 1).

Effect of PAME on cardiac total protein, SOD, GSH, MDA, and NO levels

Cardiac SOD and GSH levels in the AS control rats were significantly lower ($p<0.001$) than those in the sham rats. SOD and GSH levels in the cardiac tissue of lisinopril (15 mg/kg)-treated rats were significantly higher ($p<0.001$) than those in the AS control rats. Treatment with PAME (100 and 200 mg/kg) significantly and dose-dependently attenuated ($p<0.01$ and $p<0.001$) AS-induced decreased levels of SOD and GSH compared to those in AS control rats (Table 3).

There was a significant increase ($p<0.001$) in cardiac total protein, MDA,

Table 1. Effect of PAME on pressure overload-induced alterations in absolute heart weight, relative heart weight, serum CK-MB, LDH, and ALP.

Parameters	Sham	AS control	L (15 mg/kg)	PAME (50 mg/kg)	PAME (100 mg/kg)	PAME (200 mg/kg)
Body weight (in g)	236.20 ± 4.00	241.00 ± 2.99	239.70 ± 3.90	242.80 ± 4.08	240.30 ± 4.46	240.20 ± 4.76
Heart weight (in g)	0.30 ± 0.02	0.90 ± 0.05$^{###}$	0.40 ± 0.05***	0.85 ± 0.03	0.61 ± 0.03**	0.47 ± 0.04***
Heart weight/Body weight (X10-3)	1.27 ± 0.06	3.76 ± 0.21$^{###}$	1.65 ± 0.19***	3.52 ± 0.15	2.56 ± 0.13**	1.96 ± 0.15***
Serum CK-MB (in IU/L)	1057.00 ± 56.33	2102.00 ± 66.24$^{###}$	1268.00 ± 39.52***	1952.00 ± 37.08	1658.00 ± 50.04**	1317.00 ± 50.54***
Serum LDH (in IU/L)	1356.00 ± 73.39	2733.00 ± 62.75$^{###}$	1666.00 ± 71.75***	2748.00 ± 51.49	2025.00 ± 51.74**	1601.00 ± 110.9***
ALP (in mg %)	117.60 ± 4.98	341.70 ± 5.21$^{###}$	137.20 ± 12.82***	318.20 ± 10.28	259.10 ± 7.15**	154.80 ± 11.5***

Data are expressed as mean ± SEM (six rats per group) and analyzed by one-way variance analysis followed by Tukey's multiple range test. **$p<0.01$ and ***$p<0.001$ as compared to the AS control rats, $^{###}p<0.001$ as compared to the sham rats. AS: aortic stenosis control rats; L (15): lisinopril (15 mg/kg)-treated rats; PAME (50, 100, and 200 mg/kg); *Phyllanthus amarus* methanolic extract-treated rats. The numbers in parentheses in the table header represent the doses of the respective treatments in mg/kg. ALP: alkaline Phosphatase; AS: aortic stenosis; CK-MB: creatine Kinase-MB; g: gram; IU/L: international units per liter; kg: kilogram; L: lisinopril; LDH: lactate dehydrogenase; mg: milligram; PAME: *Phyllanthus amarus* methanolic extract; SEM: standard error means.

Table 2. Effect of PAME on pressure overload-induced alterations in electrocardiographic and hemodynamic.

Parameters	Sham	AS control	L (15 mg/kg)	PAME (50 mg/kg)	PAME (100 mg/kg)	PAME (200 mg/kg)
Heart Rate (in BPM)	363.70 ± 13.70	271.00 ± 5.82$^{###}$	321.00 ± 10.64***	276.20 ± 7.80	300.70 ± 11.90**	344.5.00 ± 13.72***
QRS interval (in ms)	12.33 ± 0.67	34.17 ± 0.87$^{###}$	16.17 ± 0.54***	30.00 ± 0.93	23.33 ± 1.17***	21.33 ± 0.88***
QT Interval (in ms)	47.33 ± 2.77	92.00 ± 2.62$^{###}$	60.50 ± 3.14***	85.00 ± 3.45	69.67 ± 2.46***	64.00 ± 1.29***
QTc Interval (in ms)	130.30 ± 4.61	177.80 ± 4.74$^{###}$	143.50 ± 1.46***	168.70 ± 3.72	148.20 ± 5.26***	144.50 ± 6.16***
PR interval (in ms)	14.00 ± 0.58	29.50 ± 0.76$^{###}$	17.33 ± 0.99***	28.67 ± 0.56	24.33 ± 1.12***	22.00 ± 0.68***
RR interval (in ms)	151.70 ± 4.55	215.50 ± 4.00$^{###}$	160.80 ± 4.35***	206.70 ± 5.54	177.50 ± 5.57***	171.70 ± 5.43***
ST interval (in ms)	12.00 ± 0.58	35.50 ± 0.76$^{###}$	15.33 ± 0.99***	32.67 ± 0.56	26.33 ± 1.12***	24.00 ± 0.68***
SBP (in mmHg)	152.50 ± 3.76	106.30 ± 2.91$^{###}$	151.30 ± 4.42***	116.50 ± 1.34	131.00 ± 1.84**	137.80 ± 2.86***
DBP (in mmHg)	116.00 ± 2.99	88.33 ± 3.54$^{###}$	111.30 ± 3.75***	95.67 ± 4.57	97.17 ± 4.19**	107.50 ± 3.53***
MABP (in mmHg)	120.50 ± 2.41	93.83 ± 1.72$^{###}$	116.00 ± 1.29	101.30 ± 2.86	105.00 ± 2.63**	110.00 ± 2.00***

Data are expressed as mean ± SEM (six rats per group) and analyzed by one-way variance analysis followed by Tukey's multiple range test. **$p<0.01$ and ***$p<0.001$ as compared to the AS control rats, $^{###}p<0.001$ as compared to the sham rats. AS: aortic stenosis control rats; L (15): lisinopril (15 mg/kg)-treated rats; PAME (50, 100, and 200 mg/kg); *Phyllanthus amarus* methanolic extract-treated rats. The numbers in parentheses in the table header represent the dose of the respective treatment in mg/kg. AS: aortic stenosis; BPM: beats per minute; DBP: diastolic blood pressure; kg: kilogram; L: lisinopril; MABP: mean arterial blood pressure; mg: milligram; mmHg: millimeters of mercury; ms: millisecond; PAME: *Phyllanthus amarus* methanolic extract; SBP: systolic blood pressure; SEM: standard error means.

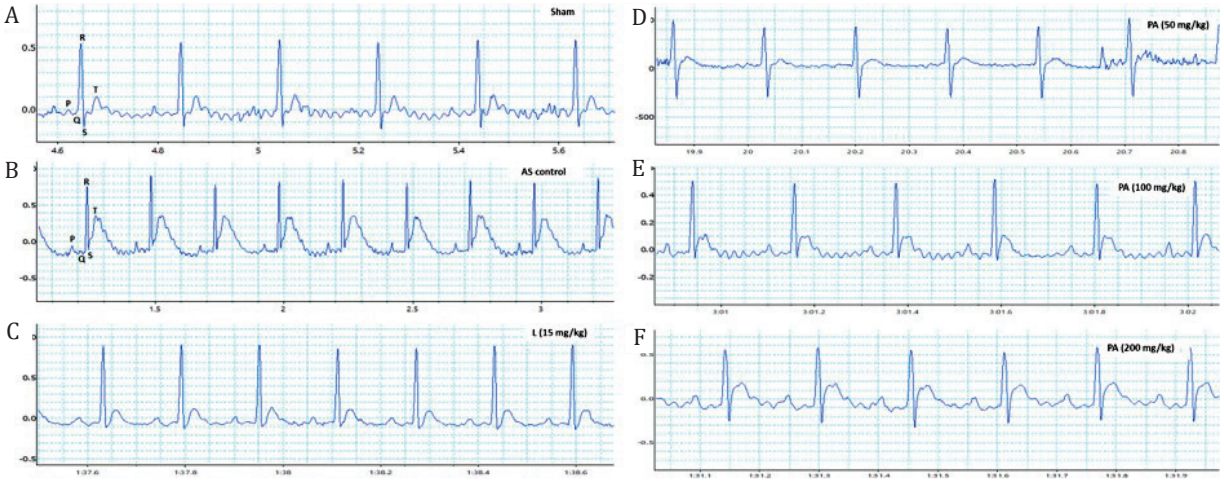


Fig. 2. Effect of PAME on pressure overload-induced alterations on electrocardiograms. A representative electrocardiographic tracing from **A** – sham rats; **B** – AS control rats; **C** – lisinopril (15 mg/kg)-treated rats; **D** – PAME (50 mg/kg)-treated rats; **E** – PAME (100 mg/kg)-treated rats; and **F** – PAME (200 mg/kg)-treated rats. AS: aortic stenosis; kg: kilogram; L: lisinopril; mg: milligram; PAME: *Phyllanthus amarus* methanolic extract.

Table 3
Effect of PAME on pressure overload-induced alterations in cardiac oxido-nitrosative stress.

Parameters	Sham	AS control	L (15 mg/kg)	PAME (50 mg/kg)	PAME (100 mg/kg)	PAME (200 mg/kg)
SOD (in U/mg of protein)	9.29 ± 0.44	4.08 ± 0.61$^{###}$	6.66 ± 0.62***	4.08 ± 0.68	5.60 ± 0.61***	6.25 ± 0.87***
GSH (in μg/mg protein)	0.35 ± 0.02	0.22 ± 0.01$^{###}$	0.34 ± 0.02***	0.25 ± 0.02	0.26 ± 0.02**	0.36 ± 0.02***
MDA (in nmol/L/mg of protein)	2.46 ± 0.32	7.17 ± 0.29$^{###}$	3.47 ± 0.29***	6.56 ± 0.35	4.67 ± 0.30**	3.35 ± 0.24***
NO (in μg/mg of protein)	212.90 ± 15.22	604.20 ± 14.52$^{###}$	307.60 ± 10.31***	552.60 ± 7.66	493.20 ± 17.84**	349.90 ± 6.36***
Total protein (in mg/mL of tissue)	24.32 ± 3.06	60.35 ± 3.47$^{###}$	33.76 ± 3.57***	56.89 ± 2.71	49.98 ± 3.15**	38.14 ± 2.60***

Data are expressed as mean ± SEM (six rats per group) and analyzed by one-way variance analysis followed by Tukey's multiple range test. **$p<0.01$ and ***$p<0.001$ as compared to the AS control rats, $^{###}p<0.001$ as compared to the sham rats. AS: aortic stenosis control rats; L (15): lisinopril (15 mg/kg)-treated rats; PAME (50, 100, and 200 mg/kg); *Phyllanthus amarus* methanolic extract-treated rats. The numbers in parentheses in the table header represent the dose of the respective treatment in mg/kg. μg: microgram; AS: aortic stenosis; GSH: glutathione peroxidase; kg: kilogram; L: lisinopril; MDA: malondialdehyde; mg: milligram; mL: milliliter; nmol: nanomole; NO: nitric oxide; PAME: *Phyllanthus amarus* methanolic extract; SEM: standard error means; SOD: superoxide dismutase.

and NO levels in AS control rats compared to sham rats. Administration of lisinopril (15 mg/kg) significantly ($p<0.001$) decreased total protein, MDA, and NO levels in cardiac tissue compared with those in AS control rats. Treatment with PAME (100 and 200 mg/kg) also significantly and dose-dependently ($p<0.01$ and $p<0.001$, respectively) decreased the cardiac total protein, MDA, and NO levels compared to AS control rats (Table 3).

Effect of PAME on cardiac ACE and collagen-I mRNA expressions

Compared with sham rats, cardiac ACE and collagen-I mRNA expressions were sig-

nificantly upregulated in AS control rats. Compared to AS control rats, ACE and collagen-I mRNA expression in the cardiac tissue of lisinopril (15 mg/kg)-treated rats was significantly downregulated ($p<0.001$). Treatment with PAME (50 mg/kg) failed to significantly downregulate ACE and collagen-I mRNA expression compared to AS control rats. However, administration of PAME (100 and 200 mg/kg) significantly and dose-dependently ($p<0.01$ and $p<0.001$) downregulated ACE and collagen-I mRNA expression compared to AS control rats (Fig. 3).

Effect of PAME on pressure overload-induced alterations in cardiac histopathology

Histopathological observations of the heart from sham rats revealed a well-maintained architecture with sham myocardial fibers and muscle bundles with well-defined boundaries and mild infiltration of neutrophils (Fig. 4A). Hearts from AS control rats showed significant ($p<0.001$) myocardial degeneration, congestion, edema, and infiltration of inflammatory cells with a disorganized arrangement of muscle bundles with no well-defined boundaries (Fig. 4B). Administration of lisinopril (15 mg/kg) protected against AS-induced myocardial damage, as reflected by a significant ($p<0.001$) reduction in myocardial necrosis, inflammatory infiltration, and congestion without any edema (Fig.4C). Heart sections from PAME (50 mg/kg)-treated rats showed severe myocardial necrosis, inflammatory cell infiltration, congestion, and edema (Fig. 4D). However, administration of PAME (100 and 200 mg/kg) significantly ($p<0.001$) reduced AS-induced myocardial aberrations, as reflected by the presence of mild to moderate myocardial necrosis, inflammatory cell infiltration, congestion, and edema (Fig. 4E and 4F). (Fig. 4G).

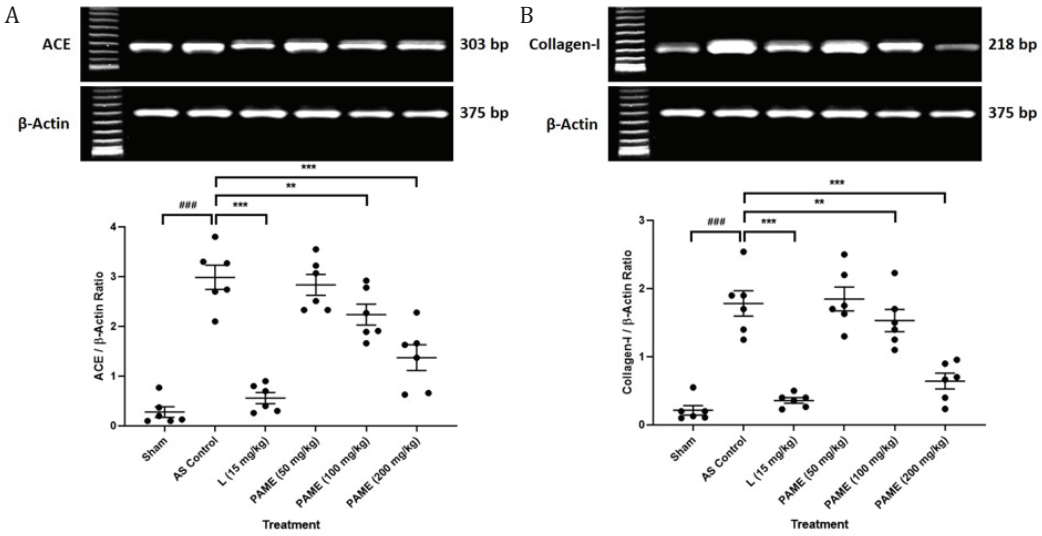
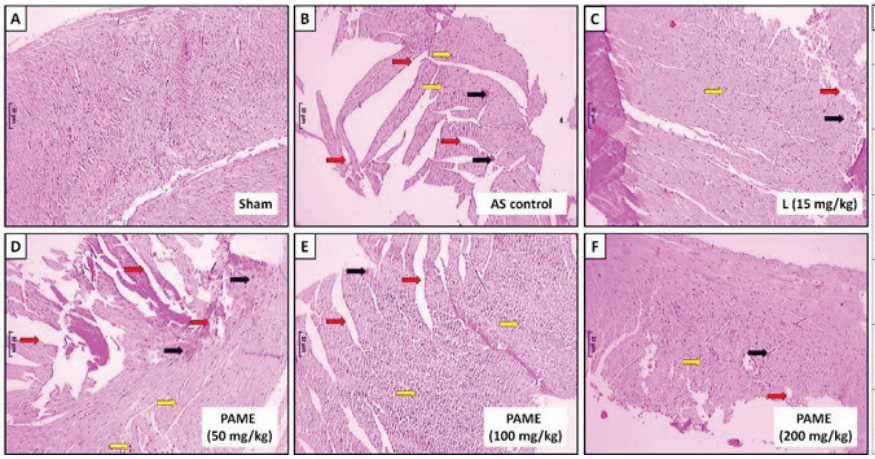


Fig. 3. A – Effect of PAME on pressure overload-induced alterations in cardiac ACE mRNA expression; and **B** – Effect of PAME on pressure overload-induced alterations in cardiac collagen-I mRNA expression.

Data are expressed as mean ± SEM (six rats per group) and analyzed by one-way variance analysis followed by Tukey's multiple range test. **p<0.01 and ***p<0.001 as compared to the AS control rats, ###p<0.001 as compared to the sham rats. L (15): lisinopril (15 mg/kg)-treated rats; PAME (50, 100, and 200 mg/kg); *Phyllanthus amarus* methanolic extract-treated rats. The numbers in parentheses on the x-axis represent the doses of the respective treatments in mg/kg. ACE: angiotensin-converting enzyme; AS: aortic stenosis; bp: base pair; kg: kilogram; L: lisinopril; mg: milligram; mRNA: messenger ribonucleic acid; PAME: *Phyllanthus amarus* methanolic extract; SEM: standard error means.



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Treatment	Myocardial degeneration	Interstitial inflammation	Interstitial hemorrhage
Sham	0 (1, 0)	1 (1, 0)	0 (1, 0)
AS Control	4 (4, 3)###	4 (4, 3)###	4 (4, 3)###
L (15 mg/kg)	0 (1, 0)***	1 (1, 0)***	1 (1, 0)***
PAME (50 mg/kg)	4 (4, 3)	3 (4, 3)	3 (4, 3)
PAME (100 mg/kg)	2 (3, 2) **	2 (2, 1)**	1 (2, 1)**
PAME (200 mg/kg)	1 (2, 1)***	1 (1, 0)***	2 (2, 1)***

Fig. 4. Effect of PAME on pressure overload-induced alterations in cardiac histopathology. Representative photomicrographs of heart sections from **A** – sham rats; **B** – AS control rats; **C** – lisinopril (15 mg/kg)-treated rats; **D** – PAME (50 mg/kg)-treated rats; **E** – PAME (100 mg/kg)-treated rats; **F** – PAME (200 mg/kg)-treated rats; and **G** – quantitative representation of the histological scores.

The sections were stained with hematoxylin-eosin, and images were captured at 40X. Data are expressed as the median (Q1, Q3) (three rats per group) and were analyzed using the non-parametric test. **p<0.01 and ***p<0.001 as compared to the AS control rats, ###p<0.001 as compared to the sham rats. Microscopic changes in cardiac histopathology include myocardial degeneration (red arrows), interstitial inflammation (yellow arrows), and interstitial hemorrhage (black arrows). The numbers in parentheses represent the doses of the respective treatments in mg/kg.

AS: aortic stenosis; kg: kilogram; L: lisinopril; mg: milligram; PAME: *Phyllanthus amarus* methanolic extract; Q1: first quadrant; Q3: third quadrant; SEM: standard error means.

DISCUSSION

This study investigated the potential of phyllanthin isolated from *P. amarus* to prevent pressure overload-induced cardiac hypertrophy using various *in vivo* and *ex vivo* parameters in experimental rats. This study employed a comprehensive set of methodologies to assess the impact of *P. amarus* on a spectrum of parameters, ranging from serum biochemistry and electrocardiographic function to molecular markers and histopathological alterations. Assessment of serum LDH, CK-MB, AST, ALT, and ALP levels provides insights into the systemic effects of constricted abdominal aorta and the potential mitigating role of *P. amarus* [12]. Concurrently, evaluating electrocardiographic and hemodynamic parameters allows for gauging the functional impact of *P. amarus* on pressure overload-induced cardiac alterations. This study investigated the levels of SOD, GSH, MDA, and nitric oxide in the cardiac tissue homogenates to unravel the molecular underpinnings [12]. The quantification of cardiac markers, such as ACE and collagen-I mRNA expressions, sheds light on the specific influence of *P. amarus* on the molecular pathways implicated in aortic stenosis-induced cardiotoxicity. Finally, histopathological evaluation of cardiac tissues elucidated the morphological changes induced by aortic stenosis and the efficacy conferred by *P. amarus*. By addressing these knowledge gaps, our study provides robust evidence that phyllanthin from *P. amarus* confers cardioprotective efficacy against pressure overload-induced cardiac hypertrophy, and can be considered as an alternative and complementary therapeutic strategy for managing ischemic heart diseases.

Current circulating biomarkers used to detect myocardial damage are classified as (a) biomarkers with elevated levels directly in the blood circulation due to systemic reactions after the myocardial toxicity events

viz. interleukins (IL-1β, IL-6), growth factors (Insulin-like Growth Factor-1, and vascular endothelial growth factor), (b) biomarkers originating from damaged myocardial tissues that are ultimately released into the blood circulation, such as LDH and CK-MB; and (c) biomarkers with abnormal serum levels before the occurrence of myocardial infarction event viz. ALP, AST, glucose, heparanase, copeptin [17,18]. Specific biomarkers directly involved in myocardial injury were investigated in the current study, including ALP, CK-MB, and LDH. According to previous research, patients with myocardial damage showed increased ALP, CK-MB, and LDH levels, suggesting their importance during IHD [19]. In the current study, stenotic rats showed elevated serum levels of ALP, CK-MB, and LDH; however, PAME treatment effectively attenuated these elevations, suggesting its cardioprotective potential.

Oxidative stress is critical in chronic inflammatory conditions, such as diabetes, cancer, cardiovascular diseases, neurodegenerative diseases, and infections [20,21]. The imbalance between pro-oxidants and antioxidants disrupts tissue homeostasis, causing the overproduction of harmful reactive oxygen and nitrogen species and leading to cell toxicity [22]. Numerous studies have documented the crucial role of oxidative stress in pressure overload-induced cardiac hypertrophy [1,13]. A redox imbalance was observed, as measured by increased levels of MDA and nitric oxide, along with a reduction in GSH and SOD activity [23,24]. GSH is an important intracellular antioxidant system pivotal in neutralizing lipid peroxides via glutathione peroxidase (GPx)-mediated inactivation, generating glutathione disulphide as a byproduct [24,25].

Moreover, GSH is crucial for conjugation with glutathione S-transferase (GST) to detoxify reactive species from lipid peroxidation and other xenobiotics [26,27]. Consequently, GSH depletion compromises cellular integrity, induces macromolecular damage, and fosters the accumulation of its oxidized form, further contributing to electrical and contractile dysfunction. A sudden influx of blood into the cardiac tissue precipitates cardiac GSH depletion, perpetuating the continual generation of oxygen-free radicals. Similarly, SOD plays a pivotal role in counteracting aortic stenosis-induced oxidative stress [12,28]. Superoxide radicals generated at the injury site may modulate SOD levels, potentially fostering superoxide anion accumulation and the consequent myocardial damage[29]. The current findings demonstrate that rats with aortic stenosis exhibit elevated MDA and nitric oxide activities and reduced SOD and GSH activities in their cardiac tissues. However, pretreatment with the *P. amarus* extract effectively restored these imbalances by regulating cardiac oxidative stress markers. Lignans have been shown to inhibit oxidative stress [30]. In addition, extensive research has highlighted the antioxidant potential of *P. amarus* in both *in vitro* and *in vivo* studies [8]. Other studies have highlighted the considerable antioxidant capacity of *P. amarus* extract against renal oxidative stress markers induced by streptozotocin in diabetic rats [9] and its ability to protect rat liver mitochondria from oxidative damage [31]. Moreover, the methanolic extract of *P. amarus* showed antioxidant properties against cyclophosphamide-induced toxicity in mice by augmenting cellular GSH and GST levels [32]. These results emphasize and confirm the promising antioxidant efficacy of *P. amarus*, suggesting its potential against stenosis-induced cardiac hypertrophy, which may be attributed to the presence of its major bioactive lignan, phyllanthin.

Mammalian homeostasis is maintained by the renin-angiotensin system, which mainly comprises renin, Ang II, angiotensin-1 (AT1) receptors, angiotensinogen, and ACE [33]. Clinical and experimental studies have established a link between angiotensin-converting enzyme inhibitors and blood pressure regulation [34]. Additionally, mounting evidence suggests that the binding of Ang II to AT1 receptors initiates ROS generation, which stimulates inflammation influx in cardiac tissue, and their synergistic action results in cardiac damage during ventricular hypertrophy [35]. Accordingly, researchers

have demonstrated that the administration of ACE inhibitors to hypertensive patients significantly decreases systemic vascular resistance, thus reducing the risk of cardiac failure and IHD [36]. Furthermore, reduced blood flow to the cardiac tissue causes a significant drop in hydrostatic pressure in the afferent arteriole, a major factor in the release of renin [37].

Moreover, long-term occlusion of the cardiac aorta causes increased expression of renin cells in the renal tissue, which are further released into the systemic circulation, where they interact with angiotensinogen. Renin-induced cleavage of angiotensinogen to AT1 and its further conversion to Ang II by ACE is responsible for increased blood pressure. In the present study, increased cardiac ACE expression caused a significant elevation in blood pressure in AS control rats. However, PAME treatment might counteract ACE activation, thereby protecting against cardiac hypertrophy.

Extensive research has suggested that *Phyllanthus niruri* is effective against pulmonary tuberculosis [38-40], vaginal candidiasis [41], urolithiasis [42], and shockwave lithotripsy for renal lithiasis [43] in various randomized controlled trials. This validation supports using *Phyllanthus* in treating hepatitis and other chronic ailments. Clinical studies have highlighted the efficacy of *P. amarus* in the management of acute viral hepatitis [44,45]. Thus, based on the findings of the present investigation, *P. amarus* should be considered further to determine its clinical efficacy in managing ischemic heart diseases.

Our investigation revealed that phyllanthin identified from *P. amarus* showed cardioprotective effects against pressure overload-induced cardiac hypertrophy, likely through mechanisms involving (a) ameliorating the alterations in electrocardiographic and hemodynamic parameters and serum biochemical markers (CK-MB, LDH, and ALP), (b) antioxidant effects by modulating the alteration in the cardiac oxide-nitrosative stress markers, (c) inhibiting ACE and collagen-I formation pathways to ameliorate hypertension and fibrosis, and (d) preserving the histological integrity of cardiac tissue against AS-induced damage. These findings suggest that P. amarus is a promising therapeutic agent for managing ischemic heart diseases.

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Data availability

The raw data underlying this article will be shared with the corresponding author upon reasonable request.

Ethical statements

The Qingdao Central Hospital approved the experimental protocol (Protocol Number: 559974002). All surgeries were performed under sodium thiopental anesthesia, and efforts were made to minimize suffering.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Author contributions

Each author has made significant contributions to the development of this manuscript. C.Z. conceived and designed the evaluation, performed parts of the statistical analysis, and drafted the manuscript; Z.L. performed data acquisition and drafted the manuscript. Y.G.: Performed parts of the statistical analysis and drafted the manuscript. All authors read and approved the final version of this manuscript.

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The role of 5-fluorouracil in Wnt/β-catenin signalling in human papillomavirus-positive cervical cancer cells.

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Keywords: 5-fluorouracil; high-risk HPV-positive cervical cancer cells; T lymphocytes; Wnt/β-catenin signalling pathway; apoptosis.

Abstract. Human papillomavirus (HPV) infection is a major risk factor for cervical cancer, especially persistent infection with high-risk HPV. 5-fluorouracil (5-FU) is a widely used antimetabolite chemotherapeutic agent that inhibits the proliferation of tumour cells by interfering with ribonucleic acid and deoxyribonucleic acid synthesis; however, its mechanism of action has not been fully elucidated. This study aimed to investigate the role of Wnt/β-catenin signalling in patients with high-risk HPV with cervical cancer treated with 5-FU. Patients with high-risk HPV-positive cervical cancer treated with surgery were taken as the research participants, and lesion tissues were collected during surgery. Human HPV-positive cervical cancer cells were isolated and cultured in vitro by the enzyme combined digestion method, and the obtained cells were divided into a control group, a paclitaxel group and a 5-FU group. A 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was used to measure the proliferation of high-risk HPV-positive cervical cancer cells under different treatment conditions. Western blotting was used to evaluate the protein expression level of the Wnt/β-catenin signalling pathway in cells, and flow cytometry was used to analyse the level of T lymphocytes in the patients' blood. The results of the MTT assay showed that the proliferation rate of cervical cancer cells in the control group was significantly higher than that in the paclitaxel group and the 5-FU group at all detection time points ($p<0.05$). The expression levels of Wnt/β-catenin protein in the 5-FU group were lower than those in the paclitaxel and the control groups ($p<0.05$). The results of the T lymphocyte level comparison showed that the ratios of $CD3^+$ T cells, $CD4^+$ T cells and $CD4^+/CD8^+$ cells affected by 5-FU were higher than those before treatment ($p<0.05$). 5-fluorouracil can significantly reduce the expression level of Wnt/β-catenin protein and increase the T lymphocyte levels in cervical cancer cells.

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Función del 5-fluorouracilo en la señalización Wnt/β-catenina en células de cáncer de cuello uterino positivas al virus del papiloma humano.

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Palabras clave: 5-fluorouracilo; células de cáncer de cuello uterino positivas al VPH de alto riesgo; linfocitos T; vía de señalización Wnt/β-catenina; apoptosis.

Resumen. La infección por el virus del papiloma humano (HPV) es un factor de riesgo importante para el cáncer de cuello uterino, especialmente la infección persistente con HPV de alto riesgo. El 5-fluorouracilo (5-FU) es un agente quimioterapéutico antimetabólico ampliamente utilizado que inhibe la proliferación de las células tumorales al interferir con la síntesis de ARN y ADN, pero su mecanismo de acción no ha sido completamente elucidado. Para investigar el papel de la señalización Wnt/β-catenina en los pacientes con cáncer de cuello uterino de alto riesgo HPV tratados con 5-FU. Los pacientes con cáncer de cuello uterino positivo para HPV de alto riesgo tratados con cirugía fueron tomados como objetos de investigación, y se recolectaron tejidos lesionales durante la cirugía. Se aislaron y cultivaron in vitro células de cáncer de cuello uterino positivas para HPV humano mediante el método de digestión combinada enzimática, y las células obtenidas se dividieron en un grupo de control, un grupo de paclitaxel y un grupo de 5-fluorouracilo. El MTT se utilizó para medir la proliferación de las células de cáncer de cuello uterino positivas para HPV de alto riesgo bajo diferentes condiciones de tratamiento. La técnica de western blot se utilizó para evaluar el nivel de expresión proteica de la vía de señalización Wnt/β-catenina en las células. La citometría de flujo se utilizó para analizar el nivel de linfocitos T en la sangre del paciente. Los resultados del ensayo MTT mostraron que la tasa de proliferación de las células de cáncer de cuello uterino en el grupo de control fue significativamente mayor que en el grupo de paclitaxel y el grupo de 5-fluorouracilo en todos los puntos de detección ($p<0,05$). Los niveles de expresión de la proteína Wnt/β-catenina en el grupo de 5-fluorouracilo fueron inferiores a los del grupo de paclitaxel y el grupo de control ($p<0,05$). Los resultados de la comparación del nivel de linfocitos T mostraron que las proporciones de células T CD3+, células T CD4+ y células CD4+/CD8+ afectadas por el 5-fluorouracilo fueron más altas que antes del tratamiento ($p<0,05$). El 5-fluorouracilo puede reducir significativamente el nivel de expresión de la proteína Wnt/β-catenina y aumentar la actividad del nivel de linfocitos T en las células de cáncer de cuello uterino.

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INTRODUCTION

Cervical cancer is a common type of malignant tumour in women worldwide and has become a major disease that affects and threatens the survival status of women in various countries. The incidence of cervical cancer in China has been rising in recent years, with approximately 500,000 new cases every year [1]. There are many factors in

the development of cervical cancer, among which human papillomavirus (HPV) infection is the main risk factor; in particular, persistent infections of high-risk HPV present a higher risk of cervical cancer [2]. Although the popularisation of the HPV vaccine and the implementation of cervical cancer screening programmes have reduced the incidence of cervical cancer to a certain extent, determining how to effectively treat HPV-positive patients or patients with cervical cancer that has progressed to an advanced stage is still a major problem for doctors [3].

As a widely used antimetabolite chemotherapy drug, 5-fluorouracil (5-FU) has been extensively used in the treatment of a variety of solid tumours. It inhibits swelling by interfering with ribonucleic acid and deoxyribonucleic acid synthesis during the proliferation of tumour cells; however, its mechanism of action has not been fully elucidated. Studies have shown [4] that 5-FU must be maintained within a certain concentration in cervical cancer, and the appropriate extension of the administration time can allow more cells to be exposed to the drugs, which helps to increase the sensitivity of the drug. In recent years, some scholars have pointed out that 5-FU may exert its anti-tumour effect by affecting signalling pathways in the tumour microenvironment, such as the Wnt/β-catenin signalling pathway. The Wnt/β-catenin signalling pathway is a key intracellular signalling network that plays a central role in regulating cell fate, maintaining tissue homeostasis and tumour development [5]. In many tumour types, aberrant activation of the Wnt/β-catenin signalling pathway is strongly associated with tumour aggressiveness, drug resistance and poor prognosis. However, research on the Wnt/β-catenin signalling pathway has focused more on colon cancer and oral cancer, and few studies are related to high-risk HPV-positive cervical cancer.

In addition, immune cells in the tumour microenvironment, especially T lymphocytes, have an important impact on tumour development and response to treatment. T lymphocytes play an immune surveillance role by recognising tumour antigens, and in some cases, they can interact with tumour cells and influence apoptosis [6]. However, the interaction between T lymphocytes and the Wnt/β-catenin signalling pathway in HPV-positive cervical cancer and its role in the treatment of 5-FU is unclear [7,8].

Given this, the mechanism of action of 5-FU in the treatment of high-risk HPV-positive cervical cancer was analysed, and the apoptosis effect of the Wnt/β-catenin signalling pathway on tumour cells and their interaction with T lymphocytes in the process of promoting apoptosis is discussed.

PATIENTS AND METHODS

Patients with high-risk HPV who underwent surgery in our hospital between June 2022 and December 2023 were selected for the experiment. A total of 78 patients with positive cervical cancer were enrolled in the study. All patients' surgeries were performed by the same team of doctors. The study was approved by the ethics committee of the hospital, and the patients and their families voluntarily signed informed consent forms; the whole experiment lasted for 2 weeks. The **inclusion criteria** were as follows: (1) patients who met the diagnostic criteria for cervical cancer and had a histopathologically confirmed diagnosis of high-risk HPV-positive cervical cancer [9]; (2) patients with no serious heart, lung, kidney or other vital organ dysfunction and who were able to tolerate surgery and follow-up treatment; (3) patients who had not received radiotherapy or chemotherapy within 3 months before and after enrolment; (4) patients who could undergo surgical treatment; (5) patients whose examination and treatment was completed under the guidance of doctors. The **exclusion criteria** were as follows: (1) patients with a history of severe allergies, especially those who were allergic to 5-FU or its adjuvant drugs; (2) pregnant or lactating

women; (3) patients with a history of other malignant tumours or who had other malignant tumours at the same time; (4) patients with a history of mental illness or cognitive dysfunction and could not cooperate with the completion of surgery and return visits; (5) patients with serious infection or immunodeficiency disease, which affected the observation of the condition and the evaluation of treatment effect; (6) patients with severe coagulation dysfunction or bleeding tendency; (7) patients who successfully completed the operation but did not cooperate with the return visits.

Main instruments and reagents

The key chemicals and laboratory equipment used in the experiments were as follows: 5-FU (Hubei Hengjingrui Chemical) and paclitaxel as chemotherapy drugs for specific biological effects; type I collagenase, which is used in the isolation process of tissues or cells; Dulbecco's modified Eagle's medium (DMEM) serves as a substrate for cell culture, providing essential nutrients [10,11]. Routine laboratory equipment – centrifuges for sample separation; fully automated enzyme label analyser for rapid and accurate determination of enzyme-linked immunosorbent assay (Nanjing Nuovezan Biotechnology, Nanjing, China); CO_2 cell culture incubator (Shanghai Jinghong Experimental Equipment Co., Ltd, Shanghai, China) to provide a suitable growth environment for cells; and a C-MAGhS10 magnetic stirrer for mixing operations during experiments.

Culture of lesion cells

The following is a description of the procedure that was adopted and the equipment used. In a sterile environment, take a small piece (volume 0.5–1 cm^3) of surgically excised tumour tissue and place it in a file containing 10% foetal bovine serum (FBS) (Guangzhou Ruite Biotechnology) with DMEM (Zhejiang Senrui Biotechnology). Rinse thoroughly three times with phosphate-buffered saline (PBS) (Zhejiang Senrui Biotechnology) to remove impurities, then briefly soak in a solution containing penicillin and streptomycin to sterilise, then rinse again with PBS. Use ophthalmic surgical scissors to cut the tissue into fine fragments (approximately 0.5 × 0.5 mm per piece). Subsequently, add 0.25% trypsin solution (Zhejiang Senrui Biotechnology) to the tissue and digest at 37°C in a 5% CO_2 incubator for 30 min, until the tissue is soft. After digestion, remove the upper layer of liquid, then add 3 ml of 0.2% collagenase type I (Zhejiang Senrui Biotechnology) and continue digestion under the same conditions for 30–60 min to ensure complete tissue breakdown. Neutralisation is performed using DMEM with 10% FBS and filtered through a 200 mesh sieve. The collected cells are further ground through a 10 ml syringe core tube to form a single-cell suspension. After 5 min of centrifugation (at a force of 1,105 g), remove the supernatant and then resuspend the cells in DMEM containing 15% FBS, adjusting the cell concentration to 1×10^6 cells per mL. Cells are seeded in 30 cm^2 flasks and cultured at 37°C and 5% CO_2. After 24 h, observe cell attachment using an inverted microscope (Thermo Fisher), change the medium after 48 h to remove unattached cells and change the medium every 72 h. When cell confluency coverage reaches 80%–90%, digestion is performed using 0.25% trypsin, and subculture is performed according to cell density. After the culture is complete, cell identification is performed to ensure that the obtained cells are high-risk HPV-positive cervical cancer cells [12,13]. Cells are cultured on microscope slides and morphological characteristics of the cells are observed using light microscopy. Cervical carcinoma cells usually present as epithelioid cells with irregular nuclei and abundant cytoplasm.

Handling of cells

Cervical cancer cells cultured to the third generation were randomly assigned to three experimental groups: a control group,

a paclitaxel group and a 5-FU group. Control group: cells were not treated with any drugs and were maintained under basal culture conditions. Paclitaxel group: cells were co-cultured with 0.7 μmol/L of paclitaxel during culture. 5-fluorouracil group: cells were co-cultured with 9.6 μmol/L of 5-FU. The number of cells in each group was consistent, and three replicate wells were set up in each well to ensure the reliability of the experimental results.

Cell proliferation rate

The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay was used to measure the proliferation of high-risk HPV-positive cervical cancer cells under different treatment conditions [5]. The experiment terminated the culture at different time points and completed the determination of the cell proliferation rate. At the end, 20 μg of MTT solution (5 mg/mL concentration) was added to each sample well and incubated at 37°C for 4 h, followed by removal of the medium. Approximately 150 μLof dimethyl sulfoxide was added and shaken at low speed for 10 min on a shaker to dissolve the previously formed formalised methazole blue crystals; finally, the absorbance of each well was measured at a wavelength of 492 nm.

Wnt/β-Catenin protein expression detection

Western blot was used to evaluate the protein expression level of the Wnt/β-catenin signalling pathway in cells [6]. The three groups of cells were added to a RIPA lysis buffer, and lysis was performed on ice to ensure complete rupture of the cell membrane and release of intracellular proteins. Cell debris was removed by centrifugation (12,000 rpm, 10 min) and the supernatant was collected to obtain protein samples. Extracted proteins were quantified using either the bicinchoninic acid assay method or the Bradford method, ensuring that the same amount of protein was loaded in each sample for comparison. Quantified protein samples were mixed with loading buffer and heated at 95°C for 5 min to denature. Samples were loaded into polyacrylamide gels and subjected to sodium dodecyl-sulfate polyacrylamide gel electrophoresis. Depending on the molecular weight of the protein, the appropriate concentration of gel (typically 10%–15%) was selected. Following the completion of electrophoresis, proteins from the gel were transferred onto polyvinylidene difluoride (PVDF) membranes. Following membrane transfer, the PVDF membranes were blocked with 5% non-fat dry milk and incubated for 1 h at room temperature to prevent nonspecific binding. The membranes were incubated with specific primary antibodies and incubated overnight at 4°C. The following day, the membranes were washed to remove unbound primary antibodies and washed three times for 5 min each using tris-buffered saline with 0.1% Tween® 20 detergent buffer. The membranes were incubated with horseradish peroxidase (HRP)-labelled secondary antibodies for 1 h at room temperature. The membranes were washed again to remove unbound secondary antibodies. The membranes were processed using a chemiluminescent substrate, and HRP reacted with the substrate to produce a detectable luminescent signal. Signals were captured using an imaging system and the images were recorded. Finally, the Wnt/β-catenin was developed in the gel imaging system β-actin bands and corresponding grey values, and the expression level of each group was calculated, with β-actin as the internal control.

T lymphocyte level

A 4 mL sample of fasting venous blood was drawn from patients with cervical cancer before and after surgery. The principle of aseptic operation during collection was strictly followed to avoid sample contamination. The collected blood sample was mixed with an appropriate amount of anticoagulant (heparin) to prevent blood coagulation and ensure the smooth progress of the subsequent separation process. The blood sam-

ple was centrifuged at a relative centrifugal force of 1,180 g for 15 min. During centrifugation, lymphocytes with lower density float in the upper layer of the density gradient medium, while other components with higher density, such as red blood cells and white blood cells, sink in the lower layer. At the end of centrifugation, the lymphocyte layer located in the upper layer was carefully collected to avoid disturbing other components of the lower layer. Lymphocytes were gently aspirated using a pipette and transferred to a new centrifuge tube. Collected lymphocytes were washed with sterile PBS buffer to remove residual density gradient media and other impurities. After washing, centrifugation was performed again, the supernatant was discarded and the precipitated lymphocytes were retained. The $CD3^+$, $CD4^+$, $CD8^+$ cells and the $CD4^+/CD8^+$ ratio in blood were analysed by flow cytometry (Beijing Boao Jingdian Biotechnology Co., Ltd.) [15,16].

Experimental data processing and analysis

The SPSS software package was used for statistical analysis. For the measurement data that met the normal distribution condition, the independent samples *t*-test and one-way analysis of variance method were used, and the results obtained were measured as the mean ± standard error. For categorical data, the chi-squared test (χ^2) was used for analysis. A *p*-value of <0.05 was used to indicate that the difference between the data is statistically significant.

RESULTS

Comparison of cell proliferation rates at different time points between the three groups of cells

The MTT assay showed that the proliferation rate of cervical cancer cells in the paclitaxel group and the 5-FU group was significantly lower than that in the control group ($p<0.05$) at all detection time points, indicating that the paclitaxel level was significantly lower than that of the control group ($p<0.05$). Both drug treatments with fluorouracil can effectively inhibit the proliferation ability of cervical cancer cells. In addition, the cell proliferation rate of the 5-FU group was lower than that of the paclitaxel group at all time points ($p<0.05$), indicating that 5-FU had a stronger inhibitory effect on cell proliferation (Fig. 1).

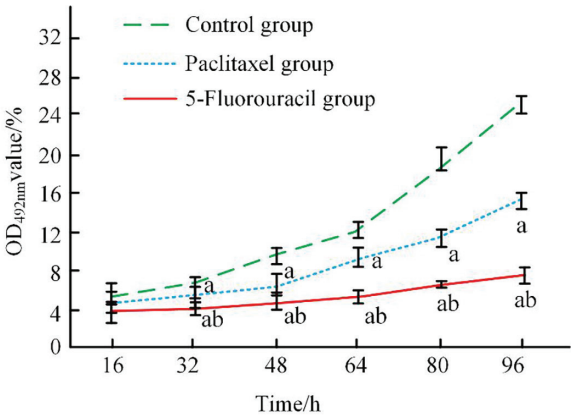

Fig. 1. Comparison of cell proliferation rates at different times. $p<0.01$ between different groups, one-way ANOVA was used for statistical analysis.

Comparison of Wnt/β-catenin in different organisations

The protein expression levels of Wnt/β-catenin in the 5-FU group and paclitaxel group were lower than those in the control group ($p<0.01$). The expression levels of Wnt/β-catenin protein in the 5-FU group were lower than those in the paclitaxel group ($p<0.01$) (Fig. 2).

Comparison of T lymphocyte levels before and after surgery in the observation group

The levels of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells and $CD4^+/CD8^+$ ratio were (68.94 ± 3.58), (43.28 ± 2.14), (24.17 ± 2.11) and (2.02 ± 1.00) in the patient after 5-FU treatment, respectively. The level of all cells was significantly higher than that before 5-FU treatment ($p<0.05$). This reflects the activation and enhancement of the intracellular immune response after treatment.

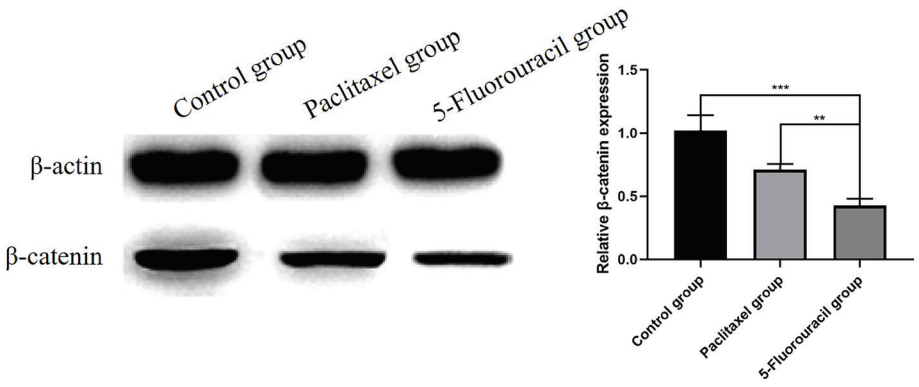

Fig. 2. Comparison of protein expression of cellsWnt/β-catenin in three groups. **: p<0.01, ***: p<0.001, one-way ANOVA was used for statistical analysis.

An increase in the $CD4^+/CD8^+$ ratio indicates an increase in helper T cells relative to cytotoxic T cells. This involves a more effective immune response. In contrast, the level of $CD8^+$ T cells (cytotoxic T cells) in patients after treatment was lower than before treatment ($p<0.05$). These results indicated that during the treatment, especially under the action of 5-FU, some cytotoxic T cells were activated and migrated into tumour tissues, participating in the direct killing of tumour cells. In addition, decreased levels of $CD8^+$ T cells may also be associated with cell activation-induced apoptosis, a natural phenomenon of T cells during sustained responses (Table 1 and Fig. 3).

DISCUSSION

Cervical cancer is the second most common malignancy in women worldwide after breast cancer, and the incidence is significantly higher in developing countries, accounting for approximately 15% of female cancers [17,18]. Although HPV vaccination and screening technology can effectively reduce and suppress the incidence of cervical cancer, HPV infection is still the main factor that induces cervical cancer in women. Most HPV infections are cleared by the body's autoimmune response; however, persistent high-risk HPV infection may activate the Wnt/β-catenin signalling pathway, which in turn promotes the development of cervical lesions [19]. Aberrant activation of the Wnt/β-catenin signalling pathway is closely related to the occurrence of a variety of tumours.

This study examined how the Wnt/β-catenin signalling pathway in 5-FU promotes apoptosis of high-risk HPV-positive cervical cancer cells, and the correlation of lymphocytes was discussed; furthermore, the role of T lymphocytes in this process was analysed,

Table 1
T lymphocyte levels in patients.

Cellular level/%	n	Before treatment	After treatment	*t*	*p*
$CD3^+$	78	58.24±4.58	68.94±3.58	17.060	<0.01
$CD4^+$		38.37±4.46	43.28±2.14	29.797	<0.01
$CD8^+$		21.49±2.12	24.17±2.11	8.090	<0.01
$CD4^+/CD8^+$		1.58±2.11	2.02±1.00	7.619	<0.01

Note: 5-FU treatment; t test was used for statistical analysis.

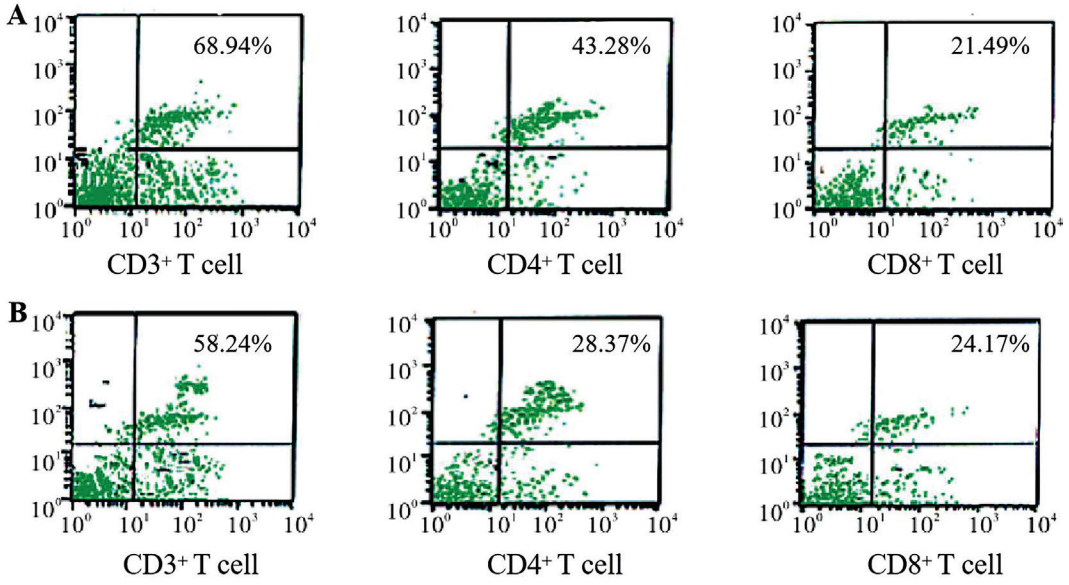

Fig. 3. Lymphocyte flow cytometry Before and after surgery. A. After surgery; B. Before surgery.

which provided a new perspective for the treatment of cervical cancer.

Broniarczyk *et al.* [20] showed that the expression level of Wnt/β-catenin protein was positively correlated with the expression level of high-risk HPV-positive cervical cancer tissues, and the high-risk HPV was positive. The content of Wnt/β-catenin protein in cervical cancer tissues was significantly higher than that in HPV-negative cervical cancer tissues [20]. The study of Li *et al.* [21] found that risperidone had a more obvious pro-apoptotic effect on bone cells, and the content of β-catenin protein was significantly reduced, which may be because risperidone has a certain inhibitory effect on β-catenin protein and slows down β-catenin. Proteins are transferred to the inner core of the cell, which upsets the balance between anti-apoptotic and pro-apoptotic protein cells [21]. The results showed that the expression level of Wnt/β-catenin protein in the paclitaxel group was higher than that in the control group after 5-FU treatment, while the expression level of Wnt/β-catenin protein in the paclitaxel group was significantly higher than that in the control group, and there was a significant difference. This indicates that the Wnt/β-catenin signalling pathway may be affected by different drugs, among which 5-FU has the most significant effect, and 5-FU can more effectively inhibit Wnt/β-catenin signalling pathways to enhance apoptosis-inducing effects on cervical cancer cells.

Shu *et al.* [22] found that after high-dose 5-FU treatment, the proliferation ability and migration ability of colorectal cancer stem cells were positively improved, while the apoptosis rate decreased significantly. These results suggest that 5-FU can induce activated stem cells by activating Wnt/β-catenin signalling cells and ultimately induce the occurrence and development of tumour diseases, which is very unfavourable to patients [22]. The study used flow cytometry to analyse the level of T lymphocytes in patients' blood and found that under the action of 5-FU, some cytotoxic T cells can be activated and migrate to tumour tissues, directly killing tumour cells, which is similar to the report of Shu et al.[22] However, it also directly shows that the decrease of T cell level has a certain correlation with cell

activation inducing apoptosis, and 5-FU can not only promote cell proliferation but also promote apoptosis. Zheng *et al*. [23] proposed that an *Artemisia annua* drug could affect and inhibit the proliferation and migration of melanoma cells by inhibiting the Wnt/β-catenin signalling pathway and ultimately induce apoptosis [23].

There are some limitations in this study; limited data were used, and the correlation between Wnt/β-catenin protein expression levels and T lymphocytes was not explored. Future studies need to expand the range of experimental data further to explore the related mechanisms of 5-FU and Wnt/β-catenin signalling in the occurrence and development of cervical cancer. Taken together, the findings support the hypothesis of a close link between the Wnt/β-catenin signalling pathway and T lymphocyte levels and reveal that 5-FU may enhance the immune response in patients with cervical cancer by modulating this signalling pathway; this offers new perspectives for the personalised treatment of high-risk HPV-positive cervical cancer.

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Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki.This study was conducted with approval from the Ethics Committee of The 305 Hospital of PLA. Written informed consent was obtained from all participants.

Competing interest

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Effect of early enteral nutrition on postoperative outcomes in pancreatic cancer patients with diabetes.

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Keywords: diabetes mellitus; early enteral nutrition; gastrointestinal function; pancreatic cancer.

Abstract. This study aimed to evaluate the impact of early enteral nutrition support in pancreatic cancer patients with diabetes mellitus following total pancreatectomy. Ninety-six patients were randomly divided into control and research groups, each with 48 patients. Both groups received parenteral nutrition, while the research group received additional enteral nutrition within the first 48 hours post-surgery. Results showed that the research group experienced faster recovery of bowel sounds, earlier first defecation, and shorter gastric tube retention times compared to the control group ($p<0.05$). Postoperative gastrointestinal function, immune function, and nutritional status were significantly better in the research group, with higher levels of gastrin, motilin, immunoglobulins G, A, and M, CD4/CD8 ratio, albumin, prealbumin, and transferrin ($p<0.05$). Furthermore, the research group had better blood glucose control from 48 hours to seven days post-surgery ($p<0.05$). The above results demonstrated a promoting impact of early nutrition support on postoperative physical functioning recovery of pancreatic cancer patients with diabetes mellitus. In conclusion, early enteral nutrition support in pancreatic cancer patients with diabetes mellitus significantly improved nutritional status, postoperative gastrointestinal recovery, gastrointestinal and immune function, and blood glucose control, leading to a better overall prognosis.

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Efecto de la nutrición enteral temprana en los resultados postoperatorios en pacientes con cáncer de páncreas y diabetes.

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Palabras clave: diabetes mellitus; nutrición enteral temprana; función gastrointestinal; cáncer de páncreas.

Resumen. Este estudio tuvo como objetivo evaluar el impacto del apoyo nutricional enteral temprano en pacientes con cáncer de páncreas y diabetes mellitus después de una pancreatectomía total. Noventa y seis pacientes fueron divididos aleatoriamente en grupos de control y de investigación, cada uno con 48 pacientes. Ambos grupos recibieron nutrición parenteral, mientras que el grupo de investigación recibió nutrición enteral adicional dentro de las primeras 48 horas posteriores a la cirugía. Los resultados mostraron que el grupo de investigación experimentó una recuperación más rápida de los ruidos intestinales, una primera defecación más temprana y tiempos de retención del tubo gástrico más cortos en comparación con el grupo de control ($p<0,05$). La función gastrointestinal posoperatoria, la función inmunológica y el estado nutricional fueron significativamente mejores en el grupo de investigación, con niveles más altos de gastrina, motilina, inmunoglobulinas G, A y M, relación CD4/CD8, albúmina, prealbúmina y transferrina ($p<0,05$). Además, el grupo de investigación tuvo un mejor control de la glucemia desde las 48 horas hasta los siete días posteriores a la cirugía ($p<0,05$). Los resultados anteriores demostraron un efecto promotor del apoyo nutricional temprano en la recuperación de la función física posoperatoria de pacientes con cáncer de páncreas y diabetes mellitus. En conclusión, el apoyo nutricional enteral temprano en pacientes con cáncer de páncreas y diabetes mellitus mejoró significativamente el estado nutricional, la recuperación gastrointestinal posoperatoria, la función gastrointestinal e inmunitaria y el control de la glucemia, lo que condujo a un mejor pronóstico general.

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INTRODUCTION

As a common malignancy, pancreatic cancer (PC) majorly occurs in the exocrine glands of patients' pancreas [1,2] we assessed serum trace element concentrations in patients with pancreatic cancer and compared the results to those of healthy controls and patients with chronic pancreatitis. We evaluated the association between trace element concentrations during cancer treatment and the risk of cancer progression and mortality in pancreatic cancer patients. Methods A retrospective cohort study was conducted at a tertiary center in Korea. Serum trace element concentrations of cobalt (Co. Generally, PC develop pretty rapidly, and patients' prognosis is unfavorable, with high morbidity and mortality in China. Moreover, PC has become a common disease that endangers

the health of the body and the quality of daily life [3,4] consequently raising the pancreatic cancer surgery rate. This study aimed to determine whether advanced age is a risk factor for morbidity and mortality following pancreaticoduodenectomy (PD. Diabetes mellitus (DM) is a metabolic disease resulting from insulin secretion or use defects [5]. Once it co-occurs with PC, it can enhance the severity and complexity of the disease and bring severe consequences to patients [6].

Currently, surgery is usually used to treat pancreatic cancer, which can effectively clear focus, prevent metastasis of focus, and prolong the survival period of patients. Total pancreatectomy refers to reconstruction and anastomosis of the digestive tract after removing the entire pancreas, duodenum, a significant part of the stomach, lower segment of common bile duct, gallbladder, large and small omentum and spleen [7]. PC patients suffer from malnutrition and poor immune function, and surgical trauma can put patients in a state of stress and immunosuppression; thus, their nutritional status and immune function will further deteriorate [8,9] due to the complexity of nutrition assessment, only 30-60% of patients with nutritional risks receive nutritional treatment at present. It is important to identify biomarkers that may be used to improve management of PDAC-associated malnutrition. Serum insulin-like growth factor binding protein 2 (IGFBP2. Enteral nutrition (EN) and parenteral nutrition (PN) are fundamental nutritional methods for postoperative PC patients [10]. Early EN has received wide recognition in clinical practice; early administration of nutrients in the gastrointestinal tract after surgery can stimulate intestinal peristalsis and related cytokine secretion, which helps protect intestinal mucosal barrier function [11]. Due to the loss of pancreatic endocrine and exocrine function in patients after total pancreatectomy, severe glucose metabolism disorders occur; coupled with surgical trauma stress, blood glucose control has become a crucial issue worthy of medical attention [12]. Clinical research demonstrates that for PC patients with DM, timely and reasonable nutrition support in the early postoperative period can ameliorate insulin tolerance, facilitate reasonable blood glucose control, and elevate postoperative recovery [13].

This research aimed to elucidate the clinical influence of early EN support in treating PC complicated with DM in terms of nutritional status, postoperative gastrointestinal recovery, gastrointestinal and immune function, and blood glucose control, which may provide a favorable basis for patients to recover better.

PATIENTS AND METHODS

General data

The 96 patients selected with PC who underwent total pancreatectomy in our hospital from January 2021 to May 2023 were randomly divided into a control group (CG) and a research group (RG), with 48 cases each. **Inclusion criteria:** 1) Primary PC confirmed by pathological examination; 2) age ranging 18-80 years old; 3) meeting surgical indications for total pancreatectomy and undergoing surgery under general anesthesia; 4) research subjects were informed and agreed to surgical, anesthesia, nursing, and blood glucose control plans, and signed informed consent. **Exclusion criteria:** 1) DM patients (type 1 and type 2); 2) those complicated with severe organic diseases such as heart, lung, liver, and kidney; 3) those complicated with primary malignancies of other organs and systems; 4) pregnant or lactating women; 5) those with severe postoperative biliary and abdominal inflammation. The research received approval from our hospital's ethics committee.

Methods

Both groups received conventional nursing. Postoperative conventional nursing included vital sign monitoring, assisted sputum drainage, oral nursing, skin nursing, drainage nursing, and parenteral nutrition

(PN). On the day after surgery, both groups received PN support via intravenous route, with a total calorie intake of approximately 110 kj/(kg • d). Administered enteral nutrition through the NJT (nasojejunal tube) from the second day after surgery, with an initial volume of 500 mL (1 Kcal/mL, protein 4.5 g/100 mL, carbohydrate 14.3 g/100 mL, lipid 2.8 g/100 mL). Both groups received conventional blood glucose monitoring and blood glucose control.

The RG received an enteral nutrition (EN) solution to prevent infection and correct electrolyte balance. Patients were given normal saline via a nasogastric tube two days after surgery. The nursing staff observed patients' reactions and continued to provide infusion if there was no abdominal discomfort. Three days after surgery, patients received Enteral Nutrition Suspension total protein-medium chain triglycerides (TP-MCT) via a nasogastric tube, with a dose gradually increasing from 250 mL. Nursing staff controlled the amount of nutrient solution used between 1000-1500 mL/d based on the patient's condition. The EN supply was reduced gradually after patients returned to a regular diet.

Observation indicators

1. Nutritional status: The serum albumin (ALB), prealbumin (PA), and transferrin (TF) levels between both groups before and seven days after surgery were compared.
2. Postoperative recovery: The bowel sound recovery time, anus exhaust time, defecation time, and gastric tube retention time between both groups after surgery received comparison.
3. Gastrointestinal function: A 5 mL venous blood sample was extracted from both groups at dawn before and seven days after surgery. The levels of gastrin (GAS) and motilin (MTL) were detected with radioimmunoassay.
4. Immune function: A 5 mL venous blood sample was extracted from both groups at dawn before and seven days after surgery. The serum immunoglobulin G (IgG), serum immunoglobulin A (IgA), and serum immunoglobulin M (IgM) levels were detected with immunoturbidimetry. Before and seven days after surgery, 5 mL of peripheral venous blood was extracted from both groups at dawn. The ratio of $CD4^+$ cells to $CD8^+$ cells (CD4/CD8) was detected with flow cytometry.
5. Blood glucose level: The fasting blood glucose (FBG) levels in both groups before surgery, 12 h, 24 h, 36 h, 48 h, 72 h, five days and seven days after surgery were compared.

Statistical analysis

The IBM® SPSS® 27.0 software was used for analyzing data. Quantitative data following a normal distribution received expression as mean ± standard deviation (mean ± SD), followed by t-tests for intergroup comparisons. Counting data received expression in percentages (%), followed by χ^2 test for intergroup comparisons, $p<0.05$ indicated a statistically significant difference.

RESULTS

General data shows no differences between the control group and the research group

RG: 24 males and 24 females; mean age of 56.30 ± 6.20 years old; body mass index (BMI): 23.10 ± 2.30 kg/m^2; tumor types: 27 cases of total pancreatic cancer, and 21 cases of pancreatic head cancer invading the pancreatic body. CG: 28 males and 20 females; mean age of 55.00 ± 6.70 years old; BMI: 23.30 ± 2.00 kg/m^2; tumor types: 30 cases of total pancreatic cancer and 18 cases of pancreatic head cancer invading the pancreatic body. Both groups exhibited no statistical significance in general data ($p>0.05$; Table 1).

Table 1
General data in both groups.

Groups	N	Gender [n (%)]		Age (years)	BMI (kg/m^2)	Tumor types [n (%)]	
		Male	Female			Total pancreatic cancer	Pancreatic head cancer invading pancreatic body
CG	48	24 (50.00)	24 (50.00)	56.30±6.20*	23.10±2.30*	27 (56.25)	21 (43.75)
RG	48	28 (58.33)	20 (41.67)	55.00±6.70*	23.30±2.00*	30 (62.50)	18 (37.50)
$\chi 2/t$		0.671		0.167	1.047	0.389	
p		0.413		0.868	0.298	0.533	

Abbreviations: CG = Control Group; RG = Research Group; BMI = Body Mass Index; * mean ± standard deviation. χ^2 test was used for categorical variables (gender, tumor types), while an independent-samples t -test was used for continuous variables (age, BMI).

Enteral nutrition ameliorates nutritional status in the research group

Before surgery, there were no statistically significant differences in ALB, PA, and TF levels between both groups ($p>0.05$); seven days after surgery, ALB, PA, and TF levels in both groups were elevated relative to those in the same group before surgery; and ALB, PA, and TF levels in the RG were elevated relative to those in CG during the same period, indicating statistical significance ($p<0.05$; Fig. 1).

Enteral nutrition accelerates postoperative gastrointestinal recovery in the research group

The bowel sound recovery time, anus exhaust time, defecation time, and gastric tube retention time in RG exhibited depletion relative to those in CG, indicating statistical significance ($p<0.05$; Fig. 2).

Enteral nutrition enhances gastrointestinal function in the research group

Before surgery, there was no statistical significance in GAS and MTL levels between both groups ($p>0.05$); seven days after surgery, GAS and MTL levels in both groups exhibited elevation relative to those in the same group before surgery, and GAS and MTL levels in RG exhibited elevation relative to those in CG during the same period, indicating statistical significance ($p<0.05$; Fig. 3).

Enteral nutrition enhances immune function in the research group

Before surgery, there was no statistical significance in IgG, IgA, IgM, and CD4/CD8 levels between both groups ($p > 0.05$); seven days after surgery, IgG, IgA, IgM, and CD4/CD8 levels in both groups exhibited elevation relative to those in the same group before surgery, and IgG, IgA, IgM, and CD4/CD8 levels in RG exhibited elevation relative to those in CG during the same period, indicating statistical significance ($p<0.05$; Fig. 4).

Enteral nutrition attenuates fasting blood glucose levels in the research group

Before surgery and 12-24 h after surgery, there were no statistically significant differences in FBG levels exhibited between SG and CG during the same period ($p< 0.05$); 48 h to seven d after surgery, FBG level in RG exhibited depletion relative to that in CG during the same period, indicating statistical significance ($p<0.05$; Fig. 5).

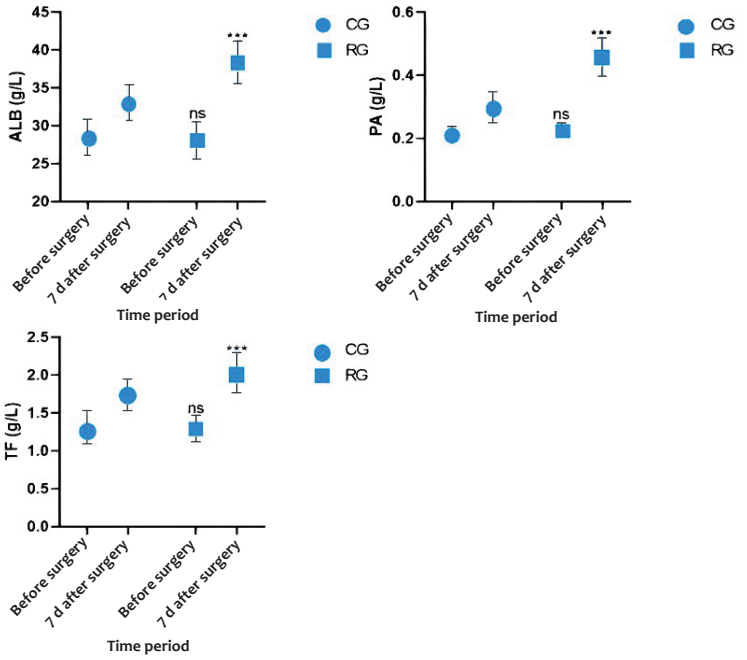

Fig. 1. Nutritional indicators in both groups.
RG versus CG, ns = no significance, ***p<0.05. CG = Control Group; RG = Research Group.
Values are mean ± SD. Statistical analyses were conducted using paired t-tests for within-group comparisons and independent-sample t-tests for between-group comparisons. **Abbreviations:** ALB: Serum Albumin, PA: Prealbumin, TF: Transferrin.

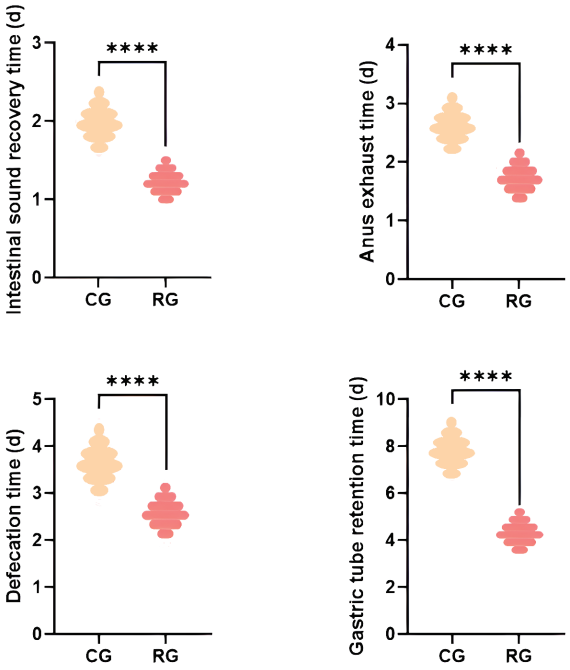

Fig. 2 Postoperative recovery indicators between both groups.
RG versus CG, RG = Research Group; CG = Control Group. ****p<0.05.
Values are mean ± SD. All statistical analyses were performed using Mann-Whitney U tests because of the non-normal distribution of the data.

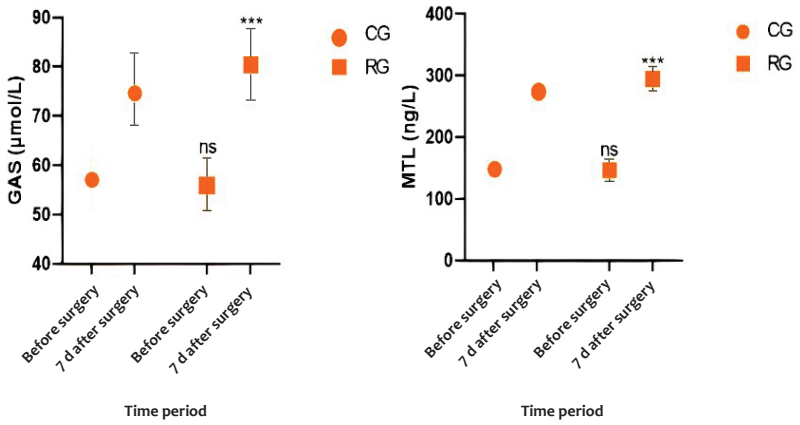

Fig. 3. Gastrointestinal function indicators in both groups.
RG versus CG, RG = Research Group; CG = Control Group. ns = no significance, ***p<0.05.
Values are expressed as mean ± SD. Statistical analyses were conducted using paired t -tests for within-group comparisons and independent-sample t-tests for between-group comparisons. **Abbreviations:** GAS: Gastrin, MTL: Motilin.

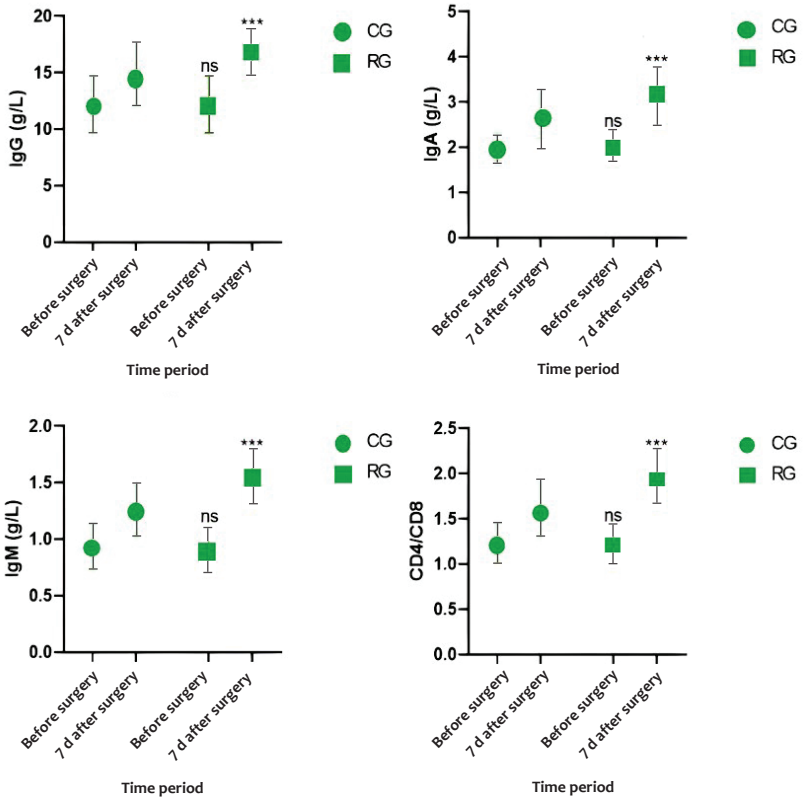

Fig. 4. Immune function indicators in both groups.
RG versus CG, RG = Research Group; CG = Control Group. ns = no significance, ***p<0.05.
All values are shown as mean ± SD. Statistical analyses were conducted using paired t-tests for within-group comparisons and independent-sample t-tests for between-group comparisons.

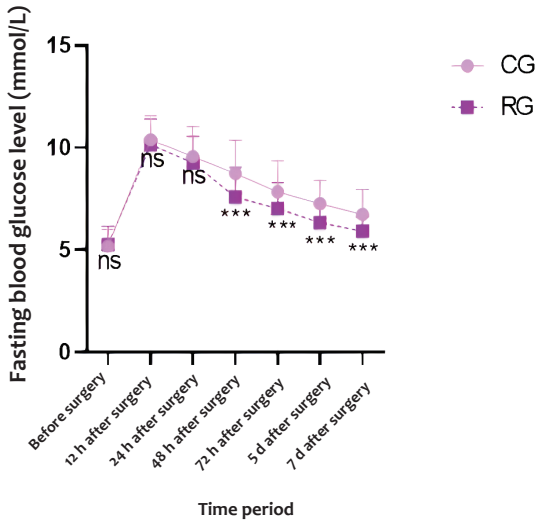

Fig. 5. Changes in blood glucose level in both groups.

RG versus CG, ns = no significance, ***p<0.05.

All values are presented as mean ± SD. Repeated-measures ANOVA and Bonferroni post hoc tests were used for statistical analyses.

DISCUSSION

PC is a common digestive system malignancy, and total pancreatectomy is the most effective treatment for early PC [14]. Nevertheless, due to the complexity of radical resection of PC, which involves resection of multiple organs and reconstruction of the digestive tract, it will cause more serious injuries to patients, and the risk of postoperative complications for patients is high, which may affect life safety in severe cases [15]. Thus, it is particularly crucial to implement effective postoperative nutritional treatment for PC patients undergoing total pancreatectomy.

All life activities and physical functions of living organisms are inseparable from the support of amino acids. The crucial physiological active substances in the human body, including enzymes, hormones, antibodies, and others, are proteins. Lack of protein can lead to malnutrition. Serum protein levels are the most commonly applied indicators reflecting the nutritional status of patients, including ALB, PA, and TF, among others [16]. Herein, after surgery, ALB, PA, and TF levels in both groups exhibited elevation relative to those in the same group before surgery, and ALB, PA, and TF levels in RG were elevated relative to those in CG during the same period, indicating that early EN support can facilitate visceral protein synthesis and elevate the overall nutritional status of patients. In the study by Mękal *et al.* [17], which confirmed the results of the present study, it was shown that Early Enteral can improve the nutritional status of patients after surgery. PC patients are often accompanied by severe gastrointestinal dysfunction, which can easily lead to malnutrition [18]. Nutritional intervention is one of the critical factors affecting the prognosis of surgical treatment. EN has gradually become a preferred method of clinical nutrition due to its advantages, such as economy, ease of maintenance, and compatibility with patients' physical characteristics. Early EN support can overcome PN deficiency, meet patients' early postoperative nutritional needs, and have advantages such as protecting the intestinal mucosal barrier, facilitating recovery of intestinal peristalsis function, and enhancing gastrointestinal hormone secretion [19]. In the study by ME Hamaker *et al.* [20], similar to this study, it was shown that EN can improve the patient's bowel function and nutritional status. Clinical reports have depicted that patients who receive early EN after surgery have a lower incidence of long-term related intestinal complications, indicating that early EN after surgery is more in line with patients' nutritional and gastrointestinal needs [21]. Herein, bowel sound recovery time, anus exhaust time, defecation time, and gastric tube retention time in RG exhibited depletion relative to those in the CG; after surgery, GAS and MTL levels in both groups exhibited elevation relative to those in the same group before surgery, and GAS and MTL levels in RG exhibited elevation relative to those in

CG during the same period. This indicates that early EN support can accelerate postoperative recovery and ameliorate patients' gastrointestinal function. The study by Yuan and Xiu 22 also showed that EN can reduce intestinal complications and problems in patients. The reasons are that early EN support can facilitate recovery of intestinal motility and absorption function in patients, accelerate organ blood circulation, improve mucosal blood flow, and prevent occurrence of mucosal acidosis and osmotic disorders; enteral nutrients can protect integrity of patients' intestinal mucosa, avoid dysbiosis of gastrointestinal microbiota, and facilitate regeneration of intestinal mucosal cells, enhance secretion of gastrointestinal hormones, thereby elevating patients' gastrointestinal function and enabling rapid recovery of gastrointestinal activity [23]. The study by Chakaroun *et al.* 24 also showed that EN favors the gastrointestinal microbiota and facilitates the regeneration of intestinal mucosal cells.

Due to the influence of PC itself and the trauma of pancreatectomy, the postoperative immune function of patients will be reduced to varying degrees, and postoperative malnutrition will also aggravate the degree of their immune dysfunction [25]. Herein, after surgery, IgG, IgA, IgM, and CD4/CD8 levels in both groups exhibited elevation relative to those in the same group before surgery, and IgG, IgA, IgM, and CD4/CD8 levels in RG exhibited elevation relative to those in CG during the same period. This indicates that early EN can enhance the immune function of patients, may be because early EN support can facilitate the absorption of nutrients in the body, enhance patient's physical fitness, and elevate their postoperative immunity; enteral nutrients can protect damaged gastrointestinal tissue, maintain the function of gastrointestinal microbiota, reduce the impact of gastrointestinal microbiota on damaged tissue, and block occurrence of inflammatory responses, thereby effectively elevating patients' immune function [26]. Negative nitrogen balance during the perioperative period, elevated insulin resistance due to surgical trauma, depleted glucose absorption in peripheral tissue, and elevated endogenous glucose production, coupled with stress hyperglycemia due to nutrition, fasting, hunger, pain, and long-term bed rest, can lead to complications such as wound infection and delayed wound healing, affecting patient prognosis [27]. Herein, 48 h to 7 d after surgery, FBG level in RG exhibited depletion relative to that in CG during the same period, indicating that EN intervention strategies effectively elevated blood glucose control efficacy 48 h after surgery. The study by Liu *et al.* (2025) [28] also showed that Early Enteral can improve immune system strengthening and blood sugar control. Thus, based on PN, combined with EN, improving short-term prognosis is vital.

Early postoperative EN support for PC patients complicated with DM can elevate the nutritional status of patients after surgery, speed up the recovery of patients, improve their gastrointestinal function and immune function, and facilitate more reasonable blood glucose control, which is conducive to a better prognosis of patients. The clinical application effect is significant.

Conflict of interest

The authors declare no conflict of interest.

Founding

None

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Authors' Participation

All authors participated in this study; XW, XLW: Contributed to the conception of the work, data collection, conducting the study, and data analysis. YZ, ZW: Contributed to the conception of the work, conducting the study, revising the draft, and approving the final version of the manuscript. YJ, LL: manuscript writing, translation and editing. Final approval of the manuscript.

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Hiperplasia endometrial: una revisión narrativa sobre su patogénesis, factores de riesgo y diagnóstico.

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Palabras clave: hiperplasia endometrial; patogenia; factores de riesgo; diagnóstico, biopsia endometrial, ultrasonido.

Resumen. La hiperplasia endometrial con o sin atipia, es un diagnóstico ginecológico común y un precursor del carcinoma endometrial. Durante los años reproductivos, el riesgo de presentar una hiperplasia endometrial está relacionado con los trastornos ovulatorios. En la menopausia y posmenopausia, la hiperplasia endometrial está asociada con factores como la obesidad, la terapia de reemplazo de estrógeno y otros. El objetivo de este estudio narrativo fue revisar y analizar la patogenia, los factores de riesgo y los diferentes métodos de diagnóstico de la hiperplasia endometrial. Se revisaron los años comprendidos entre 1970 y noviembre de 2024 en la bibliografía latinoamericana e internacional, usando los sitios electrónicos como Pub-Med, Google Scholar, Springer, The Cochrane Library, Embase, Scielo, Imbiomed-L, Redalyc y Latindex, entre otros.

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Endometrial hyperplasia: a narrative review on its pathogenesis, risk factors and diagnosis.

Invest Clin 2025; 66 (1): 101 – 115

Keywords: endometrial hiperplasia; pathogenesis; risk factors; endometrial biopsy; ultrasound.

Abstract. Endometrial hyperplasia with or without atypia is a common gynecological diagnosis and serve as precursor to endometrial carcinoma. During the reproductive years, the risk of developing endometrial hyperplasia is related to ovulatory disorders. In menopause and postmenopause, endometrial hyperplasia is linked to factors such as obesity, estrogen replacement therapy, and others. This narrative study aims to review and analyze the pathogenesis, risk factors, and different diagnostic methods related to endometrial hyperplasia. The literature from 1970 and November 2024 was reviewed in the Latin American and international bibliography using electronic databases such as Pub-Med, Google Scholar, Springer, the Cochrane Library, Embase, Scielo, Imbiomed-L, Redalyc, and Latindex, among others.

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INTRODUCCIÓN

El sangramiento por causa de pre malignidad o malignidad representa el 10% del total de los sangramientos uterinos anormales (SUA). La patología premaligna del endometrio es la hiperplasia endometrial (HE), la cual es una condición patológica caracterizada por cambios hiperplásicos en las estructuras glandulares y estromales endometriales [1]. Montgomery y col. [2] la definen como una proliferación de las glándulas de tamaño y forma irregulares con un aumento de la relación glándulas/estroma. Como se mencionó, la HE es una enfermedad precancerosa, no fisiológica y no invasiva que resulta de la proliferación del endometrio, ocasionando un aumento del volumen de tejido endometrial con alteraciones de la arquitectura glandular (forma y tamaño) y de la glándula endometrial al estroma, en una relación superior a 1:1 [3,4].

La mayoría de los casos de las HE son el resultado de niveles elevados de estrógenos (Es), combinados con niveles insuficientes de progesterona (P) [1,5]. La estimulación estrogénica, sin oposición en el endometrio, provoca cambios epiteliales glandulares proliferativos, incluida la remodelación glandular, lo que da lugar a glándulas de forma variable y de distribución irregular [1]. Armstrong y col. [6] reportaron la presencia de HE en el 10% de mujeres premenopáusicas y en el 6% de las mujeres postmenopáusicas con SUA. Se estima que la incidencia de la HE es al menos tres veces mayor que la del cáncer de endometrio (CE). Las estimaciones actuales indican que la incidencia de HE se encuentra alrededor de los 133 a 208 casos por 100.000 mujeres-año en los países occidentales[7,8]. Las tasas de incidencia de los subtipos de HE son 121 casos por 100.000 mujeres x años para la HE no atípica y 16,8 casos por 100.000 años-mujer para la HE atípica [7]. El cáncer de endometrio es la neoplasia maligna ginecológica más común en el mundo occidental, en especial en países desarrollados [9]. Según Globocan [10] y el Fondo Mundial para la Investigación del Cáncer (World Cancer Re-

search Fund International) [11] para el año 2022 se reportaron 420.368 nuevos casos de CE en todo el mundo, lo cual represento el 4,34%, ocupando el puesto 6 de todos los canceres femeninos; asimismo, provocó la muerte de 97.723 mujeres; es decir, un 2,3% de mujeres a nivel global. Igualmente, según Globocan [10], en el 2022 se diagnosticaron 233.582 casos de CE en el hemisferio occidental; es decir, el 55,6% y provocó la muerte de 53.103 (22,7%). De acuerdo con la Sociedad Americana del Cáncer (American Cancer Society) [12] para el año 2024 se diagnosticaron 67.880 casos de CE y se produjeron 13.250 muertes en los Estados Unidos de Norteamérica.

Históricamente, el CE rara vez aparece en mujeres premenopáusicas; sin embargo, con el aumento de la obesidad y la creciente prevalencia del síndrome metabólico, los cuadros de HE y de CE han aumentado notablemente en frecuencia [9]. La prevalencia del CE aumenta con la edad, cerca de una cuarta parte (21,3%) de nuevos diagnósticos ocurren en pacientes menores de 55 años [13,14]. La exposición prolongada a Es sin oposición es el principal factor de riesgo [15]. La hiperplasia endometrial sin atipia tiene el riesgo de progresar entre el 1 al 3% mientras que la HE con atipias o neoplasia intraepitelial endometrial (NIE) tiene el riesgo de progresar entre un 14 a 45% [4]; la incidencia de HE con atipias es < 1% [6,16].

Los factores de riesgo para desarrollar la HE y/o el CE que influyen en los niveles de Es y/o P pueden ser: a) patologías tales como obesidad, diabetes y otras anomalías metabólicas; b) iatrogénicos, tales como la terapia de reemplazo hormonal (THR), uso de medicamentos para la fertilidad; c) relacionados con eventos menstruales y reproductivos como la menopausia tardía; d) hábitos de vida como el tabaquismo. Los factores de riesgo para desarrollar la HE y/o el CE son similares.

Sin embargo, se ha sugerido que los factores de riesgo para estas dos condiciones difieren con respecto a la historia reproductiva, ya que la paridad parece ser un factor protector para el CE, pero no para la HE [5].

El objetivo de esta revisión de la HE es analizar la patogenia, los factores de riesgo, y los métodos para realizar el diagnóstico de la HE en forma temprana con el propósito de prevenir la evolución y el avance de esta patología a CE.

MATERIAL Y MÉTODO

En la presente revisión narrativa se investigador y analizaron los estudios más recientes y relevantes en relación con la patogenia, los factores de riesgo y el diagnóstico de la HE. Se buscaron, revisaron y analizaron publicaciones en los idiomas español e inglés. Siguiendo las guías de PRISMA, se realizó una búsqueda sistemática por vía electrónica de publicaciones sobre el tema en PubMed, Medline, ISI, DOAJ, Springer, Embase, Web of Knowledge, DOAJ, Google Scholar y the Cochrane Library para artículos originales escritos en el idioma inglés y en Scielo, Lantidex, Imbiomed-L, Redalyc y Google Scholar, para artículos originales escritos en el idioma español. La búsqueda incluyó palabras claves, tales como sangramiento uterino anormal, hiperplasia endometrial, factores de riesgo, y diagnóstico seguido de términos como: hiperplasia endometrial y patogenia o factores de riesgo o diagnóstico. Se incluyeron los artículos publicados en revistas médicas indexadas y fueron excluidas aquellas publicaciones que no pudieron ser abiertas electrónicamente; asimismo, fueron excluidos de la revisión: cartas al editor, reportes de casos, estudios sin control y resúmenes de congresos. Se revisaron los artículos publicados desde el año 1970 hasta noviembre de 2024. La búsqueda electrónica, la escogencia, el evalúo y el análisis de las publicaciones fue realizado por el autor.

Patogénesis

Durante la fase proliferativa del ciclo menstrual normal se produce aumento de la expresión del oncogén bcl-2, oncogén situa-

do en el cromosoma 18; el cual fue identificado por primera vez en el linfoma folicular[17,18]. La expresión de bcl-2 inhibe parcialmente la apoptosis celular lo que permite a una célula prolongar su supervivencia [19]. La expresión de bcl-2 parece ser parcialmente regulado a través del control hormonal y su expresión disminuye notablemente al inicio de la fase secretora del ciclo menstrual [20,21]. La disminución de la expresión de bcl-2 se correlaciona con la aparición de células apoptósicas dentro del endometrio, observada durante la fase secretora del ciclo menstrual [22]. Se ha demostrado que la expresión de bcl-2 aumenta en la HE [19,23]; sin embargo, este aumento en la expresión de bcl-2 parece limitarse a la HE compleja. Sorprendentemente, la expresión disminuye en la HE atípica y el CE al compararla con el endometrio proliferativo [23]. Recientemente también se ha investigado el papel del gen Fas/FasL, en el desarrollo de la HE [22]. El Fas es un miembro de la familia del factor de necrosis tumoral/factor de crecimiento nervioso que se une a FasL (ligando de Fas) e inicia la apoptosis. La expresión del Fas y del FasL aumenta en muestras de endometrio después del tratamiento progestacional[24]. Maruo y col. [25] señalan que una interacción entre la expresión de Fas y bcl-2 podría contribuir al desarrollo de HE; es decir, una disminución de la expresión del Fas y aumento de la expresión del bcl-2. Estos estudios han comenzado a proporcionar alguna idea sobre los cambios moleculares que conducen al desarrollo clínico de la HE y el CE [2].

La hiperplasia endometrial se clasifica en simple y compleja, basada en la complejidad y el hacinamiento del marco glandular. La hiperplasia endometrial simple, anteriormente denominada hiperplasia quística o leve, es una lesión proliferativa de mínima complejidad y amontonamiento glandular con abundante estroma entre glándulas. La hiperplasia endometrial compleja, anteriormente denominada HE moderada, es una lesión proliferativa con severa complejidad y amontonamiento glandular; en la HE compleja, las glándulas pueden variar en tamaño, y se observa un estroma mínimo entre las glándulas [2]. Igualmente, la HE también se clasifica según la presencia de atipia citológica (AC). La atipia citológica se caracteriza por ser células epiteliales agrandadas, hipercromáticas con nucléolos prominentes y aumento de la relación núcleo-citoplasmática. La atipia citológica es considerada el factor pronóstico más importante para la progresión al CE. La hiperplasia endometrial es un continuo proceso histológico evolutivo, comenzando desde HE simple sin atipia a HE compleja con atipia, seguido del CE [26]. Basado en esto, se ha establecido y recomendado una clasificación de la HE muy sencilla, tomando en cuenta la AC: HE simple y compleja sin atipia e HE simple y compleja con atipia (esta última conocida anteriormente como HE severa o adenomatosa) [2,6,27]. Menos del 2% de las HE sin atipia progresan a CE y la duración media de la progresión a CE es de casi 10 años; la HE con atipia progresa a CE en el 23% de los casos, con una duración media de 4 años [28]. La hiperplasia endometrial simple y compleja sin atipia usualmente regresan espontáneamente en el 69% a 80% de los casos; el 19% de las HE simples sin atipias y el 17% de las pacientes con HE compleja sin atipia permanecen sin cambios. La hiperplasia endometrial simple y compleja con atipias regresan en el 69% y el 57%, y progresan en el 14% y el 23%, respectivamente. Como se mencionó anteriormente, menos del 2% de la HE simple sin atipias progresan primero a HE con atipia antes de desarrollar el CE [2,28,29] y el 3% de las pacientes con HE compleja sin atipia progresan a CE; el 8% de las pacientes con HE atípica simple y el 29% de pacientes con HE atípica compleja progresan a CE [2,28,29-31].

En 2014, la Organización Mundial de la Salud [1,32] respalda una nueva clasificación de la HE: 1.- HE no atípica que reemplaza la HE benigna sin atipias y 2.- la HE atípica, también denominada neoplasia intraepitelial endometrial/endometroide (NIE), la cual ha sido reconocida como la previamente deno-

minada HE con atipias y es considerada como la lesión precursora del CE. La NIE no debe confundirse con el carcinoma intraepitelial endometrial (CIE), que es una lesión precursora del carcinoma uterino seroso papilar, el cual es más agresivo y representa entre el 5 al 10% de los CE y el 40% de las muertes producidas por los CE [4,32]. Según Ring y col. [4], esta clasificación binaria permite un pronóstico más sólido; no obstante, algunos patólogos y oncólogos ginecológicos están más familiarizado con el esquema de 1994 (HE simple y compleja, con y sin atipias) y continúan haciendo referencia a él.

Factores de riesgo

Los factores de riesgo generalmente se asocian con un aumento de Es circulante en relación con la P[6]. Entre los factores de riesgo para el desarrollo de una HE y/o un CE se pueden mencionar: edad mayor de 45 años, nivel educacional elevado, oligomenorrea/amenorrea, menarquia temprana, menopausia tardía, nuliparidad, índice de masa corporal elevado u obesidad, diabetes mellitus, hipertensión arterial, síndrome de ovarios poliquísticos, terapia hormonal de reemplazo estrogénica sin oposición progestacional, terapia con tamoxifeno, antecedentes familiares, Síndrome de Cowden y de Lynch [5,33]. Esto es importante a tener en cuenta porque entre el 10% y el 20% de los CE ocurren antes de la menopausia, principalmente en mujeres de edad entre los 40 a 50 años. En 2011, Opolskiene y col. [34] reportaron una incidencia de HE sin atipia del 4%, con atipia del 2% y CE del 24% en mujeres mayores de 45 años.

Obesidad. La obesidad se asocia con niveles elevados circulante de Es en sangre en relación con la P por varios mecanismos, incluidos el aumento de la conversión de androstenediona en estrona en el tejido adiposo y la disminución de la globulina transportadora de hormona sexuales, lo que ayuda a que persista la anovulación crónica [35,36]; la magnitud de la obesidad parece ser proporcional al riesgo tanto de la HE como del CE[6]. Epplein y col. [37] demostraron que las mujeres obesas (IMC >30 kg/m^2) presentan un riesgo 4 veces mayor de presentar HE con atipias y aquellas con un IMC ≥ 40 kg/m^2 tienen 13 veces de riesgo mayor de desarrollar una HE con atipias y 23 veces mayor de presentar un HE sin atipias [26,37].

Anovulación crónica y síndrome de ovarios poliquísticos: Las mujeres anovulatorias pueden tener un riesgo hasta 3 veces mayor de sufrir CE[38]. Aunque la edad promedio en el momento del diagnóstico de la HE con atipia es a partir de la 5ª década de la vida, las mujeres con amenorrea crónica están en riesgo entre los 20 y 30 años[39]. La afección más común asociada con la anovulación crónica es el síndrome de ovarios poliquísticos (SOP), aunque la anovulación también puede ocurrir en el período perimenopáusicas o periodo de transición. El síndrome afecta entre el 8% y el 13% de las mujeres en edad reproductiva y, según la OMS [40], hasta el 70% de las mujeres con esta patología no son diagnosticados y es la causa de anovulación e infertilidad más común en esa población [41]; entre las comorbilidades comúnmente asociadas con el SOP están la obesidad, infertilidad, nuliparidad y diabetes, factores estos de riesgo para desarrollar HE [6].

Nuliparidad e infertilidad. La nuliparidad y la infertilidad parecen ser factores de riesgo independientes para la HE y el CE, con odds ratios (OD) de 2,8 y un intervalo de confianza (IC) del 95%, 1,1–7,2 para la nuliparidad y de un OD 3,6 con IC del 95%, 1,3 –9,9 para la infertilidad[15]. Esta asociación tiende a estar incrementada cuando se ajustan o se comparan con el estado marital, sugiriendo que la infertilidad más que la nuliparidad es el factor de riesgo para desarrollar HE y/o CE [5,42]. Además, ambas afecciones están asociadas con otros factores de riesgo de HE, incluidos la anovulación crónica, la obesidad y el SOP. El riesgo de presentar una HE compleja con atipia es inversamente proporcional con el número de partos [34].

Moduladores selectivo de receptores estrogénicos. Algunos moduladores selectivos de los receptores de estrógenos (SERM) aumentan el riesgo de HE. Los SERM tienen actividad mixta, agonista o antagonista del receptor de estrógeno (RE), dependiendo del tejido diana. El tamoxifeno es un SERM que actúa como antagonista del RE en el tejido mamario; por lo tanto, se usa comúnmente para prevenir y tratar el cáncer de mama [6]. Sin embargo, el tamoxifeno actúa como un agonista de los RE en el útero por lo que su uso está asociado con un mayor riesgo para desarrollar una HE y el riesgo de desarrollar un CE es 2,5 veces mayor [43,44]. Otro SERM, el raloxifeno, actúa como antagonista del RE tanto en mama como en tejido endometrial; por lo tanto, no aumenta el riesgo de CE [45]. Runowicz y col. [44], en un estudio comparativo en mujeres posmenopáusicas, compararon el raloxifeno con el tamoxifeno y demostraron una incidencia significativamente menor de HE en que usaron raloxifeno (OD: 0,19; IC 95%, 0,12–0,29) y CE (OD: 0,55; IC 95%, 0,36–0,83).

Cáncer colon rectal no polipoideo hereditario o síndrome de Lynch. Esta condición genética autosómica dominante está asociada con un mayor riesgo de presentar una variedad de cánceres vinculados a mutaciones hereditarias relacionadas con la reparación del ADN. Las mujeres con el síndrome de Lynch tienen un riesgo de por vida del 40% a 60% de desarrollar CE, en comparación con los no portadores de estas mutaciones [46,47].

Diabetes La asociación entre la diabetes y varios tipos de cáncer ha sido reconocida durante más de un siglo [48]. La diabetes aumenta el riesgo de CE en aproximadamente el doble que en la población no diabética [49]; es probable que haya más de un mecanismo para explicar esta asociación. Una posibilidad es la resistencia a la insulina y la hiperinsulinemia asociadas a la diabetes tipo 2 ya que la insulina estimula la proliferación celular [6,48,50].

Factores genéticos Las alteraciones genéticas más comunes en la HE atípica y en el CE son la inestabilidad de microsatélites (IMS), las mutaciones PTEN, la mutación K-ras, la mutación beta-catenina y la mutación de la subunidad catalítica alfa de la fosfatidilinositol 3-kinasa (PIK3CA) [51-55]. PTEN está implicado en la patogénesis de las lesiones endometriales y puede preceder al desarrollo de la HE [50]. Un estudio inmunohistoquímico reveló un papel importante de los genes reparadores de errores de coincidencia (hMLH1 y hMSH2) en el desarrollo de la HE atípica y en el CE [56]. En pacientes con diagnóstico de HE se ha hallado un desequilibrio genómico importante y la ausencia frecuente del brazo corto del cromosoma 8 [56,57]. Igualmente, se ha observado una desregulación de CTNNB1/β-catenina en la HE simple atípica, en la HE compleja con atipia y en la NIE [58]. Más aún, se ha asociado los alelos mutantes de los polimorfismos rs1800716 de CYP2D6 con una mayor probabilidad de tener un endometrio con un espesor de ≥5 mm en mujeres posmenopáusicas que toman tamoxifeno [59]. También se ha encontrado que el polimorfismo CYP17 tiene una correlación con la HE y el CE. Del mismo modo, se ha reportado un aumento significativo del genotipo A1/A1 y una disminución del genotipo A1/A2 en pacientes con HE atípica [60]. Un reciente estudio mostró el papel de los polimorfismos funcionales de un solo nucleótido tales como: CYP2D6, CYP17, COMT, APOE y HFE, en los genes de la catecol-O metiltransferasa, de la apolipoproteína E y de la hemocromatosis en HE y CE [61]. Gerard [62] indica que el 54% de CE y 49% de adenocarcinomas endometroides de ovario presenta mutación en la subunidad catalítica alfa de la PIK3CA; este hallazgo sugiere que su inhibición pudiera servir como agente terapéutico en potencia.

Terapia hormonal de reemplazo. Las mujeres posmenopáusicas que toman suplementos de Es tienen mayor riesgo de HE si no se utiliza P, o una progestina o progestágeno

(Ps) para oponerse a la acción de los Es[2]. El riesgo de desarrollar una HE aumenta con la dosis y la duración del tratamiento con los Es [63,64]. En el estudio denominado PEPI (Postmenopausal Estrogen/Progestin Interventions), se encontró que las mujeres que recibieron solo estrógenos equinos conjugados (EEC) tenían más probabilidades de desarrollar HE simple (28% a 1%), HE compleja (23% a 1%) y HE con atipia (11,8% a 0%) al compararlas con las mujeres que recibieron EEC con una Ps en forma cíclica o continua [65].

Citocinas y marcadores inflamatorios. El endometrio posee un sistema de citocinas equilibrado durante la fase proliferativa y secretora del ciclo menstrual. La inflamación representa un factor importante en el desarrollo de la HE [26]. Zhdanov y col. [66] reportaron en 2003 un desequilibrio en el sistema de citosinas en la HE atípica, la cual se asoció con una producción reducida del factor de necrosis tumoral-α (TNF-α), del antígeno nuclear celular proliferativo y ARNm del factor de crecimiento epitelial, así como también con un aumento de la producción del ARNm del Fas. De igual modo, se encontró que la expresión de los genes del receptor 1 del TNF, interleucina-1β (IL-1β) e IL 12 disminuyó solo en la HE quística glandular, mientras que la expresión del gen del factor de crecimiento similar a la insulina-1 (IGF-1) disminuyó solo en la HE adenomatosa [67].

La producción de IGF-1 es inducida por el estradiol (E_2) y este factor está implicado en los efectos proliferativo que tiene los Es en el útero [68]. Igualmente, se ha encontrado que el receptor de IGF-1 (IGF-1R) se expresa en niveles más altos en la HE y en el CE, en comparación con el endometrio proliferativo [69]. Se ha demostrado que el TNF se expresa en el endometrio normal asi como en HE simples y complejas, pero es regulado negativamente en la HE atípica y en el CE. Asimismo, el factor de transcripción nuclear factor-κB también se encontró expresado en el endometrio en proliferación y en la HE, pero su expresión fue menor en el CE [70].

Tumores suprarrenales secretores de andrógenos. Es una causa muy rara; los andrógenos secretados por estos tumores son metabolizados periféricamente a Es[2].

Tumores de las células de la granulosa del ovario

Los tumores de las células del granuloma (TCG) se mencionan como causantes de HE y CE por su capacidad de secretar Es. El TCG puede ocurrir a cualquier edad, pero se presenta más comúnmente durante el período perimenopáusico o posmenopáusico temprano, con una edad media de diagnóstico entre 50 y 54 años [71].

En el 2001 la Sociedad de Obstetras y Ginecólogos de Canadá [72] publicó una elevada significancia estadística de los diferentes factores de riesgos capaces de ocasionar una HE y/o un CE en mujeres que presenten uno o más de estos factores, como se muestra en la Tabla 1.

Diagnóstico

Antes de proceder al estudio de la causa de la SUA, debe ser descartado un embarazo. Una historia clínica detallada y un examen físico cuidadoso permite realizar un buen diagnóstico; sin embargo, es importante tener en cuenta la edad de la paciente ya que la HE y CE se encuentran más frecuentemente en mujeres perimenopáusicas, menopaúsicas y postmenopáusicas.

Biopsia de Endometrio

La biopsia del endometrio (BE) no requiere ser realizada en todas las pacientes con SUA, por lo que es necesario identificar a aquellas mujeres que si la requieran [73]. La elección de las pacientes que son seleccionados para realizar la BE se basa, sobre todo, en el riesgo de presentar una HE atípica o CE. Una buena anamnesis permite determinar los factores de riesgo y el estudio de ultrasonido por vía transvaginal (UTV) muestra el grosor del endometrio. Varios autores emplean diferentes pautas tales como la

Tabla 1
Prevalencia y Probabilidades de Riesgo
Hiperplasia y cáncer de endometrio.

Factor	Prevalencia	RP 95% IC	Valor p
Todas las pacientes	4,9%	-	-
Peso > 90 Kg	12,7%	5.5 (2.9-10.6)	0.0001
Edad > 45 años	7,9%	3.1 (1.5-6.1)	0.0016
Peso>90 Kg y Edad >45 años	22,2%	-	-
Peso>90 Kg y Edad<45 años	2,3%	-	-
Historia familar de Ca de Colon	-	5.0 (1.3-19.1)	0.0182
Infertilidad	-	3.6 (1.3-9.9)	0.0127
Nuliparaidad	-	2.8 (1.1-7.2)	0.0267
Historia familiar de Ca de Endometrio	-	5.8 (1.1-28.6)	0.0392

RP: Rata de probabilidades y 95% de Índice de Confiabilidad; Ca: cáncer.

Tomado de [72] Vilos G, Lefebvre G, Graves G. SOGC Clinical Practice Guidelines Committee Members. Guidelines for the management of abnormal uterine bleeding. J Obstet Gynaecol Can 2001; 106:704-709.

edad, patologías asociadas, factores genéticos, y la detección del espesor o grosor del endometrio para determinar qué pacientes deben someterse a una BE [74-76]. Aunque Ash y col. [77] refieren que la edad no es un factor importante para realizar la BE, la mayoría sugiere que la BE debe ser tomada en las pacientes con SUA mayores de 45 años [75].

Los reportes o diagnósticos anatomopatológicos de las BE tomados en el consultorio son correctos entre un 87 a un 97% en muestras adecuadas y, detectan 67 a 96% de CE [75,78,79]. Aunque la elección del método para la toma de la BE puede afectar la precisión diagnostica, ningún método existente es capaz de obtener un muestreo de todo el endometrio [80].

La otra técnica para obtener una BE es la dilatación y curetaje (DyC), considerado hasta no hace mucho como el método "Gold Standard". El problema de la DyC es que se requiere sedar o anestesiar a la paciente. En el 10 a 25% de las mujeres que se les practica una DyC no se les descubre patología endometrial. En el proceso se la DyC entre un 0,6 a 1,3% se complica con una perforación uterina y en 0,4% con hemorragia, esto se debe a que es un procedimiento a ciegas; debe reservarse para situaciones en las cuales no se pueda realizar la BE o la histeroscopia en el consultorio [72].

La toma de la BE por medio de la histeroscopia, en especial a nivel de consultorio, permite detectar un mayor porcentaje de anomalías aparte de la toma de la BE, en comparación con la DyC. Las muestras obtenidas por este procedimiento son 87,3% [81] adecuadas para el estudio histológico.

La BE puede excluir HE o CE en mujeres de 40 años o más con SUA ya que a partir de ese grupo etario es considerado un factor de riesgo de presentar dicha patología debido a que es más probable conseguir una HE o CA en mujeres mayores, perimenopáusicas menopaúsicas o post-menopaúsica que en mujeres jóvenes [73].

Imágenes

La ecografía o UTV brinda una información bastante confiable sobre el tamaño, ubicación, forma y consistencia del útero sobre la existencia de patologías como miomas, fibrosis uterina y adenomiosis e igualmente sobre alguna patología endometrial tales como pólipos, miomas submucosos o sospecha de CE.

Igualmente, nos puede informar si el endometrio es probablemente proliferativo o secretor; también nos puede determinar el grosor endometrial, un endometrio de ≤ 4 es un endometrio lineal que no justifica, es decir, es innecesaria la toma de una muestra de endometrio, en especial en mujeres perimenopáusicas y posmenopáusicas debido a las posibilidades de presentar una HE o CE son muy poco probables [73,82,83].

Parecería lógico aplicar este mismo criterio a las mujeres menstrualmente activas con SUA, pero no existe evidencia que sustente este criterio por lo que la decisión de la toma de una BE en estas pacientes se basará en la historia clínica y factores de riesgo [70]. Paraskevaidis y col. [84] refieren que un endometrio con un grosor de ≥ 12 mm aumenta el riesgo de una patología premaligna o maligna del endometrio.

Natarajan y col. [85] concluyen que la resonancia magnética (MRI) tiene un valor diagnóstico potencial para mostrar la transformación maligna de la HE con atipias; sin embargo, la MRI no es muy adecuada para determinar la invasión del miometrio en pacientes con CE.

CONCLUSIÓN

La hiperplasia endometrial es una afección ginecológica relativamente común que afecta a mujeres en la adolescencia, en la menopausia y en la posmenopausia. Es importante entender y comprender el proceso de origen y de su evolución, los factores de riesgo que provocan el desarrollo de esta patología endometrial, realizar el diagnóstico correcto para aplicar el tratamiento adecuado y evitar la progresión de las lesiones premalignas a lesiones malignas.

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1) Contribución sustancial a la concepción y diseño del estudio, obtención de datos o su análisis e interpretación, 2) Revisión crítica del artículo y 3) Aprobación de la versión final a ser publicada. La obtención de fondos, la recolección de datos o la supervisión del grupo de investigación, por sí solos, no justifican la autoría. Aquellos miembros del grupo que no cumplan con los criterios para ser autores, deben ser mencionados en la sección de Agradecimientos. Ni **el número ni el orden de aparición de los autores se podrá modificar una vez que el trabajo haya sido aceptado.** En la carta de presentación, es imprescindible colocar: financiamiento, contribución de cada uno de los autores y su número ORCID, y si hubiere algún conflicto de competencia.

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Para el proceso de arbitraje se utilizará la vía electrónica. Todos los trabajos serán sometidos a la consideración del Comité Editorial de la Revista, el cual decidirá si deben ser enviados a arbitraje, o si se rechazan por no cumplir las normas editoriales o no tenerla calidad suficiente. El autor de correspondencia recibirá una carta de recepción con un código numérico.

*Entrarán en vigencia el 01-04-2025

El arbitraje de Trabajos Originales y Reporte de Casos, será realizado por dos expertos en el área objeto de la comunicación y en el caso de las Revisiones, solo por uno. Los árbitros tendrán un plazo máximo de dos meses para enviar su respuesta. Si las opiniones de los dos árbitros coinciden, el Comité Editorial podrá tomar una decisión; encaso de discrepancia, esperará la opinión deun tercer árbitro. Si la situación lo amerita, se podrán solicitar otras opiniones. **La Revista sigue la evaluación doble ciego, el nombre de los** árbitros **y el de los autores del tra bajo, serán estrictamente confidenciales.** Los autores recibirán, tanto en el caso de modificaciones como en el de rechazo, las opiniones completas respecto al trabajo. El plazo para responder a las recomendaciones de los árbitros, tendrá un máximo de dos meses, pasados los cuales el trabajo será rechazadoo readmitido como nuevo.

Costo de la publicación. Todo trabajo aceptado tendrá un costo por concepto de Manejo Editorial, dependiendo del país, origen del trabajo. Este costo se aplicará de manera fraccionada. El aporte inicial (20% del monto total) se exigirá una vez asignados los árbitros y el restante, al momento de ser aceptado para publicación. En caso de no publicarse, por concepto de rechazo o retiro, no se reembolsará el aporte inicial.

Normas Editoriales

Los trabajos deben estar escritos a doble espacio, con amplios márgenes y numeración de las páginas en "Word for Windows" y, preferiblemente, en "Times New Roman 12".

Los **Trabajos Originales**, las **Revisiones** y los **Reportes de Casos,** deben ser contribuciones inéditas de importancia para el avance del conocimiento en el tema objeto de estudio. En la primera página deben incluir:

El Título del trabajo con letra mayúscula al inicio o cuando se trate de nombres propios; luego el primer nombre, la inicial del segundo y el apellido completo de cada uno de los autores; si usaran dos apellidos deberán separarlos con un guion. El nombre de cada autor llevará superíndices de números consecutivos que correspondan a cada una de las instituciones a las que está afiliado. No repetir si pertenecen a la misma institución, solo colocar el superíndice respectivo. No colocar títulos profesionales. **El título no debe llevar abreviaturas**.

Un **Título corto** de no más de 75 caracteres, en el idioma en que fue realizado el manuscrito.

Palabras clave. En renglón aparte, se escribirán de tres a seis palabras clave en español y en inglés. Se recomienda colocar palabras que aparezcan en el resumen y evitar aquellas que se encuentren en el título.

Autor de correspondencia. Colocar Nombre completo sin títulos académicos, dirección institucional, ciudad, país, teléfono y correo electrónico.

A continuación, se presentarán un **Resumen** en español; el título y el resumen (**Abstract**), en inglés. Si el autor no está capacitado en el idioma inglés, es importante que consulte a un especialista en lengua inglesa, antes de enviar los trabajos oresúmenes en ese idioma. Se requiere el uso del inglés americano. Un trabajo puede ser rechazado, si requiere de muchas correcciones lingüísticas.

Los **Trabajos Originales**, estarán constituidos por: Resumen en español e inglés, Introducción, Material y Métodos o Pacientes y Métodos (si el trabajo se refiere a seres humanos), Resultados, Discusión, Tablas, Figuras, Agradecimientos y Referencias. El texto de los trabajos debe finalizar con una conclusión y no redactarla en una sección aparte. Las Tablas y Figuras deberán presentarse al final del manuscrito. Una copia de estas debe incluirse en archivos separados.

El **Resumen**, debe constar de un máximo de 250 palabras y establecer los objetivos, la metodología, los hallazgos

originales y las conclusiones basadas en los resultados presentados. No debe contener referencias **ni ser estructurado**. Se deben evitar las abreviaturas y si son necesarias, se deben definir en la primera mención.

El **Abstract** debe estar redactado en inglés americano y cumplir las mismas indicaciones del Resumen.

Introducción. Debe incluir antecedentes y generalidades sobre el tema objeto del estudio, hallazgos controversiales, e interrogantes y aportaciones propias y, finalmente, **el objetivo principal de la investigación**.

Material y Métodos. En esta sección se debe informar sobre las características y tamaño de la muestra. En los estudios con humanos se debe incluir el consentimiento informado, señalar los criterios de inclusión y de exclusión, así como la aprobación por parte del Comité de Ética de la institución donde se realizó la investigación y seguir los lineamientos de la Declaración de Helsinki de 1975, revisada en 2024. Se debe evitar el uso de iniciales o números de Historia de los Hospitales y no se aceptarán fotografías del rostro del paciente, sin su consentimiento escrito. Aquellos estudios que involucren animales, también deben seguir el Código de Ética correspondiente, que cumpla con los estándares internacionales establecidos para el uso, cuidado y tratamiento de los animales de laboratorio. Los proce dimientos deben ser descritos en tiempo pretérito y con suficiente detalle para permitir que el trabajo pueda ser duplicado. Los métodos no originales deberán tener su referencia; los equipos y reactivos utilizados deben ir acompañados del nombre y país de la compañía proveedora.

Análisis estadístico. Se debe informar cual fue la plataforma usada y mencionar la versión y las pruebas estadísticas empleadas.

Los **Resultados**, deben ser presentados en tiempo pretérito, en una secuencia lógica en el texto, Tablas y Figuras. Solo se deben resaltar las observaciones importantes. Los valores de laboratorio y las unidades deben ser expresados en el Sistema Internacional (SI). No repetir en el texto lo mostrado en las Figuras o Tablas, solo expresarlo. Las Tablas y figuras se presentarán en páginas separadas. **Las Tablas deben estar en formato editable**. La numeración de las Tablas y Figuras será en caracteres arábigos. No colocar siglas en los títulos.

Discusión. Mencionar los hallazgos principales del estudio, luego comparar los resultados con otros de la literatura, sus aportaciones y fortalezas, mencionar las limitaciones del trabajo y sugerir lineamientos de futuras investigaciones. El texto de los trabajos debe finalizar con una conclusión acorde con los resultados y no redactarla en una sección aparte.

Limitar a un máximo de 50 referencias para los artículos originales y 100 para las Revi siones Narrativas y Sistemáticas o Metanaálisis. Se recomienda revisar cuidadosamente el último número de la Revista (http://sites.google.com/site/revistainvestigacionesclinicas) como guía para la preparación del manuscrito

Las **Revisiones Narrativas** deben estar escritas por especialistas en el campo objeto de las mismas, y contener las contribucio nes del autor, ya sea en las referencias o con una discusión del tema revisado. **El número máximo de autores es de cuatro.** No se acep tarán revisiones que consistan meramente de una descripción bibliográfica, sin incluir un análisis. El cuerpo de las revisiones es libre, aunque es conveniente subdividirlo en secciones. Las **Revisiones Sistemáticas yMetaanálisis** deben seguir las indicaciones internacionales establecidas por PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) o por cualquier otro método similar.

Los **Reportes de Casos** se refieren a la presentación de casos clínicos poco frecuen tes en la práctica médica. Deben incluir una breve introducción sobre la patología a ser presentada, la descripción del caso y una discusión con el apoyo bibliográfico correspondiente. Limitar la discusión a lo más notable del caso.

El **Editorial** será presentado por un Miembro del Comité Editorial de la Revista o por un invitado, propuesto por este cuerpo, seleccionado entre los asiduos colaboradores.

Las **Cartas al Editor**, deben ser comentarios sobre publicaciones recientes en la revista y, en lo posible, no deben exceder de dos páginas, incluidas las referencias.

Tablas. Las Tablas deben ocupar una página cada una y estar numeradas en caracteresarábigos. Deben contener un título descriptivo, colocado en el centro, bajo el número de la Tabla. Las columnas no deben separarse con líneas. Las notas referentes a lo expresado en el cuerpo de la Tabla, deben ser escritas al pie de la misma, precedidas de los símbolos correspondientes. **Las tablas deben ser analizadas sin necesidad de recurrir al texto por ende: se deben describir las abreviaturas; colocar el tipo de análisis estadístico usado y hacer referencia a cuales grupos se refiere la significancia estadística (p).**

La revista no acepta la expresión "Fuente de información", cuando se refiere a resultados presentados en el mismo artículo, solo si provienen de otro material. Si el artículo está escrito en español, los números decimales se deben separar con una coma y si está escrito en inglés, con un punto.

Figuras. Se deben seguir los siguientes puntos generales: cada figura debe ser enviada en un archivo separado, en el programa donde fue generada (por ejemplo GraphPad Prism®). El número de la Figura debe ser arábigo y estar de acuerdo con la secuencia en el texto. Debe asegurarse que el tipo de letra y el tamaño, sean uniformes. Todas deben tener como mínimo 300 dpi. Las figuras en color deben ser enviadas en formato TIFF o RGB (sigla en inglés de *red, green, blue*). Estas deben ser presentadas con contraste adecuado. Las leyendas de las figuras se deben enviar por separado, con suficiente información para no tener que recurrir al texto. Las imágenes radiográficas no deben contener leyendas que identifiquen al paciente.

Las Fotografías pueden ser en blanco y negro o en color, deben tener un contraste adecuado para su reproducción y estar en formato JPG o TIFF, con las siguientes condiciones: las fotografías en color o en gradaciones de gris, deben tener un mínimo de 600 dpi. En el caso de las microfotografías electrónicas, debe extremarse el cuidado de la nitidez de los hallazgos reportados y señalarlos por medio de símbolos. También se debe indicar el aumento utilizado, de preferencia colocar una barra que indique el valor que representa (micras, milimicras, nanometros etc.). Las leyendas no deben estar incorporadas a la fotografía y estas deben presentarse en página aparte, en forma lo suficientemente explicativa, sin tener que acudir al texto, y cuidar la descripción si se trata de la figura a color o en tonos de gris. La Revista no aceptará fotografías o figuras tomadas de otras revistas, sin la respectiva autorización.

Referencias. Todas las referencias deben aparecer en el texto con un número en superíndice sin paréntesis y citadas por orden de aparición, según las normas internacionales "*Recommendations For the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals*", actuali- zadas en enero de 2025 (http:/www. icmje. org); es decir, primero el apellido con la letra inicial en mayúscula, seguido de las iniciales del nombre, también en mayúscula (sin puntos), de todos los autores**. Los nombres de los autores deben ir en negritas** y separados entre sí por comas. No se aceptarán los términos "y col." o "*et al.*" en la sección de las referencias. En aquellos trabajos con un número muy elevado de autores se permitirá colocar solo los seis primeros, seguido de "y col." o "*et al.*"

El título completo del trabajo tendrá mayúsculas solo al inicio y en los nombres propios. El título de la revista debe ser abreviado de acuerdo al Index Medicus (http:/ www.nlm.nih.gov), seguido del año de publicación; volumen: y primera y

última páginas, separadas por un guión. Seguidamente, se debe colocar el doi de dicho trabajo. No se aceptarán como referencias, observaciones no publicadas, comunicaciones personales o trabajos enviados a publicación; sin embargo, estos podrán aparecer citados entre paréntesis en el texto. Si el autor es una organización, se coloca el nombre de la misma como referencia.

Ejemplos:

Referencias de publicaciones periódicas: Jaspe RC, Sulbaran Y, Hidalgo M, Loureiro CL, Moros ZC, Garzaro D, Rangel HR, Pujol FH. A simple method for detecting of mutations in amino acid 452 of the Spike protein ofSARS-CoV-2 using restriction enzyme analysis. Invest Clin 2021; 62(4): 371-377. *https://doi.org/10.22209/IC.v62n4a07.*

Referencias de libros: Kandel ER, Schwartz JH, Jessell TM. Principles of neural science. New York: McGraw Hill; 2001, p 1227-1246.

Artículos en libros: Molina-Vilchez R, Diez-Ewald M, Fernández G. Anemia y Embarazo. En: Zighelboim I, Guariglia D, Eds. Clínica Obstétrica. Caracas: Disinlimed; 2000. P570-577.

Memorias de Congresos: Metabolic benefits of lifestyle intervention in the clinical setting: a pilot study in Latinos with prediabetes from Venezuela, South America. Victo ria Stepenka, Yoleida Rivas, Juan Casal, Rober to Gutiérrez, Elena Ryder, Hermes Florez. 70th. Scientific Sessions. American Diabetes Association. 25-29 junio, 2010, Orlando, USA.

Tesis: León-N I. Caracterización de aislamientos del complejo *Sporothrix* spp. provenientes de diferentes regiones de Venezuela [Tesis de Maestria] Caracas: IVIC; 2013.

Revista en formato electrónico: Calvo B, Melo A, Perozo A, Hernández M, Francisco E, Hagen F, Meis J, Colombo J. First report of *Candida auris* in America: clinical and microbiological aspects of 18 episodes of candidaemia. J Infect 2016 [citado, 2017 febrero 10] *Disponible en: http://dx.doi.org/10.1016/j.inf.2016.07.008.*

Lista de Verificación

- ☐ Carta firmada por todos los autores, donde se indique el autor de correspondencia, la participación de cada autor en la elaboración del trabajo, y se manifieste que este no ha sido publicado con anterioridad, ni está siendo enviado a otra revista para publicación.
- ☐ Páginas numeradas en forma secuencial.
- ☐ Título en español.
- ☐ Título en inglés.
- ☐ Título corto en el lenguaje principal utilizado en el manuscrito.
- ☐ Lista de Autores con nombres completos, sin títulos profesionales.
- ☐ Resumen **no estructurado** en inglés y español de no más de 250 palabras, que incluya introducción, procedimientos básicos, resultados y conclusión.
- ☐ Introducción breve, referida al objeto de estudio.
- ☐ Material y Métodos o Pacientes y Métodos, descritos con precisión y con referencias adecuadas.
- ☐ Especificación del análisis estadístico (cuando se requiere).
- ☐ Resultados presentados en forma clara y en orden lógico sin discusión de los mismos.
- ☐ Discusión basada en los hallazgos obtenidos.
- ☐ Referencias presentadas en orden de aparición, en superíndices sin paréntesis, citadas en el texto y de acuerdo a las especificaciones de la revista.
- ☐ No se aceptan comunicaciones personales, ni presentaciones en congresos cuyos resúmenes no hayan sido publicados.
- ☐ Tablas numeradas en arábigos, con las notas en la parte inferior.
- ☐ Las ilustraciones y fotografías de acuerdo a las especificaciones de la revista.
- ☐ Leyendas de las ilustraciones, figuras y fotografías en páginas separadas.
- ☐ Número ORCID de todos los autores.
- ☐ Fuente de financiamiento.
- ☐ Participación de los autores en el trabajo.
- ☐ Conflictos de interés.

Author Instructions*

Investigación Clínica publishes Original Papers, Reviews and Clinical Case Reports in Spanish and English, which contribute to advancing human or animal biology knowledge. It also includes **an Editorial** and a "Letters to the Editor" section.

Submission of the manuscript

The manuscript (Word for Windows®), with its corresponding checklist and accompanied by a cover letter to the editor, must be sent by email to the address *riclinicas@gmail.com*

If any, Tables and Figures must be at the end of the work, with their corresponding legend, and simultaneously be sent as separate files. In addition to the manuscript, the names of three possible referees and their respective institutional and electronic addresses may be included. The Editorial Committee reserves the right to decide whether to use any suggested reviewers. All matters relating to correspondence, including the opinion of the referees, the requirements resulting from the review of the work and the notification of the decision of the Editorial Committee, will be communicated by email. **The correspondence to follow up on the work must include the code assigned by the Journal in the receipt letter.**

Cover letter

The manuscript must be accompanied by a letter signed by all authors, stating that they have actively participated in the execution of the work, that they have not used "Artificial Intelligence" to prepare it, that it has not been previously published, that they are aware that it is being sent to "Investigación Clínica" for publication, and that it has not been sent to another journal for consideration.

Authorship must be based on the following:

1) Substantial contribution to the conception and design of the study, data collection or its analysis and interpretation, 2) Critical review of the article and 3) Approval of the final version to be published. Obtaining funds, collecting data, or supervising the research group alone do not justify authorship. Those group members who do not meet the criteria to be authors must be mentioned in the **Acknowledgements section**. **Neither the number nor the order of appearance of the authors may be modified once the work has been accepted.** In the cover letter, it is essential to include funding, the individual contribution of each of the authors, their ORCID number, and if there is any conflict of interest. Also, in this case, the referee may recommend some experiment or unique statistical analysis that requires allocating another author, which is why we say it is when it is accepted.

Peer-review Process

An electronic arbitration process will be used throughout this procedure. All works will be submitted for consideration by the Journal's Editorial Committee, which will decide whether they should be sent to arbitration or rejected for not complying with the editorial standards or not being of sufficient quality. The corresponding author will receive a letter of receipt with a numerical code.

*These will take effect from April 1st, 2025

The review of Original Works and Case Reports will be carried out by two experts in the scientific area of communication and, in the case of Reviews, by only one. The reviewers will have a maximum of two months to send their evaluations. If the opinions of the two referees coincide, the Editorial Committee may decide; in case of discrepancy, it will wait for the opinion of a third reviewer. If the situation warrants it, other opinions may be requested. **The Journal follows the double-blind evaluation; the names of the arbiters and the authors of the work will be strictly confidential and anonymous to each other.** Authors will receive, both in the case of modifications and rejection, the complete opinions regarding the work. The deadline to respond to the referees' recommendations will be a maximum of two months, after which the work will be rejected or readmitted as new.

Publication fees. All accepted works will be charged for Editorial Management, depending on the country's origin of the work. This fee will be applied in installments. The initial contribution (20% of the total) will be required once the referees have been assigned and the remainder at the time of acceptance for publication. This initial contribution will not be reimbursed due to rejection or withdrawal of the communication.

Editorial Standards

The papers must be double-spaced, with wide margins and page numbering in "Word for Windows"® and, preferably, in "Times New Roman 12pt".

Original papers, reviews, and case reports must be unpublished contributions that advance knowledge on the subject under study. The first page must include:

Indicate the **paper's title** with capital letters at the beginning or when it refers to a proper name. Then, the first name, the initial of the middle name, and the full surname of each of the authors are used; if they use two surnames, they must be separated with a hyphen. Each author's name will have superscripts of consecutive numbers that correspond to each of the institutions to which they are affiliated. Do not repeat if they belong to the same institution; only place the respective superscript. Do not use professional titles. The title should not contain abbreviations.

A **short title** of no more than 75 characters in the language in which the manuscript was written.

Keywords. Three to six keywords should be written in Spanish and English on a separate line. Including words that appear in the abstract and avoiding those in the title is recommended.

Corresponding author. Enter the full name without academic titles, including the institutional address, city, country, telephone number, and email.

Next, an **abstract** should be presented in Spanish; then, the title and the abstract should be presented in English. If the author is not trained in English, it is important to consult an English language specialist before sending the papers or abstracts in that language. The use of American English is required. A paper may be rejected if it requires many linguistic or grammar corrections.

Original works will consist of an Abstract in Spanish and English, an Introduction, Material and Methods or Patients and Methods (if the work refers to human beings), Results, Discussion, Tables, Figures, Acknowledgements and References. The text of the works must end with a conclusion and not be written in a separate section. Tables and Figures must be presented at the end of the manuscript. A copy of these must be included in separate files.

The abstract (in Spanish) should consist of a maximum of 250 words and establish the objectives, methodology, original findings, and conclusions based on the results presented. It should not contain references or be structured. Abbreviations should be avoided, and if necessary, they should be defined in the first mention.

The **abstract** (in English) should be written in American English and follow the same guidelines as the abstract in Spanish.

The **Introduction** should include background and generalities on the subject of the study, controversial findings, questions and contributions of the author, and finally, **the main objective of the research**.

Material and Methods. This section should report on the characteristics and size of the sample. In studies with humans, informed consent must be provided, inclusion and exclusion criteria must be indicated, and approval must be obtained by the institution's Ethics Committee where the research was conducted. The guidelines of the 1975 Declaration of Helsinki, revised in 2024, must be followed. Using initials or hospital history numbers must be avoided, and photographs of the patient's face will not be accepted without written consent. Studies involving animals must also follow the corresponding Code of Ethics, which complies with the international standards for using, caring and treating laboratory animals. The procedures must be described in past tense and with sufficient detail to allow the work to be duplicated. Non-original methods must be referenced; the name and country of the supplier company must accompany the equipment and reagents used.

Statistical analysis. The statistics platform and the version and statistical tests used must be reported.

Results should be presented in the past tense, in a logical sequence in the text, tables and figures. Only important observations should be highlighted. The International System (SI) should express laboratory values and units. Do not repeat what is shown in the figures or tables in the text; express it. **Tables and figures** will be presented on separate pages. Tables must be in editable format. Tables and Figures will be numbered in Arabic characters. Do not include acronyms in the titles that have not been previously identified.

Discussion. Mention the study's main findings, then compare the results with others in the literature, including their contributions and strengths. Mention the limitations of the work and suggest guidelines for future research. The text of the works should end with a conclusion following the results and not write it in a separate section.

References. Limit to a maximum of 50 references for original articles and 100 for Narrative and Systematic Reviews or Meta-analysis. It is recommended that the latest issue of the Journal (*http://sites.google.com/site/revistainvestigacionesclinicas*) be carefully reviewed as a guide for the preparation of the manuscript.

Narrative Reviews must be written by specialists in the reviewed field and contain the author's contributions, either in the references or with a discussion of the topic considered. **The maximum number of authors is four.** Reviews that consist merely of a bibliographic description without including an analysis will not be accepted. The body of the reviews is free, although it is convenient to subdivide it into sections. **Systematic Reviews and Meta-Analyses** should follow the international guidelines established by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) or any other similar method.

Case Reports present clinical cases that are not common in medical practice. They should include a brief introduction to the pathology, a case description, and a discussion with the corresponding bibliographical support. Limit the discussion to the most notable aspects of the case.

The **Editorial** will be presented by a Member of the Editorial Committee of the Journal or by a guest proposed by this body, selected from among the regular contributors.

Letters to the Editor should be comments on recent publications in the Journal and, if possible, should not exceed two pages, including references.

Tables. Tables should occupy one page each and be numbered in Arabic characters. They should contain a descriptive title in the center, under the Table number. Lines should not separate columns. Notes referring to what is expressed in the body of the Table should be written at the bottom of the Table, preceded by the corresponding symbols. Tables should be analyzed without having to resort to the text; therefore, abbreviations should be described; the type of statistical analysis used should be indicated, and references should be made to the groups to which the statistical significance (p) refers.

The Journal does not accept the expression "Source of information" when referring to results presented in the same article, only if they come from another material. If the article is written in Spanish, decimal numbers should be separated by a comma and if it is written in English, by a period.

Figures. The following general points must be observed: each figure must be sent in a separate file in the generating program (for example, GraphPad Prism®). The figure number must be Arabic and follow the sequence in the text. The font and size must be uniform. All figures must be at least 300 dpi. Color figures must be sent in TIFF or RGB (red, green, blue) format. Figure captions must be sent separately, with sufficient information to avoid resorting to text.. Figures must be presented with adequate contrast. Radiographic images must not contain captions that identify the patient.

Photographs may be black and white or color, must have adequate contrast for reproduction and be in JPG or TIFF format, with the following conditions: color or grayscale photographs must be at least 600 dpi. In the case of electron micrographs, extreme care must be taken to ensure the sharpness of the findings reported and to indicate them using symbols. The magnification must also be indicated, preferably with a bar indicating its value (microns, millimicrons, nanometers). The legends must not be incorporated into the photograph and must be presented on a separate page, in a sufficiently explanatory manner, without having to refer to the text, and care must be taken to describe the figure in color or shades of gray. The Journal will not accept photographs or figures from other journals without authorization.

References. All references must appear in the text with a superscript number without parentheses and cited in order of appearance, according to the international standards "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals", updated in January 2025 (*http:/www.icmje.org*); that is, first the last name with the initial letter capitalized, followed by the initials of the first name, also capitalized (without periods), of all the authors. **The authors' names must be in bold** and separated from each other by commas. The terms "y col." or "et al." will not be accepted in the references section. Only the first six will be allowed in those works with many authors, followed by "y col." or "et al.".

The work's full title must be capitalized only at the beginning and in proper names. The title of the Journal must be abbreviated according to the Index Medicus (http:/ www.nlm.nih.gov), followed by the year of publication, volume; and first and last pages, separated by a hyphen. Next, the DOI of the work must be included. Unpublished observations, personal communications or works submitted for publication will not be accepted as references; however, these may be cited in parentheses in the text. The organization's name must be included as a reference if the author is an organization.

Reference Examples

References to periodical publications: Jaspe RC, Sulbaran Y, Hidalgo M, Loureiro CL, Moros ZC, Garzaro D, Rangel HR, PujolFH. A simple method for detecting mutations in amino acid 452 of the Spike protein of SARS-CoV-2 using restriction enzyme

analysis. Invest Clin 2021; 62(4): 371-377. *https://doi.org/10.22209/IC.v62n4a07.*

Book references: Kandel ER, Schwartz JH, Jessell TM. Principles of neural science. New York: McGraw Hill; 2001, p 1227-1246.

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