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Rational evaluation of patients with COVID-19-related hair loss

Hudson Dutra Rezende[1,2], Gabriela Minari[1*], Marcele Cunha[1], Sandra Dinato[1], Maria Fernanda Reis Gavazzoni Dias[3], Ricardo Romiti[2]

Hair loss has been noted to be a frequent complaint during or after infection caused by the coronavirus disease 2019 (COVID-19). During the current viral pandemic, it has become frequent for dermatologists to evaluate hair loss after infection caused by COVID-19)[1]. Following systemic illness, especially after febrile diseases, patients commonly experience a diffuse, but self-limited hair shedding, probably due to the impact of cytokines over the hair follicle circle, picturing what is called telogen effluvium (TE)[1]. Even though there is no pattern of alopecia attributed to TE on physical examination, reduced hair density over the temporal areas is typically seen in severe cases[2].

Hair loss has been addressed by several authors during the current pandemic, and cases of alopecia areata, trichotillomania (TTM), and androgenetic alopecia have somehow been linked to COVID-19 infection, either in association with or as being possibly triggered by the viral infection[3,4]. Nonetheless, scientific discussions regarding differential diagnosis and initial management for such cases are still scarce in the published literature.

In fact, not all cases of diffuse hair loss after COVID-19 are simply TE. Since hair shedding can be seen in many other medical conditions and considering that establishing a diagnosis does not automatically rule out others, it is wise to consider possible associations for every patient.

For instance, TTM, one of diseases listed in the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*), can be sparked by social and environmental stressors and may present as any given type of hair loss[2]. Recently, Dutra Rezende et al. have commented on the psychological impacts caused by the pandemic on female patients who suffer from hair loss and how stressful it can be for them to cope with hair shedding while facing pandemic issues[5].

Dutra Rezende and co-authors have also described a case of a middle-aged woman with acute and diffuse hair loss who was being treated for isolated TE for over 8 months with no clinical response[6]. Careful examination of the scalp with dermoscopy and trichogram showed black dots, broken hairs, and dystrophic hair shafts pointing to the diagnosis of diffuse alopecia areata, which was confirmed by histopathology. Diffuse alopecia areata pictures yet another possibility for patients with diffuse hair loss and may be mistreated as TE. In addition, alopecia areata is also related to stress, though the exact mechanism by which it affects the hair follicle cycle remains unclear[2].

In 2012, the World Health Organization has implemented the pre-exposure prophylaxis (PREP) in order to control HIV infection. Despite the good results that this strategic plan seems

Table 1. How can dermoscopy help differentiate diffuse hair loss during the COVID-19 pandemic.

Clinical condition	Dermoscopic findings
Androgenetic alopecia	- Anisotrichia
	- Miniaturized hairs
	- Yellow dots
	- Single-hair units
Secondary syphilis	- Yellow dots
	- Black dots
	- Broken hairs
Trichotillomania	- Hairs broken at different lengths
	- Trichoptilosis
	- Coiled hairs
	- Black dots
Diffuse alopecia areata*	- Black dots
	- Exclamation mark hairs
	- Yellow dots
	- Dystrophic hairs
	- Pig tail hair (repilation)

*Diagnosis lies on histopathologic findings.

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Table 2. Proposed initial blood screening for patients with diffuse hair loss following COVID-19 infection.

Blood tests
Complete blood count
Iron tests
Erythrocyte sedimentation rate (ESR)
Thyroid function tests
Serum creatinine and urea
Vitamins D and B12
Glucose test
Liver enzymes
Treponemal and nontreponemal tests

to be reaching, PREP does not prevent patients from having syphilis, chancroid, and other sexually transmitted diseases[2]. As already known, diffuse hair loss can be the only clinical manifestation of secondary syphilis and the diagnosis is likely to be overlooked if proper laboratory examinations are not taken.

Up to now, no dermoscopic clue is enough for the diagnosis of TE[2]. In addition, most patients with isolated acute TE will show no remarkable dermoscopic features. In contrast, if TE is associated with androgenetic alopecia, syphilis, diffuse alopecia areata, or TTM, other visual elements are very likely to be found by dermoscopy (Table 1). Moreover, a general blood screening is right at any given new-onset diffuse hair loss so that comorbidities and hormonal imbalances, especially in women, can be ruled out, as shown in Table 2.

Finally, hair loss following COVID-19 infection is frequent and should not be evaluated solely based on clinical grounds. If the attending physician is not acquainted with dermoscopy, an initial blood screening should be requested, and the patient should also visit a dermatologist for specialized help.

AUTHORS' CONTRIBUTIONS

HDR: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation Visualization, Writing – original draft, and Writing – review & editing. **GM**: Data curation, Investigation, Methodology, Project administration, Software, Validation Visualization, Writing – original draft, and Writing – review & editing. **MC**: Data curation, Investigation, Methodology, Project administration, Software, Validation Visualization, Writing – original draft, and Writing – review & editing. **SD**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing. **MFRGD**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing. **RR**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing.

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Malignant distal biliary obstruction – palliative treatment-modality of endoscopic stent: metal stent × plastic stent

Maria Vitória Cury Vieira Scatimburgo[1*], Vitor Massaro Takamatsu Sagae[1], Mateus Pereira Funari[1], Eduardo de Moura[1], Wanderley Bernardo[1]

The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct and to assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical condition of each patient.

Guideline conclusion: April 2021.

Societies: Sociedade Brasileira de Endoscopia Digestiva.

Group AMB: Wanderley Marques Bernardo

INTRODUCTION

Although malignant bile duct tumors are uncommon and estimated to have an incidence of 8000 new intrahepatic and extrahepatic cases per year according to the American Cancer Society[1], these neoplasms are associated with a very poor overall prognosis. In many cases, these lesions have no curative perspective by the time of diagnosis. Thus, palliative treatment methods to achieve bile duct clearance play a major role, providing a longer life expectancy and improved quality of life[2].

Endoscopic stenting, percutaneous transhepatic bile duct drainage (PTBD), and surgical bile derivation (i.e., surgical bypass) are established methods to achieve bile duct drainage. Endoscopic biliary stenting was first described by Soehendra[3] in 1979, and is currently considered the treatment of choice in the palliative care of unresectable or inoperable malignant distal biliary obstruction (MDBO). Additionally, endoscopic biliary drainage may be considered an alternative or as a combined approach method to PTBD[4]. Endoscopic drainage has been shown to be associated with a decreased mortality and lower complication rate, as well as a higher clinical success rate, compared to a traditional surgical approach; however, there does appear to be a higher rate of recurrent biliary obstruction[4,5].

Two types of stents may be utilized to achieve successful endoscopic biliary drainage: plastic stent (PS) and self-expanding metal stent (SEMS) placement. Each of these stent types possess different characteristics regarding stent patency, need for reintervention, potential for stent dysfunction, and other adverse events.

METHODS

A systematic review and meta-analysis of the literature (Medline, Central Cochrane, Embase, LILACS/VHL, and grey search) was carried out according to the recommendations of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) using the PICO system, including more patients aged 18 years with indication of palliative drainage of the biliary duct. The intervention and control were SEMS and PS, respectively, and the SEMS group was divided into subgroups, uncovered metal stent (uSEMS), partially and fully covered metal stent (pcSEMS/cSEMS), and third subgroup, SEMS that do not specify (SEMS not specified).

We screened all studies comparing PS versus SEMS placement among patients with inoperable MDBO, due to unresectability or poor patient status (after evaluation by the surgeon or anesthesiologist). The outcomes were assessed as follows: stent dysfunction rate, reintervention rate, duration of stent patency, median survival, complications (e.g., cholecystitis, bleeding, pancreatitis, perforation, and liver abscess), and clinical success.

Risk of bias was evaluated through the individual randomized controlled trials (RCTs) by Cochrane's risk assessment tool for randomized trials, available as ROB-II[18]. The quality of the evidence was analyzed using the Recommendation Classification, Development, and Evaluation (GRADE) working group[19]. The data from the selected works were analyzed through the software Review Manager version 5.4 (RevMan 5.4). The results were exposed as Forest plot and are available as Appendix.

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RESULTS

The search strategy identified 4378 articles. After excluding the duplicates, retrospective studies, and applying the eligibility criteria, 12 RCTs were selected, with a total of 1005 patients[6-17] (Figure A1).

The risk of bias analysis for each individualized study is shown in Table A1.

Results exposed by comparison were obtained as follows:

CLINICAL SUCCESS

→ Clinical success was evaluated in eight studies[6,8,9,11-13,16,17], evaluating a total of 765 patients.

There was no difference between the two groups (RD=0.03, 95%CI -0.01, 0.07). There was also no difference in the uncovered SEMS (RD=0.04, 95%CI -0.05, 0.13). Partially/fully covered SEMS (RD=0.03, 95%CI -0.03, 0.10), and SEMS not specified subgroups (RD=0.01, 95%CI -0.04, 0.06) (Figure A2).

The quality of evidence was moderate.

Mean survival

→ The mean survival analysis was performed in days and documented in six studies[7-9,11,14,16], evaluating a total of 610 patients.

There was no difference between the two groups (MD=0.63, 95%CI -18.07, 19.33). Regarding the subgroups, uSEMS (MD=65 days, 95%CI -18.44, 148.44) and SEMS not specified (MD=14.10 days, 95%CI -22.43, 50.63) were not different from PS placement. However, pcSEMS/cSEMS revealed an increase in mean survival (MD=-17.45 days, 95%CI -32.68, -2.21) (Figure A3).

The quality of evidence was low.

Complications

Analysis of 10 studies[6,8,9,11-17], totaling 1005 patients.

There was no difference between the two groups (RD=-0.03, 95%CI -0.10, 0.03). Subgroup analyses revealed no differences by specific SEMS type (uSEMS: RD=-0.09, 95%CI -0.21, 0.03; pcSEMS/cSEMS: RD=-0.00, 95%CI -0.09, 0.09; and SEMS not specified: RD=-0.06, 95%CI -0.21, 0.08) (Figure A4).

The quality of evidence was very low.

Stent dysfunction

→ Analysis of 11 studies[7-17], totaling 465 patients in the PS group and 542 patients in the SEMS group.

The rate of stent dysfunction was 24% lower in the SEMS group (RD=-0.24, 95%CI -0.33, -0.15) (Figure A5). Performing a subgroup analysis by type of SEMS revealed no difference in stent dysfunction rate between uSEMS and PS placement (RD=-0.08, 95%CI -0.56, 0.39). In the other two subgroups, there was a statistically significant difference: in the pcSEMS/cSEMS subgroup, the stent dysfunction rate was 21% lower than in the PS group (RD=-0.21, 95%CI -0.32, -0.1), and in the SEMS not specified subgroup, there was 29% less dysfunction than in the PS group (Figure A5).

The quality of evidence was very low.

Stent patency

→ Data from seven studies[7-9,11,12,14,16] were evaluated in a total of 720 patients.

The duration of patency was longer in the SEMS group (MD=125.77, 95%CI 77.5, 174.01).

In all subgroups, there was a longer time for stent dysfunction compared to PS (Figure A6).

The quality of evidence was very low.

Reintervention

→ The reintervention analysis was divided into two analyses, one evaluating studies in which the result was expressed in dichotomous variables and the other in continuous variables.

Dichotomous variables

It was possible to evaluate four studies[11,12,14,15], totaling 443 patients. The reintervention rate was 34% lower in the SEMS group, with statistical difference (RD=-0.34, 95%CI -0.46, -0.22).

In both the pcSEMS/cSEMS subgroup and the SEMS not specified subgroup, there was a lower reintervention rate than in the PS group. In the first subgroup, the intervention rate was 29% lower (RD=-0.29, 95%CI -0.41, -0.17), and in the second group, it was 39% lower than PS group (RD=-0.39, 95%CI -0.63, -0.15) (Figure A7).

The quality of evidence was very low.

Continuous variables

Three studies[10,16,17] were evaluated, with 176 patients.

The reintervention rate was 67% lower in the SEMS group (MD=-0.67, 95%CI -0.85, -0.50).

The uSEMS subgroup revealed no difference versus the PS group (RD=-0.76 95%CI -1.53, 0.01); however, the SEMS not specified subgroup had a reintervention rate 67% lower than in the PS group (RD=-0.67, 95%CI -0.85, -0.49) (Figure A8).

The quality of evidence was low.

DISCUSSION

Despite promising therapies that are the subject of studies and clinical trials, most of the time, at the time of diagnosis, these tumors are unresectable and present obstruction of the bile duct. Thus, endoscopic drainage using stents plays an important role in this condition.

In the comparisons between SEMS and PS, SEMS was associated with a longer duration of patency, lower rate of stent dysfunction, and decreased need for reintervention. This may be explained by two factors. First, SEMS is self-expanding and reaches a larger diameter when compared to PS placement, allowing for a greater flow and consequently better drainage of the bile duct. Furthermore, SEMS possess less surface for bacterial multiplication and fixation, which may lead to the formation of biofilm and deposition of bile sludge, responsible for earlier obstruction of the PS[10,16,17].

In the subgroup of uncovered metal stents, the main cause of obstruction was internal tumor growth ("ingrowth"), making replacement extremely challenging in cases of obstruction. In the subgroups of partially covered or covered metal stents, due to their covering, the main complication is migration. This is due to the fact that this type of stent applies a greater expandable force that, associated with tumor growth, leads to its migration. However, partially or fully covered SEMS allows for a greater possibility of stent removal or replacement in case of failure/clogging compared to uSEMS[10,16].

Regarding survival, there was no difference between SEMS and PS. However, when analyzing the subgroups, the pcSEMS/cSEMS placement outperformed PS.

This guideline presents as limitation the heterogeneity present in the RCTs analyzed, such as the presence of metastatic and non-metastatic patients, the use of different metal stents (i.e., fully cSEMS, pcSEMS, or uSEMS), the difference in diameters and subjective definitions for inoperable patients or for dysfunction. However, to minimize these limitations, we divided the SEMS groups into subgroups, in addition to evaluating a large number of studies, standardizing the location and approach method, maintaining relative homogeneity between the compared groups.

The limitations of this guideline and the difficulty of availability of the recommended resources are factors that can hinder the dissemination of the exposed recommendations. In contrast, the high level of evidence facilitates the dissemination of the content covered.

RECOMMENDATIONS

For MDBOs, the use of SEMS has a longer time for stent dysfunction (showing a longer patency time), a lower rate of reintervention, and a lower rate of dysfunction when compared to the use of PS in patients with MDBO. In the analysis of survival, there is no statistical difference between two groups; however when assessing the subgroups, pcSEMS / cSEMS showed higher survival compared to the PS. Regarding clinical success and rate of complications, there was no difference between the methods.

Thus, the SEMS presented favorable results in relation to the PS. However, the patient's survival time should always be taken into account, since those with an average survival of less than 4–6 months, the use of PS is more indicated, due to its lower initial cost.

The level of evidence varies from very low to low depending on the outcome analyzed.

AUTHORS' CONTRIBUTIONS

MVCVS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, and Writing – review & editing. **VMTS**: Conceptualization, Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft, and Writing – review & editing. **MPF**: Data curation, Investigation, and Visualization. **EM**: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Supervision, Validation, and Visualization. **WB**: Data curation, Methodology, Software, Supervision, and Visualization.

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APPENDIX

Protocol and registration

This study was performed in conformity with the PRISMA guidelines and was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under file number CRD42020191234.

Eligibility Criteria

We analyzed all RCTs that compared the placement of PS versus SEMS, only through endoscopy in patients with inoperable/unresectable MDBO or poor condition of the patient (after evaluation by the surgeon or anesthesiologist). No restrictions were set for the publication date or language.

Literature search strategy, study selection, and data extraction

A comprehensive search was performed in MEDLINE, Cochrane, Embase, LILACS, and grey literature, from their inception to December 2020.

Search

We used this search strategy: ((Neoplasia OR Neoplasias OR Neoplasm OR Neoplasms OR Tumors OR Tumor OR Cancer OR Cancers OR Malignancy OR Malignancies) AND (Biliary Tract OR Biliary Tree OR Biliary System OR Bile Duct OR Bile Ducts)) OR (Bile Duct Neoplasms OR Bile Duct Neoplasm OR Bile Duct Cancer OR Bile Duct Cancers OR Biliary Tract Neoplasm OR Biliary Tract Neoplasm OR Biliary Tract Cancer OR Biliary Tract Cancers)) AND ((Prostheses and Implants) OR Prosthetic OR Implants OR Implant OR Prostheses OR Prosthesis OR Endoprosthesis OR Endoprostheses OR Stent OR Stents).

Statistical analysis

The data from the selected works were analyzed through the software Review Manager version 5.4 (RevMan 5.4).

For dichotomous end points, the difference was calculated by the risk difference, using the Cochran-Mantel-Haenszel test, with 95% confidence interval (CI). For continuous variables, the inverse variance test was applied. Statistically, we considered the 95%CI and $p<0.05$. The results were exposed in the form of a forest plot.

The inconsistency index was evaluated through I^2, in which it is possible to observe the presence of heterogeneity. The I^2 varies from 0% to 100%, and when it presents heterogeneity, >50% is considered high and >75% is considered very high. The sensitivity test (Egger) was performed whenever the heterogeneity was high in the search for publication bias (outlier)[20].

Risk of bias

Risk of bias was evaluated through the individual RCTs study by Cochrane's risk assessment tool for randomized trials, available as ROB-II[18].

The quality of the evidence was analyzed using the Recommendation Classification, Development, and Evaluation (GRADE) working group[19] (Table A2).

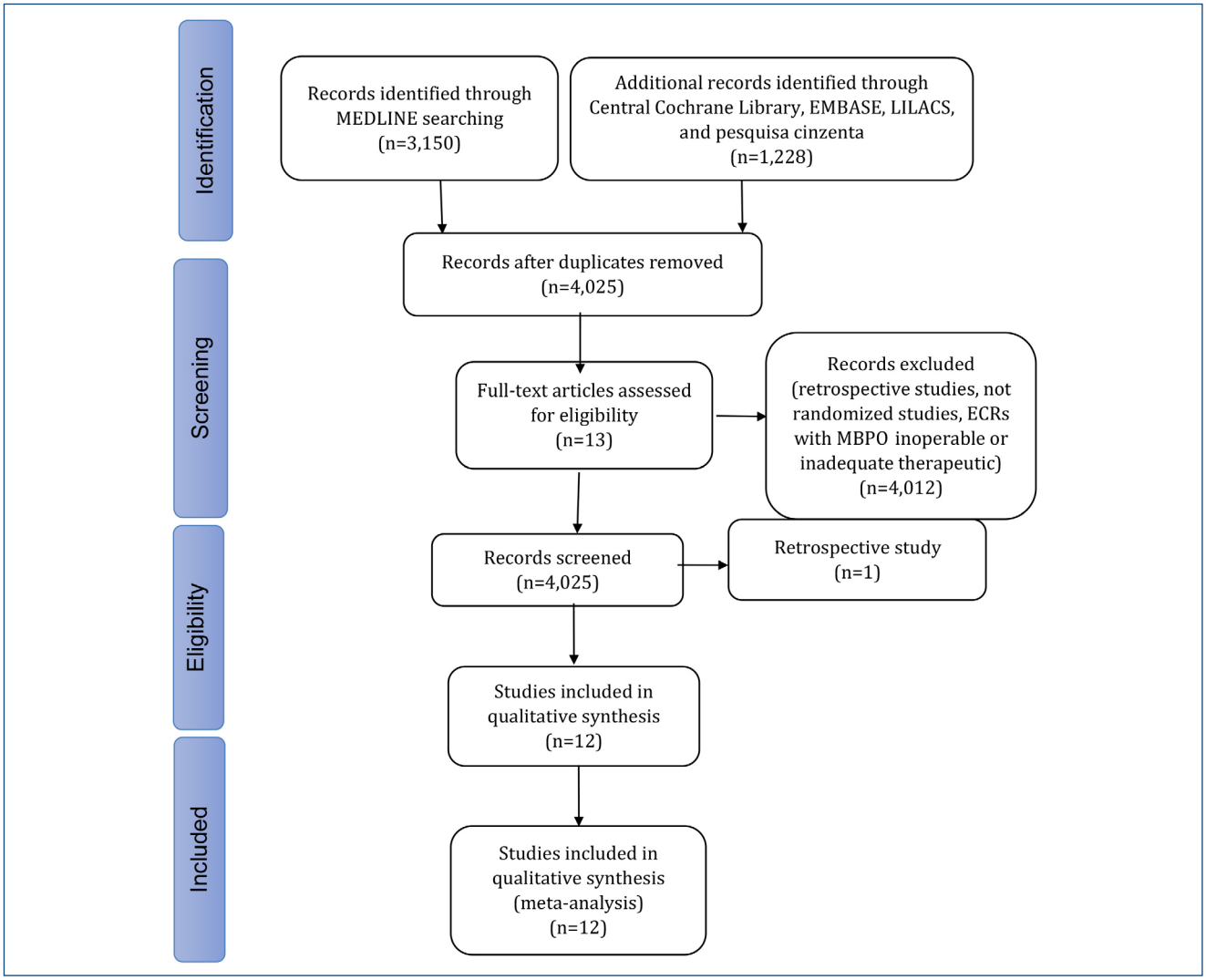

Figure A1. Flow diagram showing the article selection process.

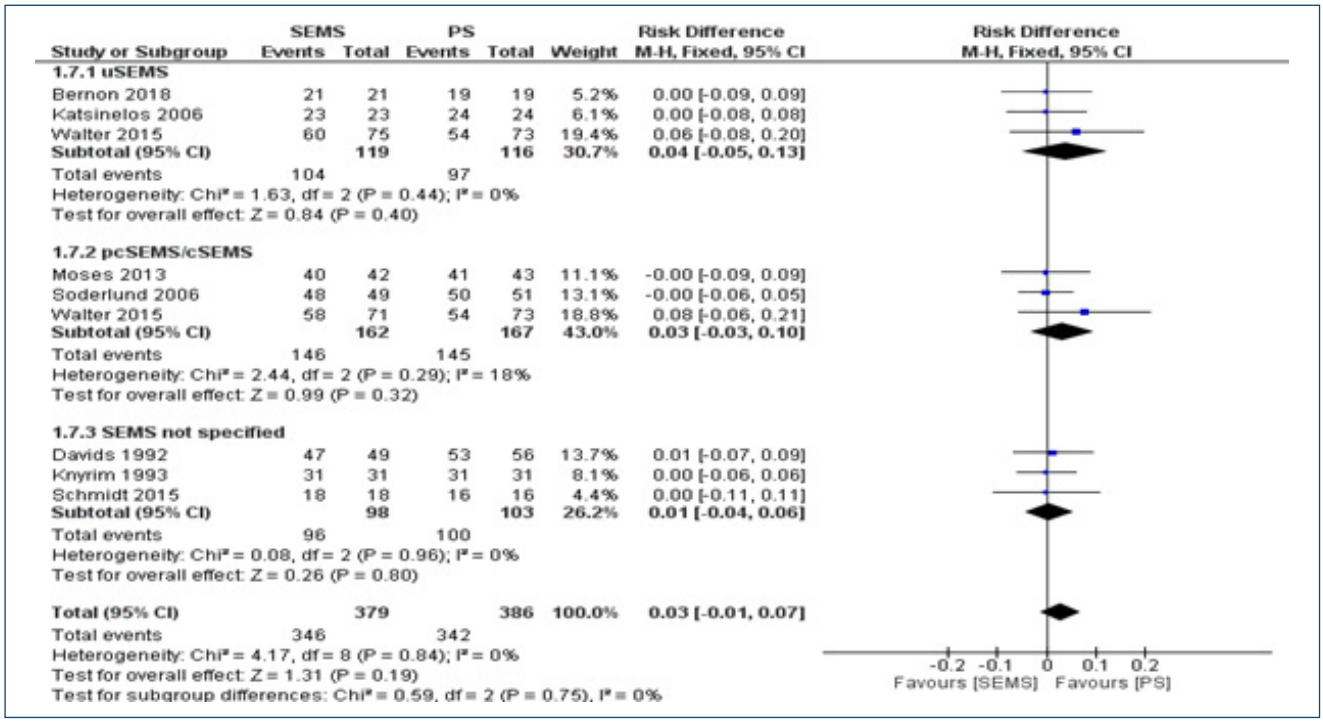

Figure A2. Clinical success – forest plot.

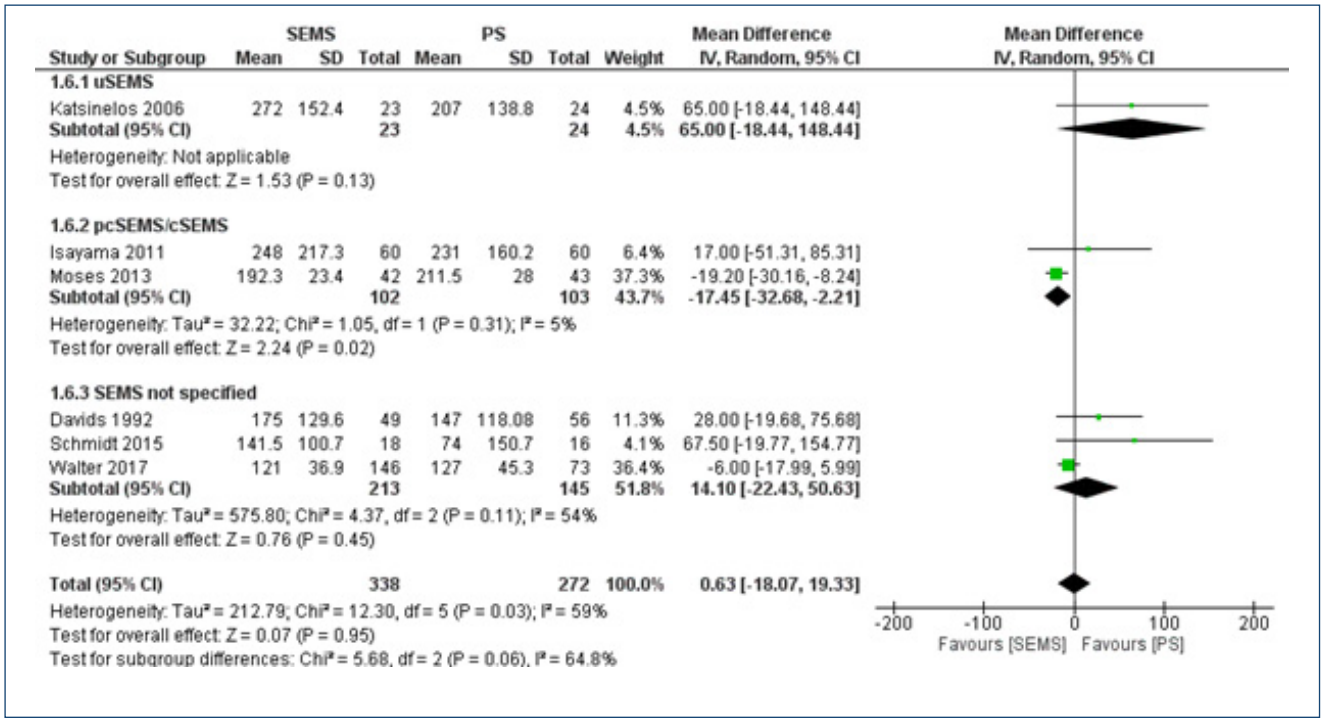

Figure A3. Mean survival (days) – forest plot.

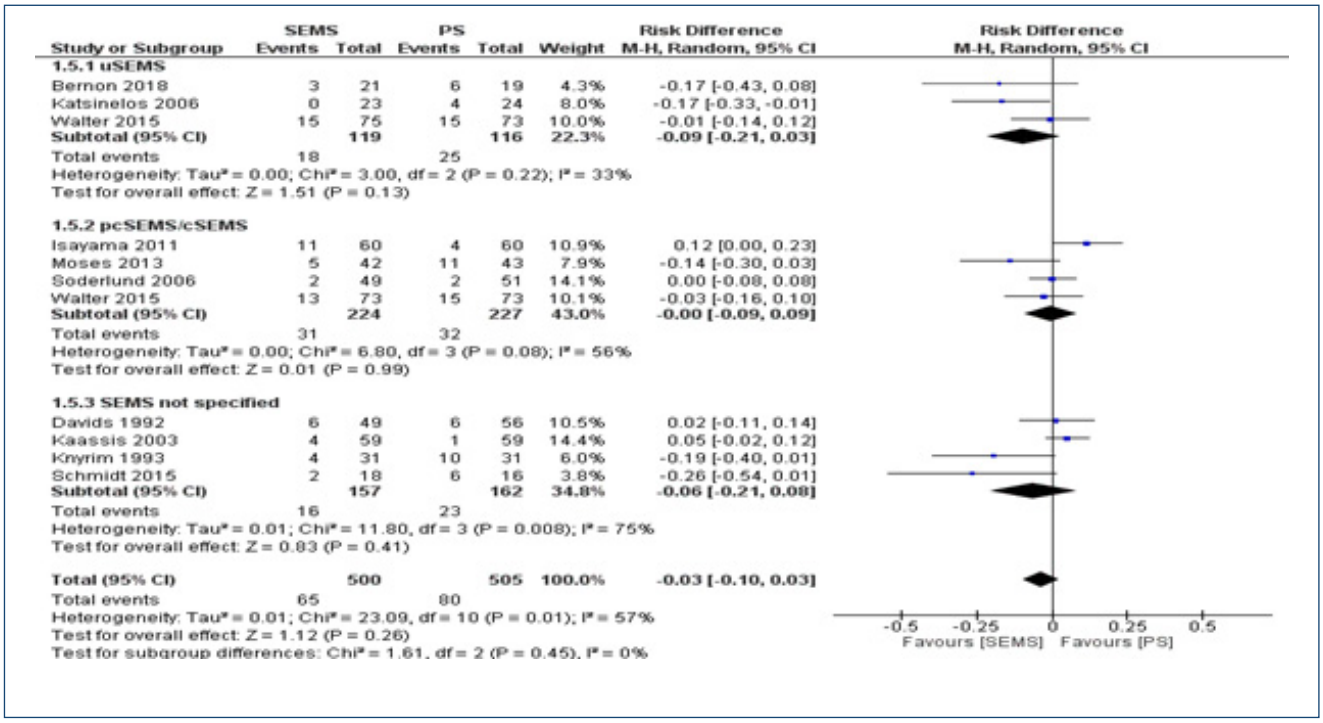

Figure A4. Complications – forest plot.

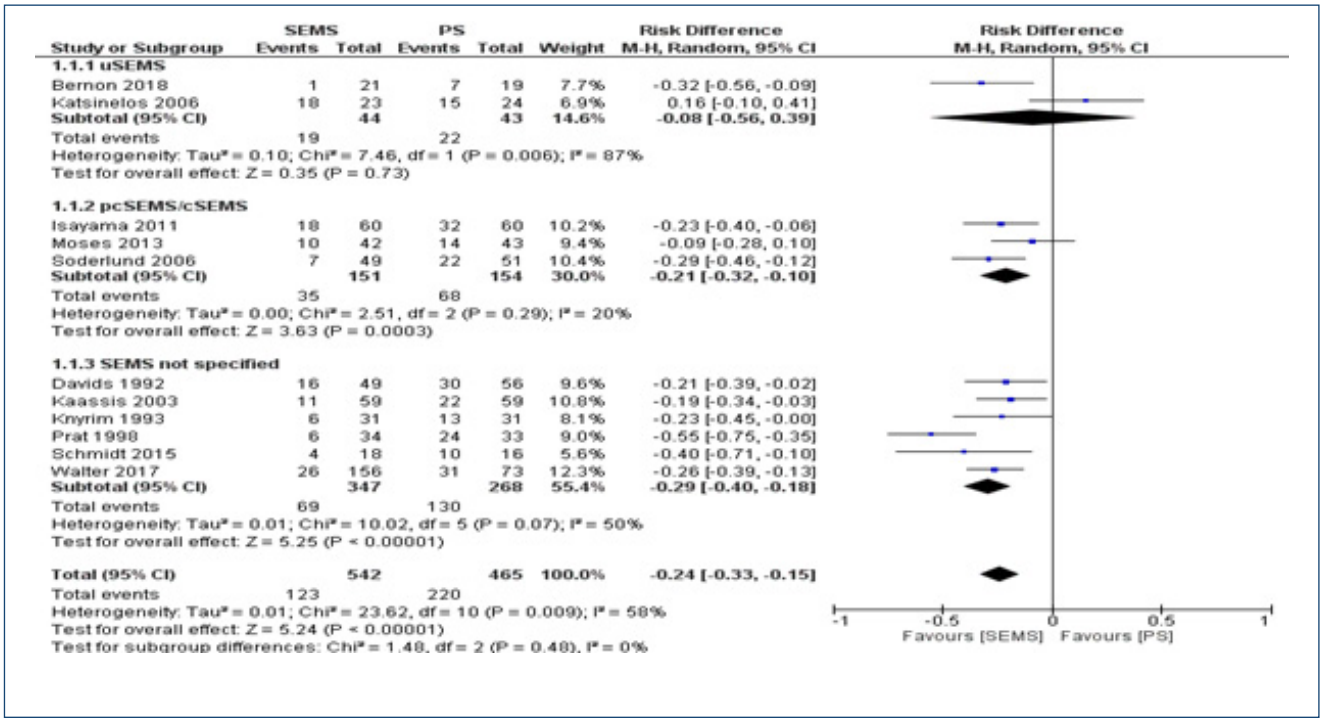

Figure A5. Stent dysfunction – forest plot.

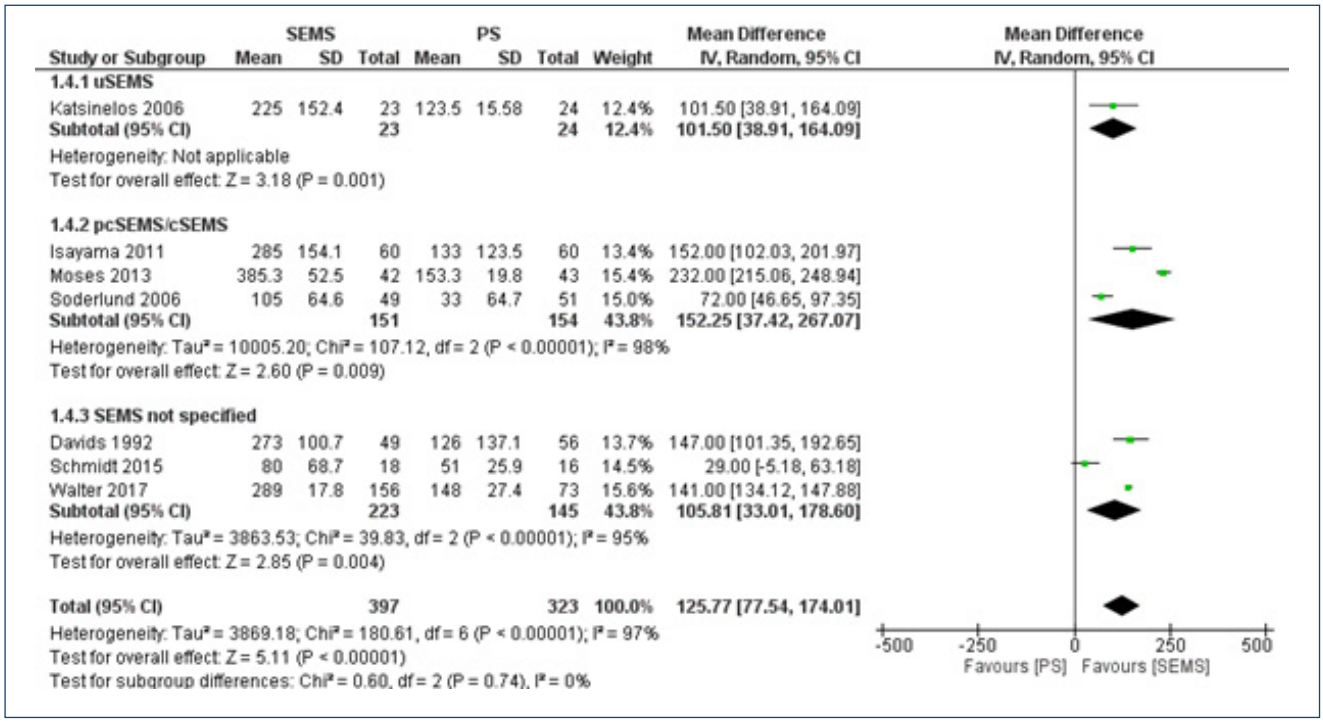

Figure A6. Stent patency (days) – forest plot.

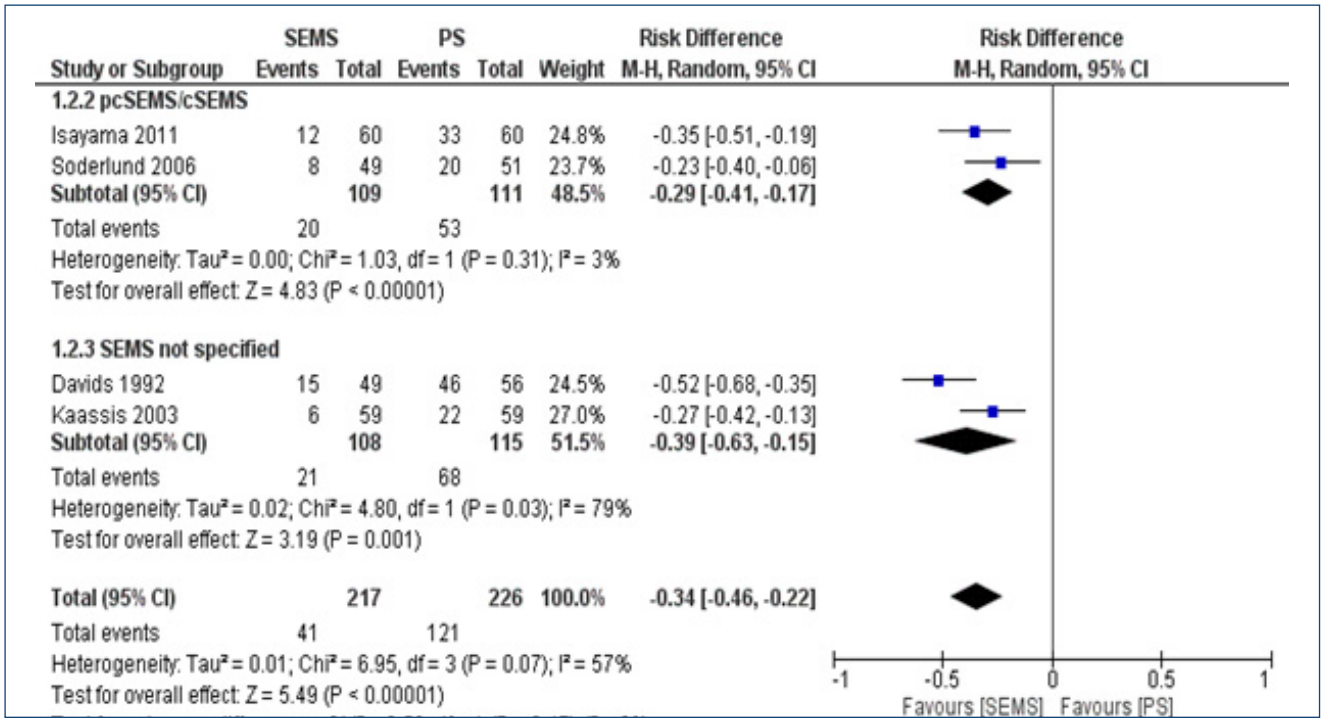

Figure A7. Reinterventions (dichotomic) – forest plot.

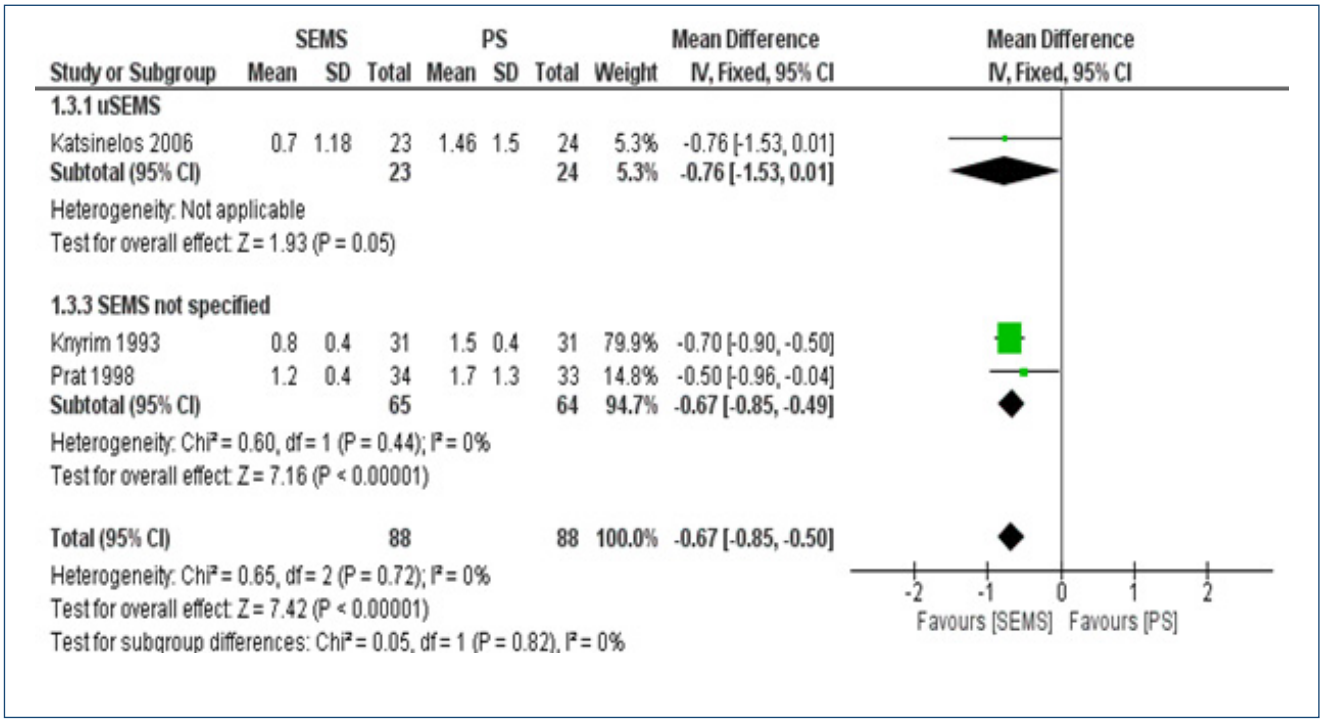

Figure A8. Reinterventions (continuous) – forest plot.

Table A1. Description of risk of biases in therapeutic study (ROB-II).

RCT	APPROPRIATE RANDOMIZATION	BLINDFOLDED ALLOCATION	DOUBLE-BLIND	BLIND AND BLINDED EVALUATOR	LOSS >20%	PROGNOSTIC CHARACTERISTICS	ANALYSIS BY INTENTION OF TREATMENT	SAMPLE CALCULATION	EARLY INTERRUPTION
Davis et al. (1992)	🟢	🟡	🔴	🟡	🟢	🟢	🟢	🟢	🟢
Knyrim et al. (1993)	🟢	🟡	🔴	🟡	🟢	🟢	🔴	🟢	🟢
Prat et al. (1998)	🔴	🟡	🔴	🟡	🟢	🟢	🟢	🟢	🟢
Kaassis et al. (2003)	🔴	🟡	🔴	🟡	🟢	🟢	🟢	🟢	🟢
Katsinelos et al. (2006)	🟢	🟢	🔴	🟡	🟢	🟢	🟢	🟢	🟢
Soderlund et al. (2006)	🔴	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢
Isayama et al. (2011)	🟢	🟡	🔴	🔴	🟢	🟢	🟢	🟢	🟢
Moses et al. (2013)	🔴	🟢	🔴	🔴	🟢	🟢	🟢	🟢	🟢
Schmidt et al. (2015)	🟢	🟡	🔴	🔴	🟢	🟢	🟢	🟢	🟢
Walter et al. (2015)	🟢	🟡	🔴	🔴	🟢	🟢	🟢	🟢	🟢
Walter et al. (2017)	🟢	🟡	🔴	🔴	🔴	🟢	🟢	🟢	🟢
Bernon et al. (2018)	🟢	🟢	🔴	🟡	🟢	🟢	🟢	🟢	🟢

🟢 Appropriatea

🟡 Not available

🔴 Improper

Table A2. Quality of evidence was evaluated by Recommendation Classification, Development, and Evaluation criteria.

Certainty assessment							Summary of findings				
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	Study event rates (%)		Relative effect (95%CI)	Anticipated absolute effects	
							With metal stent	With plastic stent		Risk with metal stent	Risk difference with plastic stent
COMPLICATIONS											
930 (10 RCTs)	Very serious[a,b]	Serious[c]	Not serious	Not serious	None	⊕◯◯◯ Very low	65/432 (15.0%)	65/498 (13.1%)	RR 0.80 (0.58–1.10)	150/1,000	30 fewer per 1,000 (from 63 fewer to 15 more)
STENT DYSFUNCTION											
1007 (11 RCTs)	Very serious[a,b,d]	Serious[c]	Not serious	Not serious	None	⊕◯◯◯ Very low	220/465 (47.3%)	123/542 (22.7%)	RR 0.50 (0.42–0.60)	473/1,000	237 fewer per 1,000 (from 274 fewer to 189 fewer)
SURVIVAL											
610 (6 RCTs)	Serious[a,d]	Serious[c]	Not serious	Not serious	None	⊕⊕◯◯ Low	272	338	–	The mean SURVIVAL was 0	MD 10.33 lower (18.18 lower to 2.47 lower)
DRAINAGE SUCCESS											
692 (8 RCTs)	Serious[a]	Not serious	Not serious	Not serious	None	⊕⊕⊕◯ Moderate	288/313 (92.0%)	346/379 (91.3%)	RR 1.02 (0.98 to 1.07)	920/1,000	18 more per 1,000 (from 18 fewer to 64 more)
REINTERVENTIONS											
443 (4 RCTs)	Very serious[a]	Serious[c]	Not serious	Not serious	None	⊕◯◯◯ Very low	121/226 (53.5%)	41/217 (18.9%)	RR 0.36 (0.27–0.48)	535/1,000	343 fewer per 1,000 (from 391 fewer to 278 fewer)
REINTERVENTIONS											
176 (3 RCTs)	Very serious[a,b]	Not serious	Not serious	Not serious	None	⊕⊕◯◯ Low	88	88	–	The mean REINTERVENTIONS was 0	MD 0.67 lower (0.85 lower to 0.5 lower)
TIME FOR STENT DYSFUNCTION											
710 (7 RCTs)	Very serious[a,d]	Very serious[e]	Not serious	Not serious	None	⊕◯◯◯ Very low	323	387	–	The mean TIME FOR STENT DYSFUNCTION was 0	MD 144.97 higher (138.99 higher to 150.95 higher)

CI: confidence interval; RR: risk ratio; MD: mean difference. [a]Inappropriate randomization; [b]Intention to treat analysis; [c]Heterogeneity >50%; [d]Lost to follow-up >20%; [e]Heterogeneity >75%.

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Side effects and antibody response of an inactive COVID-19 vaccine: correspondence

Rujittika Mungmunpuntipantip[1*], Viroj Wiwanitkit[2]

Dear Editor,

We would like to share ideas on the publication "Side effects and antibody response of an inactive severe acute respiratory syndrome coronavirus 2 vaccine among health care workers[1]." Gümüş et al. concluded that "*vaccination by two-dose CoronaVac could elicit a specific humoral response, and it was well tolerated in health care workers. The high seropositivity developed after the second dose attracted attention. Our study will be useful in terms of showing short-term immunity and side effects*[1]." We agree that the inactivated COVID-19 vaccine can help stimulate immunity against COVID-19. The results from this report is consistent with a recent report in our setting, a developing Asian region, that the inactivated vaccine can provide favorable immunogenicity result[2]. As a classical vaccine produced by a classical vaccinology principle, the vaccine should be safer comparing to other type of vaccines produced by newly implemented biotechnology. The evidence from the present report can confirm the antibody response after vaccination. Nevertheless, it is interesting to further study cellular immune response if possible. Additionally, a long-term follow-up might provide a clear view on the protective utility of the studied vaccine. If there is a dataset of other vaccines in the same setting for comparison, it will also be very interesting.

AUTHORS' CONTRIBUTIONS

RM: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **VW:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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2. Jantarabenjakul W, Chantasrisawad N, Puthanakit T, Wacharapluesadee S, Hirankarn N, Ruenjaiman V, et al. Short-term immune response after inactivated SARS-CoV-2 (CoronaVac®, Sinovac) and ChAdOx1 nCoV-19 (Vaxzevria®, Oxford-AstraZeneca) vaccinations in health care workers. Asian Pac J Allergy Immunol. 2021. https://doi.org/10.12932/AP-250721-1197

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Is atrial fibrillation a preoperative risk factor in elderly patients without heart failure after hip fracture surgery?

Daoyun Lei[1], Jie Sun[1*]

Dear Editor,

We read with great interest the study by Ahmet Lütfullah Orhan[1]. It revealed that the incidence of atrial fibrillation (AF) was significantly higher in the non-surviving group after long-term postoperative follow-up. Using multivariate Cox regression analysis, the authors found that AF, advanced age, and blood urea levels were identified as independent predictors of all-cause long-term mortality. The authors concluded that AF was an independent predictor for long-term death in hip fracture cases above 65 years of age who were free from heart failure. This has important implications for improving the safety of hip surgery and reducing mortality in elderly patients with atrial fibrillation. However, in our opinion, more factors should be taken into consideration for drawing this conclusion.

Firstly, some laboratory parameters and echocardiographic parameters were included to measure cardiac functional status, but laboratory indicators only included blood routine indicators. Adding some myocardial infarction indicators such as troponin, BNP, pro-BNP, etc. and activity tolerance were more conducive to evaluating the cardiac function in elderly patients. Additionally, the author claimed to include patients over 65 years of age, but Table 1 indicated that the mean age of the patients included was over 80 years. Finally, the authors did not state the inclusion and exclusion criteria.

Atrial fibrillation may be associated with high or low blood pressure, fast or slow heart rate, and unknown thrombosis in the perioperative period. However, the effect of these factors on survival was not investigated in this study. Therefore, more clinical data should be collected to exclude the effect of AF in elderly patients with hip fracture so that a conclusion can be reached.

AUTHORS' CONTRIBUTIONS

DL: Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review & editing. **JS:** Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing.

REFERENCE

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ORIGINAL ARTICLE

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Relationship between different body composition and bone mineral density in Qinhuangdao city

Yujian Zhang[1], Xiaojiao Jia[1], Xin Liu[1], Wen An[1], Jiaqi Li[1], Wenli Zhang[2*]

SUMMARY

OBJECTIVE: This study aimed to explore the correlation between different body components and bone mineral density in healthy adults.

METHODS: A total of 306 non-manual subjects, 161 males and 145 females, were selected from the physical examination center of our hospital from June to September 2019. They were divided into control group, overweight group, and obese group according to body mass index. The muscle mass and fat mass, body fat content, trunk fat mass, upper limb and thigh fat mass, bone density of femoral neck and lumbar vertebra, and bone mineral salt content of the whole body were measured by dual-energy X-ray absorptiometry.

RESULTS: Body mass index, systolic blood pressure, diastolic blood pressure, femoral neck bone mineral density, bone mineral salt content, fat mass, muscle mass, upper limb fat mass, thigh fat mass, and trunk fat mass in the overweight group and obese group were all higher than those in the control group ($P<0.05$). The fat mass, muscle mass, upper limb fat mass, and trunk fat mass were positively correlated with the femoral neck bone mineral density, total lumbar vertebra bone mineral density, and bone mineral salt content ($P<0.05$). In addition, thigh fat mass was positively correlated with femoral neck bone mineral density and total lumbar spine bone mineral density, whereas body fat content was negatively correlated with bone mineral salt content.

CONCLUSION: Body composition was related to bone mineral density and bone mineral salt content, and the correlation between different body composition indexes, and bone mineral density, and bone mineral salt content was different.

KEYWORDS: Bone density. Muscles. Body composition.

INTRODUCTION

The human body is composed of water, protein, fat, and inorganic salts and their composition ratio is an essential health indicator. Thus, an unbalanced body composition ratio is an important cause of the development of many diseases. The measurement and analysis of body composition can provide an understanding of the general health and nutrition level of individuals, and to some extent, can also reflect factors such as gender, age, genetics, geographical location, growth, and development. It can also provide valuable information for the diagnosis and treatment of many diseases[1].

Osteoporosis is a systemic skeletal disorder characterized by osteopenia and deterioration of bone microarchitecture, leading to increased bone fragility and fracture risk[2]. The diagnosis of osteoporosis relies on clinical examination, bone mineral density (BMD) assessment, and dual-energy X-ray absorptiometry (DEXA). The measurement of BMD combined with identification of clinical risk factors is currently the gold standard for the diagnosis of osteoporosis[3].

This study aimed to investigate the correlation between body composition, bone mineral content (BMC), and BMD by performing physical examination in a healthy population using DEXA to provide a basis for the comprehensive assessment of body composition and a theoretical basis for the prevention and treatment of osteoporosis.

METHODS

Subjects

The data of 306 subjects who underwent physical examination at the Health Examination Center of the First Hospital of Qinhuangdao between June to September 2019 were retrospectively analyzed, and they all voluntarily underwent DEXA for body composition measurement and analysis. The subjects included 161 males and 145 females (excluding pregnant or lactating women). The study was approved by the Ethics Committee of the First Hospital of Qinhuangdao, and all subjects signed an informed consent form.

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Inclusion criteria

Inclusion criteria were as follows: (1) age 21–65 years; (2) non-manual worker; (3) no history of alcoholism or excessive smoking; (4) no severe liver and kidney disease or chronic obstructive pulmonary disease; (5) no cancer requiring treatment in the past 5 years; (6) no recent history of continuous bed rest for more than 3 months; (7) not taken drugs affecting bone metabolism and body composition (glucocorticoids, estrogen, thyroid hormone, parathyroid hormone, calcitonin, or bisphosphonates); and (8) not taken calcium, vitamin D supplements, or any drugs affecting calcium and vitamin D metabolism.

Grouping

The subjects were divided into three groups according to their body mass index (BMI): subjects with a BMI between 18.5 and 24 kg/m^2 were included in the normal control group (n=107); those with a BMI between 24 and 28 kg/m^2 were included in the overweight group (n=120); and those with a BMI greater than 28 kg/m^2 were included in the obese group (n=79), in accordance with the criteria issued by the National Health and Family Planning Commission of China[4].

Measurements

The measurements were taken using a Discovery QDR 4500 DEXA from Hologic. The instrument was calibrated before the examination to reduce the error. All the subjects were placed in the supine position and the body composition of the patients, including their total body muscle mass and fat mass, body fat content, trunk fat mass, upper limb and thigh fat mass, femoral neck and lumbar spine BMD, and total body BMC, were measured using a bone densitometer.

Statistical analysis

SPSS 13.0 statistical software was used in this study, and the measurement data were expressed as ± s. Analysis of variance (ANOVA) was used for the comparison of the three groups (a Student-Newman-Keuls (S-N-K) test was also used for comparison between the groups). Mono-factor analysis was performed using the Pearson correlation. P<0.05 was considered a statistically significant difference.

RESULTS

General information

A total of 306 subjects were included in this study, of which 161 were males and 145 were females. There were 56 males and 51 females in the normal control group with a mean age of 48.67 ± 7.49 years, 63 males and 57 females in the overweight group with a mean age of 48.91 ± 8.46 years, and 42 males and 37 females in the obese group with a mean age of 47.15 ± 9.91 years. The differences were not statistically significant in age, fasting blood glucose, total cholesterol, and low-density lipoprotein cholesterol among the three groups (see Table 1).

A comparison of body composition and levels of bone mineral density and bone mineral content

Compared with the control group, BMI, systolic blood pressure, diastolic blood pressure, femoral neck BMD, BMC, fat mass, muscle mass, upper limb fat mass, thigh fat mass, and trunk fat mass were all higher in the overweight and obese groups (p<0.05). Moreover, BMI, body fat content, fat mass, muscle mass, thigh fat mass, trunk fat mass, total lumbar spine BMD, and BMC were higher in the obese group than in the overweight group (p<0.05) (see Table 1).

The correlation of body composition with bone mineral density and bone mineral content at different sites

Fat mass, muscle mass, upper limb fat mass, and trunk fat mass were positively correlated with femoral neck BMD, total lumbar spine BMD, and BMC (p<0.05). Thigh fat mass was positively correlated with femoral neck BMD and total lumbar spine BMD, but not with BMC. In addition, body fat content was negatively correlated with BMC, but not with femoral neck BMD and total lumbar spine BMD (see Table 2).

DISCUSSION

In this study, we identified differences in body composition among obese, overweight, and normal-weight healthy subjects, and found correlations between different body composition indexes with BMD and BMC.

Body mass, BMI, and body composition are all important determinants of BMC and the occurrence of osteoporosis[5]. Most studies have concluded that BMD is positively correlated with BMI and that obesity can reduce the risk of osteoporosis, but some studies have shown that obese individuals are more likely to develop osteoporosis[6]. Fat mass has a positive role in peak bone mass acquisition in young male adults, but it accounts for only 1.8% of total body BMD change. In the presence of excess adipose tissue, low-grade inflammation is associated with a greater number of inflammatory cytokines, which negatively affects bone metabolism[7]. Therefore, it is uncertain whether adipose tissue plays

Table 1. Comparison of body composition and levels of bone mineral density and bone mineral content in different groups ($\bar{x}\pm s$).

Variables	Control group	Overweight group	Obese group	F	P
N (M/F)	107(56/51)	120(63/57)	79(42/37)	–	–
Age (years)	48.67±7.49	48.91±8.46	47.15±9.91	1.338	0.276
BMI (kg/cm^2)	22.05±1.55	25.89±1.12*	31.49±3.66*#	406.896	0.000
Systolic blood pressure (mmHg)	123.15±15.13	134.05±18.14*	134.56±18.03*	14.142	0.000
Diastolic blood pressure (mmHg)	76.70±12.61	84.53±12.56*	84.88±11.32*	13.950	0.000
Fasting plasma glucose (mmol/L)	5.73±0.70	6.16±1.74	5.98±1.18	2.948	0.054
Total cholesterol (mmol/L)	4.87±1.01	4.89±0.92	4.59±0.96	2.228	0.110
Low-density lipoprotein cholesterol (mmol/L)	2.84±0.88	2.91±0.81	2.70±0.96	1.244	0.290
Femoral neck BMD	0.74±0.12	0.77±0.11*	0.86±0.11*#	20.928	0.000
Total lumbar spine BMD	1.11±0.12	0.99±0.13	1.06±0.14*#	6.848	0.001
BMC (kg)	2.14±0.36	2.35±0.36*	2.48±0.33*#	18.498	0.000
Body fat content (%)	23.90±5.03	25.44±5.16	27.19±4.62*	3.713	0.039
Fat mass (kg)	15.82±3.72	18.66±3.36*	24.30±4.56*#	97.250	0.000
Muscle mass (kg)	43.53±7.33	52.96±7.37*	62.90±9.48*#	120.713	0.000
Upper limb fat mass (kg)	1.76±0.62	1.95±0.47*	2.75±0.73*#	56.064	0.000
Thigh fat mass (kg)	5.32±1.66	5.61±1.54	7.17±2.05*#	24.321	0.000
Trunk fat mass (kg)	7.53±2.14	9.63±1.94*	12.87±2.69*#	114.724	0.000

*P<0.05 compared with control group; #P<0.05 compared with overweight group.

Table 2. Correlation of body composition with bone mineral density and bone mineral content at different sites.

Variables	Femoral neck BMD		Total lumbar spine BMD		BMC	
	r	p	r	p	r	p
Body fat content (%)	-0.099	0.089	-0.052	0.374	-0.299	<0.001
Fat mass (kg)	0.297	<0.001	0.188	0.001	0.230	<0.001
Muscle mass (kg)	0.476	<0.001	0.279	<0.001	0.652	<0.001
Upper limb fat mass (kg)	0.252	<0.001	0.180	0.002	0.119	0.041
Thigh fat mass (kg)	0.208	<0.001	0.187	0.001	0.063	0.280
Trunk fat mass (kg)	0.280	<0.001	0.124	0.033	0.272	<0.001

a role in BMD. Our study showed that the level of femoral neck BMD and BMC was higher in the overweight and obese groups than in the control group ($p<0.05$). In addition, some studies have shown that blood lipid, blood glucose, and blood pressure have varying degrees of influence on BMD[8,9], but in this study, BMD and BMC have no correlation with blood glucose, blood pressure, and blood lipid, which may be related to the small sample size.

Iwaniec et al.[10] suggested that increased muscle mass and function may increase the positive effects of BMI on bone health. Bone cells can sense the increased mechanical load in obese patients and adapt to this change by increasing the BMD. Fracture risk in obese patients can be prevented by the increased bone load and the prevalence of hip fracture decreases with an increase in BMI in both men and women. Weight gain partially counteracts age-related bone loss. Higher body weight can increase bone tissue load, stimulate bone formation, and reduce bone resorption, which facilitates the increase in BMD and BMC and delays the occurrence of osteoporosis[11]. However, when the BMC is too high, it leads to increased bone stiffness, increased bone fragility, and a relative decrease in bone flexibility, making it prone to fracture. The results of our study found that with the increase of BMI, fat mass and muscle mass tended to increase, especially the

trunk fat mass. In addition, the femoral neck BMD increased in people with a high BMI, but the total lumbar spine BMD did not follow this pattern.

Muscle strength has been reported to be an indicator of muscle mass and function in people of either sex and any age, and muscle strength also correlates with BMC and BMD[12]. Makovey et al.[13] found that the correlation between muscle mass and BMD was stronger than fat mass and BMD in both sexes. Fat mass and muscle mass and their distribution in the body were differentially associated with regional BMD and differed with age. For example, prior to menopause, left upper arm BMD in women was associated with muscle mass, whereas after menopause it was associated with fat mass[14]. Cheng et al.[15] concluded that muscle mass was the main factor affecting the lumbar spine, femoral neck, and total body BMD in all age groups, whereas fat mass was associated only with older men and postmenopausal women. After eliminating the effects of age, gender, and BMI, BMD was correlated with muscle mass but not with fat mass, and upper limb BMD was negatively correlated with the upper limb body fat rate[16].

Bone mineral content was correlated with total body fat in women (regardless of age) and young men, but not in older men. The proportion of lean soft tissue to total bone in the skeleton decreases with age in women but remains stable in men[17]. A study[12] showed that BMC/BMD decreased by 30% between the ages of 20 and 80 years in females, whereas it decreased by only 16% in males from the same age group, with their muscle mass decreasing by 18 and 17%, respectively. A longitudinal study including overweight and normal-weight children aged 9–11 years showed that changes in bone strength were associated with muscle mass, but not with fat mass[18]. The association between body fat content and bone mass was weak or did not exist[19]. Our results showed that fat mass, muscle mass, upper limb fat mass, and trunk fat mass were positively correlated with femoral neck BMD, total lumbar spine BMD, and BMC ($p<0.05$). Thigh fat mass was positively correlated with femoral neck BMD and total lumbar spine BMD, but not with BMC. In addition, body fat content was negatively correlated with BMC but not with femoral neck BMD or total lumbar spine BMD.

The decrease in the amount of activity in men as they age is more likely to lead to lower BMD[20], but effective exercise in youth can increase bone mass and help reduce bone loss due to aging. After exercise, such as weight-bearing aerobics and strength and resistance exercises, BMD loss is significantly reduced in the elderly, although both BMD and muscle strength decrease[20]. Howe et al.[21] concluded that exercise has a relatively small effect on BMD in postmenopausal women. However, another study[22] indicated that a 9-month high-impact jumping intervention can significantly increase leg BMC and bone mass and improve bone stiffness.

In summary, body composition is associated with BMD and BMC, but the increase in BMD and BMC is more closely related to muscle mass than fat mass. Exercise should be advocated as a way of preventing osteoporosis since such an improvement in lifestyle can reduce fat content and increase muscle content. Nevertheless, there are some limitations to this study, including the small number of subjects, the limitation to only one city, Qinhuangdao, and the lack of follow-up data. More studies are therefore needed to confirm the relationship between body composition and BMD to provide a firm basis for the prevention and treatment of osteoporosis.

ETHICS APPROVAL

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Qinhuangdao First Hospital (2019C009).

AUTHORS' CONTRIBUTIONS

YJZ, XJJ: Conception and design of the work. These authors have contributed equally to this study. **XL, WA:** Data collection. **YJZ, XJJ, WLZ:** Supervision. **JQL, WLZ:** Analysis and interpretation of the data. **YJZ, XJJ:** Statistical analysis. **YJZ, XJJ:** Drafting the manuscript. **YJZ, XJJ, WLZ:** Critical revision of the manuscript. All authors: Approval of the final manuscript.

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ORIGINAL ARTICLE

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Relationship between the number of comorbidities, quality of life, and cardiac autonomic modulation in patients with coronary disease: a cross-sectional study

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate if there is a relationship between the number of comorbidities, autonomic modulation, and quality of life in patients diagnosed with coronary artery disease.

METHODS: A cross-sectional study was conducted at an outpatient rehabilitation center in Presidente Prudente-SP, Brazil. A total of 27 participants (65.33±9.23 years) diagnosed with coronary artery disease were assessed, from a cardiac rehabilitation program, independent of sex or age. The number of comorbidities was evaluated using the Self-Administered Comorbidity Questionnaire, and quality of life was evaluated using the Medical Outcome Study 36-Item Short Form Health Survey (SF-36) (eight domains: functional capacity, physical aspects, pain, general health status, vitality, social aspects, emotional aspects, and mental health). To evaluate the cardiac autonomic modulation, the heart rate was registered beat to beat using an heart rate monitor in the supine position during rest for 30 min. A total of 1000 RR intervals were considered to calculate linear (time domain: RMSSD, SDNN; frequency domain: LF, HF, LF/HF) and nonlinear indices (SD1, SD2, SD1/SD2) of heart rate variability.

RESULTS: A negative correlation was observed between the aggregation of comorbidities and the pain domain of the SF-36 (r=-0.427; p=0.03). No significant correlations were observed between other variables (p>0.05).

CONCLUSION: The number of comorbidities is inversely related to the pain domain of the SF-36, suggesting that a higher pain level is related to a higher number of comorbidities in coronary artery disease patients.

KEYWORDS: Comorbidity. Chronic disease. Coronary artery disease. Heart rate. Quality of life.

INTRODUCTION

Chronic disease aggregation, defined as comorbidity[1], promotes impaired functional capacity, reduced quality of life (QoL), and increased mortality[2], and it represents a challenge to health systems, due to the increase in costs and utilization of services[3].

Among chronic diseases, coronary artery disease (CAD) is the main cause of mortality and morbidity in the world[4]. CAD is associated with various chronic diseases, such as osteoarthritis, peripheral arterial disease, chronic obstructive pulmonary disease, diabetes mellitus, asthma, and depression[2].

Reduced QoL[5] and impaired autonomic modulation[6] in CAD patients have been reported in the literature. Previous studies found that reduced QoL could be related to the presence of comorbidities in CAD patients[7-9]. However, although the literature has suggested that a greater number of comorbidities causes longer hospital stays and mortality[10], only one study[8] considered the number of comorbidities to evaluate the relationship between QoL and comorbidities. Likewise, CAD patients with comorbidities are more likely to present reduced heart rate variability (HRV)[11], which indicates autonomic modulation impairment. However, to date, knowledge about the relationship between the presence of comorbidities and autonomic modulation in CAD patients is limited to specific chronic conditions, such as depression[11].

Therefore, it is relevant to investigate if the number of comorbidities associated with CAD is also related to the impairment of cardiac autonomic modulation and QoL. Understanding these aspects may help in the development of public policies for health prevention and promotion and in the identification of patients with worse prognoses, who need greater support during treatment.

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This study aimed to evaluate if there is a relationship between the number of comorbidities, autonomic modulation, and QoL in CAD patients. We hypothesized that a higher number of comorbidities in CAD patients is related to a greater autonomic and QoL impairment.

METHODS

Study design and setting

This was a cross-sectional study, conducted from 2018 to 2019, at the Center for Physical Therapy and Rehabilitation Studies and Treatment of São Paulo State University (UNESP), Faculty of Sciences and Technology, Presidente Prudente (SP), Brazil.

The experimental procedure was divided into two steps. In the first step, an initial assessment was performed, composed of personal data collection, anthropometric evaluation, and application of the Self-Administered Comorbidity Questionnaire[12], to assess the number of comorbidities, and the Medical Outcome Study 36-Item Short Form Health Survey (SF-36)[13] was performed to assess QoL. In the second step, a cardiac autonomic modulation assessment was performed at rest, by recording the heart rate (HR) beat to beat using an HR monitor.

All procedures were approved by the research ethics committee of the institution (CAAE: 79213417.0.0000.5402). Participants were previously informed about the aims and procedures of this study and provided a written informed consent.

This cross-sectional study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations[14].

Participants

A total of 27 patients from a cardiac rehabilitation program (CRP) performed at the Center for Physical Therapy and Rehabilitation Studies and Treatment of São Paulo State University (UNESP), Faculty of Sciences and Technology, Presidente Prudente (SP), Brazil, were invited to participate in the study regardless of sex and age.

The participants met the following eligibility criteria: (1) a medical diagnosis of CAD, regardless of sex and age, and (2) agreed to participate in the study. Exclusion criteria were as follows: participants with atrial fibrillation, who had a pacemaker or cardiac transplant, did not understand the questionnaires or refused to participate in the study, or participants who presented errors >5% on the HRV record.

Sample characterization

For sample characterization, the personal data of the participants (e.g., sex, age, and medications) were collected, and the body weight; height; and waist, abdominal, and hip circumferences were measured.

Exposure variable

Comorbidities evaluation

The "Self-Administered Comorbidity Questionnaire"[12] was used to verify the number of comorbidities. This questionnaire presents 13 previously selected medical conditions (i.e., heart disease, high blood pressure, lung disease, diabetes, stomach disease, kidney disease, liver disease, anemia or another blood disease, cancer, depression, osteoarthritis, back pain, and rheumatoid arthritis) as well as the option to add up to three additional conditions in an open-ended manner. For each condition, the participants were instructed to answer the following questions: "Do you have any of the following problems?" "Do you receive treatment for it?" and "Does it limit your activities?" For each affirmative answer, the participant received 1 point. Considering the 13 defined medical problems and 3 optional conditions, the maximum score is 48 points.

Outcomes

Quality of life evaluation

QoL was assessed using the "Medical Outcome Study 36-Item Short Form Health Survey (SF-36)"[13]. The questionnaire consists of eight multi-item dimensions, namely, functional capacity, physical aspects, pain, general health status, vitality, social aspects, emotional aspects, and mental health. For each dimension, item scores are coded, summed, and transformed on a scale ranging from 0 (worst health) to 100 (best health).

Cardiac autonomic modulation

The analysis of cardiac autonomic modulation was performed using HRV indices. A capture strap was placed on the participants' chest in the region of the distal third of the sternum, and the HR monitor Polar RS800CX (Polar Electro OY, Finland) was placed on the wrist to record HR beat to beat. The participants were instructed to remain awake, without speaking, spontaneously breathing, at rest, in the supine position for 30 min.

All the procedures were performed in a room with a temperature between 21 and 23°C and humidity between 40 and 60%, between 2:00 and 6:00 p.m., to avoid variations in the circadian cycle. Participants were instructed not to consume substances that stimulate the autonomic nervous system for at least 12 h before the evaluation.

For the HRV analysis, the RR interval series was transferred to Polar Precision Performance software (Kempele, Finland)[15].

After digital and manual filtering of the data to eliminate premature ectopic beats and artifacts, 1000 consecutive RR intervals from the period of greatest signal stability were selected. Only series with more than 95% sinus beats were used in the analyses[16]. HRV was analyzed by linear, in the time and frequency domains, and nonlinear methods, calculated using the software Kubios HRV version 2.0 (Kubios, Biosignal Analysis, and Medical Image Group, Department of Physics, University of Kuopio, Finland)[16].

In the time domain, the RMSSD (root mean square of the differences between adjacent normal RR intervals, in a time interval, expressed in milliseconds) and SDNN indices (standard deviation of all normal RR intervals recorded in a time interval, expressed in milliseconds)[15] were calculated.

In the frequency domain, the spectral components of low frequency (LF: 0.04–0.15 Hz) and high frequency (HF: 0.15–0.40 Hz) were used, expressed in milliseconds squared (ms^2) and normalized units (nu). Fourier Fast Transform (FFT) was used as an algorithm for the spectral analysis[15].

For nonlinear HRV analysis, the indices were calculated using quantitative analysis of the Poincaré plot: SD1 (dispersion of the points perpendicular to the line of identity and representing the instantaneous record of the beat-to-beat variability), SD2 (dispersion of points along the identity line and representing HRV in the long-term records), and the SD1/SD2 ratio (the ratio between the short and long duration variations in RR intervals)[15].

Data analyses

For the sample characterization, the descriptive statistical method was used and the results are presented as mean and standard deviation (parametric data) or median and interquartile interval (non-parametric data), minimum and maximum (continuous data), and absolute numbers and frequencies (categorical data).

The normality of the data was assessed by the Shapiro-Wilk test. The relationship between the number of comorbidities and HRV indices and the scores of the SF-36 components was evaluated by the Pearson or Spearman correlation, according to the normality of the data.

The level of significance was set at <5%, and the SPSS statistical package was used (version 22.0) (SPSS Inc., Chicago, IL, United States).

RESULTS

Table 1 presents the characterization of the participants. The sample was composed predominantly of older males (masculine sex: 66.67%, n=18; older people: 74.07%, n=20).

Table 2 shows the results of the Self-Administered Comorbidity Questionnaire. Hypertension (66.7%, n=18), back pain (37%, n=10), and diabetes mellitus (33.3%, n=9) were the main comorbidities reported by the participants.

Table 3 shows the correlation between the number of comorbidities, HRV indices, and QoL components evaluated by the SF-36. A significant negative correlation was found between the number of comorbidities and the pain domain (r=-0.427; p=0.03). No significant correlation was observed between the other SF-36 domains or HRV indices and the number of comorbidities.

DISCUSSION

The present study investigated the relationship between the number of comorbidities, autonomic modulation, and QoL in patients diagnosed with CAD. The main findings suggest that a higher number of comorbidities is related to a higher pain level. Furthermore, the number of comorbidities is not related to cardiac autonomic modulation in CAD patients.

Table 1. Sample characterization.

Variables		
Age (years)	65.33±9.23	44.00–83.00
BMI (kg/m2)	27.12±3.67	19.90–37.83
AC (cm)	96.91±10.13	79.00–120.00
WHR	0.94 [1.12]	0.53–1.72
Medications in use		
Anxiolytic	3 (11.1)	
Platelet antiaggregant	25 (92.6)	
Antiarrhythmic	1 (3.7)	
Anticonvulsants	2 (7.4)	
Antidepressant	4 (14.8)	
Anti-ischemic	3 (11.1)	
Beta-blockers	22 (81.5)	
Diuretic	4 (14.8)	
Hypoglycemic	7 (25.9)	
Hypolipidemic	25 (92.6)	
Proton-pump inhibitors	9 (33.3)	
Levothyroxine	1 (3.7)	
Others	8 (29.6)	
Vasodilator	21 (77.8)	

Data represented as mean±standard deviation; minimum – maximum, median [interquartile range], and number (percentage). BMI: body mass index; AC: abdominal circumference; WHR: waist-to-hip ratio.

The main comorbidities reported were hypertension, back pain, and diabetes mellitus. Hypertension and diabetes mellitus are risk factors for CAD[17,18]. The interaction between a variety of pathophysiological, genetic, and environmental mechanisms is responsible for the genesis of hypertension and the development of related target-organ damage, including CAD[17]. Furthermore, prolonged exposure to the elevated blood glucose levels, associated with other risk factors such as hypertension and dyslipidemia, is responsible for microvascular and macrovascular diabetic complications, such as CAD[18].

Previous studies have also found a strong association between the presence of back pain and the occurrence of CAD[19]. The adoption of a sedentary lifestyle as a result of pain makes the individual more susceptible to the occurrence of cardiovascular diseases such as CAD[19]. Furthermore, data in the literature show that the presence of inflammation, elevated cortisol levels, and sympathetic – parasympathetic imbalances may be common factors between heart disease and back pain[19].

Our results showed a negative correlation between the number of comorbidities and the pain domain of the SF-36, which suggests that a higher number of comorbidities associated with CAD is related to a higher pain level, since a lower score in the SF-36 amounts to a worse condition. This result corroborates with the findings of Assari et al.[8], who also found a negative correlation between the total comorbidity score and the pain domain of the SF-36 in individuals with CAD.

The high prevalence of back pain may justify, at least in part, the correlation observed between the number of comorbidities and the pain domain of the SF-36. According to

Table 3. Correlation between the number of comorbidities, the HRV indexes, and SF-36 domains.

Variables	r	p	
Functional capacity	-0.355	0.07	–
Physical aspects	-0.319	0.11	–
Pain	-0.427	0.03	Moderate
General health status	-0.180	0.37	–
Vitality	-0.185	0.36	–
Social aspects	-0.132	0.51	–
Emotional aspects	-0.140	0.49	–
Mental health	-0.250	0.21	–
Mean RR	0.340	0.08	–
RMSSD	0.211	0.30	–
SDNN	0.015	0.94	–
HF (ms2)	0.246	0.22	–
HF (un)	0.236	0.24	–
LF (ms2)	0.073	0.72	–
LF (un)	-0.230	0.25	–
LF/HF	-0.226	0.26	–
SD1	0.211	0.29	–
SD2	-0.001	1.00	–
SD1/SD2	0.281	0.16	–

Bold indicates statistically significant value. rMSSD: square root of the mean of the square of the differences between adjacent normal RR intervals, expressed in ms2; SDNN: standard deviation of all normal RR intervals recorded in a time interval, expressed in milliseconds; LF: low frequency component; nu: normalized units; HF: high frequency component; LF/HF: LF/HF ratio; SD1: standard deviation of the variability of RR intervals in short term; SD2: standard deviation of RR intervals in long term.

Table 2. Self-Administered Comorbidity Questionnaire results.

Comorbidities	Patients who received treatment				Limitation	
	n	%	n	%	N	%
Heart disease	27	100	26	96.3	10	37
Hipertension	18	66.7	18	100	6	33.3
Lung disease	0	0	0	0	0	0
Diabetes	9	33.3	9	100	2	22.2
Stomach disease	2	7.4	2	100	0	0
Kidney disease	1	3.7	0	0	0	0
Liver disease	0	0	0	0	0	0
Blood disease	0	0	0	0	0	0
Cancer	1	3.7	1	100	0	0
Depression	3	11.1	3	100	0	0
Osteoarthritis	3	11.1	3	100	2	66.7
Back pain	10	37	4	40	6	60
Rheumatoid arthritis	1	3.7	0	0	1	100
Other	5	18.5	2	40	2	40

Vlaeyen et al.[20], the presence of back pain generates negative repercussions on QoL. In addition, other comorbidities that also promote chronic pain, such as osteoarthritis and rheumatoid arthritis[21], were also reported by the study participants.

The presence of pain represents a limiting factor to perform daily life tasks, which contributes to a negative perception of QoL, especially in older people[22]. More than half of all participants were diagnosed with back pain or osteoarthritis, and 100% of participants with rheumatoid arthritis reported having an activity limitation, which corroborates with the literature[20,21]. For this reason, the number of comorbidities should be considered at the time of decision-making regarding the treatment of CAD patients.

No significant correlations were found for the other SF-36 domains. In general, individuals diagnosed with CAD present reduced QoL when compared to individuals without the disease[23]. However, it is important to highlight that exercise-based CRP improves the QoL of CAD patients[24]. Therefore, it is possible to suggest that the participation of study participants in a CRP motivated an improvement in their perception of QoL, despite the number of associated comorbidities.

Regarding cardiac autonomic modulation, no correlation was observed between the number of comorbidities and HRV indices. It has already been well established in the literature that CAD patients present reduced cardiac autonomic modulation compared to the general population[6]. This may have influenced our results, confounding the changes promoted by chronic diseases associated with CAD. Furthermore, data from previous studies suggest that physical exercise programs, such as CRP, can modulate cardiac autonomic control, through the promotion of reduced sympathetic influence and increased parasympathetic tone and, consequently, HRV improvement[25].

Cardiovascular dynamics present a complex structure defined by non-stationary, intermittent, scale-invariant, and nonlinear behaviors[26]. In this context, previous studies have suggested that traditional linear HRV indices are not able to characterize the complex dynamics of heartbeats generation[27]. Also, it has been shown that nonlinear HRV indices can discover new information not obtained by linear HRV indices[28]. Thus, it is possible to suggest that the nonsignificant results that have been found in this study may be due to effect of the limitations of the methods used. Therefore, future studies to determine if the number of comorbidities is related to cardiac autonomic modulation assessed through nonlinear HRV indices in CAD patients may be interesting.

Another point to be discussed is that the HRV analysis may be influenced by different factors, such as age, gender, and body composition[29], and has some limitations for assessing cardiac autonomic dysfunction[30]. However, it is important to highlight that HRV is a validated and widely used method for ANS assessment, and the necessary procedures[29] for an appropriate assessment of autonomic modulation by means of HRV were followed in this study. Future research using other methods, such as the study of the interaction between the regulation of the heart and peripheral blood flow[31], to evaluate the relationship between the number of comorbidities and cardiac autonomic modulation in CAD patients may proportionate relevant information about this topic.

There are some limitations in our study that should be considered. It is important to point out that more than 80% of the participants used beta-blockers, which may alter cardiac autonomic modulation. Niemelä et al.[32] studied the influence of beta-blocker therapy on HRV in individuals with stable CAD and observed improvement in linear indices in the experimental group compared to placebo. Furthermore, the information about comorbidities in this study was self-reported, which could represent a source of error. Finally, the small sample size may also be reported as a limitation. Despite these limitations, to the best of our knowledge, this is the first study to evaluate the correlation between the number of comorbidities and impaired cardiac autonomic modulation in CAD patients.

CONCLUSION

The results suggest that the number of comorbidities is inversely related to the pain domain of the SF-36, which suggests that a higher pain level is related to a higher number of comorbidities in CAD patients. Furthermore, the number of comorbidities is not related to cardiac autonomic modulation or the other SF-36 domains.

AUTHORS' CONTRIBUTIONS

HBV: Conceptualization, Methodology, Investigation, Supervision, Formal Analysis, and Writing – original draft. **VESS:** Conceptualization, Methodology, Investigation, Supervision, and Writing – original draft. **TRMB:** Conceptualization, Methodology, Investigation, and Writing – original draft. **FMV:** Data curation, Formal Analysis, and Writing – review & editing. **MJLL:** Investigation, Data curation, and Writing – review & editing. **AFBB:** Conceptualization, Methodology, and Writing – review & editing. **LMV:** Conceptualization, Methodology, and Writing – review & editing. **ACB:** Investigation and Writing – review & editing. **LCMV:** Conceptualization, Methodology, Project administration, Supervision, and Writing – review & editing.

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ORIGINAL ARTICLE

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Long non-coding RNA HOTAIR induces the PI3K/AKT/mTOR signaling pathway in breast cancer cells

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SUMMARY

OBJECTIVE: The phosphoinositide 3-kinase/protein kinase AKT/mammalian target of rapamycin signaling pathway is essential for proper cellular metabolism and cell growth. However, aberrant activation of this pathway has been linked to the progression and metastasis of breast cancer. Recently, the role of long non-coding RNAs in interfering with the cell signaling pathways involved in cell growth and metabolism has been identified. HOX antisense intergenic RNA is an long non-coding RNA whose abnormal expression has been associated with development, therapy resistance, and metastasis of breast cancer. The purpose of this study was to investigate whether the long non-coding RNA HOX antisense intergenic RNA is linked to the phosphoinositide 3-kinase/protein kinase AKT/mammalian target of rapamycin signaling pathway in breast cancer cells.

METHODS: HOX antisense intergenic RNA was silenced in the breast cancer cell line MCF-7 using siRNAs. Subsequently, the gene expression level of HOX antisense intergenic RNA, PI3K, AKT, and mTOR was assessed using real-time RT-PCR. Also, the 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl-tetrazolium bromide) assay was used to analyze cell proliferation.

RESULTS: The results revealed that HOX antisense intergenic RNA knockdown can downregulate the expression of PI3K, AKT, and mTOR RNAs compared to negative control in MCF-7 cells. In addition, the proliferation of breast cancer cells was significantly reduced following the HOX antisense intergenic RNA silencing.

CONCLUSION: This study may introduce HOX antisense intergenic RNA as a molecule involved in the upregulation of the phosphoinositide 3-kinase/protein kinase AKT/mammalian target of rapamycin signaling pathway in breast cancer cells that may contribute to breast cancer cell proliferation.

KEYWORDS: MCF-7 cells. HOTAIR long non-coding RNA. RNA. Long non-coding. Gene expression.

INTRODUCTION

Breast cancer (BC), the most prevalent malignancy in women, is classified as a heterogeneous group of disorders with extremely varied clinical outcomes[1,2]. Although the specified process and related cellular mechanisms driving BC progression are not fully understood, new technologies and molecular research have significantly increased the understanding of cancer biology and discovered potential anticancer treatment targets.

The significance of non-coding RNAs in the development of various malignancies has recently been addressed in several studies[3,4]. Long non-coding RNA (lncRNA) is a kind of RNA molecule that has a length of more than 200 nucleotides and does not code for proteins[5]. HOX antisense intergenic RNA (HOTAIR) is an lncRNA whose aberrant expression is shown to be linked to BC progression, treatment resistance, and cancer cell metastasis[6]. It has been indicated that HOTAIR can alter the expression of many of the essential genes related to cell signaling pathways, including TGF-β, JAK/STAT, and PTEN pathways, which ultimately leads to increased cancer cell invasion as well as metastasis[7].

The phosphoinositide 3-kinase/protein kinase AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway

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plays a critical role in controlling normal cellular metabolism and cell proliferation[8]. However, abnormal activation of this pathway is shown to be associated with growth, metabolism, and survival of cancer cells in a variety of human cancers, including BC[9]. The activated mTOR signaling pathway has been shown to be associated with decreased patient survival and correlated with a worse prognosis in BC[10]. Understanding the mechanisms involved in regulating this pathway could be a step toward finding effective therapeutic agents to inhibit cancer progression. In this study, we aimed to evaluate whether lncRNA HOTAIR is associated with the PI3K/AKT/mTOR signaling pathway in BC cell line MCF-7.

METHODS

Cell culture

The BC cell line MCF-7 was purchased from the National Cell Bank, Pasteur Institute (Tehran, Iran). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, Grand Island, NY, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2.

RNA interference and transfection

Smart pool siRNA-HOTAIR and scramble sequences (negative control siRNA) were purchased from Dharmacon. Smart pool siRNA-HOTAIR consisted of a mixture of four siRNAs targeting HOTAIR. Target sequences for siRNA-HOTAIR were as follows: 5'-AGACGAAGGUGAAAGCGAA-3', 5'-CAAUAUAUCUGUUGGGCGU-3', 5'-GGGACUGGGAG GCGCUAAU-3', and 5'-CAGUGGAAUGGAACGGAUU-3'.

MCF-7 cells were transfected with siRNA-HOTAIR or scrambled sequences using Attractene transfection reagent (Qiagen) according to the manufacturer's instructions. Briefly, 3×10^4 MCF-7 cells/well were seeded into 24-well plates. The transfection medium was prepared by adding 1.5 µl of Attractene transfection reagent to 100 µl of serum-free DMEM containing siRNA-HOTAIR or scrambled sequences at a final concentration of 100 nM. The tube was incubated at room temperature for 20 min. The mixture was then added drop-wise to cells maintained in serum-free media in 24-well plates. The medium was replaced with DMEM 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin after 5 h. Cells were maintained in a humidified incubator at 37°C with 5% CO_2 and were evaluated 48 h after transfection.

RNA extraction and cDNA synthesis

RNA was extracted from MCF-7 cells transfected with siRNA-HOTAIR and control groups, including MCF-7 cells without transfection or cells transfected with siRNA-scramble according to the manufacturer's instruction (RNX Plus Isolation Kit; Sinaclon, Iran). The RNA purity was assessed by 1% agarose gel electrophoresis, and its concentration was measured using a NanoDrop 2000 UV–Vis Spectrophotometer (Thermo Scientific, USA). The extracted RNA was kept at –70°C until use. The cDNA synthesis was performed using the Prime Script RT reagent kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's instruction. Briefly, 50–100 ng of total RNA in a 10-µl sample volume was reverse-transcribed using both oligo-dT/random hexamer primers. cDNA was kept at -20°C until analysis of gene expression.

Measurement of mRNA expression

Real-time RT-PCR analysis was performed in a total volume of 20 µl, including 1 µl of cDNA, 0.5 µM of each forward and reverse primer, 10 µl of SYBR® Premix Ex Taq™ II (Takara Bio, Inc.), and 8 µl of H_2O. The qPCR reactions were performed on the Rotor-Gene 6000 machine (Corbett Research, Australia) using the universal thermal cycling parameters, including an initial denaturation at 95°C for 30 s, and 40 cycles including a denaturation at 95°C for 5 s, an annealing at 60°C (for HOTAIR, PI3K, and mTOR) and 57°C (for AKT) for 20 s, and an extension at 72°C for 30 s. Finally, a melting curve analysis was performed at 60–95°C.

The primer sequences are mentioned in Table 1. The relative expression levels were normalized to the endogenous control β-actin and were expressed as $2^{-\Delta\Delta Ct}$[11]. Data were expressed as fold changes in the amount of mRNA.

Cell proliferation assay

Cell growth was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) (MTT) assay 48 h post transfection. Briefly, 2×10^3 cells/well were seeded in 96-well plates. MTT solution with a concentration of 5 mg/ml in PBS was added to each well, and plates were incubated at 37°C for 3 h. Thereafter, the supernatants were discarded and the formazan crystals were solubilized by dimethyl sulfoxide (DMSO). The absorbance was measured using a microplate reader (Awareness Stat Fax 2100) at 570 nm.

Statistical analysis

For statistical analysis, the Prism software version 6.07 and Excel version 16.0 were used, and all data were expressed as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the statistical significance of

Table 1. Primer sequences.

Gene name	Forward (5'à3')	Reverse (5'à3')
HOTAIR	GGTAGAAAAAGCAACCACGAAGC	ACATAAACCTCTGTCTGTGAGTGCC
PI3K	GAACGAGTGGTTGGGCAATG	CCTCGCAACAGGTTTTCAGC
AKT	ACAGGTGGAAGAACAGCTCG	ACAGGTGGAAGAACAGCTCG
mTOR	GCTTGATTTGGTTCCCAGGACAGT	GTGCTGAGTTTGCTGTACCCATGT
β-Actin	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA

the results. A $p<0.05$ was considered to show significant differences between groups.

RESULTS

Transfection and gene-silencing efficiency

To find the optimal time for HOTAIR silencing, RNA isolation and analysis of the HOTAIR expression were performed at different time points, including 12, 24, 48, and 72 h (data not shown). Then, RNAs were extracted from both transfected and control groups 48 h post transfection as the optimal time. The interference efficiency of siRNA-HOTAIR was determined by real-time RT-PCR through measurement of the expression of HOTAIR in the transfected cells group compared to the negative control group. Our findings showed that the expression of HOTAIR was significantly decreased in cells transfected with siRNA-HOTAIR compared to cells transfected with siRNA-scramble or cells without transfection ($p<0.05$) (Figure 1A).

HOX antisense intergenic RNA silencing could downregulate phosphoinositide 3-kinase/protein kinase AKT/mammalian target of rapamycin signaling pathway in MCF-7 cells

To investigate the effect of HOTAIR silencing on the PI3K/AKT/mTOR signaling pathway in MCF-7 cells, the expression of *PI3K*, *AKT*, and *mTOR* genes was assessed in both transfected and control groups using real-time RT-PCR. As shown in Figure 1, the mRNA levels of PI3K, AKT, and mTOR in MCF-7 cells were significantly decreased in cells transfected with siRNA-HOTAIR compared to cells transfected with siRNA-scramble or cells without transfection ($p<0.05$ for PI3K and mTOR and $p<0.01$ for AKT).

HOX antisense intergenic RNA silencing decreased the proliferation of MCF-7 cells

To determine the effect of the HOTAIR knockdown on cell growth, the MTT assay was performed. The optical density (OD) values were used to assess the proliferation rate. As shown in Figure 2, HOTAIR silencing resulted in a significant decrease in the percent of MCF-7 cells compared with the cells transfected with scrambled sequences or cells without transfection ($p<0.05$). This finding may confirm that knockdown of lncRNA-HOTAIR could result in suppression of the proliferation of MCF-7 cells.

DISCUSSION

In recent years, significant efforts have been made to enhance the diagnosis and treatment of BC. Nevertheless, the pathogenic mechanism involved in BC progression remains largely unknown. Identifying the molecular mechanisms underlying cancer development and progression may be important in clinical prognosis, patients' survival, and application of efficient therapies. Recently, the role of lncRNAs, a major class of newly identified non-coding transcripts, in the pathogenesis of several malignancies, including BC, has been shown to have great importance[3]. lncRNAs exert their regulatory effects at both transcriptional and post-transcriptional levels. They can regulate a variety of cellular processes by influencing or interacting with different elements, including protein, DNA, and RNA molecules[12]. In this study, we aimed to evaluate the potential role of lncRNA HOTAIR in regulating the PI3K/AKT/mTOR signaling pathway in BC cells.

HOTAIR was shown to be one of the most upregulated cancer-associated lncRNAs in BC. It has been demonstrated that HOTAIR interacts with the Polycomb Repressive Complex 2 (PRC2) to alter chromatin state and promote cancer metastasis[7,13]. Accumulating evidence suggests that high level of HOTAIR is correlated with a worse prognosis of patients with BC[14,15]. A study by Yu et al.[16] showed that HOTAIR silencing could significantly inhibit MCF-7 cell proliferation and increase apoptosis of MCF-7 cells through regulating the P53/AKT/JNK signaling pathway. Accordingly, the present study indicated a decreased proliferation of MCF-7 cells following knockdown of HOTAIR. However, HOTAIR silencing in other cancer cells, including oral squamous cell carcinoma[17],

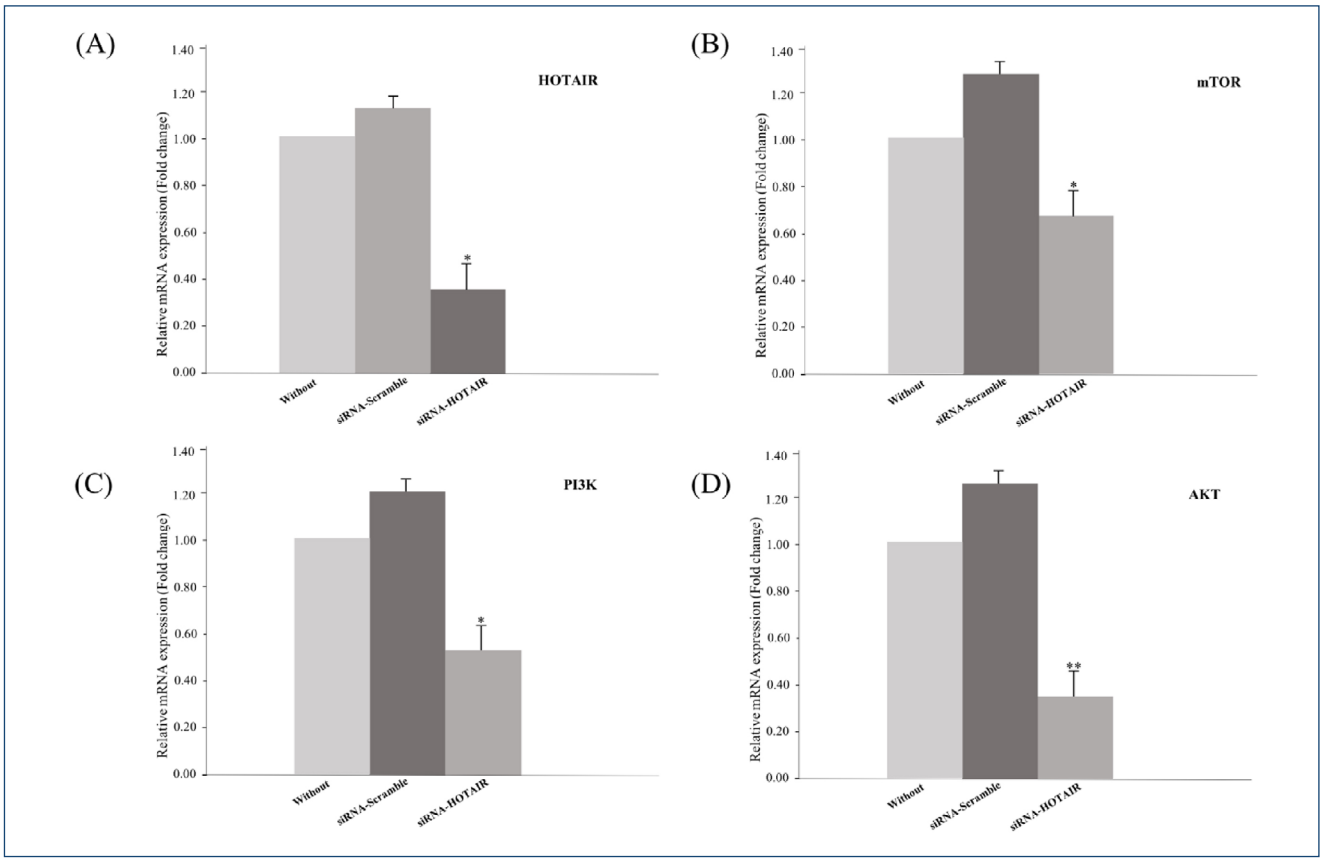

Figure 1. The expression level of HOTAIR, mTOR, PI3K, and AKT in MCF-7 cells without transfection and in MCF-7 cells transfected with siRNA-scramble or siRNA-HOTAIR. Silencing of HOTAIR by siRNAs downregulated the expression of *HOTAIR* (A), *mTOR* (B), *PI3K* (C), and *AKT* (D) genes in MCF-7 cells. Data presented as relative gene expression changes in the treated cells compared to the controls (*p<0.05, **p<0.01). HOTAIR, HOX antisense intergenic RNA; lncRNA, long non-coding RNA; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinases; AKT, protein kinase AKT.

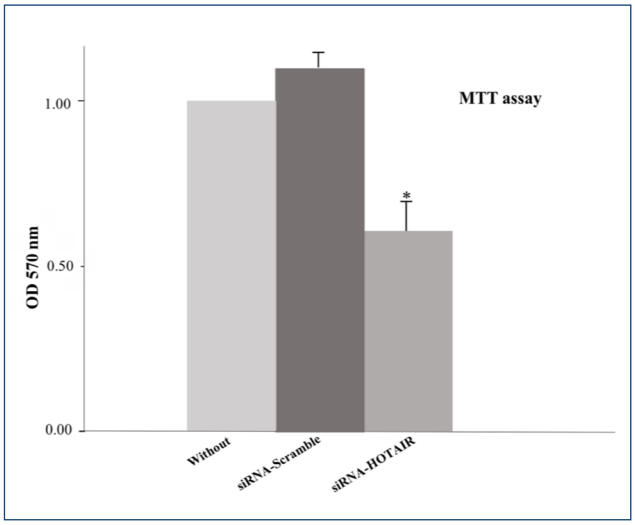

Figure 2. HOTAIR silencing decreased the proliferation of MCF-7 breast cancer cells. The MTT assay was performed in MCF-7 cells without transfection and in MCF-7 cells transfected with siRNA-scramble or siRNA-HOTAIR. Values are expressed as the mean±standard deviation (n=3). *p<0.05 compared to the control group. HOTAIR, HOX antisense intergenic RNA; lncRNA, long non-coding RNA; OD, optical density.

gastric cancer[18], and lung cancer[19], has reduced the invasion and metastasis of cancer cells. These data support the hypothesis that HOTAIR could be considered an important mediator in cancer cell proliferation, survival, and metastasis.

Among the tumor-associated signaling pathways, the PI3K/AKT/mTOR signaling pathway plays a central role in regulating the expression of a variety of signaling molecules involved in cell proliferation, survival, apoptosis, and metastasis[20,21]. On the other hand, this pathway itself can be regulated by many mediators in the tumor microenvironment as well as some hormones in BC. Interestingly, prolactin-mediated activation of the PI3K pathway may be involved in proliferation and cytoskeletal dynamics, leading to the progression of mammary tumors[22]. Therefore, identifying the regulators of this pathway may be an important step toward inhibiting cancer progression through targeted therapies.

Since the significant role of HOTAIR has been shown in increasing the proliferation of several types of cancer cells, in this study, we hypothesized whether this lncRNA could serve a role

in inducing the PI3K/AKT/mTOR signaling pathway. In this study, we suggested the lncRNA HOTAIR as an upstream molecule involved in the upregulation of the PI3K/AKT/mTOR signaling pathway (Figure 3). HOTAIR silencing resulted in a significantly reduced expression level of PI3K, AKT, and mTOR molecules. In accordance with the present study, previous studies have also shown the important role of HOTAIR in inducing the mTOR signaling pathway in several types of cancers. Li et al. revealed that HOTAIR could enhance osteosarcoma cell growth through activation of the AKT/mTOR signaling pathway. In their study, silencing of HOTAIR using siRNAs reduced the phosphorylation of AKT/mTOR signaling pathway proteins[23]. Furthermore, a previous study demonstrated that the knockdown of HOTAIR decreases doxorubicin resistance in BC cells via the PI3K/AKT/mTOR signaling pathway, demonstrating that HOTAIR could be considered a therapeutic target for BC therapy[24]. Furthermore, a study by Hui et al. showed the association between the upregulated HOTAIR and

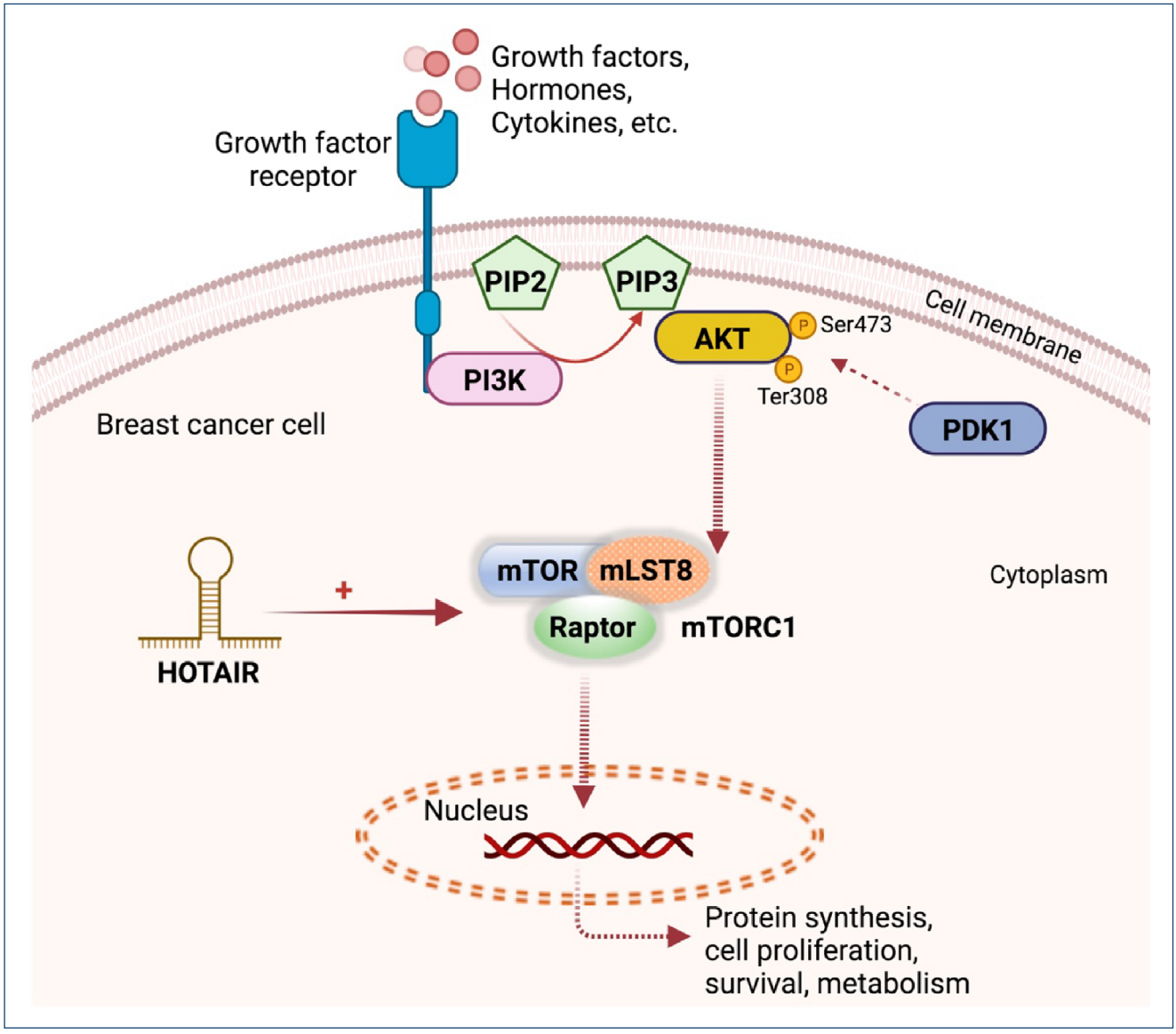

Figure 3. Role of lncRNAs HOTAIR in inducing the mTOR signaling pathway. The mTOR pathway, consisting three principal molecular components, namely, PI3K, AKT, and mTOR, plays an important role in regulating several pivotal cellular processes such as cell proliferation, survival, apoptosis, metabolism, and metastasis. Thus, the lncRNAs HOTAIR silencing using siRNAs, as a therapeutic approach, may result in decreasing cancer progression. HOTAIR may act as a molecule upstream of the PI3K/AKT/mTOR signaling pathway in BC cells, which may ultimately be involved in inducing tumor growth. HOTAIR, HOX antisense intergenic RNA; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinases; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PDK1, phosphoinositide-dependent kinase 1. Image created with BioRender.com.

abnormal activated PI3K/AKT pathway in adenocarcinoma of the esophagogastric junction[25].

Consequently, based on the obtained findings, it can be concluded that lncRNA HOTAIR can play a role in BC progression, maybe through upregulation of the PI3K/AKT/mTOR signaling pathway. Although the exact mechanism of HOTAIR in inducing the gene expression of PI3K, Akt, and mTOR molecules is not yet known, it is thought that HOTAIR can directly or indirectly affect the activity of transcription factors and their expression. It is possible that HOTAIR regulates the gene expression of PI3K, AKT, and mTOR by interaction with the regulators of this pathway.

Therefore, further studies are required to investigate the interaction between this lncRNA and regulators of this pathway, as well as the activation state of proteins under the influence of HOTAIR.

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AUTHORS' CONTRIBUTIONS

MS: Conceptualization, Data curation, Formal Analysis, Validation, Experimental work, Writing – original draft. **KM:** Conceptualization, Writing – review & editing, Validation. **HRMM:** Conceptualization, Formal Analysis, Writing – review & editing. **FN:** Conceptualization, Formal Analysis, Writing – review & editing. **AM:** Conceptualization, Formal Analysis, Writing – review & editing. **SA:** Conceptualization, Formal Analysis, Writing – review & editing. **NR:** Conceptualization, Data curation, Formal Analysis, Validation, Writing – review & editing, Project Administration.

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ORIGINAL ARTICLE

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Risk factors for mediolateral episiotomy at a tertiary hospital: a cross-sectional study

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SUMMARY

OBJECTIVE: The main aim of this study was to assess the associated factors for selective mediolateral episiotomy at a tertiary, academic hospital.

METHODS: A retrospective cohort analysis between 2017 and 2019 was performed. The primary outcome was the prevalence of selective mediolateral episiotomy. Independent variables were maternal, intrapartum, and neonatal characteristics. A significance level of 5% was established, and univariate and multivariate analyses with logistic regression models were performed.

RESULTS: From 2,761 vaginal deliveries eligible for inclusion during this period, the prevalence of selective mediolateral episiotomy was 18.7%. Univariate analysis has shown that non-white women were protective factors (OR=0.77 [0.63–0.96]; p=0.02) for episiotomy; primiparity (OR=2.61 [2.12–3.21]; p<0.01), number of vaginal examinations between 6–10 repetitions (OR=3.16 [2.48–4.01]; p<0.01) and 11–20 repetitions (OR=5.40 [3.69–7.90]; p<0.01), longer second stage duration (OR=1.01 [1.00–1.02]; p<0.01), and women with gestational age more than 37 weeks were risk factors. Multivariate analysis reported that second stage duration (AOR=1.01 [1.00–1.03]; p<0.01), primiparity (AOR=2.03 [1.34–3.06]; p<0.01), and number of vaginal examinations between 6–10 repetitions (AOR=2.36 [1.50–3.70]; p<0.01) and 11–20 repetitions (AOR=3.29 [1.74–6.20]; p<0.01) were remained as risk factors for selective mediolateral episiotomy.

CONCLUSION: A higher number of vaginal examinations during labor (over six repetitions), longer duration of second stage labor, and primiparity were risk factors associated with selective mediolateral episiotomy.

KEYWORDS: Episiotomy. Risk factors. Second stage labor. Childbirth. Cervical dilatation.

INTRODUCTION

Episiotomy is defined as an incision in the vagina and perineum carried out by a trained attendant to enlarge the vaginal opening[1]. Most of the current guidelines agree that episiotomy should not be performed routinely and that, when indicated, mediolateral episiotomy (MLE) should be the option of choice[2]. In cases where instrumental delivery is not planned, selective episiotomy results in fewer women with severe perineal trauma. Moreover, the World Health Organization (WHO) has recommended a 10% rate for episiotomy, and these suggestions have an impact on the rate of this procedure worldwide[3].

However, there is no consensus about evidence-based, specific clinical indications for performing selective episiotomy. Most commonly specified reasons are fetal distress, shoulder dystocia, and perineal trauma prevention[4]. An U.S. study has found that private attending, prolonged second stage deliveries, fetal macrosomia, and epidural analgesia were associated with episiotomy[5]. Despite decreasing episiotomy rates in several countries (the United States with 11.6% in 2012), several demographic characteristics were associated with the receipt of this technique, such as white women and commercial insurance; rural and academic hospitals were associated with less use[6]. In Canada, these rates have dropped to 6.5% for spontaneous vaginal deliveries[7]. It is possible that providing adequate knowledge on this topic will help in reducing these rates. In Brazil, less than one-third of obstetricians reported that they perform episiotomies in less than 20% of their cases[8]. It is important to understand the associated factors with selective episiotomy so that preventive measures can be implemented if higher rates are found. We sought to assess the factors associated with selective episiotomy in a tertiary, referral, and academic hospital.

METHODS

We performed a retrospective cohort analysis of 2,846 singleton vaginal births between April 2017 and February 2019. The study occurred in a tertiary maternity hospital and received the approval of the Institutional Review Board from Women's Hospital,

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University of Campinas – Brazil (CAAE 88954218.2.0000.5404 – June 6, 2018). Electronic medical records and printed medical charts from the maternity database were thoroughly analyzed. After this process, the data collected were organized into a spreadsheet for the assessment of incorrect typing and missing data. Women submitted to cesarean section and twin pregnancies were excluded from the present analysis. This study followed the STROBE (strengthening the reporting of observational studies in epidemiology) statement[9].

MLE is the standard technique for performing this procedure in our institution. No midline episiotomies were found in the retrieved records. We could not obtain more specific details of the technique (e.g., length, depth, and angle) as this was a retrospective chart review. However, the surgical technique is standardized, and careful attention is provided to all these topics. The procedure is generally performed under the supervision of the head of the obstetric ward and the chief resident. Local and/or regional anesthesia is provided. No specific instruments are used to perform an episiotomy (e.g., Epi-Scissors™). In cases of instrumental delivery and severe perineal trauma (third and fourth degree), prophylactic antibiotics are usually performed.

Diagnosis of perineal trauma was performed by trained obstetricians according to the Royal College of Obstetricians and Gynecologists guidelines. Details of the study methodology were recently published by our research group[10]. In this study, the primary outcome was the presence of selective MLE (yes/no). The secondary outcomes were defined as follows: maternal outcomes (i.e., age, ethnic, marital status, gravidity, parity, gestational age during labor, and amniotic fluid index); intrapartum outcomes (i.e., induced or spontaneous labor, forceps, number of vaginal examinations during labor, fetal presentation, and duration of the second stage), severe perineal trauma (i.e., third and fourth degree), and neonatal outcomes (i.e., birthweight, 1- and 5-mine Apgar, and head circumference).

Statistical analysis

For statistical analysis, we used Intercooled Stata version 13.0 software (StataCorp, LLC, College Station, TX, USA). Continuous and categorical variables were compared by the Student's t-test and chi-square or Fisher's test, respectively. Significance level was set at 5%. Logistic regression models for univariate and multivariate analysis were performed, and odds ratio (OR) with 95% confidence intervals (CI) were built. The cutoff point for including the variables at the multivariate analysis was every variable whose p-value was <0.05. Missing data from patients that were more than 50% were not included in the study. Imputation methods were not applied to missing data variables. Considering a study power of 90%, a 5% alpha level, and a suggested prevalence of 10% by WHO, we would need 1,046 women to study this variable (G.Power version 3.1.9.4, Germany).

RESULTS

Between April 2017 and February 2019, we selected 2,846 records of women who delivered in the maternity, 85 of whom were excluded due to incomplete electronic medical records (Figure 1). A total of 2,761 women were included for further analysis. In our study, the episiotomy rate was 18.7%. Maternal, intrapartum, and neonatal outcomes are presented in Table 1.

More than 88% of women were below 35 years of age, and 66.3% were classified as white. Most women presented a gestational age between 37 and 40 weeks (65.9%), followed by <37 weeks (25.7%) and >40 weeks (8.4%). Primiparity represented more than half of the evaluated cases (52.3%), and instrumental delivery was performed in 192 (6.95%) cases (all forceps-assisted deliveries). Obstetric and anal sphincter injuries (third- and fourth-degree perineal tear) were noted in 517 cases. Of these, 506 (18.32%) occurred in women who did not undergo an episiotomy and 11 (0.39) occurred in women who underwent an episiotomy. Head circumference was predominantly ≥33 cm (79.4%), and macrosomia was found in 65 (2.5%) newborns.

A higher duration of second stage was noted in the episiotomy group (p<0.01). In the univariate analysis, gestational age between 37–40 weeks (OR 1.75; 95%CI 1.32–2.33; p<0.01) and >40 weeks (OR 1.87; 95%CI 1.20–2.90; p<0.01) was associated with episiotomy. This trend was not observed in the multivariate analysis. However, the number of digital vaginal examinations was associated with episiotomy in univariate and multivariate analyses. Women who received 6–10 digital vaginal examinations increase the odds of undergoing an episiotomy by threefold (OR 3.16; 95%CI 2.48–4.01; p<0.01). When the number of digital vaginal examinations reached 11–20 repetitions, the odds of women being submitted to episiotomy increased by above fivefold (OR 5.40; 95%CI 3.69–7.90; p<0.01). After adjusting to maternal age, parity, gestational age, race, number of vaginal examinations, newborn sex and weight, head circumference, and the number of digital vaginal examinations in 6–10 and 11–20 repetitions remained associated with episiotomy (AOR 2.36; 95%CI 1.50–3.70; p<0.01 and AOR 3.29; 95%CI 1.74–6.20; p<0.01, respectively). A higher duration of the second stage also remained in the final analysis for episiotomy. Finally, primiparity increased the odds of undergoing episiotomy by twofold in both univariate and multivariate analyses (Table 2).

Figure 1. Flowchart describing the steps of the study.

DISCUSSION

This retrospective cohort analysis has found a prevalence of selective MLE of 18.7%. Significant differences were observed regarding race, gestational age more than 40 weeks, primiparity, and intrapartum outcomes (i.e., use of instrumental delivery, number of digital vaginal examinations, and the duration of the second stage of labor) between the presence and absence of episiotomy. In univariate analysis, gestational ages (37–40 weeks and >40 weeks), primiparity, duration of the second stage, and number of digital vaginal examinations

Table 1. Sociodemographic, maternal and neonatal variables according to the presence of mediolateral episiotomy.

Variables	No episiotomy	Episiotomy	p-value*
Age (years), n (%)			0.06
<35	1,964 (80.76)	468 (19.24)	
≥35	280 (85.11)	49 (14.89)	
Race, n (%)			**0.02**
White	1,466 (80.02)	366 (19.98)	
Non-white	778 (83.75)	151 (16.25)	
Marital status, n (%)			0.53
Without partner	865 (80.69)	207 (19.31)	
With partner	1,397 (81.65)	310 (18.35)	
Presentation, n (%)			0.07
Cephalic	2,215 (81.11)	516 (18.89)	
Pelvic	23 (95.83)	1 (4.17)	
Gestational age (weeks), n (%)			**<0.01**
<37	465 (87.08)	69 (12.92)	
37–40	1,090 (79.33)	284 (20.67)	
>40	137 (78.29)	38 (21.71)	
Parity, n (%)			**<0.01**
Primiparity	1,080 (74.69)	366 (25.31)	
2–3 Gestations	878 (86.76)	134 (13.24)	
≥4 Gestations	286 (94.39)	17 (5.61)	
Amniotic fluid index, n (%)			0.20
Oligohydramnios	44 (89.80)	5 (10.20)	
Normal	1,859 (81.18)	431 (18.82)	
Polyhydramnios	17 (89.47)	2 (10.53)	
Type of delivery, n (%)			0.44
Spontaneous	1,484 (82.22)	321 (17.78)	
Induced	607 (80.93)	143 (19.07)	
Instrumental delivery, n (%)			**<0.01**
No	2,237 (87.08)	332 (12.92)	
Yes	7 (3.65)	185 (96.35)	
Number of vaginal examinations, n (%)			**<0.01**
0–5	1,045 (90.87)	105 (9.13)	
6–10	936 (75.91)	297 (24.09)	
11+	105 (64.81)	57 (35.19)	
Fetal macrosomia, n (%)			0.36
No	2,044 (81.37)	468 (18.63)	
Yes	50 (76.92)	15 (23.08)	

Continue...

Table 1. Continuation.

Variables	No episiotomy	Episiotomy	p-value*
Duration of the second stage (min)	37.07±32.75	54.93±37.19	**<0.01**
Head circumference (cm), n (%)			0.06
<33	443 (84.06)	84 (15.94)	
≥33	1,640 (80.47)	398 (19.53)	
Newborn sex, n (%)			0.06
Male	1,015 (79.80)	257 (20.20)	
Female	1,077 (82.72)	225 (17.28)	
Apgar 1 min, n (%)			0.22
7–10	1,940 (81.48)	441 (18.52)	
0–6	144 (77.84)	41 (18.78)	
Apgar 5 min, n (%)			0.15
7–10	2,057 (81.11)	479 (18.89)	
0–6	30 (90.91)	3 (9.09)	
OASIS, n (%)			0.21
No	2,213 (98.62)	31 (1.38)	
Yes	506 (97.87)	11 (2.13)	

*Chi-square test for binomial variables and Student's t-test for continuous variables. OASIS: obstetric anal sphincter injuries. Bold indicates statistically significant values.

were associated with episiotomy. After multivariate analysis, higher number of digital vaginal examinations, higher length of second stage duration, and primiparity remained associated with selective MLE.

Episiotomy rates around the world varies considerably. There are low numbers such as 9.7% in Sweden and countries achieving as high as 100% in Taiwan[11]. This large differences in the rates of episiotomy is related to the episiotomy policies applied worldwide[2]. In our service, the performance of episiotomy is restricted to the selective episiotomy policy, in which the clinical judgment is applied to determine the need to perform it and to certify if the benefits outweigh the harms in critical situations[12].

The number of digital vaginal examinations increased the risk of performing episiotomy by twofold (6–10 examinations) and threefold (11–20 examinations) in the present multivariate analyses. The labor progress assessment is one of the main tools carried out in intrapartum care, combined with different assessments in the partograph including the dilatation of the cervix os, fetal descent, and fetal position[13]. Although fetal descent and position may be assessed externally, the digital vaginal examination is routinely used for the assessment of the cervix

Table 2. Univariate and multivariate analysis for obstetrical and neonatal variables associated with mediolateral episiotomy.

Variables	Crude OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value
Age (>35)	0.73 (0.53–1.01)	0.06		
Non-white color	0.77 (0.63–0.96)	0.02		
Gestational age 37–40 weeks	1.75 (1.32–2.33)	**<0.01**	1.65 (0.93–2.93)	0.08
Gestational age 40+ weeks	1.87 (1.20–2.90)	**<0.01**	1.38 (0.59–3.21)	0.46
Aminiotic fluid index	1.30 (0.69–2.44)	0.42		
OASIS (Yes)	1.55 (0.77–3.11)	0.21		
Duration of second stage	1.01 (1.00–1.02)	**<0.01**	1.01 (1.00–1.03)	**<0.01**
Marital status (with partner)	0.94 (0.77-1.14)	0.53		
Type of delivery	1.09 (0.87–1.35)	0.44		
Fetal macrosomia (Yes)	1.31 (0.73–2.35)	0.37		
Female newborn sex	0.82 (0.68–1.01)	0.06		
Head circumference	1.27 (0.99–1.66)	0.06		
Apgar 1 min	0.95 (0.89–1.01)	0.12		
Apgar 5 min	1.02 (0.93–1.13)	0.63		
Number of vaginal examinations (6–10)	3.16 (2.48–4.01)	**<0.01**	2.36 (1.50–3.70)	**<0.01**
Number of vaginal examinations (11–20)	5.40 (3.69–7.90)	**<0.01**	3.29 (1.74–6.20)	**<0.01**
Primiparity	2.61 (2.12–3.21)	**<0.01**	2.03 (1.34–3.06)	**<0.01**

OR: odds ratio; CI: confidence interval; OASIS: obstetric and anal sphincter injuries; adjusted for: Maternal age, parity, second stage duration, gestational age, race, number of vaginal examinations, newborn sex and weight, head circumference; n=762. Bold indicates statistically significant values.

os. A vaginal examination is recommended in case of uncertainty whether the woman is in established labor[14]. In the first stage of delivery, vaginal examination is recommended every 4 h and hourly in the second active stage, or in response to the woman's wishes[15].

There is evidence that vaginal examination may interfere with labor progress in some women by causing pain and distress and raising their anxiety compared with less invasive tools for the assessment of labor progress, digital vaginal examination was found to cause negative experiences[16]. In a study comparing ultrasonography and digital vaginal examination, the latter consistently over-estimated cervical dilation when compared with ultrasonography[17].

Moreover, intrapartum digital vaginal examination presented a higher median pain score than intrapartum transtibial ultrasound (4.5 against 0), with no difference in pain scores obtained for digital vaginal examination by clinicians with different experiences[18].

To the best of our knowledge, only one study reported that the episiotomy rate was increased in the digital vaginal examination group. This randomized controlled trial showed that episiotomy was performed more frequently in the digital vaginal examination group (9.8%) than in the transperineal ultrasound group (7.1%); however, the difference between these two groups was not statistically significant (p=0.66)[19].

The rates of episiotomy and the frequency of advanced perineal trauma seem to be higher in primiparous women[20]. In the present study, primiparity increased the risk of performance of episiotomy by twofold in both univariate and multivariate analyses. Episiotomy was performed in 77.2% in the first delivery in a retrospective study.

Interestingly, the study reported that the risk of undergoing a spontaneous perineal tear or an episiotomy in the second delivery is increased by the performance of episiotomy in the first one (AOR 3.27, 95%CI 2.37–4.51)[21]. In contrast with our study, a systematic review found no clear evidence of a difference between primiparity–multiparity and episiotomy in a subanalysis[2]. Selective episiotomy also seems to have a protective effect in primiparous women, lowering the risk of severe perineal trauma[22].

Our results found, in univariate and multivariate analyses, an association between the duration of the second stage of labor and episiotomy. A prolonged second stage of labor increases the risk of perineal trauma[23]. The second stage of labor for more than 2 h increased the risk of perineal trauma by 1.42 (AOR 1.42; 95%CI 1.28–1.58)[24].

Gestational age between 37–40 weeks and above 40 weeks increased the risk of performance of episiotomy in 1.75 and 1.87 times, respectively, in univariate analysis. After adjusting for confounders in multivariate analysis, this trend could not be observed. Similar to our findings, a large retrospective cohort study found that gestational age was a risk factor for episiotomy in both nulliparous (AOR 1.07) and multiparous (AOR 1.06) women[25].

As a strength of this study, it was performed in a large tertiary hospital in the southeast region of Brazil with a considerable number of included women. This study also raised a critical discussion regarding the role of digital vaginal examination in the performance of episiotomy. Prospective, controlled studies are necessary to investigate whether vaginal examination should be performed with caution in the intrapartum scenario. Limitations concerning the study design of retrospective analysis should be taken into consideration. Finally, our analysis is related to one single-center practice, and it might have interfered in our results.

AUTHORS' CONTRIBUTIONS

GMVP: Conceptualization, Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft. **RCA:** Conceptualization, Data curation, Formal Analysis, Investigation, Visualization. **AGL:** Conceptualization, Data curation. **MAN:** Conceptualization, Data curation. **GJL:** Conceptualization, Data curation. **LGOB:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, and Writing – review & editing.

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ORIGINAL ARTICLE

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Tumorigenic and immunological roles of Heat shock protein A2 in pancreatic cancer: a bioinformatics analysis

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SUMMARY

OBJECTIVE: Heat shock protein A2 has been reported to be tightly associated with tumorigenesis and tumor progression. This study aimed to determine the oncogenic and immunological roles of Heat shock protein A2 in pancreatic cancer by bioinformatics.

METHODS: Expression of Heat shock protein A2 in tumorous and normal specimens of pancreatic cancer was analyzed using the Cancer Genome Atlas and the Cancer Genome Atlas + Genotype-Tissue Expression data sets, respectively. Relationships of Heat shock protein A2 expression with immune infiltrates in pancreatic cancer were assessed. Heat shock protein A2-associated coexpressed genes in pancreatic cancer were obtained, followed by the implementation of enrichment analysis.

RESULTS: The data demonstrated that Heat shock protein A2 was significantly overexpressed in tumorous samples compared with normal samples. Heat shock protein A2 expression was remarkably positively interrelated with CD8+ T cell, neutrophil, dendritic cell, and macrophage, but not with CD4+ T and B cells. Heat shock protein A2 expression was markedly positively relevant to both cancer-associated fibroblast and endothelial cell. Enrichment data revealed that Heat shock protein A2 was intimately involved in the tumorigenesis and progression of pancreatic cancer.

CONCLUSION: Heat shock protein A2 is upregulated in pancreatic cancer and is closely associated with tumor immunity and aggressive progression.

KEYWORDS: HSPA2. Pancreatic cancer. Carcinoma. Pancreas. Gene expression.

INTRODUCTION

Pancreatic cancer (PC) is a fatal solid malignancy that seriously endangers human health and is the seventh leading cause of malignancy-related death worldwide[1]. Due to the rapid progression of the disease, most patients first identified have reached an advanced stage, losing the best time for curative resection. Moreover, PC is insensitive to radiotherapy and chemotherapy. Targeted therapy and immunotherapy are currently the most promising adjuvant anticancer therapies. Therefore, the search for biomarkers associated with tumor immunity and aggressiveness is a significant insight for the development of new targeted therapies or immunotherapies for PC.

Heat shock-associated 70-kDa proteins (HSP70s) are a family of stress proteins with an approximate 70-kD molecular weight that have antioxidative, antiapoptosis, and immunoregulatory functions[2]. HSPA2, also known as HSP70-2, is one of the elements of the HSP70s group, initially identified in male germ cells and is associated with spermatogenesis[3]. Former publications have demonstrated that HSPA2 is highly expressed in many cancers, including malignancies of the cervix, bladder, esophagus, lung, liver, colorectum, breast, and pancreas[4-15], suggesting that HSPA2 is involved in cancer development. Moreover, immunoinformatics analysis has showed an intimate association of HSPA2 with immune responses in several human tumors, revealing the potential of HSPA2 as a molecular biomarker for cancer immunotherapy[16].

Although HSPA2 overexpression has been reported, the immunological role and biological function of HSPA2 have not been elucidated in PC. Therefore, this study evaluated the expression, clinical value, immunological effect, biological role, and potential mechanisms of HSPA2 in PC through multiple bioinformatics platforms. These data contribute to our understanding of the oncogenic and immunological roles of HSPA2 in PC from a cancer omics perspective.

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METHODS

Data acquisition and gene expression analysis

The mRNA expression data of 178 tumor specimens and 77 normal specimens were downloaded from the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). These specimens were obtained from 234 cases, including 177 TCGA-PAAD, 49 ORGANOID-PANCREATIC, and 8 HCMI-CMDC. Some cases provided multiple specimens. The tumor specimens were all from TCGA-PAAD cases. Among the 77 normal specimens, 4 were from TCGA-PAAD cases, 55 were from ORGANOID-PANCREATIC cases, and 18 were from HCMI-CMDC cases. The HSPA2 mRNA data were then extracted using Perl software, and the difference in expression between tumor and normal tissues was analyzed using R software.

The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) (http://gepia2.cancer-pku.cn/), an open platform matching TCGA and Genotype-Tissue Expression (GTEx) data, was used for analyzing the expression difference of HSPA2 mRNA between tumorous and normal tissues[17]. The cutoff values for log2FC and p were designated as 0.5 and 0.01, respectively. The 171 normal specimens combined the tissues of TCGA-PAAD and GTEx. Moreover, the association of HSPA2 expression with pathological staging and survival was estimated by the GEPIA2 server.

Assessment of Heat shock protein A2 in relation to immune infiltrates

We evaluated the correlations between HSPA2 with the abundance of infiltrating immune cells such as T cell (CD8+ and CD4+), neutrophil, dendritic cell (DC), macrophage, and B cell in TCGA-PAAD tumor tissues using the Tumor IMmune Estimation Resource version 2.0 (TIMER 2.0; http://timer.cistrome.org/), an open platform for comprehensive estimation of tumor-infiltrating immune cells based on the TIMER algorithm[18]. In addition, we analyzed the associations of HSPA2 with infiltrating stromal cells such as cancer-associated fibroblast (CAF) and endothelial cell based on the EPIC algorithm using the TIMER 2.0 server. The results were displayed as scatterplots. Purity-adjusted Spearman's test was used to obtain correlation (Cor) coefficients.

Acquisition of Heat shock protein A2-related coexpressed genes

LinkedOmics (http://www.linkedomics.org/login.php) is a free access database containing multi-omics data from 32 TCGA tumors, designed with three modules, namely, LinkFinder, LinkInterpreter, and LinkCompare, depending on their functionality[19]. In this study, the LinkFinder module of the LinkedOmics webserver was used to obtain coexpressed genes associated with HSPA2 in PC.

Enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (https://david.ncifcrf.gov/home.jsp) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses on HSPA2-associated coexpressed genes to understand the biological functions of HSPA2 in PC[20]. GO functional annotation includes biological process, cell component, and molecular function.

Statistical analysis

The statistical analyses were conducted using R software version 4.0.3 (https://www.r-project.org/). Differences of gene expression in different pathological stages were estimated by F-test. Survival plots were drawn by the Kaplan-Meier method. Log-rank was used to compare survival differences. Spearman's test was used for correlation analysis. Differences were considered statistically significant at $p<0.05$.

RESULTS

Expression of Heat shock protein A2 in pancreatic cancer tissues

The Cancer Genome Atlas data indicated that HSPA2 mRNA expression was evidently increased in PC specimens compared with nontumor samples ($p<0.05$; Figure 1A). Similarly, the GEPIA2 website, which integrated TCGA and GTEx data, also demonstrated that HSPA2 mRNA was significantly upregulated in tumor samples compared to normal samples ($p<0.05$; Figure 1B). However, no significant differences in HSPA2 expression were observed among the different pathological stages ($p>0.05$). Survival analysis also did not reveal significant differences in disease-free survival (DFS) and overall survival (OS) between patients with high and low HSPA2 expression ($p>0.05$).

Associations of Heat shock protein A2 with immune infiltrates in pancreatic cancer

To explore the immunological role of HSPA2 in PC, we evaluated the correlations between HSPA2 expression and immune infiltrates. First, we evaluated the associations of HSPA2 expression with infiltrating immune cells through the TIMER 2.0 website based on the TIMER algorithm. The results presented that HSPA2 expression was evidently positively interrelated with CD8+ T cell, neutrophil, DC, and macrophage, but not

with CD4+ T and B cells (Figure 2). We then evaluated the correlations of HSPA2 with infiltrating CAF and endothelial cell based on the EPIC algorithm through the TIMER 2.0 platform. The results revealed a remarkable positive associativity between HSPA2 expression and both CAF and endothelial cell infiltration abundance (Figure 2).

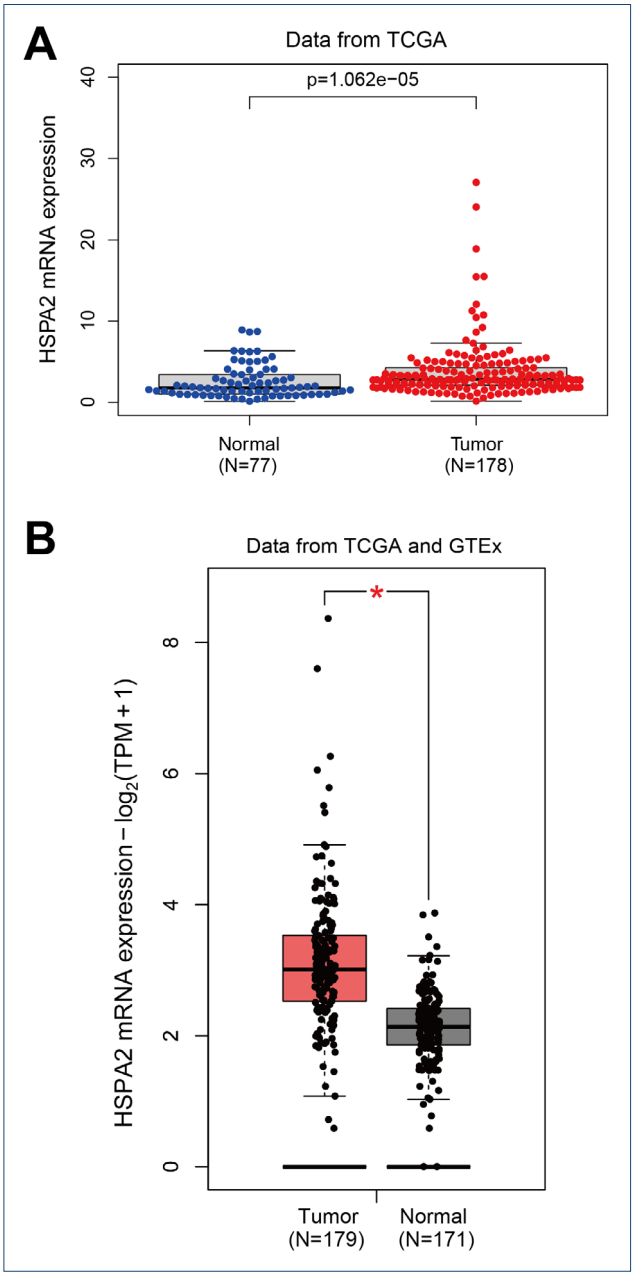

Figure 1. Expression of HSPA2 in PC. (A) Differential expression of HSPA2 between tumor and normal specimens in the TCGA data set. (B) Expression difference of HSPA2 between tumor and normal specimens after combining TCGA and GTEx data sets. HSPA2, heat shock protein A2; PC, pancreatic cancer; TCGA, the Cancer Genome Atlas; GTEx, Genotype-Tissue Expression. *p<0.05.

Gene ontology and Kyoto encyclopedia of genes and genomes pathway analyses data

Coexpression analysis of the LinkedOmics program revealed that there were 1,240 and 342 genes with distinct positive and negative association with HSPA2, respectively (FDR<0.05). The GO functional annotation revealed that HSPA2-related coexpressed genes were principally involved in the extracellular matrix, cell adhesion, focal adhesion, extracellular matrix binding, collagen catabolic process, extracellular space, extracellular region, and so on (FDR<0.001; Figure 3A). The KEGG pathway analysis indicated that these genes were enriched in multiple tumor-related signaling pathways, including PI3K-Akt signaling pathway, MAPK signaling pathway, TGF-β signaling pathway, and so on (FDR<0.05; Figure 3B).

DISCUSSION

In this study, we comprehensively evaluated the expression, clinical significance, immunological role, and biological function of HSPA2 in PC through different bioinformatics platforms. Our results demonstrated that HSPA2 was markedly upregulated in PC specimens compared to non-tumorous specimens. The TIMER algorithm revealed that HSPA2 expression was positively correlative with CD8+ T cell, neutrophil, DC, and macrophage, but not with CD4+ T and B cells. It is worth noting that the EPIC algorithm in the TIMER2.0 server presented a positive correlativity between HSPA2 and infiltrating CAF and endothelial cell. Enrichment analysis revealed that HSPA2 was strongly linked to multiple cancer-related biological processes and signaling pathways.

Many studies have demonstrated that HSPA2 was overexpressed in diverse malignancies, including PC[4-15]. HSPA2 expression has also been reported to increase with progressive tumor stage in PC. Enhanced HSPA2 expression resulted in an obvious reduction of DFS and OS in PC. Consistent with previous reports, the present study confirmed that HSPA2 expression was remarkably elevated in PC samples versus non-cancerous samples. Inconsistently, we did not find any correlation between HSPA2 expression and the pathological stage and survival of PC. The reason for these different results may be that the sample size of PC in the TCGA database is not large enough. Additionally, the gene expression data in the TCGA project were obtained by RNA sequencing, which may differ from the PCR and immunohistochemistry results in the two studies that have been reported[14,15]. Therefore, further investigations are needed to determine the prognostic effect of HSPA2 on PC.

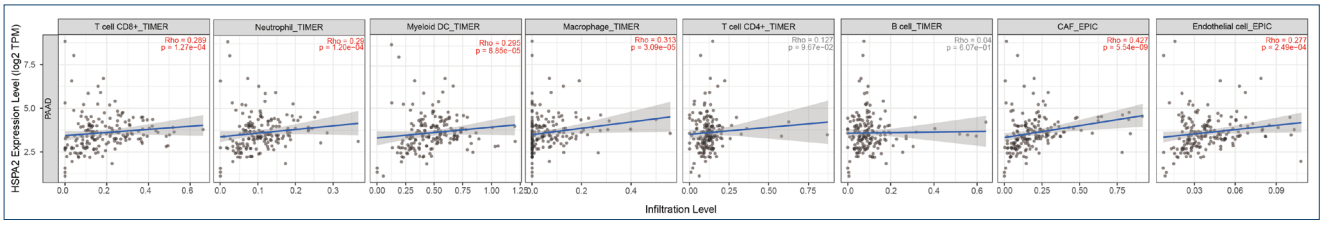

Figure 2. Association of HSPA2 with the abundance of immune infiltrates in PC. HSPA2, heat shock protein A2; PC, pancreatic cancer; DC, dendritic cell, CAF, cancer-associated fibroblast; TIMER, Tumor IMmune Estimation Resource. PAAD represents the TCGA abbreviation for pancreatic cancer.

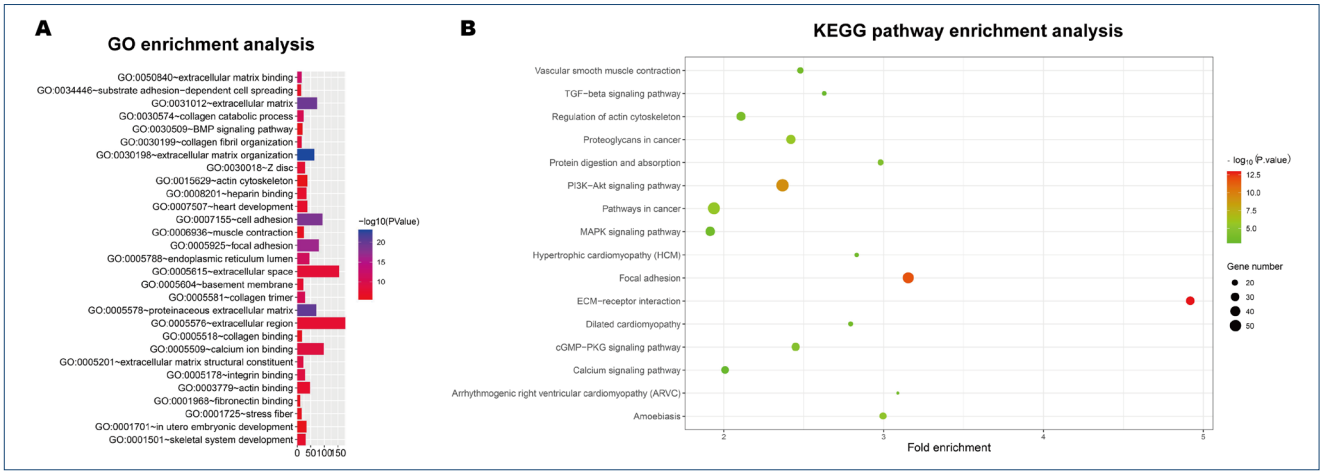

Figure 3. GO and KEGG pathway enrichment analyses of HSPA2-associated coexpressed genes in PC. (A) Bar graph of strongly enriched GO annotation for HSPA2-associated coexpression genes (FDR<0.001). The y-axis indicates the GO annotation item and the x-axis indicates the number of genes enriched in the GO item. (B) Bubble plot of the prominently enriched KEGG pathway for HSPA2-associated coexpressed genes (FDR<0.05). The y-axis indicates the KEGG pathway name and the x-axis indicates the fold enrichment (GeneRatio divided by BgRatio). Higher values of fold enrichment indicate higher levels of enrichment. The size and color of the bubbles in the graph represent the number of enriched genes and the p-value, respectively. HSPA2, heat shock protein A2; PC, pancreatic cancer; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

The components and abundance of immune infiltrates in tumor tissues are known to be strongly related to cancer progression. In this study, we investigated for the first time the relationships between HSPA2 and immune infiltrates in PC. We found that HSPA2 expression was positively linked to CD8+ T cell, neutrophil, DC, and macrophage, but not with CD4+ T and B cells. These different results warrant further in-depth exploration. Of interest, the EPIC algorithm yielded an apparent positive relativity between HSPA2 and CAF and endothelial cell, which are key stromal cells in the tumor microenvironment and contribute to tumor growth and metastasis via immunosuppression[21,22]. Collectively, these findings suggest that HSPA2 is strongly involved in tumor-associated immune responses in PC.

Several studies have demonstrated that HSPA2 regulates the biological behavior of tumor cells. In cervical, bladder, colorectal, and breast cancers, HSPA2 expression deficiency diminished the viability and invasiveness of cancer cells *in vitro* and repressed tumor growth *in vivo*[4,5,10-13,23]. Additionally, in ovarian and lung carcinomas, ablation of HSPA2 led not only to impaired proliferation and motility of cancer cells but also to cell-cycle arrest[24,25]. The role of HSPA2 in the biologic behavior of PC cells is currently unknown. The current study explored for the first time the potential biological functions and mechanisms of HSPA2 in the pathogenesis and progression of PC by enrichment analysis. Enrichment data demonstrated that HSPA2 upregulation was strongly involved in the aggressive behavior of PC. Our data, combined with previous results, reveal a momentous role of HSPA2 in the malignant biologic features of tumor cells.

The present study provides the first meaningful insights into the oncogenic and immunogenic role of HSPA2 in PC. The data also provide a basis for further exploration of the role and molecular mechanisms of HSPA2 as an oncogene in the pathogenesis and progression of PC. However, there are still several limitations. First, the results in this study were obtained

based on bioinformatics and can only be interpreted from the perspective of cancer omics, which requires further verification by biological experiments. Moreover, this study failed to confirm the clinicopathological and prognostic significance of HSPA2 expression, and further studies with large sample sizes are thus needed.

CONCLUSION

In summary, our findings suggest that HSPA2 is upregulated in PC and is strongly associated with tumor immunity and aggressive progression.

AUTHORS' CONTRIBUTIONS

L-LZ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **P-PQ:** Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **Y-SS:** Data curation, Formal Analysis, Validation, Writing – original draft, Writing – review & editing. **T-FJ:** Formal Analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Z-GT:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Comparison of severe acute respiratory syndrome coronavirus 2 (COVID-19) vaccine side effects by age groups

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SUMMARY

OBJECTIVE: This online survey aims to compare the side effects that may occur after inactivated severe acute respiratory syndrome coronavirus 2 (COVID-19) vaccination by age groups.

METHODS: A total of 411 participants aged 18–100 who received inactivated coronavirus disease 2019 vaccine were included in the study.

RESULTS: Participants were divided into four groups according to their ages (i.e., 20–35, 36–50, 51–65, and over 65 years old). Vaccine-related side effects were primarily seen in the 20–35 age group and at least in the >65 age group ($p<0.001$). The most common side effects were pain, redness, swelling, and numbness at the injection site. Fatigue and headache were other common side effects. After vaccination, 3 (0.73%) participants had hypertension, and 1 (0.24%) had an asthma attack and was admitted to the hospital. No severe side effects were observed in any of the patients. The most critical factors determining the development of side effects were female gender and young age.

CONCLUSION: According to the results of this study, different types and rates of side effects are seen in all age groups after the inactivated coronavirus disease 2019 vaccine. Since the 20–35 age group and female gender are at risk of side effects, it would be more appropriate to follow up the side effects after vaccination according to gender and age.

KEYWORDS: Age groups. SARS-CoV-2. Inactivated vaccine. Adverse effects.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), which was declared a pandemic by the World Health Organization on March 11, 2020, is the most important health problem of the 21st century[1]. The high contagiousness of the virus and the absence of treatments that can improve the prognosis of the disease increase the importance of effective and safe vaccines against this disease. For this reason, different types of vaccines have been developed against COVID-19 around the world.

Inactivated COVID-19 vaccine is a non-living virus vaccine that can create an immune response in the body, obtained by eliminating the disease-causing feature of the SARS-CoV-2 virus that causes COVID-19 through chemical and physical applications[2]. In our country, mass vaccination was initiated for the COVID-19 pandemic with this inactivated COVID-19 vaccine as of January 14, 2021, with a total of two doses at 4-week intervals for people aged 18 years and above[3]. A phase 3 study conducted in our country with this vaccine shows that it can be used effectively and reliably against COVID-19[4]. Despite the fact that there are sufficient scientific studies on the side effects, risks, and efficacy of this new vaccine, there are some hesitations in the society for vaccine-related side effects. This study aimed to compare the side effects that may occur after vaccination according to age groups by analyzing the safety and more detailed side-effect profile of the inactivated COVID-19 vaccine using an online questionnaire to respond to these hesitations.

METHODS

Ethics committee approval was received for our study from both the Ministry of Health (approval number: 2021-02-26T10_33_23) and Adiyaman University Clinical Research Ethics Committee (date: March 16, 2021, protocol number: 2021/03-11). A total of 411 participants who were given the COVID-19 vaccine (CoronaVac, Sinovac Life Sciences, Beijing, China) and lived in Adiyaman between the ages of 18 and 100 years participated in the study. Participants were divided into

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four groups (i.e., 20–35, 36–50, 51–65, and above 65 years) according to age.

In our study, an online questionnaire was used as the data source. The consent of the volunteers who filled out and approved the online questionnaire was accepted. The researchers shared the online questionnaire form on the web for about 1 month (March 17, 2021, to April 10, 2021). During this period, 614 people who were previously vaccinated were reached via telephone, email, and WhatsApp. The participation rate in the study was 67%.

Online survey forms the age, gender, systemic diseases, medications used, allergic conditions, profession of people who have received COVID-19 vaccine, whether the COVID-19 test was performed during the epidemic, or whether there was contact with someone who had a positive COVID-19 test, it was composed of a total of 16 questions, including the absence of the disease, thoughts about the vaccine and the side effects after the vaccination, and whether he/she had COVID-19 disease after vaccination.

Statistical analysis

SPSS program (Statistical Package for Social Science version 21, IBM Corp., Chicago, IL, USA) was used to analyze the data. The chi-square test was used to compare categorical data by groups. Kruskal-Wallis test and Mann-Whitney U test were used to compare the continuous data. Continuous data were given as mean±standard deviation or median (range), and categorical data were presented as n (%). Logistic regression analysis was performed to predict vaccine-related side effects. 95% of confidence intervals (CIs) were used; $p<0.05$ was considered statistically significant.

RESULTS

Detailed sociodemographic characteristics of the participants by age groups are given in Table 1. Accordingly, gender distribution, allergic disease history, COVID-19 disease history in the past 6 months, post-vaccination COVID-19 infection status, and the number of vaccine-related side effects were similar in all four age groups ($p>0.05$). However, vaccine-related side effects were most common in the 20–35 age group (118, 68.2%), while they were least common in the >65 age group (31, 38.8%) ($p<0.001$). During the pandemic period, the status and results of the COVID-19 test differed between the groups ($p<0.001$). As expected, when the groups were compared in terms of occupations, most of the >65 age group were retired or housewives. In addition, there was an increase in systemic disease rates with age ($p<0.001$).

The incidence of vaccine-related side effects according to age groups is shown in Table 2. Accordingly, the most common side effects are pain in the vaccinated area, redness, swelling and numbness, fatigue, headache, a tendency to sleep, and muscle joint pain. When the vaccine-related side effects were compared according to the groups, pain, redness, swelling and numbness complaints, fatigue, and tendency to sleep were primarily seen in the 20–35 age group; headache was mostly seen in the 36–50 age group and fever was primarily seen in the 51–65 age group ($p<0.05$). There was no statistically significant difference between the groups in terms of other vaccine-related side effects ($p>0.05$).

In Table 3, the parameters (i.e., age, gender, systemic diseases, and allergic conditions) of the participants in the study that may affect the occurrence of side effects against the vaccine were compared. Accordingly, lower mean age ($p<0.001$), more female gender ($p<0.001$), and more allergic diseases ($p=0.037$) were detected in the group with side effects. In the binary logistic regression analysis conducted to evaluate whether age, gender, systemic disease, and allergic condition can be used to predict vaccine-related side effects, it was found that only age and gender could be used in prediction ($p<0.001$, Nagelkerke R^2: 0.181). The odds ratio was 1.04 (95%CI 1.02–1.05, $p<0.001$) for age and 3.2 (95%CI 2.1–4.9, $p<0.001$) for gender.

DISCUSSION

The COVID-19 pandemic is a serious public health emergency due to the high contagiousness of the virus and the lack of effective treatment[5]. For this reason, vaccines in different categories have been developed in many countries and started to be applied in our country and all over the world. With the onset of vaccination, there have been some hesitations in society since there are not enough scientific studies on the side effects, allergy risks, and efficacy of these new vaccines. In this study conducted to respond to these hesitations, the distribution of the detailed side-effect profile of the inactivated COVID-19 vaccine applied in our country according to age groups was evaluated. In the literature, the incidence of mild and moderate side effects is between 17% and 47% in studies conducted with inactivated COVID-19 vaccine[6-10]. In only one of these studies, serious side effects such as hypersensitivity reaction have been reported. None of the participants had severe vaccine-related side effects in our study, and approximately 60% of the participants had mild or moderate side effects. The high rate of mild side effects in our study may be due to the heterogeneity of the participant population. The incidence of moderate side effects was similar to the studies in the literature.

Of the 4 (0.9%) participants who developed mild side effects, 3 (0.7%) had to apply to the hospital because hypertension developed, and 1 (0.2%) had an asthma attack. However, it is necessary to investigate whether these disease exacerbations are related to vaccination in the literature, primarily as side effects attributed to vaccination, but not proven[11]. Post-vaccination fatigue, headache, fever, tremor, muscle/joint pain, vomiting, diarrhea, and systemic and local side effects such as pain, redness, and swelling in the vaccinated area can be seen in patients who received inactivated COVID-19 vaccine[12]. The frequency of side effects may vary according to the demographic characteristics of the study population. In a phase I study conducted with a total of 192 people with BBIBP-CorV, an inactivated COVID-19 vaccine, 42% of side effects were observed in the 18–59 age group and 17% in the >60 age group in the first 7 days after vaccination[10]. Unlike this study, although the incidence of side effects was higher in all age groups (59.9%), the highest was found in the 25–35 age group (68.2%) and the lowest in the >65 age group (38.8%), similar to the literature.

Although there are different results in the literature about the most common types of vaccine-related side effects, local side effects are more common than systemic side effects. In phase I and phase II studies conducted with inactivated COVID-19 vaccines, injection site pain, the most common local side effect in the young age group (<60 years), varies between 5% and 35.0%[6,7,10,13]. Similarly, the most common side effect in the >60 age group is injection site pain[9,10]. However, this side effect is seen at lower rates in the >60 age group compared to

Table 1. Characteristics of patients by age groups.

	Age	20–35 years	36–50 years	51–65 years	>65 years	p
	n	173	119	39	80	
Gender (n, %)	Female	84 (48.6)	48 (40.3)	19 (48.7)	36 (45.0)	0.552
	Male	89 (51.4)	71 (59.7)	20 (51.3)	44 (55.0)	
Professions (n, %)	Retired	0 (0.0)	0 (0.0)	6 (15.4)	42 (52.5)	<0.001
	Housewife	0 (0.0)	0 (0.0)	13 (33.3)	34 (42.5)	
	Health professions	76 (43.9)	88 (73.9)	18 (46.2)	0 (0.0)	
	Others	97 (56.1)	31 (26.1)	2 (5.1)	4 (5.0)	
Systemic diseases (n, %)	None	151 (87.3)	93 (78.2)	10 (25.6)	16 (20.0)	<0.001
	Hypertension	2 (1.2)	4 (3.4)	14 (35.9)	22 (27.5)	
	Cardiovascular disease	2 (1.2)	2 (1.7)	4 (10.3)	16 (20.0)	
	Asthma	6 (3.5)	4 (3.4)	2 (5.1)	7 (8.8)	
	Diabetes	1 (0.6)	5 (4.2)	3 (7.7)	9 (11.3)	
	Goiter	4 (2.3)	4 (3.4)	4 (10.3)	7 (8.8)	
	Others	7 (4.0)	7 (5.9)	2 (5.1)	3 (3.8)	
Continuous drug use (n, %)		26 (15.0)	19 (16.0)	26 (66.7)	56 (70.0)	<0.001
Allergic disease (n, %)		36 (20.8)	27 (22.7)	9 (23.1)	11 (13.8)	0.430
Has the COVID test been done in the pandemic? (n, %)	None	91 (52.6)	43 (36.1)	19 (48.7)	60 (75.0)	<0.001
	Positive	29 (16.8)	20 (16.8)	6 (15.4)	7 (8.8)	
	Negative	53 (30.6)	56 (47.1)	14 (35.9)	13 (16.3)	
Have you had COVID in the last 6 months? (n, %)	No	131 (75.7)	89 (74.8)	33 (84.6)	71 (88.8)	0.067
	Unknown	6 (3.5)	8 (6.7)	0 (0.0)	1 (1.3)	
	Treatment in home	26 (15.0)	20 (16.8)	6 (15.4)	7 (8.8)	
	Treatment in hospital	1 (0.6)	1 (0.8)	0 (0.0)	1 (1.3)	
	Other*	9 (5.2)	1 (0.8)	0 (0.0)	0 (0.0)	
Side effect (n, %)		118 (68.2)	75 (63.5)	22 (56.4)	31 (38.8)	<0.001
How many side effects were there? (Median, range)		2 (1–15)	2 (1–9)	2 (1–8)	1 (1–5)	0.575
Have you had COVID-19 post-vaccination? (n, %)		2 (1.2)	4 (3.4)	0 (0.0)	1 (1.3)	0.882

*I think I have the infection, but I have not been tested.

the younger age group (9–13% vs. 5–35.0%). Similar to the literature, in our study, the most common side effect in all age groups was redness and pain at the vaccine site. According to our results, the incidence of this side effect decreases with age. This finding is consistent with the literature. However, we think the possible reason for the higher incidence of local side effects than the literature is that we expressed all the side effects (e.g., complaints of pain, redness, swelling, and numbness in the vaccine area) developing at the vaccine site in a single question.

There are different results in the literature regarding the incidence of systemic side effects due to the inactivated COVID-19 vaccine. In the study conducted by Xia et al.[10], it was found

Table 3. Factors that may affect the development of side effects.

		With side effects	No side effects	p
		246	165	
Age (mean±SD)		39.4+16.5	48.4+19.9	<0.001
Gender (n, %)	Female	139 (56.5)	48 (29.1)	<0.001
	Male	107 (43.5)	117 (70.9)	
Systemic diseases (n, %)		79 (32.1)	62 (37.6)	0.253
Allergic disease (n, %)		58 (23.6)	25 (15.2)	0.037

SD: standard deviation.

Table 2. Distribution of side effects by age groups.

	Age	20–35 years	36–50 years	51–65 years	>65 years	p
	n	173	119	39	80	
Local side effects	Complaints of pain, redness, swelling, and numbness in the vaccine area (n, %)	116 (67.1)	62 (52.1)	13 (33.3)	23 (28.7)	<0.001
Systemic side effects	Fatigue (n, %)	55 (31.8)	24 (20.2)	6 (15.4)	8 (10.0)	0.001
	Headache (n, %)	35 (20.2)	29 (24.4)	8 (20.5)	6 (7.5)	0.025
	Somnolence (n, %)	26 (15.0)	15 (12.6)	4 (10.3)	2 (2.5)	0.033
	Muscle-joint pain (n, %)	20 (11.6)	15 (12.6)	5 (12.8)	3 (3.8)	0.180
	Dizziness (n, %)	9 (5.2)	6 (5.0)	3 (7.7)	5 (6.3)	0.917
	Fever (n, %)	6 (3.5)	0 (0.0)	4 (10.3)	2 (2.5)	0.011
	Back pain (n, %)	9 (5.2)	3 (2.5)	2 (5.1)	0 (0.0)	0.054
	Lumbago (n, %)	8 (4.6)	5 (4.2)	1 (2.6)	0 (0.0)	0.088
	Throat ache (n, %)	6 (3.5)	1 (0.8)	0 (0.0)	3 (3.8)	0.186
	Sweating (n, %)	4 (2.3)	3 (2.5)	1 (2.6)	1 (1.3)	0.934
	Abdominal pain (n, %)	5 (2.9)	2 (1.7)	0 (0.0)	2 (2.5)	0.517
	Dryness of throat (n, %)	7 (4.0)	1 (0.8)	1 (2.6)	0 (0.0)	0.068
	Tremor (n, %)	8 (4.6)	2 (1.7)	3 (7.7)	1 (1.3)	0.163
	Difficulty breathing (n, %)	3 (1.7)	1 (0.8)	2 (5.1)	1 (1.3)	0.468
	Loss of taste (n, %)	4 (2.3)	1 (0.8)	1 (2.6)	0 (0.0)	0.291
	Hypertension (n, %)	0 (0.0)	2 (1.7)	2 (5.1)	2 (2.5)	0.077
	Palpitation (n, %)	2 (1.2)	2 (1.7)	0 (0.0)	1 (1.3)	0.765
	Itching (n, %)	1 (0.6)	0 (0.0)	0 (0.0)	1 (1.3)	0.524
	Vomiting (n, %)	4 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0.073
	Nasal Discharge (n, %)	1 (0.6)	3 (2.5)	0 (0.0)	0 (0.0)	0.194
	Cough (n, %)	3 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0.156
	Diarrhea (n, %)	2 (1.2)	1 (0.8)	0 (0.0)	0 (0.0)	0.546
	Hallucination (n, %)	2 (1.2)	0 (0.0)	1 (2.6)	0 (0.0)	0.223
	Fainting (n, %)	1 (0.6)	0 (0.0)	1 (2.6)	2 (2.5)	0.098
	Skin rash (n, %)	1 (0.6)	1 (0.8)	0 (0.0)	2 (2.5)	0.623
	Speech disorder (n, %)	1 (0.6)	0 (0.0)	1 (2.6)	0 (0.0)	0.297
	Anosmia (n, %)	3 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0.156

that the incidence of fever, which is the most common systemic side effect, was 6% in the <60 age group and 1% in the 60-age group and above. In the phase II study of the same vaccine, fever was detected in 2% of the participants in all age groups. In other inactivated COVID-19 vaccine studies, it has been shown that the incidence of fatigue, which is seen as the most common systemic side effect, varies between 6% and 10%[7,13,14]. In another inactivated COVID-19 vaccine study conducted with healthy people over the age of 60, the most common systemic side effect was fever (3%)[8]. Since the comparison of COVID-19 vaccine side effects by age groups in the literature was not made in detail as in our study, sufficient comparison could not be made. In our study, fatigue was the most common systemic side effect in the 20–35 age group and >65 age group, while headache was more common in the 36–50 age group and 51–65 age group, and fever was more common in the 51–65 age group. Somnolence, muscle joint pain, and dizziness were less common systemic side effects. The fact that systemic side effects are seen at different rates according to age groups fits the purpose of our study.

The development of post-vaccination anaphylaxis is one of the most severe side effects of vaccines[15,16]. Current reports from the Centers for Disease Control and Prevention (CDC) show that anaphylactic reactions to the BNT162b2 mRNA vaccine may occur more frequently than other vaccines[17]. On December 14–23, 2020, 21 cases of anaphylaxis were identified after the administration of the reported 1,893,360 first doses of Pfizer-BioNTech COVID-19 vaccine (11.1 cases per million doses). Similarly, in a review of COVID-19 vaccines by Kaur et al.[18], serious vaccine side effects were not associated with inactivated vaccines. In our study, none of the participants developed severe side effects such as anaphylaxis supports this information.

The most important limitation of our study was due to involuntary errors that may occur due to the way the data were collected. In addition, the selection of cases from a single region, the time of onset of symptoms after vaccination, and the inability to provide specific data on the duration of symptoms were among our other limitations. However, it was the superiority of our study to investigate the factors affecting the side effect against inactivated COVID-19 vaccines. Another advantage of our study was that side effects were examined in detail according to age groups, unlike other studies. The most important factors determining the development of side effects were found to be female gender and young age.

CONCLUSIONS

According to our study, side effects after inactivated COVID-19 vaccine were most common in female gender and 20–35 age group, while vaccine-related side effects were least common in >65 age group. The most common vaccine-related side effects were pain, redness, swelling, and numbness in the vaccinated area, while the most common systemic side effect was fatigue. Most of the reported side effects were mild, and no severe side effects such as anaphylaxis were observed. According to these findings, it may be necessary to be more careful about side effects, especially in women and young people, after inactivated COVID-19 vaccine administration.

ETHICAL ASPECTS

Approval for this study was obtained from the Ministry of Health (decision no: 2021-02-26T10_33_23) and Adıyaman University Clinical Research Ethics Committee (date: March 16, 2021, decision no: 2021/03-11).

AUTHORS' CONTRIBUTIONS

FT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **MB:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **İT:** Conceptualization, Data curation, Project administration, Resources, Software, Validation, Visualization.

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ORIGINAL ARTICLE

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Quality of primary health care for quilombolas' Afro-descendant in Brazil: A cross-sectional study

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SUMMARY

OBJECTIVE: The aim of this study was to assess the quality of primary health care services through self-reports by caregivers of children and adolescents living in quilombola communities in Brazil.

METHODS: This is a cross-sectional study in accordance with the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology). Exposure variables included sociodemographic characteristics; and outcome variable was the quality of primary health care offered to quilombola children and adolescents.

RESULTS: A total of 68 individuals participated in the survey. Quilombolas have a low income, a lower level of education, do not work, and receive government benefits. Our results showed that the quality of primary health care, measured by the experience of caregivers of quilombola children and adolescents, generally presents satisfactory values.

CONCLUSION: The quality of primary health care has generally satisfactory values. However, as these results differ from most studies, more research should be conducted.

KEYWORDS: Primary health care. Vulnerable populations. Public health.

INTRODUCTION

Access to health care is a moral imperative for all countries. Equitable distribution of health care is essential to making the gains established by the Sustainable Development Goals (SDG) and contributing to the realization of the right to health. According to the World Health Organization, efforts must be made to strengthen health systems and improve services with an emphasis on universal health coverage. When countries seek universal health coverage, progressive universalism includes vulnerable populations from the beginning, which is the most effective way to reach vulnerable groups in society. Thus, primary health care (PHC) represents one of the most promising avenues for responding to growing health needs, demography, environmental challenges, and emergencies[1].

Brazil, which has undergone several transformations in the past 40 years, instituted universalism through the Unified Health System. The Family Health Program, created in 1994, was a reorientation strategy for the development of primary care that is carried out in the actions of multidisciplinary teams, responsible for the health of a given territory among these territories, we find the quilombolas' communities[2] (has self-attributed ethnic and racial characteristics and a trajectory of black ancestry related to slavery)[3].

In 2004, the Brazil Quilombola Program was launched (Decree 6261); the estimate of the quilombola population in Brazil was 2,14,000 families in 2012 (approximately 1.17 million people)[4]; however, these data are imprecise and underestimated. PHC in quilombola communities is already recognized; however, the investment was reduced in this scenario[5] (e.g., public resources for the quilombola territories in 2017 was almost four times lower compared to the 3 previous years). Health inequality is caused by different impacts, such as wars for ethnic-racial reasons, religious, territorial, radical political-economic changes, and revolutions[6].

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Evidence registers inequities in the health services provided to children and adolescents — which become even more evident when these subgroups are from quilombola communities — in which health actions aimed at this vulnerable segment have to consider different needs[7]. A way to protest and to help this population is through scientific evidence; however, there are no studies aimed at the perception of historically excluded groups in PHC.

We believe that improvements in the health and well-being of this population will drive progress toward meeting the SDG health goals; this is the justification for conducting this study. Our aim was to assess the quality of PHC services through self-reports by caregivers of children and adolescents living in quilombola communities in Brazil.

METHODS

Design

This is a cross-sectional study conducted in accordance with the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology). Exposure variables included sociodemographic characteristics; outcome variable was the quality of PHC offered to quilombola children and adolescents.

Setting

The study was carried out between 2015 and 2017 in quilombolas' communities in the state of Tocantins (Brazil); we collect data from clinically set sites in communities. The teams were previously trained, and the collections were monitored by senior researchers who have experience in studies with vulnerable communities.

Participants

Sample is composed of caregivers of children and adolescents up to 17 years of age, residing in five quilombolas' communities in the state of Tocantins/Brazil. Due to the few references on the evaluation of the quality of PHC to quilombola children and adolescents, this is a convenience sampling.

Inclusion criteria were as follows: being the main caregiver of the child/adolescent under the age of 17, being able to answer the questionnaire in question, and knowing the unit used for the research.

Variables

We used the Primary Care Assessment Tool (PCATool — Brazil), child version, developed by the Johns Hopkins Population Care Policy Center[8,9]. We assessed the extent of attributes classified as essential (i.e., first contact access, coordination, longitudinally, comprehensiveness, and coordination of care) and derivatives (i.e., community guidance and family guidance), investigating the "Degree of Affiliation" the regular source of care.

Data sources/ measurement

PCATool — Brazil has Likert-type answers with an interval of 1 to 4 for each attribute (4=definitely yes, 3=probably yes, 2=probably not, 1=definitely not, and 9=do not know/do not remember). The degree of evaluation of the caregiver to the health service was calculated using the structured algorithm according to the different possibilities of the caregiver's response to the three initial questions[9].

Bias

We reduced the information bias by conducting prior training to apply the instruments. Also, we created an electronic data collection form (Epi info 7.2®) to build the database. All data were validated in duplicate (in cases of divergence between data, a third researcher was consulted).

Study size

The communities have about 106 families with 205 children and teenagers up to 17 years old.

Quantitative variables

We calculated the scores of each attribute of PCATool — Brazil, obtained by the average of the items that compose it. All scores for each of the attributes were later transformed into a continuous scale between zero and 10, using the formula: Adjusted score = [(obtained score–1)/(4–1)] × 10, so that it could be performed the calculation of the "Essential Score" and "General Score" of PHC.

The "Essential Score" was measured by the sum of the average scores of the components belonging to the "Essential Attributes" plus the value attributed to the "Degree of Affiliation" and divided by the number of components. The calculation of the "General Score," in turn, followed the same principle, having added, however, the average scores of the three attributes derived from PHC[8,9] (scores ≥6.6 indicate satisfactory values and adequate presence of the attributes in PHC).

Statistical methods

Statistical analysis was performed using the statistical package STATA, version 18.0, for Windows. We used bivariate statistics (chi-square) and the appropriate tests according to the adherence of the data to the Gaussian distribution (normality verified by the Shapiro-Wilk test).

Ethical principles

All procedures of the present study were previously approved by the research ethics committee (opinion: 3358190).

RESULTS

Participants

We recruited 106 families for the study; only 68 individuals who met the inclusion criteria participated in the survey. A total of 38 participants were excluded, due to the absence after three collection attempts, refusals, and giving up in any of the stages. The greatest loss of data available for analysis occurred with anthropometric data.

Descriptive data

Caregivers were the main informants with low economic status and were dependent on government benefits. Table 1 consolidates the main socioeconomic, demographic, and social support characteristics of caregivers.

Outcome data

Table 2 shows the scores for the quality of PHC, based on the experience of caregivers of children and adolescents who use PHC services in quilombola communities in Tocantins/Brazil. The mean overall score was 7.68 or above the cutoff point for good general primary care (fixed at 6.6). If we break this analysis down by attribute, we will find the reasons for these scores.

Table 1. Characteristics of the guardian of quilombolas children/adolescents in relation to health services, Tocantins/Brazil, 2017 (n=68).

Variables	n (%)
Sex (female)	50 (73.53)
Age	
Adult	61 (89.71)
Elderly	7 (10.29)
Economic class (ABEP)	
C1	4 (7.50)
C2	13 (22.50)
DE	39 (70.00)
Receipt of benefit (yes) [a]	41 (50.00)
Sons	
≤ 2	25 (36.76)
> 2	43 (63.24)

ABEP: Brazilian Association of Research Companies. [a] Receipt of benefit: municipal, state or federal government.

In general, the contribution of "affiliation," "utilization," "longitudinally," "services provided," "coordination – information system," and "coordination of care" helped to improve the score; in turn, "ease of access" and "available services" were negative. The highest average scores obtained from caregivers of children and adolescents were affiliation, use, and information system. At the other extreme, the worst overall scores were found in the ease of access, available services, and essential scores.

DISCUSSION

Key results

We describe the quality of care provided by health services through a validated instrument that allows the measurement of caregivers' perception of the quality of these services aimed at the child population.

Our results showed that the quality of PHC, measured by the experience of caregivers of quilombola children and adolescents, generally presents satisfactory values. However, evidence of access and services provided point to inequalities in the access of these vulnerable populations to high-quality health services, so more studies should be conducted.

Table 2. Average score based on self-report of guardians of quilombola children/adolescents in relation to PHC in the State of Tocantins/Brazil, 2017 (n=68).

PHC attributes	Mean (standard deviation)	95%CI
Essential attributes		
Degree of affiliation	10 (0)	
Use	8.45 (0.39)	7.63–9.26
Accessibility	6.01 (0.42)	5.13–6.88
Longitudinally	7.25 (0.34)	6.54–7.95
Care integration	7.39 (0.52)	6.33–8.45
Information system	8.24 (0.36)	7.49–8.99
Available services	6.57 (0.31)	5.93–7.21
Services provided	7.93 (0.41)	7.09–8.78
Derived attributes		
Family orientation	7.93 (0.49)	6.53–8.55
Community orientation	7.54 (0.34)	6.72–8.12
General evaluation		
PHC essential score	4.42 (0.20)	4.00–4.84
General score	7.68 (0.21)	7.24–8.12

PHC: primary health care; CI: confidence interval.

Limitations

We made several attempts to collect data from all caregivers in the communities, but there were some data losses that reduced the sample size. This small sample size may have been a limitation with regard to the ability to extrapolate the results found in this study. However, this study is the first to assess the quality of PHC in five quilombola communities from different locations in Tocantins, using an internationally developed and validated tool in Brazil, which makes this an important parameter for similar studies, as well as strengthening the goals of SDGs related to the inclusion of vulnerable populations.

Interpretation

Main socioeconomic demographic characteristics of quilombolas have lower income, less education, do not work, and receive government benefits (i.e., they are poorer and have lower purchasing power, greatly affecting the child population). This corroborates evidence from other studies[7,10] that have shown that population groups like this are less likely to access and use health services.

Similar studies[11,12] report that the identification and use of PHC as a regular source of care are directly related to social vulnerabilities (belonging to groups with a historical profile of exclusion/discrimination — quilombolas[5], indigenous[13,14], and populations with disabilities)[15]. Equitable distribution of high-quality health care is essential to achieving the goals set out in the SDGs and contributing to the realization of the right to health.

Our results demonstrate that overall satisfaction was well evaluated, possibly influenced by a number of other factors, including social, economic, and demographic aspects; this may explain some literacy-related findings (e.g., satisfaction is often high for demonstrably low-quality services, particularly for users with less education or less experience in high-quality health services)[16,17].

An Internet survey of 12 countries showed contradictory evidence between educational level and satisfaction. People with primary (or less) education consistently rated their user experience as worse than people with secondary and/or higher education. In numbers, 34% of respondents reported that the staff had treated them poorly because of their identity; 10% attributed this to their poverty[18]. Improving health literacy can reduce this mismatch, although user satisfaction provides an important perspective, other measures should be considered. These can include trust in the health system and the quality of interpersonal care provided by health professionals[19].

Scores observed for the degree of affiliation and use (i.e., the recognition and use of the health service) demonstrate that those responsible identify the PHC as a reference service for their child/adolescent and is related to the characteristics of quilombola communities such as geographic isolation, large distances from other health services, transportation difficulties, and low income[20,21] — model of the universal health system in Brazil[22] — it is also a variable that explains the good performance found.

Universal access to health is essential to comply with the SDG; however, the urgency to expand essential services to the population at any cost, without a focus on quality, does more harm than good[18,23]. Concern with heritage implies access to a minimum level of quality guaranteed for everyone; two reasons that support this hypothesis are as follows: ethical achievement of health outcomes (increased access will not translate into better health outcomes for disadvantaged people unless all people have access to high-quality services) and efficient use of resources (spending scarce resources to expand access without quality is wasteful and inefficient).

Low scores found in the accessibility sub-item show the disadvantages of health services in quilombolas' communities and portray how poverty and vulnerability increase inequalities. A study carried out in Kenya[24] showed that poor quality of maternal care was substantially more prevalent for the poor than for the richer people. Likewise, in Brazil, poor communities receive poor quality care[25].

Longitudinally implies a therapeutic relationship, characterized by the responsibility of health professionals and the patient's trust[26]; for this to be effective, the bond or affiliation between the child's family and the health service must occur. However, despite the good results found in this study, bonds in quilombolas' communities are often established with community health agents (and not with doctors and nurses). In Brazil[27], as in other countries[28,29], community health workers operate within their own community and are more likely to behave as expected by community members (establish trusting relationships with caregivers).

Our findings are similar to those of Nanyonjo et al.[30], in which continuity of longitudinal care for children under 5 years of age — treated for malaria, pneumonia, and diarrhea, continuity of longitudinal care — was greater for Community Health Workers than for professionals from the health care unit. If professionals know the child's health history and develop a family bond, this strengthens the longitudinally and quality of the service and increases user satisfaction with the health service (and, of course, the professionals involved in the work performed).

Integrality is the measure in which health services are provided and available in a complementary and coherent way[26].

Services provided component obtained a satisfactory score, demonstrating that professionals guide caregivers[31,32] on how to maintain health, growth, development, and child/adolescent safety. However, the available services component scored below ideal — this indicates inadequate facilities, lack of diagnostic and treatment tools needed for high-quality care — these are important and sensitive services (e.g., for disease management noncommunicable and other chronic conditions more common in children and adolescents)[33], as they refer to the early detection of infections such as HIV, which require continuous support from the patient.

Despite the significant decline in the incidence of vaccine-preventable diseases, several countries report delayed vaccination of children and adolescents in Asia, Eastern Europe, Africa, and South and Central America[34] (mainly in relation to socioeconomic conditions and characteristics of the health system)[35]. Formative surveys at eight facilities in Zambia revealed that only 46% of patients are on stable HIV treatment; in Brazil, the population group between 13 and 19 years old continues to show increasing incidence rates of this infection[36], despite being a national policy for patients in these countries. Similarly, lapses in tracking test results have also been reported and pose serious challenges for HIV[37,38].

It is important to highlight the need for health units to provide more services to meet the population's basic health needs (including supplies such as vaccines and medicines). Another warning to professionals about the low quality of care in health care. Research has revealed that individuals in low- and middle-income countries do not receive appropriate treatment during consultations — including preventive interventions during childcare, oral rehydration therapy for children with diarrhea, or antibiotics for those with symptoms of pneumonia[18].

Competent systems provide individuals and communities with timely health promotion and disease prevention. People have the right to rely on their conditions being detected and managed in an integrated manner. Assistance coordination presented a satisfactory assessment, which may be due to the perception of the need to combine the basic care network and the specialized services that are absent in the quilombolas' communities. Inadequate integration of the Health Care Network generates weak referral systems and undermines the ability of health systems to take care of complex and emerging conditions[39].

In spite of a new design for the coordination of care for PHC, it will be used in the past four decades as a key for health care for all[40], the continuity of two care in this way requires that the maintenance of the quality be incorporated into the DNA of all health systems. Among the actions of succession include adhering to a policy and a national quality strategy, building management capacity in all the new health systems, strengthening the regulation and provision of bills, and collecting and learning the data of the health system[41].

Thus, the coordination of care must be focused on the user, with a system that is easy to access and navigate, especially for the most vulnerable subpopulations that, in addition to communication barriers in health services, are common even among the most enlightened (who do not recognize, for example, the place right for the service)[39], face barriers of prejudice, discrimination, and exclusion in the various PHC services[42,43].

Among other essential challenges in care coordination, robust information systems for all health care platforms represent a key area for innovation. However, the use of electronic records to produce a coherent view of indicators needs to evolve into technical organizational arrangements (single information systems) that maximize quality at the system level.

Evidence shows that health system data collection is often expensive, uncoordinated, and disconnected from decision-making. Data quality in routine health information systems is poor, with vertical program evaluations often identifying a high prevalence of missing or inaccurate data[44]. Tools and indicators are fragmented by disease and source of funding, with inadequate harmonization and few plans coordination and use of data[45]. Inadequate integration between platforms and weak referral systems hampers the ability of health systems to address complex and emerging conditions. Poorly organized health systems lose lives, waste scarce resources, and the goodwill of populations.

High scores for longitudinally were also accompanied by higher scores for the attribute of family orientation. Considering that child health care presupposes greater interaction with the family, the results suggest that the Estratégia Saúde da Família/Family Health Strategy (ESF/FHS) tends to provide, in fact, a change in the care model, which starts to value the context of people's lives more (people-centered model) and the insertion of the subject in the family and in the community.

This second aspect is notably highlighted by the children's caregivers, who attributed better scores for the attribute of community guide to the family health strategy teams. Similar evidence was described, highlighting some factors that seem to be related to the better effectiveness of community guidance in care[28,29], much as the actions of home visits carried out by all professionals, but mainly by community health workers, which facilitates health surveillance and monitoring of families in quilombolas communities.

Appropriate counseling and health education are essential elements for family and community guidance, quality indicators, and derived attributes, evaluated in this study with

satisfactory scores. Similar results were observed by children assisted in PHC, showing improvements in some health indicators, such as the reduction in the number of deaths from diarrheal disease and respiratory tract infection in the postneonatal period[46]. Respiratory infections in children showed a reduction in mortality in approximately 80% in the past two decades, a fact strongly attributed to increased access to health services[47].

However, in observation of consultations of sick children in 17 countries, only 43% of professionals informed caregivers about the diagnosis of their children. Counseling is particularly important for the management of diseases characteristic of health particularities in this stage of life characterized by the greater volume of acute conditions[48]. Another shows that 2 million deaths from neonatal diseases and tuberculosis, which are amenable to health care, 56% occurred in people who used the health system but did not receive good quality care[49].

The low overall assessment of the essential score (4.42), similar to other studies[50], represents the reality of many vulnerable populations in relation to high-quality access in different countries. One explanation for this scenario would be the conceptual application of the Reverse Care Law[51], as the availability of good medical care tends to vary inversely with the need to provide it in the population served — there is evidence of this reverse care law in many health systems — for example, tuberculosis has a strong socioeconomic gradient across countries, within countries, and within communities[52].

The equitable distribution of high-quality health care is essential to reducing systematic health disparities, especially among socially disadvantaged groups such as quilombolas. Primary care is one of the main determinants of health, and equitable access to the system is, therefore, important to ensure positive results, especially those aimed at by the SDG, ultimately contributing to the realization of the right to health.

The results of our study do not allow us to ensure that the child population has received the best possible care. Health units suggest a precarious structure to provide basic clinical care and deficiencies in health promotion, disease prevention, and continuity of care. A diverse range of movements must be accommodated under the umbrella of PHC. These movements include intersectoral actions to ensure the health agenda of vulnerable populations on the agenda of all policies, governing with quality, investing in infrastructure, transforming strength, and educating community residents, especially caregivers and leaders, about rights, responsibilities, and fighting for them.

Finally, it is highlighted that the results must be interpreted considering some limitations. The lack of national studies on the subject, especially among quilombola populations, points to the need for new similar assessments. Although the scope and scope of the study portray the perception of five communities, it is difficult to draw conclusions about how generalizable these strategies are.

However, it should be noted that the same socioeconomic reality and the same history of segregation and abandonment are present in many communities in the national and international Canary Islands. We emphasize that the registered perception did not include all social actors, and the perspectives of managers and service providers must also be known, in order to better face the situation.

More broadly, the successful development of shared vision, policies, strategies, coordination, and implementation with vulnerable populations is necessary to design a care architecture that directs patients and professionals to the proper performance of PHC services.

CONCLUSIONS

Quality of PHC has generally satisfactory values. However, as these results differ from most studies, more research should be conducted.

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AUTHORS' CONTRIBUTIONS

FRPQ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing**. ESM:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing**. FA:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing. **AMB:** Validation, Visualization, Writing – original draft, and Writing – review & editing**. AP-S:** Validation, Visualization, Writing – original draft, and Writing – review & editing. **FLAF:** Visualization, Writing – original draft, and Writing – review & editing.

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Parenthood experiences of LGBT+ individuals: a systematic review

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SUMMARY

OBJECTIVE: This systematic review aims to analyze the parenthood experiences of LGBT+ individuals.

METHODS: The review was carried out between February and June 2020 and used key words about LGBT+ and/or parenthood, including, "lesbian and mother," "lesbian and parenthood," "gay and father," "gay and parenthood," and "trans and parenthood," which were published in databases, such as PubMed, Google Academic, Wiley Online Library, and ScienceDirect.

RESULTS: We reviewed 19 research articles, and the majority of these articles suggested that LGBT+s faced negative reactions and discrimination when they decided to become parents. Once they became parents, the relationship of most of the LGBT+ individuals with their partners and the surrounding social environment improved and social support for and self-esteem of the LGBT+ individuals increased.

CONCLUSIONS: Compared to the heterosexual parents, LGBT+ individuals faced with various problems in their social, family, and professional lives during parenthood. Therefore, they need more family, legal, and social support.

KEYWORDS: Homosexuality. Parents. Sexual and gender minorities. Social support.

INTRODUCTION

The concept of LGBT+ gained importance as a result of the struggles of people with different sexual identities for their rights. The concept refers to lesbian, gay, bisexual, transgender/transsexual, intersexual, and queer individuals[1]. As such, sexual orientations of not only heterosexual but also of homosexual and bisexual people are defined[2]. Given that all individuals have the right to sexual and reproductive health, we may suggest that LGBT+ individuals have the right to parenthood and have children[3].

Individuals with different sexual orientations have different methods to become parents, including egg and sperm donation, surrogate motherhood, and assisted reproductive techniques[4-6]. Besides, LGBT+ individuals may adopt children, which is a widely recognized method of parenthood.

The review of the literature on the comparison of the experiences of LGBT+ parents with their heterosexual counterparts showed conflicting findings. Some of the studies found that LGBT+ parents raised happier children and spent more time to raise their children, independent of gender roles and rules[6]. However, the review by Carneiro et al. (2017) reported no difference between the experiences of gay and heterosexual fathers in terms of involvement in their children's activities, level of intimacy, parenting problem-solving, and time spent with their children[7]. Other studies also dealt with childbearing problems in nontraditional societies, including social exclusion of the children, homophobia, and the absence of family support and legal protection[8,9].

Despite the existence of systematic reviews on the parenthood experiences of individuals with different sexual orientations in the literature, we have not found any studies that dealt with the parenthood experiences of LGBT+ individuals[7,10]. We believe that reviewing the difficulties that LGBT+ individuals experience during parenthood may help policy makers, civil society groups, and health professionals that provide care to these people, including nurses, physicians, and social service experts.

Aim

This systematic review aims to analyze the parenthood experiences of LGBT+ individuals. The research questions include the following:

- What are the sociodemographic characteristics of LGBT+ individuals?
- What are the positive parenthood experiences of LGBT+ individuals?
- What are the negative parenthood experiences of LGBT+ individuals?

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METHODS

Design

This systematic review used PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) to prepare the protocol and report the article. PRISMA statement may be accessed via http://prisma-statement.org/.

Data sources and search strategy

We conducted this systematic review between February and June 2020 and reviewed scientific articles in English version which were published in different databases (e.g., PubMed, Google Academic, Wiley Online Library, Taylor and Francis Online, and ScienceDirect). We used the key words about LGBT+ and/or parenthood, including "lesbian and mother," "lesbian and parenthood," "gay and father," "gay and parenthood," "trans and parenthood," "LGBT+ and parent," "LGBT+ and parenthood," "LGBT+ parent," and "adoption parenthood experience." We used the words "and" and "or" to search key words.

Study selection

We analyzed the qualitative, quantitative, and mixed-design studies on parenthood experiences of LGBT+ individuals that were published in English until June 2020.

Inclusion criteria

- Studies on LGBT+ individuals that had parenthood experience and lived with their children.
- Research articles that were published in English.

Exclusion criteria

- Articles on the intentions and motivations of LGBT+ individuals.
- Articles on the relations between LGBT+ individuals and their parents.
- Articles on the opinions of health professionals on the parenthood of LGBT+ individuals were excluded from the systematic review.

Methodological quality assessment

Quality assessment of the reviewed articles was independently conducted by two researchers. We used 12 criteria proposed by Polit and Beck (2009) to evaluate the quality of evidences. The two researchers independently scored 1 that met the criteria and 0 for the studies that did not meet the criteria[11]. Coherence between the researchers was assessed by using Cohen's kappa (κ), where the values of 0.41–0.60, 0.61–0.80, and 0.80–1.00 referred to moderate, substantial, and almost perfect agreement, respectively[12]. Kappa (κ) in our study was 0.54, indicating moderate agreement.

RESULTS

Study design and sample characteristics

We reviewed 4 quantitative, 14 qualitative, and 1 mixed-design scientific studies. Parenthood experiences of five gay, five lesbian, one transsexual, and eight LGBT+ people were included in our systematic review.

Demographic characteristics

Age of the participants who were included in our systematic review ranged between 22 and 59 years, and the majority of the participants were above the age of 40. Most of the participants had postgraduation degree. Besides, the majority of the participants had high-income levels (Table 1).

Parenthood experiences

This study reviewed positive and negative parenthood experiences of LGBT+ individuals and their parenthood processes (Table 1).

Positive experiences

Primary studies on LGBT+ parents found that parenthood was more important than work life and careers of LGBT+ individuals[19] and that the relationship with their partners[9] and families[7] and the levels of self-esteem and social support increased after parenthood[7]. Besides, these parents perceived themselves as more important in the society and stated that legal processes on adoption made their work easier[20] (Table 1).

Negative experiences

Although participants in some of the studies expressed that the society was more tolerant of LGBT+ parents, the majority of studies reported that social discrimination continued even after parenthood[7,11,21-25].

Problems of LGBT+ individuals in work life included precariousness, discrimination, and being unable to benefit from the rights of heterosexual employees, such as maternity and paternity leave, breastfeeding leave, unpaid leave, or flexibility in working hours[7,19,21].

DISCUSSION

This study, which reviewed positive and negative parenthood experiences of LGBT+ individuals, analyzed 19 qualitative and quantitative research articles. Findings of these studies on sociodemographic characteristics of the participants and their negative and positive experiences are discussed below.

Table 1. Studies on parenthood experiences of LGBT+ individuals.

Author and year	Participants	Country	Research design	Methods to become parents	Sociodemographic characteristics	Findings
Blake et al.[4]	74 gay fathers	The United States	Qualitative	Surrogacy	*Average age was 47.2 years 98% university or above *A high income	*Some of the gay couples who chose surrogacy as a path to parenthood received negative feedback about the ethical dimensions of surrogacy. *Negative attitudes before the birth changed into positive ones after birth.
Lindheim et al.[5]	78 gay couples	The United States	Cross-sectional	Surrogacy, IVF	*44.7% aged between 40 and 49 years * 76.6% were university graduates and 69.9% had high income	*Parents received negative reactions of their children/society about their family structures. *Participants faced with difficulties in their professional life after becoming parents, such as use of annual leave and changes in working hours. *Children of the participants were disappointed for not having a mother. After explaining about their unique family structures, participants asked their children to choose one of the parents as mother.
Erera and Engelchin[17]	9 gay fathers	Israel	Qualitative	Pregnancy with heterosexual relationship	*Age ranged from 35 to 56 years *8 is high education *Economic status (2 poor, 7 good)	*Respondents stated the importance of the presence of both father and mother for their children. *Most of the respondents underlined the functions of mothers in raising their children, including affection, care, and love. Due to this reason, they formed a family with a co-parent biological mother.
Bergman et al.[15]	40 gay fathers	The United States	Qualitative	Surrogacy	*Age ranged from 27 to 52 years *No data on education level. *37 respondents had a mean annual income of US$270,000.	*Parenthood brought losses and missed opportunities in the work lives of most of the participants. *After having children, frequency of business and leisure travels decreased and travels mostly involved visits to families of origin and domestic family-oriented trips. *Relationship with families and self-esteem improved after the participants became fathers. *Participants felt themselves more valuable after becoming fathers.
Goldberg[16]	29 lesbian couples	The United States	Mixed method	IVF	*Mean age of biological and nonbiological mothers was 35 and 37.7 years *Most were highly educated *Mean annual income was US$100,600.	*41% stated that the desire to experience pregnancy and childbirth and have a biological connection to the child were determining factors in their decisions. *Most couples found it relatively easy to decide on who would carry the children. Fertility reasons, health, career plans, and age of the couples influenced their decisions. *Perceived family support increased after the transition to parenthood.
Forenza et al.[19]	4 lesbian, 1 bisexual, and 4 gay parents.	The United States	Qualitative	Adoption	* No data on age, education, and income levels of the participants	*Legal regulations were the primary obstacles for their parenthood. * After becoming parents, participants de-emphasized their LGB+ identities in favor of self-categorization as a parent.
Bos et al.[22]	100 lesbian and 100 heterosexual families	Netherlands	Qualitative	...	* No data on age, education, and income level of the participants	*Lesbian social mothers reported significantly more than heterosexual fathers that they felt the need to justify the quality of their parenthood. *Lesbian biological mothers were more satisfied with their partner than heterosexual mothers.

Conitnue...

Table 1. Continuation.

Author and year	Participants	Country	Research design	Methods to become parents	Sociodemographic characteristics	Findings
Van Ewyk and Kruger[6]	10 lesbian couples	South Africa	Qualitative	8 donor insemination, 2 opted for adoption.	*Age of parents ranged from 25 to 49 years. *Education level: high school to tertiary degree and middle class.	*Lesbian couples reported a decrease in their sexual activities and socialization after parenthood. * Participants reported that co-parenting alleviated much of stress and prevented exhaustion and that sharing the responsibilities prevented frustration.
Van Rijn-Van Gelderen et al., 2018	38 gay, 61 lesbian, and 41 heterosexual	The United Kingdom, France, the Netherlands	Cross-sectional	Surrogate carriers, sperm donors, and IVF without sperm or egg donation	* Age ranged from 22 to 59 years. * Nearly two-thirds were employed full time and most families had good income	*Parents reported low levels of parental stress, anxiety, and depression regardless of family type. *There was no significant difference between the family groups in terms of parental stress, depression, anxiety, partner relationship satisfaction, and caregiver role. *Parents in all family types were satisfied with intimate relationships.
Faccio et al., 2013	14 transsexuals and 14 men	Italy	Qualitative	-----	*Age ranged between 39 and 58 years *Socioeconomic and education levels were high	*Most of the parents did not receive help for child care. *Transsexual parents considered themselves as competent parents who spent sufficient time for child care.
Lévesque et al., 2020	19 heterosexual and 4 same-sex couples	Canada	Qualitative	Pregnancy with heterosexual relationship	*Age ranged from 27 to 49 years *47.8% completed university degree *Majority were employed full time with satisfactory financial situation	*Participants reported fatigue, lack of sleep, social isolation, financial precariousness, discrimination at work, and balancing work and family life as main difficulties. *Parents spent less time on social activities and families and more time to meet the needs for children. *Lesbians expressed social norms influenced their relations with the children and created anxiety.
Ryan and Whitlock, 2007	96 lesbian parents	The United States	Cross-sectional	adoption	*Mean age ranged from 41 years to 45 years. *Majority held bachelor's degree or above *Mean annual income was US$95,000.	*Major sources of counseling were adoption agencies, friends, adoption books, and social services and the participants found counseling service to be helpful. *Majority of the parents considered adoption process as a positive experience. * Legal sources of information on adoption supported the decision and helped them to feel good.
Brown et al., 2009	183 gay and lesbian parents	The United States	Qualitative	adoption	*Mean age was 45 years for women and 43 years for men *Majority had bachelor's degree or above and high annual household income	*Parents experienced difficulties to access LG adoptive families and felt the need for role models for their children. *Parents struggled with how to address children's emotional and behavioral difficulties. *Financial difficulties, finding time for family/relationship, and social acceptance were problems. *Parents considered children as the center of their lives and source of joy. *Parents expressed they were supported by their families and friends during the adoption process.

Conitnue...

Table 1. Continuation.

Author and year	Participants	Country	Research design	Methods to become parents	Sociodemographic characteristics	Findings
Peterson et al.[40]	3 gay fathers	The United States	Qualitative	Surrogacy, adoption	*Ages ranged from 30 to 50 years. *Participants had college or university degree *High income	*Family support and support within workplace were influential on their decisions to become parents. *Although parenthood was exhausting, they expressed that sharing experiences with their children was very important.
Goldberg[35]	84 parents (17 lesbian, 13 gay, and 12 heterosexual)	The United States	Qualitative	Adoption	*Mean age was 38.5.	*Legal challenges to adoption were the major problems during the adoption process. *Participants believed that social agencies could help adoptive parents to overcome the problems they experienced during the processes of adoption and preparation to parenthood.
Jennings et al.[38]	41 gay, 40 lesbian, and 49 heterosexual parents	The United Kingdom	Qualitative	Adoption	*Mostly around the age of 40 years *No data on education level *Most were working	*Parental decision was framed by societal prejudice, both as to whether their children would be discriminated against and whether they as parents would be supported. *Legal changes, visibility of gay and lesbian parents in the media, and the prompts of family and friends encouraged the participants to adopt children.
McConnachie et al.[39]	30 gay father, 29 lesbian mother, and 38 heterosexual parent families	The United Kingdom	Cross-sectional	Adoption	*Mean age was 46 years and above *Education and income levels were relatively high	*Gay fathers adopted older children compared to heterosexual parents. *Children in gay father families showed greater attachment security due to the characteristics of parents, the characteristics of the children, or the combination of two factors. *Secure-autonomous attachment of the children in gay father families was higher than children in heterosexual parent families. *Gay fathers were lower in depression and parenting stress than heterosexual parents.
Park et al., 2016	24 gay men, 24 lesbians, and 3 bisexual women	California and Nebraska states of the United States	Qualitative	Donor insemination, adoption, surrogacy IVF	*Mean age was 41 years. *Majority had college degree or above and annual income was high	* Parents wanted a safe and supportive environment for their children, which could be maintained by balancing the legal conditions with other factors, including job security, connection to family and friends, and a desire to create social change. *Participants reported their plans to move to another state, which had more legal protections for same-sex families. *Majority of the couples in both states were supported by their families, but only a few families were not supported their decision to parenthood.
Gartrell et al.[37]	131 lesbian parents	The United States	Qualitative	Donor insemination	*Mean age was 59.8 years *Most had a college degree or higher education and were employed full time	Distress about their children's experience of exclusion, nonacceptance of lesbian parent family by their family of origin, homophobia or hostility toward their nontraditional families, and lack of legal protections were the most challenging parenthood experiences of lesbian parents. *Other difficulties included dissatisfaction with the role of known donor in the family and disregarding the co-mother as parent.

Demographic characteristics

Marriage is an affirmative action to raise children. Although current average age for marriage and having the first children for heterosexual parents ranges from 30 to 35 years, it is mostly higher for LGBT+ individuals[13,14]. This delay is primarily related to the fact that it takes a longer period for LGBT+ individuals to make themselves accepted, have a profession, and reach their career goals, compared to their heterosexual counterparts[15,16]. This situation is positively considered in the literature since it prevents adolescent pregnancies, which have a negative impact on the health status of mother and children. Besides, our review revealed that education and income status of the LGBT+ individuals were higher than their heterosexual counterparts. This finding may be explained with reference to the need for higher economic status in order to be an LGBT+ parent[15,17,18].

Parenthood experiences

Positive experiences

Best parenthood experiences of LGBT+ individuals include acceptance by their families and the society that they live in and increase in their self-esteem[4,15,16]. As such, their identity as parent may be accepted by the society irrespective of their sexual identities[19]. Besides, various studies noted that same-sex parents had better social relations with their co-workers after the transition to parenthood[4,15,16,20,21].

Studies on lesbian mother families found that exhaustion and parental stress were lower for these families since parental responsibilities were equally shared[6,16]. This positive effect was evident in other studies that compared lesbian mother families with gay, bisexual, and heterosexual parent families[18,22-24]. This finding may be explained with reference to the absence of heteronormative codes in lesbian mother families.

Some of the studies on LGBT+ parents focused on the relationship between children and their parents and found that the LGBT+ parents, who spent more time on their children, considered their children a source of joy and the center of their lives[20,25]. Our review also found that social lesbian mothers felt the need to express the quality of their parenthood compared to their heterosexual male counterparts[22]. This finding may be related to the intention to overcome social prejudice and the need for self-expression.

Most of the LGBT+ individuals reviewed in our study considered parenthood a positive experience. Adopted same-sex parents used adoption agencies, friends, adoption books, and health professionals as sources of counseling and information and believed that the counseling and information services were sufficient[26,27]. Today, LGBT+ individuals do not have the right to marriage in most of the countries[28]. However, satisfaction of the same-sex parents with the recognition of the right to marriage and parenthood in some of the countries may be considered a positive dimension of this study.

Negative experiences

This review found that same-sex parents in various studies faced with various negative reactions due to their sexual identities when they decided to become parents[5,15,19]. Primary studies noted that parenthood of LGBT+ individuals was socially questioned, homophobia was common in their family and social environment, and LGBT+ individuals were under the pressure to form healthy parent-child relationship[7,15,29-32]. These studies also found that heterosexual parents suffered from problems, such as fatigue, lack of sleep, social isolation, and inability to balance work and family lives[7,15,29].

In addition to these negative experiences, LGBT+ individuals opted for costly assisted reproductive techniques compared to their heterosexual counterparts[4-6]. This situation, coupled with the increase in the costs, resulted with financial difficulties. In some cases, one of the same-sex parents left their job to care for the child, which, in turn, forced the other parent to seek for additional employment[5,15].

Child adoption is one of the most common methods of parenthood among the LGBT+ individuals[33]. Individuals who chose adoption as a way to become parents mostly suffered from legal problems, including procedures on adoption and legal recognition[34,35]. However, participants in the study by Ryan and Whitlock (2008) did not express any problems during the transition to parenthood[27]. These differences are closely related to the variety of legal regulations on parenthood of same-sex couples across the countries, even the states of same countries[27].

Implication of practice

This review may raise social awareness on parenthood experiences of LGBT+ individuals and provide the basis to solve their negative parenthood experiences. Besides, it may be guiding for health professionals, civil society organizations, legislators, and policy makers.

Strength and limitations

The review of the studies on the parenthood experiences of LGBT+ individuals reveals the existence of different studies for each groups[7,10]. The strength of our study is related to the fact that it systematically analyzed the parenthood experiences of all same-sex couples under the banner of LGBT+. Methodological dimensions and analysis methods of the studies reviewed within the scope of this research constitute both the strength and the limitation of this research.

Given the fact that most of the studies were conducted in the United States, a country that had higher income and education levels, the findings of this research may not be generalizable.

CONCLUSIONS

Although LGBT+ individuals are prone to discrimination due to their sexual identities, social acceptance increases after their transition to parenthood, which has a positive impact on their mental wellbeing. Once they become parents, LGBT+ individuals suffer from various difficulties, including financial problems and the burden of care.

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AUTHORS' CONTRIBUTIONS

NEB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing. **SOA**: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, and Writing – review & editing. **GD**: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing. **NŞ**: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, and Writing – review & editing.

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ORIGINAL ARTICLE

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Translation and cross-cultural adaptation of the Brazilian version of BREAST-Q©: breast reconstruction expectations module

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SUMMARY

OBJECTIVE: This study aimed to translate the BREAST-Q© — Breast Reconstruction Expectations Module (preoperative) 2.0 into Portuguese and adapt it to the Brazilian cultural context.

METHODS: Authorization for translation and cross-cultural adaptation of the questionnaire was obtained from the holders of the instrument's distribution rights. The questionnaire was translated and retro-translated. For cultural adaptation, the instrument was applied to 40 patients who had breast reconstruction surgery scheduled. Cronbach's alpha was used to assess the internal consistency.

RESULTS: The mean age of the patients was 53.5 years, and the majority (72.5%) was undergoing reconstruction with implants. Good and excellent internal consistencies were observed for the Coping and Appearance expectations scales (Cronbach's alpha values of 0.878 and 0.909, respectively). For the Pain scale, the internal consistency was moderate (0.738), and it was acceptable (0.587) for the Medical team.

CONCLUSION: The BREAST-Q© — Breast Reconstruction Expectations Module (preoperative) 2.0 was successfully translated and adapted to the Brazilian context.

KEYWORDS: Breast neoplasms. Mammaplasty. Quality of life. Validation studies. Surveys and questionnaires.

INTRODUCTION

Breast reconstruction plays a major role in the quality of life improvement, and it is considered a part of the breast cancer treatment[1-3]. Patient satisfaction and quality of life measures are essential in the assessment of surgical outcomes after breast reconstruction[4-6].

Patients have expectations regarding the effectiveness of their treatment and postoperative recovery, which are built from information received in the preoperative period[7-9]. Therefore, exploring patients' expectations is important to establish actions in order to avoid misconceptions regarding treatment and to improve satisfaction with the surgical care[10,11].

The BREAST-Q© is a procedure-specific patient-reported outcome (PRO) measure, which was designed to assess patient satisfaction and health-related quality of life following breast surgery[12,13]. The Breast Reconstruction Expectations Module[14] is not yet available for use in Brazil.

This study aimed to translate into Portuguese and adapt the BREAST-Q© — Breast Reconstruction Expectations Module[14] (preoperative) 2.0 to Brazilian cultural context.

METHODS

The Institutional Ethics Committee approved the study, and written informed consent was obtained from all the participants. A convenience sample of 40 women was selected at the Breast Center's Philanthropy Clinics of Sírio Libanês Hospital. Patients diagnosed with breast cancer, aged between 18 and 65 years, and candidates for breast reconstruction after mastectomy by any technique were eligible.

The instrument

The BREAST-Q© — Breast Reconstruction Expectations Module consists of scales developed to be administered preoperatively only[14]. These scales assess expectations for Support from Medical Team (how much time and emotional support the patient expects to receive from the medical team and the surgeon), Pain (magnitude of pain the patient expects to face in the first postoperative week), Coping (how the patient is anticipating she will cope with the process of breast reconstruction during the first postoperative year), and Breast Appearance (expectation with appearance and sensation of the reconstructed breast 1 year after surgery)[14]. The scales can be used either independently or together, and each of them generates a score ranging from 0 to 100.

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Translation and linguistic validation

Initially, we obtained authorization from the holder of the distribution rights to translate, validate, and culturally adapt the instrument into Brazilian Portuguese. The methodology for translation and cultural adaptation was based on the model recommended by Beaton et al.[15].

The original version of the instrument, i.e., in English, was translated into Brazilian Portuguese by two translators, independently. A multidisciplinary team, which was composed of two plastic surgeons, a psychologist, and a physiotherapist, reviewed the two translations and a consensual version in Portuguese was produced. The linguistic context was appropriately adapted, and all the essential characteristics of the original questionnaire in English were maintained. The idiomatic, semantic, conceptual, and cultural equivalences were preserved.

Two other translators translated the Brazilian version back to English. The same multidisciplinary committee compared the original English version with the translated and back-translated versions of the questionnaire, resulting in another consensual version of the instrument in Portuguese. This new Portuguese version, together with the back-translated version, was sent to the author of the original instrument who approved it.

Cultural adaptation or pretest

The Portuguese version was applied to a group of 20 patients to verify the understanding of the instrument (pretest group 1). All patients were asked to express their understanding of each item and to suggest changes if deemed necessary. Items not understood by 20% or greater of patients were reviewed by the multidisciplinary team, and the instrument was reformulated. A second Portuguese version was produced when all items were understood by at least 80% of the participants.

This Portuguese version 2 was administered to a second group of 20 patients (pretest group 2), who fully understood the content of the instrument and, therefore, version 2 was considered the final version.

Statistical analysis

The scores of each expectation scale (i.e., Medical team, Pain, Coping, and Appearance) were compared by patient´s characteristics via analysis of variance (ANOVA). When there were differences in the means, these were identified by means of the Duncan's multiple range test. The linear association between scores and ages was assessed using Pearson's correlation.

The comparison of mean scores of the expectation scales was performed using Friedman's nonparametric test, due to the violation of the assumption of normality in the data distribution. The internal consistency of the scales was assessed using Cronbach's alpha.

The statistical analysis was performed by using the Statistical Package for Social Sciences (SPSS) version 20.0, and an alpha level of 5% ($p \leq 0.05$) was adopted.

RESULTS

The mean age of the 40 patients was 53.5±9.0 years (range 33–65 years). They were mostly white (67.5%) and nonpartnered women (55%). The education level among the patients extended from elementary school (45%), high school (22.5%), and college (32.5%). The majority (72.5%) intended to perform reconstruction using an implant.

The application of the first Portuguese version to the pretest group 1 resulted in some changes. The term "razoavelmente provável" ("reasonably likely") raised doubts and was replaced by "pouco provável" ("unlikely") in questions 4, 5, 9, 10, 23, and 25. Question 7 was reworded to "... quanta dor você espera sentir com o expansor?" ("... how much pain do you expect to feel with the expander?"). The word "sensibilidade" ("sensitivity") was better understood than the word "sensação" ("sensation"), leading to changes in questions 14, 20, and 22. Finally, in question 24, the word "consciência" ("awareness") was replaced by "percepção" ("perception").

After these modifications, a second Portuguese version of the instrument was created. This version, applied to the pretest group 2, obtained complete cultural equivalence and was considered the final version.

About the expectations regarding information, involvement in decision-making, and the possibility of complications in surgery, 40% of the patients said that they wanted to receive all the information about the surgery, 50% said that they were very involved in decision-making, and 39.4% said that it is very unlikely that any complications will occur after surgery. In the expectations related to the medical team scale, all patients indicated that they thought it would be very likely to receive care quickly and have the surgeon and nurses available when needed, as well as receiving support from them. In contrast, more than 50% said it was unlikely to feel "as if she was unique" and that the surgeon would spend a lot of time with them.

Regarding expectations about pain, more than 57% of patients said that it was unlikely to feel hurt or experience intense pain. However, at least 50% felt very likely to experience discomfort and need a lot of pain medication. As for coping expectations, more than 80% of women believed that the situation is very likely to improve or that adaptation to the new condition is possible.

Regarding expectations with the appearance of the reconstructed breast and the scars 1 year after the surgery, 56.8% of the women said that they expected the new breast to look

beautiful, and 60.6% said that the scars would be somewhat noticeable. At least half of the patients expected that the two breasts will be similar to each other, that they will have some sensation in the new breast, that the size will be slightly different from their natural (smaller or larger), that the new breast will have less movement than the natural, that the sides of the chest will be slightly different than before surgery, and that the sides of the chest will feel normal.

In the assessment of expectations regarding the characteristics of the reconstructed breast, 63.2% of the patients said that they expect the reconstructed nipple to look similar to normal and 57.3% mentioned to believe that they will have no sensation in their nipples after reconstruction. Among women who intended to perform reconstruction using an implant, 67.9% said that the breast will feel harder than the natural and 39.3% said that the new breast will feel like a natural part of their body.

For the expectations after 10 years, 41% of the patients indicated to believe that their breasts will not be as symmetrical as they were after the reconstruction, and 62.9% expected further reconstruction procedures will be unnecessary.

The levels of expectations were not similar between the four aspects (i.e., Medical team, Pain, Coping, and Appearance). The expectation related to pain was lower than the others, which were similar to each other (Table 1).

Table 2 shows Pearson's correlations between age and expectations scores. There was only a weak significant positive association between age and pain score (r=0.351; p=0.033), indicating that the older the patient, the greater her expectation of pain.

There were no differences in mean scores of expectations regarding the Medical team, Pain, Coping, and Appearance in relation to marital status or skin color. Regarding education, differences in means were found only for the expectation score related to the Medical team (p=0.022). Higher education patients had the lowest average compared to the others.

As for the type of reconstruction, differences were found in the pain expectancy score (p=0.036). Women candidates for reconstruction using local flaps had lower average compared to the others, similar to each other.

The scales expectation with Coping and Appearance showed good/excellent internal consistencies (Cronbach's alpha values of 0.878 and 0.909, respectively). The internal consistency for the Pain expectation scale was moderate (Cronbach's alpha value of 0.738) and, for the Medical team, it was acceptable (Cronbach's alpha value of 0.587).

DISCUSSION

To increase the benefits for patients and guide the decision-making process for treatment, accurate and relevant information must be provided preoperatively in a clear, objective, and efficient manner[10,16,17]. When the information received is insufficient, it can lead to imprecise expectations, culminating in disappointment with the surgical result and the postoperative recovery[5,8].

Identifying patients' expectations when making a decision regarding surgical treatment can potentially improve the informed consent process and prepare them for their postoperative recovery and also for the possibility of complications and the need for other treatments[10]. Furthermore,

Table 2. Pearson's correlation between age and scores of expectations regarding the Medical team, Pain, Coping, and Appearance.

Scales of expectation	Pearson's Correlation	
	Estimate	p
Medical team	-0.203	0.221
Pain	0.351	0.033
Coping	-0.100	0.557
Appearance	-0.144	0.388

Table 1. Summary measures of the expectations scores regarding the Medical team, Pain, Coping, and Appearance and comparison between the scales.

	Expectations Scales			
	Medical Team[A]	Pain[B]	Coping[A]	Appearance[A]
Variation	54–100	0–80	42–100	53–100
First Quartile	78	30	100	91
Median	78	48	100	100
Third Quartile	100	59	100	100
Mean±SD	82.8±14.5	43.3±19.7	95.2±13.7	93.2±13.3
Friedman's test	p<0.001*			

*p: Descriptive level of Friedman's nonparametric test. [A, B] Different means according to multiple Dunn-Bonferroni comparisons.

exploring expectations individually can allow surgeons to recognize patients who have unrealistic expectations, in order to address misunderstandings in the preoperative moment, through better education. When multiple surgical options exist, shared decision-making can be enhanced by careful exploration of expectations[16].

The BREAST-Q© — Breast Reconstruction Expectations Module (preoperative) 2.0 was successfully translated and adapted to the Brazilian context. To the best of our knowledge, there is no other validated instrument to assess expectations regarding breast reconstruction among Brazilian patients. The availability of this instrument for use in Brazil makes it possible to measure, accurately, information about patients' expectations for use in clinical trials and clinical practice. Knowledge of Brazilian patients' expectations regarding breast reconstruction will allow identifying opportunities to improve patient education and promoting greater postoperative satisfaction and quality of life.

CONCLUSION

The BREAST-Q© — Breast Reconstruction Expectations Module (preoperative) 2.0 has been translated and adapted to the Brazilian context.

AUTHORS' CONTRIBUTIONS

IGEO: Conceptualization, Data curation, Formal Analysis, Investigation, and Writing – review & editing. **MSN**: Conceptualization, Formal Analysis, Supervision, Writing – review & editing. **LCA**: Formal Analysis, Writing – original draft, and Writing – review & editing. **HKU**: Formal Analysis, Writing – original draft, and writing – review & editing. **LMF**: Conceptualization, Formal Analysis, and Writing – review & editing. **DFV**: Conceptualization, Methodology, Formal Analysis, Supervision, Writing – original draft, and Writing – review & editing.

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ORIGINAL ARTICLE

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Lung cancer screening in clinical practice: identification of high-risk chronic obstructive pulmonary disease patients

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SUMMARY

OBJECTIVE: The NELSON study demonstrated a positive association between computed tomography scanning and reduced mortality associated with lung cancer. The COPD-LUCSS-DLCO is a tool designed to improve screening selection criteria of lung cancer for chronic obstructive pulmonary disease patients. The aim of this study was to examine and compare the discriminating value of both scores in a community-based cohort of chronic obstructive pulmonary disease patients.

METHODS: A retrospective study of chronic obstructive pulmonary disease patients followed in pulmonology consultation for a period of 10 years (2009–2019) was conducted. The NELSON criteria and COPD-LUCSS-DLCO score were calculated for each patient at the time of the study inclusion. The lung cancer incidence was calculated for each of the subgroups during the follow-up period.

RESULTS: A total of 103 patients were included in the study (mean age 64.7±9.2 years, 88.3% male). Applying the COPD-LUCSS-DLCO score, high-risk patients have a 5.9-fold greater risk of developing lung cancer versus the low risk. In contrast, there was no significant association between NELSON selection criteria and lung cancer incidence. The area under the curve was 0.69 for COPD-LUCSS-DLCO and 0.59 for NELSON criteria. Comparing test results showed no differences.

CONCLUSIONS: The use of the COPD-LUCSS-DLCO score in clinical practice can help to detect chronic obstructive pulmonary disease patients in greater risk of developing lung cancer with better performance than NELSON criteria. Therefore, models that include a risk biomarker strategy can improve selection criteria and consequently can enhance a better lung cancer prediction.

KEYWORDS: Lung cancer. Screening. Chronic obstructive pulmonary disease.

INTRODUCTION

Lung cancer (LC) is the second most common malignancy worldwide and is responsible for the highest mortality burden[1]. The considerable majority of patients are diagnosed in advanced stages and, consequently, the overall survival at 5 years remains low[2]. Therefore, it is urgent the design of strategies to identify patients at high risk of developing LC in order to detect the disease at an early and potentially curable stage. The National Lung Screening Trial (NLST) demonstrated that screening high-risk individuals with low-dose computed tomography (LDCT) is effective in detecting early stages of the disease and achieving a reduction in LC mortality of approximately 20%[3]. More recently, the largest randomized LC screening trial in Europe, the Dutch-Belgian Randomized Lung Screening Trial (NELSON), also found reduction in LC mortality by 26% in screening with LDCT[4].

Unfortunately, we do not still have the necessary conditions in our country, Portugal, to start LC screening with LDCT and, therefore, we put our focus attention on individual patients with comorbidities and habits more associated with LC, including chronic obstructive pulmonary disease (COPD). There is plenty of evidence that establishes an association between COPD and LC[5-9]. Beyond sharing smoking as a main etiological factor, several biopathogenic pathways may explain this deadly association[10]. Additionally, several authors have suggested that the presence of emphysema increases 2- to 3-fold the risk of LC, independent of tobacco history, age, sex, airway obstruction, and body mass index (BMI)[9,11,12]. Subsequently, Torres et al. developed a COPD-specific score to predict LC risk for patients with COPD (COPD-LUCSS)[13] that is determined by four parameters, namely, age, BMI, pack-years of smoking history, and the presence of emphysema in the LDCT. However, in clinical practice, most of the patients with COPD

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do not have a chest CT available and later the same authors proposed a modified version of the score, in which diffusing capacity for carbon monoxide (DLCO) is used as a surrogate marker of emphysema (COPD-LUCSS-DLCO). This system classifies patients into high-risk group with 2.4 increased risk of death by LC when compared to the second group, i.e., the low-risk group[14].

The aim of this study was to examine and compare the discriminating ability of COPD-LUCSS-DLCO and NELSON selection criteria to identify patients with the highest risk of LC in our population of COPD patients treated in pulmonology consultations.

METHODS

Study Population

An observational retrospective study was conducted on a cohort of patients diagnosed with COPD recruited from pulmonology consultations and followed over a 10-year period, between January 1, 2009 and December 31, 2019, at the Centro Hospitalar e Universitário de Coimbra (CHUC). The inclusion criteria were an age greater than 40 years and diagnosis of COPD. The exclusion criteria were the presence of chronic respiratory disease caused by something other than COPD and personal history of oncological diseases.

Clinical and Physiological Parameters Measurements

Data were retrospectively collected from the patients' medical records, including demographic information (e.g., age, sex, and cigarette smoking), pulmonary function tests, and date of diagnosis of LC. Pulmonary function tests (e.g., spirometry and diffusing capacity) were performed according to the European Respiratory Society (ERS)/American Thoracic Society (ATS) guidelines[15]. COPD was diagnosed in patients with a history of at least 10 pack-years of cigarette smoking and a post-bronchodilator forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) ratio less than 0.70[15]. COPD patients were classified using the grades of airway limitation according to Global Initiative for COPD (GOLD) strategy. Each patient was attributed a COPD-LUCSS-DLCO and NELSON score calculated at the time of study inclusion. The patients were subsequently divided into groups of high and low risk, according to the components of each system.

Statistical analysis

Descriptive statistics was used to describe the characteristics of all the participants. Quantitative data with a normal distribution were expressed as mean and standard deviation (SD) and those variables without normal distribution were expressed as median and interquartile range (P25–P75). Qualitative data were described using relative frequencies. The association between the COPD-LUCSS-DLCO and LC, and between the NELSON criteria and LC was assessed by Cox proportional hazards regression. To compare the predictive capacity of COPD-LUCSS-DLCO for LC based on the NELSON criteria, we performed a receiver operating analysis and intra model area under the curve (AUC) comparisons. An AUC varies between 0 and 1, in which a value of 1 indicates a perfect diagnostic tool with 100% sensitivity and 100% specificity, whereas an AUC of 0.5 implies no discrimination. All analyses were performed using the statistical program SPSS version 20.0, and all hypothesis tests were bilateral, with a significance level of 5%.

RESULTS

A total of 103 subjects were included in this retrospective cohort and their characteristics are described in Table 1. The mean age was 64.7±9.2 years, men constituted 88.3% of the patients, and 36.9% were active smokers. According to the GOLD classification, 5.8% of the patients were categorized as GOLD 1, 41.7% as GOLD 2, 43.7% as GOLD 3, and 8.7% as GOLD 4. The median follow-up time was 92.4 months (IQR 57–120). About 55.5% of the LC cases were diagnosed in the first 60 months and 61.0% in the first 72 months after inclusion.

Applying the COPD-LUCSS-DLCO, 52.4% of the individuals were qualified to the high-risk category and 47.6% to the low risk. Among the patients of the high-risk group, 15 (27.8%) of 54 individuals were diagnosed with LC during the follow-up, and 3 (6.1%) of 49 patients of the low-risk group. Using the NELSON criteria, 74.8% subjects were characterized as high risk and 25.2% subjects were characterized as low risk. In the high-risk group, 16 (20.8%) of 77 individuals developed LC, and 2 (7.7%) in 26 individuals developed LC in the low-risk group. The distribution of LC in each group is shown in Figures 1 and 2.

Furthermore, we conducted a Cox regression analysis and identified that COPD-LUCSS-DLCO scores were significantly associated with LC in our population. Hazard ratio (HR) for the high risk versus the low risk in

Table 1. Characteristics of patients with chronic obstructive pulmonary disease.

Variable	n=103
Age, mean±SD	64.7±9.2
Gender, n (%)	
Female	91 (88.3)
Male	12 (11.7)
BMI, mean±SD	26.6±5.5
Active smokers, n (%)	38 (36.9)
Former smokers, n (%)	65 (63.1)
Pack-years, mean±SD	50.9±29.5
FEV_1, %	51.4±18.0
FVC, %	84.3±19.7
DLCO, %	66.2±19.6
GOLD 2009 I/II/III/IV degrees, n (%)	5.8/41.7/43.7/8.7
COPD-LUCSS-DLCO score, n (%)	
Patients with high-risk score	54 (52.4)
Patients with low-risk score	49 (47.6)
NELSON criteria, n (%)	
Patients with high-risk score	77 (74.8)
Patients with low-risk score	26 (25.2)
Median follow-up time, months (IQR)	92 (57–120)
Lung cancer diagnoses in all patients	18
COPD-LUCSS high-risk score	15
COPD-LUCSS low-risk score	3
NELSON high-risk score	16
NELSON low-risk score	2

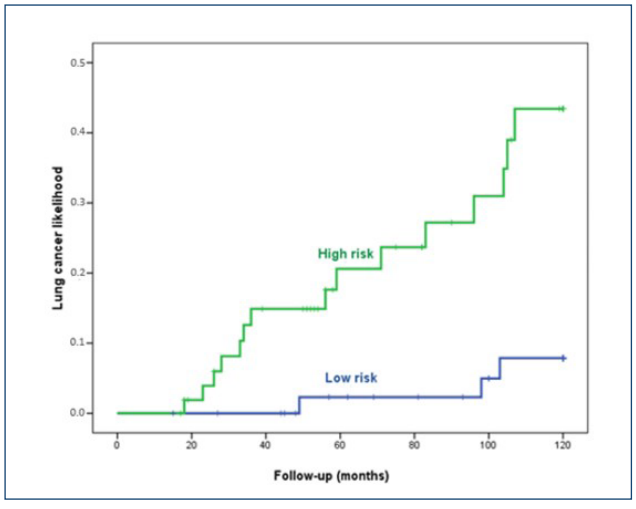

Figure 1. Incidence curves for lung cancer according to the COPD-LUCSS-DLCO score.

the COPD-LUCSS-DLCO was 5.9 (95%CI 1.71–20.44; p=0.005), showing that patients in the highest risk category have a 5.9-fold greater risk of developing LC. In contrast, there was no significant association between NELSON selection criteria and LC incidence (HR=2.8, 95%CI 0.67–12.25; p=0.168).

Concerning discriminative capacity of the two screening systems, a receiver operating characteristic analysis showed AUC values of 0.69 for the COPD-LUCSS and 0.59 for the NELSON criteria (Figure 3). However, there was no significant difference between the AUC values of two screening systems (p=0.16).

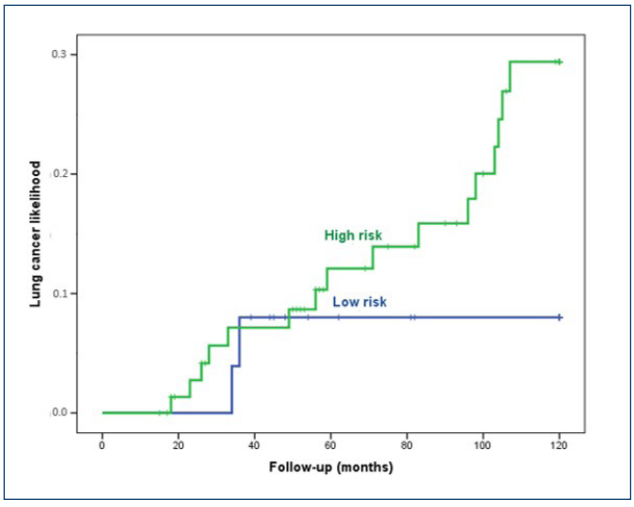

Figure 2. Incidence curves for lung cancer according to the NELSON criteria.

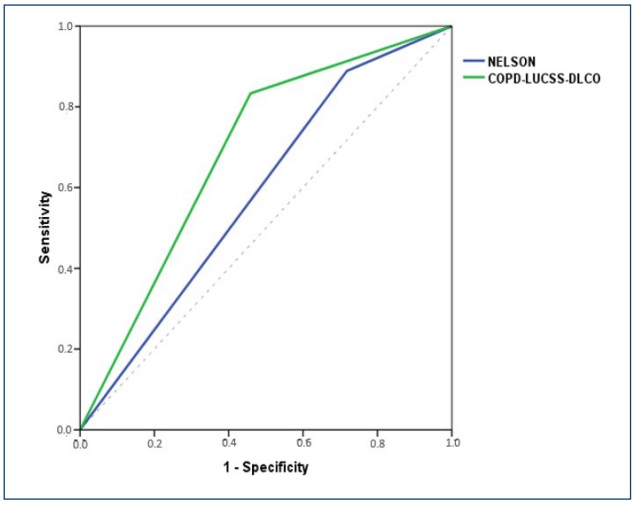

Figure 3. ROC curve of the COPD-LUCSS-DLCO and the NELSON criteria in patients with COPD.

DISCUSSION

In this study, we aimed to analyze the usefulness of COPD-LUCSS-DLCO and NELSON selection criteria to identify individuals with COPD with high risk of LC.

Our findings demonstrated that COPD-LUCSS-DLCO was significantly associated with LC and that the COPD patients in the highest risk category had a 5.9-fold greater risk of developing LC compared with the low-risk group. In opposition, the NELSON criteria indicated an incidence of LC in the high-risk patients of 2-fold than the low-risk patients, but this increase was not statistically significant.

We evaluated the discrimination capacity of the two screening systems, and we verified that both systems were identical (no statistical difference). However, COPD-LUCSS-DLCO presented a higher AUC value, approaching to the levels of acceptable discrimination (0.69). The enhanced accuracy of COPD-LUCSS-DLCO can be explained by the increased number of variables included, namely, BMI and DLCO (surrogate of emphysema). Although age and smoking criteria remain the most common metrics used to identify those eligible for screening, risk models might benefit from including other biomarkers. The use of parameters like BMI, family history of LC, occupational exposure, and genetic predictors has been previously described in the context of optimizing the selection of candidates for screening[16-19].

Our findings that the COPD-LUCSS-DLCO is associated with LC among COPD patients corroborated the findings stated by Torres et al.[14] who created this score. The authors reported a 2.4-fold increase mortality in high-risk patients compared with the low-risk group. However, Torres et al. followed their cohort for 5 years, and our study had a follow-up of 10 years, which can explain our increased mortality rate in high-risk patients. It is more likely to have LC diagnoses as the time increases.

Besides supporting previous published results, our study validates the COPD-LUCSS-DLCO use in a cohort of COPD patients recruited from pulmonology consultations. These results emphasized the utility of this score in identifying COPD patients with high risk of LC in a typical situation of standard clinical practice where, unfortunately, not all patients undergo CT.

Although the relevant results obtained, this study has some limitations that need to be considered. First, this was a retrospective study in design and, hence, we did not perform a standard protocol with CT scans in the follow-up of patients. Second, the study population belonged to a single hospital and the sample size was limited. Third, it is possible that an information bias occurred due to obtaining the variables from the patients' medical records.

CONCLUSIONS

COPD-LUCSS-DLCO score was significantly associated with LC among COPD patients, in contrast to the NELSON selection criteria. Models that include a risk biomarker strategy can improve the identification of high-risk patients and consequently can enhance a better LC prediction.

AUTHORS' CONTRIBUTIONS

SRS: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft. **JNC:** Formal Analysis, Investigation, Methodology, Writing – original draft. **CR:** Formal Analysis, Investigation, Supervision, Writing – review & editing. **AF:** Formal Analysis, Investigation, Supervision, Writing – review & editing. **FB:** Formal Analysis, Investigation, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Ozone combined with doxorubicin exerts cytotoxic and anticancer effects on Luminal-A subtype human breast cancer cell line

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SUMMARY

OBJECTIVE: We aimed to examine the potential anticancer effects of ozone applied after chemotherapeutic treatment with different concentrations of doxorubicin in Luminal-A subtype of human breast cancer cell line (MCF-7) and compare the results with effects on L929 fibroblast cell line.

METHODS: Both cell lines were incubated with increasing doses of doxorubicin (1–50 μM) for 24 h at 37°C. Then, half of groups were incubated with 30 μg/mL ozone for 25 min as combination groups. Cell viability was analyzed by MTT assay, apoptosis by flow cytometry, and levels of tumor necrosis factor alpha, transforming growth factor beta, and matrix metalloproteinase-2 and MMP-9 by immunocytochemistry.

RESULTS: Doxorubicin + ozone treatment enhanced viability of L929 ($p<0.01$) but reduced viability of MCF-7 compared to only doxorubicin-applied cells without ozone treatment ($p<0.001$). This combined treatment also enhanced apoptotic effect of doxorubicin on MCF-cells ($p<0.001$), but not on L929. It significantly increased all protein levels of L929 compared with those of other groups ($p<0.05$ for tumor necrosis factor alpha and MMP-2; $p<0.01$ for transforming growth factor beta and MMP-9). This treatment reversed the effect of doxorubicin on tumor necrosis factor alpha levels and considerably reduced MMP-2 and MMP-9 levels of MCF-7 compared with those of control group ($p<0.01$ and $p<0.001$, respectively).

CONCLUSION: Ozone treatment potentiated the apoptotic and anticancer activities of doxorubicin in MCF-7 cells and showed repairing and healing effect on healthy fibroblast cells, which were damaged from cytotoxic effects of chemotherapeutic agent. MCF-7 cells may acquire sensitivity against the doxorubicin combined with ozone treatment through activating tumor necrosis factor alpha, MMP-2, and MMP-9 expressions.

KEYWORDS: Breast cancer. Doxorubicin. MCF-7. Ozone. Fibroblast.

INTRODUCTION

Ozone therapy is an alternative treatment of remarkable clinical interest in the field of oncology[1], showing different effects at different concentrations (changing between 1 and 50 μg/mL) on different organs[1,2]. The ozone treatment was demonstrated to enhance anticancer effects of conventional anticancer drugs like 5-fluorouracile and to exert anti-inflammatory effects on colon cancer[3] and melanoma cells[4]. Moreover, ozone can alter the tumor microenvironment by reducing the production of cytokines involved in the survival and chemoresistance of cancer cell.[5]

Breast cancer is the most common type of cancer in women and second most common reasons of cancer-related mortalities. According to the reports of World Health Organization, 1.2 million new cases are diagnosed each year, with more than 500,000 deaths in all over the world[6]. Breast cancer could be classified into at least five subtypes: Luminal-A, Luminal-B, HER2, basal, and normal subtypes according to the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Luminal-A cancers are low grade, tend to grow slowly, and have the best prognosis. The immune profile of Luminal-A type breast cancer is ER+, PR+/–, and HER2–, and these cancers are responsive to the endocrine therapy[7]. The addition of chemotherapy to endocrine therapy for breast cancer generally provides little benefit. However, the value of chemotherapy in all patients with Luminal-A breast cancer is controversial[8].

Of the cell lines commonly incorporated into the in vitro models of cancers, ER-positive luminal A cell line MCF-7 has been intensively used to investigate the effectiveness of anticancer therapies[9]. However, L929 is a noncarcinogenic murine cell line that has been used as a control group, especially in cancer studies[10].

From a medical point of view, the investigation of ozone treatment could be of great interests to interpret the ozone-related

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multiple effects in the cancer patients. The aim of this study was to examine the potential in vitro anticancer effects of ozone applied after a chemotherapeutic treatment with different concentrations of doxorubicin in MCF-7 human breast cancer cell line and compare the results with the effects on L929 fibroblast cell line.

METHODS

Cell culture and experimental groups

L929 fibroblast cell line and MCF-7 human breast cancer cell line purchased from ATCC (USA) were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin–streptomycin and maintained at 37°C in a humidified atmosphere with 5% CO_2. When cells grown as adherent monolayer reached 70–80% confluency, they were passaged using trypsin EDTA (Sigma, USA).

L929 was used as a control group, while MCF-7 was used as an experimental group. Both cell lines were grown for 24 hours to reach to a number of 1×10^4 cells/well, then incubated with increasing doses of doxorubicin (ranged from 1 to 50 µM) for 24 h at 37°C. At the end of incubation, half of groups were incubated with 30 µg/mL ozone for 25 min, produced with Medical Ozone Generator (Turkozone® Blue S) in a specific setup, following the same method described in literature[5].

MTT assay

The cell viability (%) was determined with MTT assay kit (Thiazolyl Blue Tetrazolium Bromide, Sigma Aldrich, Missouri, ABD, Cas: 298-93-1). After incubation, both cell lines were washed slowly in PBS at pH 7.4, mixed with 10 µl MTT, and incubated for 3 h at 37°C. Cell viability was measured at 540 nm using a spectrophotometer (SPECTROstar® Nano [ABS]) and then calculated according to following formula: Viability = (Sample–Blank) / (Control–Blank). All experiments were repeated for three times in 3 different weeks.

Flow cytometry

Apoptosis and necrosis at 24 h after treatment with increasing doses of doxorubicin and ozone were detected using flow cytometric assay by staining with Annexin V 7-Aminoactinomycin D (7-AAD) kit (Invitrogen/Biolegend, San Diego, CA, USA). As per the manufacturer's protocol, MCF-7 and L929 cells (8×10^5 cells/well) were seeded in 6-well plates containing 2 mL of medium and incubated at 37°C for 24 h. After the cells were harvested individually into Eppendorf tubes, they were washed twice with cold PBS. Then, all cells were centrifuged at 1500 rpm (2819×*g*) for 5 min and the supernatant was removed. Annexin V binding buffer was added, and cells were counted as 10^6 cells/mL. Then, 5 µl Annexin V+5 µl 7-AAD were added, cells were incubated for 15 min at room temperature in dark. Later, 400 µl binding buffer was added on ice, and the percentages of apoptosis and necrosis were analyzed by flow cytometry (BD Accuri™ C6 Plus).

Immunocytochemistry

The immunocytochemical analysis of tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), and matrix metalloproteinase-2 (MMP-2) and MMP-9 was performed using a horseradish peroxidase/AEC Detection IHC Kit (ScyTek Laboratories, USA, cat no: ACD002) according to the literature[11]. Primary antibodies of TNF-α (Santa Cruz Biotechnology Cas: 52B83, Lot: G2018), TGF-β (ThermoFisher Scientific, MA, USA, product no: MA5-16949), MMP-2 antibody (Invitrogen, USA, MA5-13590), and MMP-9 antibody (Invitrogen, USA, MA5-15886) were diluted at 1/100, and 150 µl of diluted antibodies were added onto 1×10^4 cells. Stained cells were scored as follows: 0: no staining, 1: mild staining, 2: moderate staining, and 3: strong staining, and a total score was given between 0 and 300.

RESULTS

Ozone treatment enhanced antiproliferative effects of doxorubicin on MCF-7

The morphological analysis divulged that cell proliferation of both L929 and MCF-7 was greatly inhibited by 5 µM doxorubicin compared to the control groups, while addition of ozone to the treatment reversed the cytotoxic effect of doxorubicin in L929 but conversely enhanced this effect in MCF-7 cells. MTT assay revealed that the increasing doses of doxorubicin significantly decreased the cell viabilities of both cells, and the effective dose was 5 µM, especially for MCF-7 ($p<0.001$; Figure 1). Addition of ozone after doxorubicin treatment showed more cytotoxicity on the viability of MCF-7 cells than those of L929 cells. Interestingly, the ozone treatment after application of increasing doses of doxorubicin significantly enhanced the viability of L929 cells ($p<0.01$) but reduced the viability of MCF-7 compared to the only doxorubicin-applied cells without ozone treatment

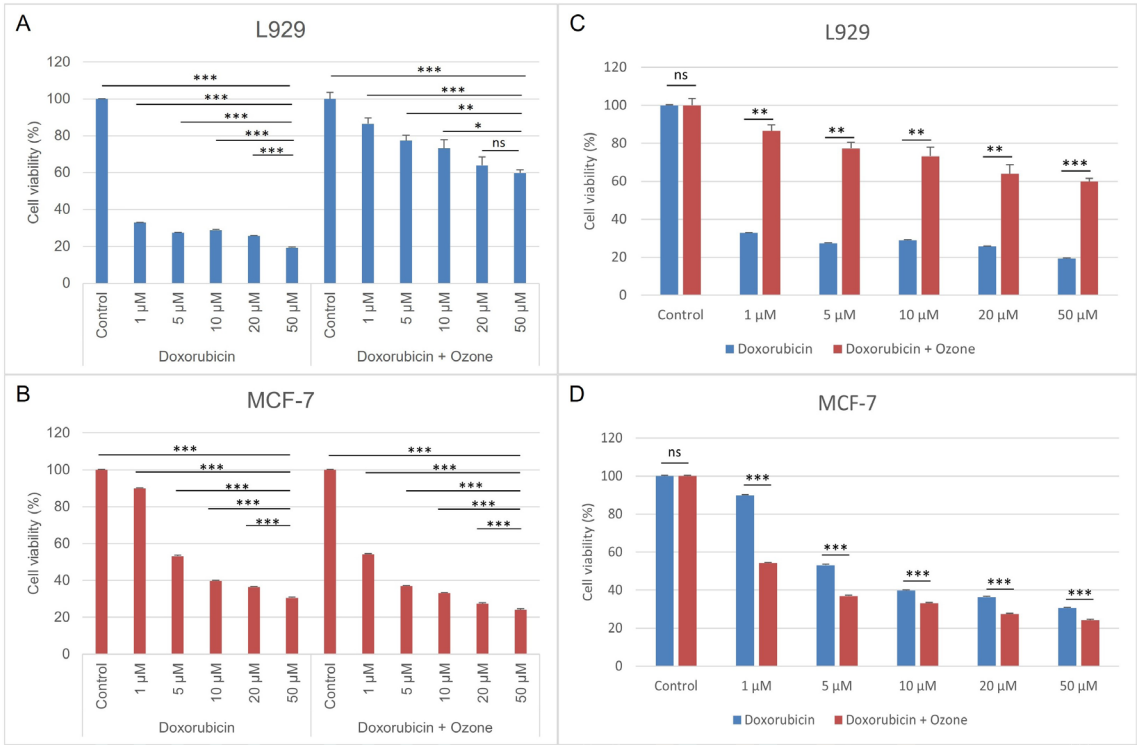

Figure 1. The cell viabilities (%) of L929 and MCF-7 cells calculated according to the results of MTT assay. The intragroup comparisons of cell viabilities of L929 (A) and MCF-7 (B) cells treated with increasing doses of doxorubicin (ranged from 1 to 50 µM). The intergroup comparisons of cell viabilities of L929 (C) and MCF-7 (D) cells treated with or without ozone. All data shown are given as mean±standard deviation of three different experiments performed independently. *p<0.05, **p<0.01, and ***p<0.001 are the statistically significance levels. ns: not significant.

(p<0.001; Figure 1). These results suggest that the doxorubicin treatment alone may inhibit the cell viability of both L929 and MCF-7 cells in a dose-dependent manner, and addition of the ozone application mainly enhanced the antiproliferative effect of doxorubicin on the MCF-7, but not on L929 cells.

Ozone treatment enhanced apoptotic effect of doxorubicin on MCF-7 cells

Flow cytometric analysis showed that MCF-7 cells were relatively more resistant to 5 µM doxorubicin alone as compared to L929 cell line (p<0.001; Figure 2). L929 cells that were treated with doxorubicin combined with ozone showed a significantly higher ratio of cell death compared to untreated cells (p<0.001) and significantly lower ratio compared to doxorubicin-alone-treated cells (p<0.001). MCF-7 cells that were treated with 5 µM doxorubicin combined with ozone showed a considerably higher ratio of cell death compared to both untreated and doxorubicin-alone-treated cells (p<0.001). These results strongly suggest that the combination of ozone with doxorubicin enhanced the apoptotic effect of doxorubicin on MCF-7 cells, but not on L929 cells.

Ozone treatment combined with doxorubicin altered the expressions of TNF-α, MMP-2, and MMP-9 in MCF-7 cells

Immunocytochemical analysis showed no significant difference between the scores of control group and 5 µM doxorubicin-alone group of L929 cells for TNF-α, TGF-β, MMP-2, and MMP-9 proteins (Table 1). However, the combination treatment significantly increased the scores of L929 cells for all proteins compared with those of the other groups (p<0.05 for TNF-α and MMP-2; p<0.01 for TGF-β and MMP-9). For MCF-7 cells, doxorubicin alone did not change the scores of TGF-β, MMP-2, and MMP-9 immunostainings but strongly increased the score of TNF-α immunostaining compared with the control

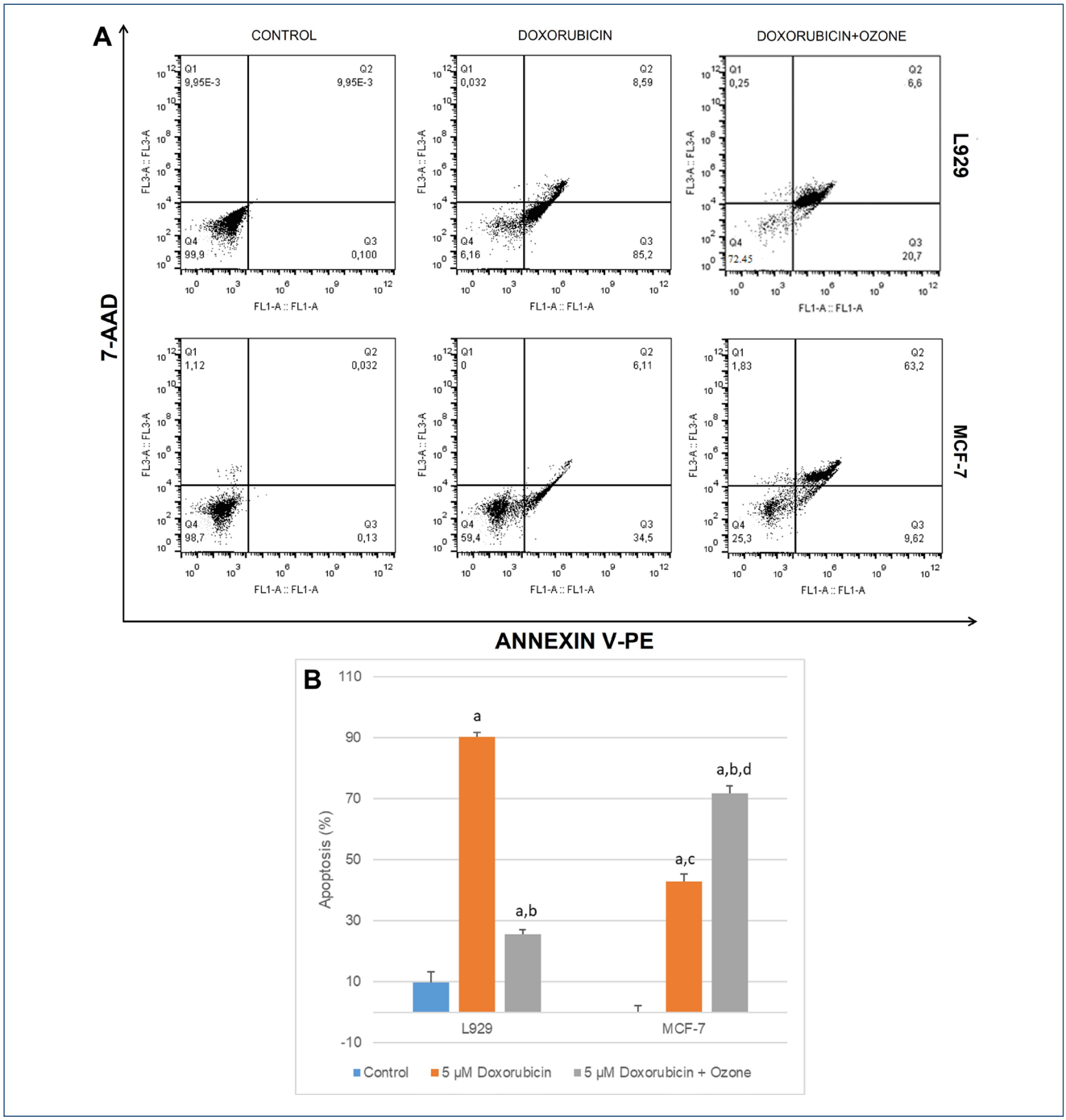

Figure 2. Flow cytometric analysis of Annexin V-PE/7-AAD-stained L929 and MCF-7 cells. A: Flow cytometry results are exhibited as dot plots; B: Bar graph representation of quantitative results of flow cytometry. Quantitative data are shown as mean±standard deviation of three different experiments performed independently. [a] p<0.0001 vs. control groups; [b] p<0.0001 vs. 5 µM Dox groups; [c] p=0.0003 vs. 5 µM doxorubicin-treated L929 cells; [d] p=0.0001 vs. 5 µM doxorubicin + ozone-treated L929 cells.

group (p<0.01). In contrast, the combination treatment with ozone reversed the effect of doxorubicin on TNF-α levels and considerably reduced MMP-2 and MMP-9 immunostaining of MCF-7 cells compared with the control group (p<0.01 and p<0.001, respectively). In addition, the combination treatment significantly reduced MMP-9 immunostaining of MCF-7 cells compared with the doxorubicin-alone group (p<0.001) (Table 1). These results suggest that the ozone treatment combined with doxorubicin altered the expressions of TNF-α, MMP-2, and MMP-9 in MCF-7 cells, but not that of TGF-β.

Table 1. Immunocytochemical findings for L929 and MCF-7 cell lines.

Cell	Protein	Control	5 μM doxorubicin	5 μM doxorubicin + ozone	p-value
L929	TNF-α	140.0±22.4	120.0±27.4	190.0±22.4[a]	0.0242
	TGF-β	180.0±27.4	220.0±27.4	300.0±0.0[b]	0.0096
	MMP-2	130.0±27.4	110.0±22.4	200.0±0.0[a]	0.0156
	MMP-9	190.0±22.4	200.0±0.0	266.0±8.9[b]	0.0087
MCF-7	TNFα	180.0±27.4	280.0±27.4[b]	230.0±27.4	0.0094
	TGF-β	300.0±0.0	300.0±0.0	294.0±13.4	0.3679
	MMP-2	300.0±0.0	250.0±0.0	220.0±27.4[b]	0.0022
	MMP-9	296.0±8.9	298.0±4.5	272.0±4.5[c,d]	<0.0001

[a]p<0.05 and [d]p<0.001 vs. 5 μM doxorubicin group. [b]p<0.01 and [c]p<0.001 vs. control group.

DISCUSSION

Several screening and diagnostic methods have been developed for the patients with breast cancer; therefore, most get their diagnoses and treatment at earlier stages. The modified radical mastectomy (MRM) and breast-conserving surgery (BCS) are standard treatment modalities in breast cancer surgery, while the sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND) could also be applied depending on the involvement of axillary lymph nodes. Although the incidence of breast cancer has been increasing, the mortality rates have decreased by developing anticancer therapies due to these developments in diagnostic and therapeutic facilities[12]. Increased efforts are being made to find a novel, effective, and safe anticancer treatment for human breast cancer[13,14]. One of these efforts uses the ozone as an innovative method for decreasing the inflammation status in preclinical and clinical experiences. Ozone therapy showed its harmless effects and increased efficiency of complex treatment of patients with radiation reactions and skin lesions on the areas of irradiation[15]. Therefore, we examined the potential success of combination of ozone with doxorubicin in treatment for Luminal-A subtype human breast cancer cell line MCF-7 and compared the results with L929 fibroblast cell line and observed the cytotoxic and anticancer effects of combination therapy on cellular models. We also analyzed the possible biological mechanisms involved in these effects and found that MCF-7 cells acquire sensitivity against the doxorubicin combined with ozone treatment through activating numerous pathways, which include TNF-α, MMP-2, and MMP-9 expressions.

Ozone was shown to be able to increase the cytotoxicity of some chemotherapeutics including 5-fluorouracile and cisplatin and to decrease interleukin secretion in human colon cancer cells[3]. A 24-h incubation with ozone was proved to decrease the cytotoxicity of doxorubicin in skin fibroblasts and cardiomyocytes by mediating its anti-inflammatory effects, and the best cytoprotective effect of ozone was reached to 30 μg/mL[5]. In the present study, same effective dose of ozone was used for combination treatment with doxorubicin and enhanced cytotoxicity of doxorubicin in breast cancer cells but reversed this effect in fibroblast cells. These results suggest that the synergistic effect of ozone treatment with chemotherapeutics may change according to the type of cells and tissues and the malignancy.

During the development of medical treatments, ER positivity in the tumor has gained importance. In the literature, ER positivity has been reported in 60–65% of breast cancers[16]. ER-positive Luminal-A-subtype breast cancers compose at least half of all new breast cancer diagnoses due to the high proliferative mitotic activity. This subtype shows a well prognosis and its metastasis is mostly limited to the bone. Several in vitro studies have reported some anticancer effects of chemotherapeutic agents used in ER-positive breast cancers, and a very good response is obtained especially in luminal tumors[17]. Mostly used Lumina-A-subtype MCF-7 cells acquire doxorubicin resistance through activating or inhibiting numerous pathways, which include apoptosis, inflammation, or metastasis. It was reported that doxorubicin resistance was induced by an increased drug efflux through upregulating expression of transporters such as P-glycoprotein and multidrug resistance protein-1[18] or phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and glycogen synthase kinase-3β (GSK-3β) in doxorubicin-resistant MCF-7 cells[19]. Increased expression levels of the antiapoptotic protein Bcl-2 along with activation of MAPK pathways have been demonstrated in doxorubicin-resistant MCF-7 cells[20]. Any treatment targeting apoptotic pathways can be promising to enhance the effectiveness of standard chemotherapeutic regimens while protecting the safety of cancer treatment. In the present study, ozone treatment after doxorubicin incubation was able to induce apoptotic cell death with

its intrinsic ability to suppress the viability of MCF-7, but not of L929. Therefore, the ozone treatment potentiated the apoptotic and anticancer activities of doxorubicin in MCF-7 cells and showed a repairing and healing effect on healthy fibroblast cells, which were damaged from cytotoxic effects of the chemotherapeutic agent.

TNF-α can be produced by tumor cells, infiltrating immune cells, and stromal cells in tumor microenvironment. Patients with different advanced cancers have elevated TNF-α expression in biopsies and in the plasma[21]. Soluble TNF-α is well known to be involved in all steps of tumor development, including tumorigenesis, proliferation, angiogenesis, metastasis, and subverting the immune responses[22]. Moreover, soluble TNF-α induces resistance to BRAF inhibitors in melanoma cells[23] and to cisplatin chemotherapy in malignant pleural mesothelioma[24]. The expression of transmembrane TNF-α was found to be correlated with the disease severity and doxorubicin resistance. Both in vitro and in vivo studies reported that suppressing transmembrane TNF-α expression increased the sensitivity of breast cancer cells toward doxorubicin[25]. In consistent with the literature, the present study demonstrated that doxorubicin alone significantly increased the levels of TNF-α but the combination treatment with ozone reduced these levels in MCF-7 cells, suggesting that ozone treatment may enhance the sensitivity of breast cancer cells against chemotherapy.

Multiple pathways contribute to cancer aggressiveness, and one of these pathways implied in the invasion by cancer cells involves the MMPs, which breakdown and remodel the extracellular matrix proteins[26]. Increased expression of MMP-2 and MMP-9 have been proposed as the prognostic marker in breast cancer[27]. Mohammed et al. investigated the effects of sublethal doxorubicin treatment in noninvasive MCF-7 cells and found that doses of 0.6 μM or less of doxorubicin led to increased migration and invasion by increasing the induction and secretion of MMP isoforms, including MMP-2 and MMP-9[28]. The present study showed that 5 μM doxorubicin alone did not change the levels of MMP-2 and MMP-9 in MCF-7 cells, but addition of ozone treatment successfully reduced these levels. These results confirm the activated invasive program in MCF-7 cells can be inhibited by a combination treatment with doxorubicin and ozone.

CONCLUSION

Overall, our results suggest that an ozone application may aid MCF-7 cells to overcome the resistance against chemotherapies. Considering the success of exposure to ozone after treatment with doxorubicin, this in vitro study is a pilot study for preclinical studies for the effective and safe treatment of human breast cancer. These effects could be of great interest in future oncologic studies for the management of the chemoresistance phenomena of malignancies against many drugs like the doxorubicin.

AUTHORS' CONTRIBUTIONS

OOK, AGY: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing.

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ORIGINAL ARTICLE

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Comparison between somatostatin analog injections

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SUMMARY

OBJECTIVE: Long-acting depot formulations of somatostatin analogs, i.e., octreotide and lanreotide, are the first-line medical therapies for patients with acromegaly to whom surgery/radiotherapy cannot be performed or who have inadequate response. In this study, we aimed to evaluate the short-term local and systemic adverse reactions developed after the somatostatin analogs injections in the patients with acromegaly, in order to compare the side effects of somatostatin analogs injections.

METHODS: Patients diagnosed with acromegaly who were referred to our endocrinology clinic for monthly somatostatin analogs injections were questionnaired. Wong-Baker Faces Pain Rating Scale was used to evaluate the injection-site pain at the time of injection. The existence of leg pain, nausea, diarrhea, and abdominal pain following the previous injection was also investigated during the next injection.

RESULTS: A total of 49 patients were included in the study. The statistical difference could not be shown between the injection-site pain, anorexia, and leg pain frequencies of the groups, while the frequency of gastrointestinal disturbances, i.e., diarrhea and abdominal pain, was significantly lower in the octreotide group ($p<0.001$ and $p=0.015$, respectively).

CONCLUSIONS: This is the first prospective study that compared the severity of the injection-site pain by using a scoring scale, following the long-acting somatostatin analogs injections. We have shown that there was no significant association of the injection-site pain severity with the somatostatin analogs regimen nor the dose differences within each somatostatin analogs treatment.

KEYWORDS: Octreotide. Lanreotide. Injection site reaction. Drug-related side effects and adverse reactions.

INTRODUCTION

Long-acting depot formulations of somatostatin analogs (SSA), i.e., octreotide (OCT) and lanreotide (LAN), are the first-line medical therapies for patients with acromegaly to whom surgery/radiotherapy cannot be performed or who have inadequate response to surgery/radiotherapy[1,2]. The comparison between both short- and long-acting formulations of SSA has been evaluated in various studies, which generally have focused on the efficacy of therapies[3-8]. To date, only one head-to-head clinical trial, of which the primary aim was to evaluate the patient-reported outcomes of the SSA injections, including the injection-site pain duration, has been reported[9].

The most commonly reported local and systemic adverse reactions of SSA injections include erythema, injection-site and leg pain, impaired glucose metabolism, biliary gallstones, diarrhea, nausea, vomiting, and abdominal pain[10]. We designed a prospective study evaluating the short-term local and systemic adverse reactions developed after SSA injections in the patients with acromegaly who have been followed up by our clinic, in order to compare the side effects of SSA injections, particularly the pain severity at the injection site.

METHODS

Patients diagnosed with acromegaly who were referred to our endocrinology clinic for monthly SSA injections between April 2021 and August 2021 were questionnaired. The outcomes of the three consecutive monthly injections that had been performed by the same nurse were evaluated. The injections had been performed at a different site (left/right), considering the previous injection site. Wong-Baker Faces Pain Rating Scale was used to evaluate the injection-site pain at the time of injection, and the patients were asked to choose the exact pain score on the scale (Figure 1)[11]. This pain scale displays a series of faces, ranging from a happy face (0) to a crying face (10). The patients were expected to choose a face that best described their level of pain. The existence of leg pain, nausea, diarrhea, and abdominal pain within 3

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consecutive days following the previous injection was investigated during the next injection.

In addition to local and systemic side effects, name and dose of SSA, the duration of the treatment, and the site location of the current injection were noted. Since patient-reported pain is a subjective entity that could be affected by the psychological status, demographic features of the patients such as, age, sex, marital, and educational status were also noted. The average of local pain scores of three injections was calculated. The occurrence of side effects was evaluated according to the reports from the patients. The presence of an adverse effect during or after any injection(s) was recorded as positive in terms of that side effect. The patients who were above 18 years old and with acromegaly were eligible for inclusion in the study if they were admitted to our clinic for at least four times consecutively for injection and able to respond to the questions by which our nurse had asked during their injections. Patients with any neuromuscular disease that could cause a sensory impairment on the lower extremities (n=1) and patients who were under the current dose of SSA for less than 3 months (n=4) and whose injections were more than a month apart, such as 45 days (n=3), were excluded.

All statistical analyses were performed using the IBM SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used to assess the assumption of normality. Continuous variables were presented depending on normal distribution with either mean ± standard deviation or (in case of no normal distribution) median (25th–75th percentile). Categorical variables were summarized as counts (percentages). Comparisons of continuous variables between groups were carried out using the independent samples t-test, the Mann-Whitney U test and the Kruskal-Wallis test, whichever was appropriate. Association between two categorical variables was examined by the chi-square test. All statistical analyses were carried out with 5% significance, and a two-sided $p<0.05$ was considered statistically significant. Ethics approval was obtained from the ethics committee of the National Ministry of Health (date: March 19, 2021, no: E-66175679-514.05.01-375015).

RESULTS

After the exclusion of 8 patients, a total of 49 patients were included in the study, consisting of 27 (55.1%) men and 22 (44.9%) women, with a mean±SD age of 51.1±11.3 years. Thirty-four (69.4%) patients were on OCT, while 15 (30.6%) were on LAN. General demographic and clinical characteristics of the patients are shown in Table 1.

Table 1. Demographic and clinical characteristics of the patients (n=49).

	Mean±SD
Age (years)	51.1±11.3
Height (cm)	172.8±11.3
Weight (kg)	87.5±17.7
Body mass index (kg/m^2)	29.26±5.05
Waist circumference (cm)	106.2±11.9
Hip circumference (cm)	105.2±16.9
Duration of treatment (month)	73.4±55.4
	n (%)
Sex	
Male	27 (55.1)
Female	22 (44.9)
Marital Status	
Married	42 (85.7)
Single	7 (14.3)
Education	
Nonliterate	4 (8.2)
Primary school	24 (49.0)
High school	15 (30.6)
College	6 (12.2)

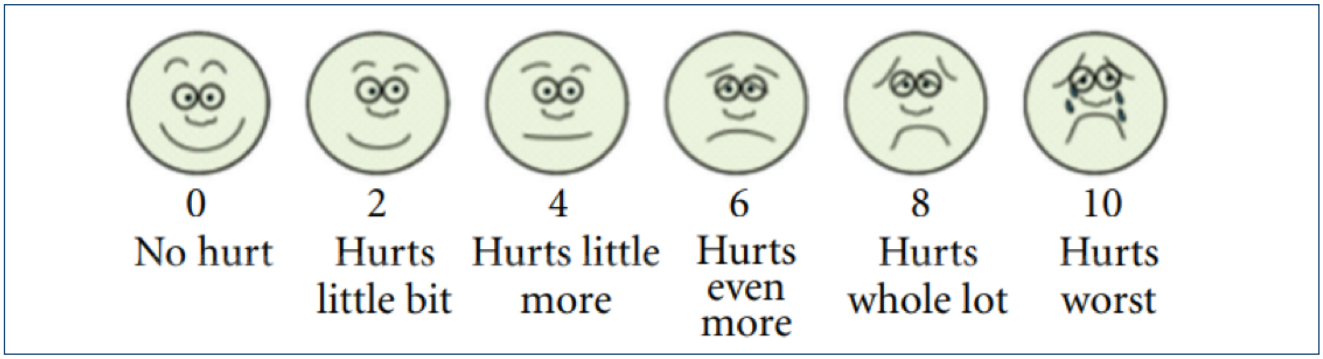

Figure 1. Wong-Baker faces pain rating scale.

As described in Table 2, there was no statistical difference between age, sex, marital, and educational status distribution of the two groups. The waist circumference medians of the groups were similar (p=0.527). The statistical difference could not be shown between the injection-site pain, anorexia, and leg pain frequencies of the groups, while the frequency of gastrointestinal (GI) disturbances, i.e., diarrhea and abdominal pain, was significantly lower in the OCT group (p<0.001 and p=0.015, respectively) (Table 2). Statistical analysis could not be performed for the comparison of the nausea frequencies,

Table 2. Comparison of demographic characteristics' distribution and the side effects of somatostatin analogs.

	Somatostatin analogs		p
	Octreotide (n=34)	Lanreotide (n=15)	
Age (years) mean±SD	50.3±10.5	52.7±12.8	0.503*
Sex, n (%)			
Male	19 (59.4)	8 (47.1)	0.601**
Female	13 (40.6)	9 (52.9)	
Marital status, n (%)			
Married	27 (84.4)	15 (88.2)	1.000**
Single	5 (15.6)	2 (11.8)	
Education, n (%)			
Nonliterate	1 (3.1)	3 (17.6)	0.212**
Primary school	18 (56.3)	6 (35.3)	
High school	10 (31.3)	5 (29.4)	
College	3 (9.4)	3 (17.6)	
Side effects, n (%)			
Diarrhea$^{\beta}$			
Yes	2 (6.3)	10 (58.8)	**<0.001****
No	30 (93.8)	7 (41.2)	
Nausea$^{\beta}$			
Yes	0 (0.0)	2 (11.8)	Not Available
No	32 (100.0)	15 (88.2)	
Leg pain$^{\beta}$			
Yes	3 (9.4)	3 (17.6)	0.405**
No	29 (90.6)	14 (82.4)	
Abdominal pain$^{\beta}$			
Yes	2 (6.3)	6 (35.3)	**0.015****
No	30 (93.8)	11 (64.7)	
Anorexia$^{\beta}$			
Yes	3 (9.4)	3 (17.6)	0.405**
No	29 (90.6)	14 (82.4)	
	Median (25th–75th)	Median (25th–75th)	
Pain at the injection siteα	1.00 (0.00–2.00)	1.00 (0.00–1.67)	0.766***
Hip circumference (cm)	102.0 (100.0–110.0)	102.0 (96.5–108.0)	0.527***
Duration of treatment	66.0 (36.0–120.0)	48.0 (21.0–84.0)	0.084***

*Evaluated by independent samples t-test. **Evaluated by chi-square test. ***Evaluated by Mann-Whitney U test. αMedian of mean injection-site pain score of three injections. $^{\beta}$Total of responses regarding 3 injection days.
Bold values indicate statistically significance.

due to insufficient number of patients who experienced nausea in the OCT group (n=0).

In the OCT group, there was no significant association between the maximum treatment doses (10 [n=6], 20 [n=9], 30 [n=17] mg) and the injection-site pain (p=0.682). Similarly, the treatment doses of LAN (60 [n=7] and 120 [n=8] mg) had no statistically significant effect on the injection-site pain (p=0.336) (Table 3). Due to insufficient number of patients, LAN 90 mg group (n=2) could not be included in the analysis.

DISCUSSION

Our prospective study has demonstrated that there was no difference in the local adverse effects such as the severity of injection-site pain and leg pain, which developed following SSA injections between OCT and LAN groups, while bowel problems, i.e., diarrhea and abdominal pain, were significantly lower in the OCT group. These results are in agreement with the only head-to-head comparative study investigating the outcomes of SSA therapies in 195 acromegalic patients published in the literature[9]. Strasburger et al. have reported that the incidence of bowel problems was significantly higher in patients on LAN (p=0.0076). The study has also evaluated the duration of pain following the injections, instead of the severity of pain, and the authors have stated that the duration of injection-site pain following OCT injection was longer than that in the LAN group (p=0.0007). In another study published in 2012, 68 patients with acromegaly have been evaluated in terms of efficacy and side effects of SSA[5]. In the LAN group, the number of patients experiencing diarrhea was higher than that in the OCT group (5/32 to 1/36). In contrast to the difference between GI side effects of SSA therapies in our study, two other studies that examined 25 and 54 patients with acromegaly have reported similar GI adverse reactions between the two treatments[4,8]. Similarly, in other two studies evaluating the efficacy and side effects of the short-acting SSA, which were designed as switching between SSA drugs, the occurrence of the side effects, including the intensity of diarrhea, was similar in both therapies[6,7].

The most frequent side effect of LAN treatment was diarrhea (58.8%) in our study, which was similar to that reported by Chanson et al.[12]. In various studies investigating the outcomes of LAN therapy, GI disturbances, most prominently diarrhea, have been reported as the most common adverse reaction of the treatment, of which the incidence rate varied between 19% and 76%[12-16]. In a comprehensive review on the adverse events associated with SSA in acromegaly by Grasso et al.[10], it has been stated that the treatment discontinuations due to the side effects were generally related to GI problems. The possible mechanism responsible for that side effect of SSA has been attributed to the drug-induced impairment of gastroenteropancreatic hormones that causes exocrine pancreatic insufficiency[10,17].

In multiple studies, local pain at the injection site has been reported as the most frequent side effect of OCT treatment[18-20]. In our study, we have evaluated the severity of pain using a scoring scale, instead of the frequency of pain, and found that the median of the average injection-site pain score of the three consecutive monthly injections was 1.00 (0.00–2.00) out of 10, with no significant difference from LAN injection (p=0.766). Vance et al. have evaluated the outcomes of short-acting OCT treatment in 189 patients with acromegaly and found local pain at the injection site as one of the common side effects[20]. In contrast, a meta-analysis assessing the efficacy and tolerability of the long-acting OCT has demonstrated that diarrhea, gallstone formation, headache, and abdominal discomfort were the general side effects of the therapy, and the local pain has not been emphasized[17]. Combining that analysis with the low injection-site pain scores reported in our study, it can be stated that the local side effects may be trivial during the long-acting SSA injections. Additionally, we have also found that the doses of SSA treatments were not significantly related to the local site pain, which may be considered another negligible factor during the management of patients with acromegaly.

The strength of our prospective study was that all 147 injections were performed by the same specialist nurse and all questionnaire forms were completed by the patients under the supervision of our nurse. The low sample size, which was 49 patients, has been the main limitation of our study. The association of the SSA doses with the side effects was one of our end points; however, the statistical analysis could not be performed for the majority of side effects other than the injection-site pain, due to the lack of enough patients experiencing each side effect.

Table 3. Association of injection-site pain with the doses of somatostatin analogs.

	Doses	Injection-site pain*	p
Octreotide	10	1.00 (0.00–2.42)	0.682¥
	20	1.00 (0.00–1.67)	
	30	1.00 (0.67–2.33)	
Lanreotide	60	1.67 (1.00–2.00)	0.336Ω
	120	0.67 (0.08–2.83)	

*Data are expressed as median of mean injection-site pain score of three injections (25th–75th). ¥Evaluated by Kruskal-Wallis test. ΩEvaluated by Mann-Whitney U test.

CONCLUSIONS

To the best of our knowledge, this is the first prospective study that compared the severity of the injection-site pain by using a scoring scale, following the long-acting SSA injections. We have shown that there was no significant association of the injection-site pain severity with the SSA regimen nor the dose differences within each SSA treatment. Additionally, the frequency of GI disturbances, i.e., diarrhea and abdominal pain, was significantly lower in the OCT group. During the management plan of patients with acromegaly, it may be beneficial to consider the GI disturbances as a possible adverse event following SSA treatments, particularly after LAN injections.

AUTHORS' CONTRIBUTIONS

EG: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft. **YD:** Investigation, Methodology, Visualization, Writing – review & editing. **AS:** Data curation, Investigation, Supervision, Visualization, Writing – review & editing. **ZC:** Data curation, Formal Analysis, Supervision, Visualization, Writing – review & editing. **BÇ:** Data curation, Formal Analysis, Software, Supervision, Writing – original draft. **MS:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **DK:** Methodology, Investigation, Visualization, Writing – original draft. **APK:** Formal Analysis, Methodology, Visualization, Writing – original draft.

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ORIGINAL ARTICLE

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Epidemiological profile of suicide attempts in a municipality in southwest Paraná, from 2017 to 2020

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SUMMARY

OBJECTIVE: This study aimed to analyze the epidemiological profile and psychological disorder of the suicide attempt cases in Francisco Beltrão, Paraná.

METHODS: This is an epidemiological descriptive/qualitative study of suicide attempts conducted between 2017 and 2020. This study used data from the Brazilian Information system for notifications involving suicide attempts (Sistema de Informação de Agravos de Notificação, SINAN) and 447 notifications were obtained. An analysis of the electronic medical record of these patients was performed in order to investigate the mental disorders, using a questionnaire.

RESULTS: Of the 447 notifications, 382 were eligible for the study. Using the 95% confidence interval, there was a higher frequency of females with 71.7% aged between 18 and 35 years representing 48.4%, with 77.2% white race/color, the singles appeared in the majority with 47.6% with a history of previous suicide attempts, using exogenous intoxication as a method in the attempt with 67.5%. Regarding mental health, 66.5% of the patients had some mental disorders, with the highest prevalence of recurrent depressive disorder found in 40.6%.

CONCLUSION: It was observed that there is a need for training of health professionals and implementation of programs and preventive measures aimed primarily at females aged between 18 and 35 years with mental disorders, especially with recurrent depressive disorder and with a history of previous suicide attempt.

KEYWORDS: Public health. Mental disorder. Depressive disorders. Suicide.

INTRODUCTION

Understanding the reasons why a person commits suicide is very complex. As a self-inflicted disease, suicide has become a big public health problem. There is no single cause of reason, and it can result from a complex multifactorial interaction. However, most suicides are preventable[1].

More than 700,000 people die globally due to suicide every year, which means that every 40 s a person dies. In 2016, the Word Health Organization (WHO) found that suicide was the fourth leading cause of death among young people aged 15–29 years. About 77% of suicides in the world occur in low- and middle-income countries[2].

It is a phenomenon with great relevance to public health, due to its magnitude, the seriousness of the cases, hospitalizations and sequelae, and emotional damage caused to the victims and their families, thereby causing numerous psychological, social, and economic problems[3].

Suicide can be understood as a deliberate act that is performed by the individual, whose main intention is death, consciously and intentionally, even if ambivalent, using means or method that the individual believes to be lethal[4,5]. The Brazilian population has been showing a prevalence of suicidal behavior, with 17% of people having thought of suicide at some point in their lives, and only 1% was seen in the emergency department[4].

The principal risk factors for suicide are as follows: history of attempted suicide and mental disorder[1,4,6]. Previous attempt is the most important predictive factor, indicating that this patient is five to six times more likely to try again[4,5,7,8]. Besides mental disorders, other factors such as social and psychological aspects and health conditions, even impulsive acts, are involved[2,4].

The most frequently mental disorders associated with suicide are depression, bipolar mood disorder, dependence on alcohol and other psychoactive drugs, schizophrenia, and personality disorder. The risk becomes even bigger for patients with multiple psychiatric comorbidities[4].

Suicide prevention efforts are mainly focused on identifying people with mental health problems and on the availability of

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care and treatment, but there are also other forms of prevention, such as those aimed at other basic needs of each individual[9,10].

Thus, given this scenario, the main purpose of this study was to analyze the epidemiological profile, identify the existence of mental disorders, and investigate factors associated with suicidal behavior in the municipality of Francisco Beltrão, Paraná.

METHODS

This is a descriptive, quantitative, cross-sectional epidemiological study of suicide attempts that occurred in the municipality of Francisco Beltrão, Paraná, between 2017 and 2020.

This study was conducted using the database on notifications involving suicide attempts, through the SINAN database, from 2017 to 2020. These data were provided by the Municipal Health Department of Francisco Beltrão. SINAN is updated through notifications realized by the multidisciplinary teams of health units that provide care to these victims, filling out the specific Interpersonal/Self-Inflicted Violence form. Through this report, the following variables were extracted: sex, marital status, age group, method used in the suicide attempt, and if it occurred other times.

Then, for the analysis of information regarding mental disorders and multidisciplinary care, data collection was carried out through the verification of the electronic medical record using the operating system of the municipal health secretary, the electronic medical record of each patient individually since 2017, using a closed questionnaire, answering questions related to diagnosis of mental disorder, suicide attempts and/or reports of suicidal ideation, use of psychotropic medication for mental disorders, monitoring by a psychiatrist, psychologist and by the referral health unit of the patient.

Mental disorders were classified as described by the WHO in relation to those with the highest prevalence in cases of attempted suicide, and using the ICD — *International Classification of Diseases*[1], as follows: F19: Mental and behavioral disorders resulting from the use of psychoactive substances; F20: Schizophrenia; F31: Bipolar affective disorder; F33: Recurrent depressive disorder; F39: Unspecified "affective" mood disorders; F41: Anxiety disorders; and F60: Personality disorders.

The methods used in the suicide attempt were classified as Gunshot wounds, Knife wounds, Hanging, Exogenous intoxication (ingestion of drug overdose or other intoxication), Precipitation from high places, and other methods.

The inclusion criteria included all patients notified as a suicide attempt in the municipality of Francisco Beltrão, Paraná, from January 2017 to December 2020. Exclusion criteria used were notifications made for cases of accidental intoxication and interpersonal violence. Thus, of the 447 notifications extracted from the SINAN for interpersonal or self-inflicted violence, only 382 were considered eligible for suicide attempts, due to some notifications that did not qualify as a suicide attempt and/or duplicate.

Being a documentary research, without direct contact with patients, it did not preset any risk to patients, as well as those related to breaches of confidentiality, safeguarding the ethical and moral precept throughout its execution, not being necessary the approval by the ethics committee.

Maintaining the ethical and legal precepts, the identity of the subjects was kept confidential and the information collated reliably as shown in the notification forms in SINAN and in the electronic records of notified patients.

The collected data were analyzed using the statistical program IBM SPSS (Statistical Package for the Social Sciences), version 21. Absolute (n) and relative (%) frequencies were calculated to describe the sample profile. The chi-square test with continuity correction was performed to compare and cross-reference patients with a diagnosis of mental disorder and categorical variables, with values that presented $p<0.05$ being considered statistically significant.

RESULTS

A total of 382 patients who attempted suicides were analyzed. The general and clinical characteristics of the patients are shown in Table 1.

Of patients diagnosed with mental disorder (n=254), recurrent depressive disorder had the highest prevalence with 40.6% of cases (n=103), followed by anxiety disorder with 20.5% (n=52), disorder affective bipolar with 15.3% (n=39), unspecified "affective" mood disorder with 10.6% (n=27), and the other disorders added up to 13% (n=33).

Regarding the means used in suicide attempts, it was found that exogenous intoxication is the most prevalent with 67.5% (n=258), followed by knife wounds with 11.3% (n=43), hanging with 10.7% (n=41), and the other methods or association of methods with 10.5% (n=40).

When correlating patients diagnosed with mental disorder with the other characteristics of the sample, a statistically significant difference was observed in relation to variables related to age, follow-up, and recurrences (Table 2).

DISCUSSION

The prevalence of cases of suicide attempts in females can be explained by the fact that in males, suicide prevails as a fait accompli, because men tend to use more lethal medhods[11].

Table 1. General and clinical characterization of patients with a suicide attempt in the municipality of Francisco Beltrão, Paraná, from 2017 to 2020.

Variable	N	%
Sex		
Female	274	71.7
Male	108	28.3
Age group		
Between 8 and 17 years	104	27.2
Between 18 and 35 years	185	48.4
Above 36 years	93	24.4
Race		
White	295	77.2
Brown	56	14.7
Others	31	8.1
Marital status		
Single	182	47.6
Married	100	26.2
Others	100	26.2
Diagnosis of mental disorder		
Yes	254	66.5
No	128	33.5
Psychiatric follow-up		
Yes	208	54.5
No	174	45.5
Psychological follow-up		
Yes	135	35.3
No	247	64.7
Use of psychotropic medications		
Yes	256	67.0
No	126	33.0
More than one suicide attempt		
Yes	190	49.7
No	149	39.0
Ignored	43	11.3

Source: The author, 2021.

A lot of factors must be evaluated, which may be associated with sex, as the fact that women may be more vulnerable to moral and/or sexual violence and even physical aggression. Other factors are linked to unemployment and the women social culture in the family and society[12]. Suicidal ideation and suicide attempts in females are also related to sexual and domestic violence, unwanted or unplanned pregnancy, and mental disorders[13]. These suicidal behaviors presented in the age group between 20 and 35 years could be due to several reasons, including emotional, family and social problems, rejection, neglect, physical and sexual abuse in childhood, depressive mood, and family history of psychiatric disorders[14].

Data on race/color as a self-declared variable showed that 77.2% of the population declared themselves white, in line with the profile of the state of Paraná, which, according to the IBGE, more than 60% of the population declared themselves white[15].

Single individuals who still live alone have a high suicide rate. With the decrease in the average number of people in the family and especially the lack socialization, they have a significant relation with the increase in the suicide rate[16].

It is noteworthy that some disorders can lead to suicidal behavior and act as a mood disorder with 36%. In suicide victims, depression has the high prevalence, and it is more prevalent in women (25%) than that in men (12–13%). Data show that 6–8% of the Brazilian population will experience at least one episode in their lifetime[4,17].

Psychiatric disorders account for a large proportion of suicides and suicide attempts, and these numbers are at least 10 times higher than in the general population. The reported percentage of suicides committed in this context varies between 60% and 98% of all suicides. Mental disorders, and more specifically depressive disorder, are more prevalent in women and have a strong association with suicide[4,6].

In Brazil, in 2014, of the notified cases, 28% had already made a previous attempt[11]. One of the most important factors

Table 2. Comparison of general variables in relation to patients with a diagnosis or patients with mental disorders

Variable	Patients with diagnosis of mental disorder		
	Yes (n=254)	No (n=128)	p-value
Female	187 (73.6%)	87 (67.9%)	0.247
Age from 18 to 35 years	110 (45.8%)	75 (58.6%)	0.001
Single	123 (48.4%)	59 (46.1%)	0.141
Attempt for exogenous intoxication	163 (64.2%)	95 (74.2%)	0.138
More than one try	143 (56.3%)	47 (36.7%)	0.001
Drug treatment	241 (94.9%)	15 (11.7%)	0.000
Psychiatric follow-up	207 (81.5%)	01 (0.8%)	0.000
Psychological follow-up	132 (51.9%)	03 (16.6%)	0.000

Source: The author, 2021.

in future suicidal behavior is that the first suicide attempt has taken place[4,18]. There are still some doctors who think that patients who talk about suicidal ideation will never do it, that talking about the subject can encourage the act, are taboos that intervene in the assessment and adequate conduct[19].

Suicide attempts using drug intoxications occur mainly in young adults and females, corroborating the data from the present study, which suggests the need to implement assistance programs aimed at young adults that allow for the identification of risk situations for the suicidal act, due to the prevalence of suicide attempts in relation to the age group of 20–39 years with the abusive use of medications with a focus on causing their own death [20,21].

Thus, the multidisciplinary treatment for patients who attempted suicide is essential for the prevention of completed suicide.

Regarding the data in Table 2, demonstrating the association of patients diagnosed with mental disorder with the other variables in the sample, it is remarkable that patients diagnosed with mental disorder, undergoing multidisciplinary and drug treatment, still attempted suicide and the chance is 56.3%. These data show a warning, since patients undergoing treatment are still trying to commit suicide, which can expose weakness in treatment protocols, lack of trained professionals in care, difficult access, especially when the patient presents clinical instability. With a focus on reducing suicide rates, it is necessary to minimize ignorance and stigma related to mental illness. Suicide risk assessment should be performed whenever the physician deems it necessary[19].

Suicide prevention requires prevention and protection strategies at all levels of society. Learn the warning signs, promote prevention, and commit to social change[22].

CONCLUSIONS

The results of the present study reveal that there is an urgent need for training and capacitate of health professionals, demystification around the topic, implementation of preventive measures, with a focus on health promotion and facilitated access to health care points in all areas, with multidisciplinary care, with an emphasis on mental health, especially for populations with a history of suicide attempt or multiple attempts, with mental disorders, especially depressive disorder, the young adults and females, as they know the risk groups and disorders that more associated, will know how to plan and implement more efficient intervention protocols.

AUTHORS' CONTRIBUTIONS

AJB: Conceptualization, Data curation, Formal Analysis, Research, Methodology, Project management, Programs, Visualization, Writing – original draft, and Writing – review & editing. **LS**: Conceptualization, Formal Analysis, and Writing – original draft. **GWW**: Conceptualization, Data curation, Formal Analysis, Methodology, Validation, Visualization, Writing – original draft, and Writing – review & editing. **GV**: Formal Analysis, Acquisition of funding, Methodology, Validation, Visualization, Writing – original draft, and Writing – review & editing. **FMB**: Data curation, Formal Analysis, Programs, Validation, and Visualization. **RY**: Formal Analysis, Validation, Visualization, and Writing – review & editing. **FACF**: Conceptualization, Data curation, Formal Analysis, Methodology, Project management, Resources, Programs, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing.

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ORIGINAL ARTICLE

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Postchemotherapy retroperitoneal residual mass resection for germ cell testicular tumors: a single-center experience

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SUMMARY

Objective: Postchemotherapy retroperitoneal lymph node dissection (PC-RPLND) plays an important role in the management of advanced germ cell testicular tumors. Bilateral template lymph node dissection is considered a standard treatment in postchemotherapy residual masses; however, modified unilateral templates have gained acceptance in patients with unilateral residual disease. In this study, we aimed to demonstrate the perioperative and oncological outcomes of the patients with advanced testicular cancer who underwent unilateral modified template PC-RPLND in our center.

Methods: This is a retrospective study in which patients who underwent PC-RPLND in a referred center between 2004 and 2021 were investigated. All patients had three or four cycles of chemotherapy and retroperitoneal residual masses. Data were retrospectively collected from medical, operative, radiology, and pathology records and analyzed.

Results: A total of 57 patients underwent PC-RPLND. The mean age was 32.7±8.1 years (19–50). According to the disease stage at presentation, there were 39 patients with stage 2 and 18 patients with stage 3. The average tumor size after chemotherapy was 57.6±2.7 mm (25–117). The overall complication rate was 35% (20/57 patients). No grade 4 and 5 complications were observed. Pathologic review demonstrated the presence of teratoma in 28 (49.1%) patients, fibrosis and/or necrosis in 15 (26.3%) patients, and viable germ cell tumor in 14 (24.5%) patients. The mean follow-up was 69.4 months (8–201). During follow-up after surgery, 14 (24.5%) deaths occurred due to advanced disease.

Conclusion: PC-RPLND is a major component of the management of advanced testicular germ cell cancer. Our study demonstrated that modified unilateral template is an effective and safe procedure in the postchemotherapy setting for selected patients.

Keywords: Retroperitoneal lymph node dissection. Testicular cancer. Germ cell tumor. Nonseminoma. Seminoma.

INTRODUCTION

Testicular cancer is the most common solid malignancy among males aged 15–35 and represents 1% of adult neoplasms and 5% of urological tumors[1]. Since the past decade, the incidence of testicular cancer has been rising in many countries. Northern European countries have the highest incidence rates, while Eastern European, Asian, African, and South American countries have the lowest[2]. The majority of malignant testicular tumors are germ cell tumors (GCTs), accounting for 95% of all cases, and GCTs are classified into seminomas and non-seminomatous GCTs[3]. In the vast majority of patients with stage 1 disease, radical orchiectomy is curative, although those with advanced stages require chemotherapy[4]. The majority of patients achieve complete remission after chemotherapy, although a significant number will still have postchemotherapy masses.

Surgical resection of postchemotherapy residual retroperitoneal masses is an essential component of multimodality treatment for patients with advanced testicular cancer receiving systemic chemotherapy. The optimal management of residual mass after chemotherapy for non-seminomatous testicular cancer is still being debated. Patients with non-seminomatous testicular cancer and residual retroperitoneal lymph nodes > 1 cm following chemotherapy should undergo a postchemotherapy retroperitoneal lymph node dissection (PC-RPLND)[5,6]. In these patients, following the first-line bleomycin, etoposide, and cisplatin (BEP) chemotherapy, only 6–10% of residual masses contain active cancer, 50% have postpubertal teratoma, and 40% comprise necrotic-fibrotic tissue only[7]. Seminomas are extremely sensitive to chemotherapy, but residual masses are detected after chemotherapy in 66–80% of patients with advanced disease. Fluorodeoxyglucose-positron emission tomography (FDG-PET) is recommended with residual masses after treatment of seminoma due to its high negative predictive value[8]. Surveillance is advised for residual lesions less than 3 cm in size or lesions larger than 3 cm in size with a negative FDG-PET. In patients with postchemotherapy residual masses <3

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cm, FDG-PET is optional[8,9]. PC-RPLND should be considered a treatment option in patients with postchemotherapy residual masses >3 cm with a positive FDG-PET scan.

The optimal approach to PC-RPLND has proven to be more contentious. In the postchemotherapy setting, bilateral nerve-sparing RPLND is the standard option. In selected patients, ipsilateral template resection with nerve preservation has been shown to produce comparable long-term oncologic results to bilateral systematic resections[4,10]. In this study, we present the results of 57 patients undergoing PC-RPLND for retroperitoneal residual mass after chemotherapy for germ cell testicular tumors. We aimed to present our surgical experience and evaluate oncological results, complications, and survival of PC-RPLND procedures performed at our institution.

METHODS

Patient population and inclusion criteria

Between May 2004 and March 2021, patients with primary non-seminomatous or seminomatous testicular tumor and history of chemotherapy after orchiectomy were enrolled in our study. All 62 patients who underwent open PC-RPLND for residual mass in the retroperitoneal area in a single center were included in this study. All of the patients underwent radical orchiectomy for primary diagnosis, and all patients received three or four cycles of chemotherapy prior to surgery according to their prognostic group. Patients with extragonadal tumor, previous RPLND prior to chemotherapy, and previous salvage chemotherapy were excluded from the study. Before PC-RPLND, all patients underwent computed tomography of the chest and abdomen 6–8 weeks following the last cycle of chemotherapy, and measurement of the serum tumor markers was taken.

Data collection

Data were retrospectively collected from medical, operative, radiology, and pathology records and analyzed. Five patients with incomplete data were excluded from the study. Follow-up data were available for 57 patients. Preoperative demographic and clinical variables included age, clinical stage, initial pathology of testicular tumor, preoperative chemotherapy status, size of the retroperitoneal mass, and time to RPLND. Operative and postoperative variables included pathology of retroperitoneal mass, intraoperative complication status, estimated blood loss, length of hospital stay (LOH), and oncologic outcomes. Intraoperative and postoperative complications were recorded according to Clavien-Dindo classification system[11]. The 2016 Tumor Node Metastasis (TNM) classification of the International Union Against Cancer is used for clinical staging and classification of prognostic groups[12].

Surgical technique

Patients were placed in supine position, and a midline incision was made. After obtaining the intra-abdominal access, a medial rotation of the colon was made to create the retroperitoneal space. Modified template resection limits for right-sided tumors consist of the ureter (lateral), the midpoint of the aorta (medial), bifurcation of iliac vessels (inferior), and and renal hilum (superior), and for left-sided tumors consist of ureter (lateral), there are midpoint of vena cava (medial), bifurcation of iliac vessels (distal), and renal hilum (superior). Lymph nodes in these areas were packed and dissected. Care was taken to avoid major vessels and sympathetic trunk injury during dissection. If the residual mass is close to the ureters, a double J ureteral catheter was placed before RPLND, in order to identify and avoid damage to the ureters.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0 (Chicago, IL, USA) program. The categorical variables were compared using the chi-square and continuous variables were evaluated by Mann-Whitney U test. Kaplan-Meier test was used to calculate the survival of patients. Statistical significance was accepted as p-value <0.05.

RESULTS

A total of 62 patients who underwent postchemotherapy open PC-RPLND were evaluated. Of them, 57 patients with a mean age of 32.7±8.1 years (range 19–50) were included in the study. The primary testicular tumor sides were in the right and left testis in 33 (57.8%) and 24 (42.2%) patients, respectively. The pathology of primary tumor demonstrated non-seminomatous germ cell (n=35, 61.4%), seminoma (n=8, 14%), and mixed GCT (n=14, 24.5%). According to the disease stage at presentation, there were 39 patients with stage 2 and 18 patients with stage 3. The primary chemotherapy regime in 41 (71.9%) patients was standard three or four cycles of BEP, 8 (14%) patients received epirubicin and cisplatin (EP) for bleomycin toxicity, 4 (7%) patients received etoposide, ifosfamide, and cisplatin (VIP), and another 4 (7%) patients received alternative individualized chemotherapy regimens. The average tumor size after chemotherapy was 57.6±2.7 mm

(25–117 mm). Baseline demographics and patient characteristics are shown in Table 1.

Open PC-RPLND via an anterior abdominal approach was performed in all patients. The mean LOH was 8.4±7.5 days. The overall complication rate was 35% (20/57 patients). There was no grade 4 and grade 5 complications (perioperative death). Eight of these complications were occurred in the intraoperative period, of which four were bleeding requiring blood transfusion and four were major vascular (inferior vena cava or aorta) injuries requiring surgical intervention. In all, 12 patients suffered from postoperative complications, of which 8 were Clavien-Dindo grades 1 and 2 and 4 were Clavien-Dindo grade 3b. Complications are summarized in Table 2.

Final retroperitoneal mass pathology demonstrated teratoma in 28 (49.1%) patients, fibrosis and/or necrosis in 15 (26.3%) patients, and viable GCT in 14 (24.5%) patients. The mean follow-up was 69.4±54.5 months (8–201). During follow-up after surgery, 14 (24.5%) deaths occurred due to advanced disease. Overall survival rate was 75.5%, with a median follow-up of 47 months. Kaplan-Meier survival curves are presented in Figure 1.

DISCUSSION

In this study, we aimed to present our surgical experience and demonstrate that modified unilateral template is an effective and safe procedure in the postchemotherapy setting. In the treatment of metastatic testicular cancer, surgical excision of remaining masses after chemotherapy is still an integral and crucial aspect of the treatment[13]. For patients undergoing RPLND after chemotherapy, a full bilateral dissection is currently recommended. Patients with advanced disease who have received chemotherapy may benefit from a unilateral, modified template RPLND[4]. Both procedures can be performed by open, laparoscopic, or robotic-assisted laparoscopic approach. In our study, we examined oncologic outcomes following postchemotherapy open unilateral modified template RPLND in patients with clinical stage II and III diseases. In these patients, RPLND is a key part of multidisciplinary treatment, but surgery requires

Table 2. Grading of surgical complications.

Grade	Complication	n
1	Ileus Wound infection	4 2
2	Blood transfusion Deep vein thrombosis Damage of IVC or aorta	4 2 4
3b	Wound dehiscence Coloileal anastomosis leakage Small intestine necrosis	2 1 1
Total (%)		20 (35)

IVC, inferior vena cava.

Table 1. Patient characteristics.

Characteristic	Non-seminomatous (n=49)	Seminomatous (n=8)	p-value
Age (years)	32.4 (19–46)	39.1 (30–50)	0.042
Site of primary tumor, n			
Right	29	4	0.660
Left	20	4	
Tumor size, cm (biggest diameter)	4.72 (1.5–9.2)	6.8 (3.5–12)	
Stage of disease, n			
Stage II	34	5	0.657
Stage III	15	3	
Histology of residual mass, n			
Teratoma	30	2	
Necrosis/fibrosis	8	4	0.215
Viable GCT	11	2	
Perioperative complication	15	5	0.136
Mean estimated blood loss	194 (140-400)	234 (115-440)	0.451
Length of hospitalization (days)	7.8 (3-50)	9.2 (6-21)	0.657
Mean follow-up (months)	68.6 (8-201)	75.6 (24-112)	0.732
Survival (death patients/total)	13/49	1/8	0.339

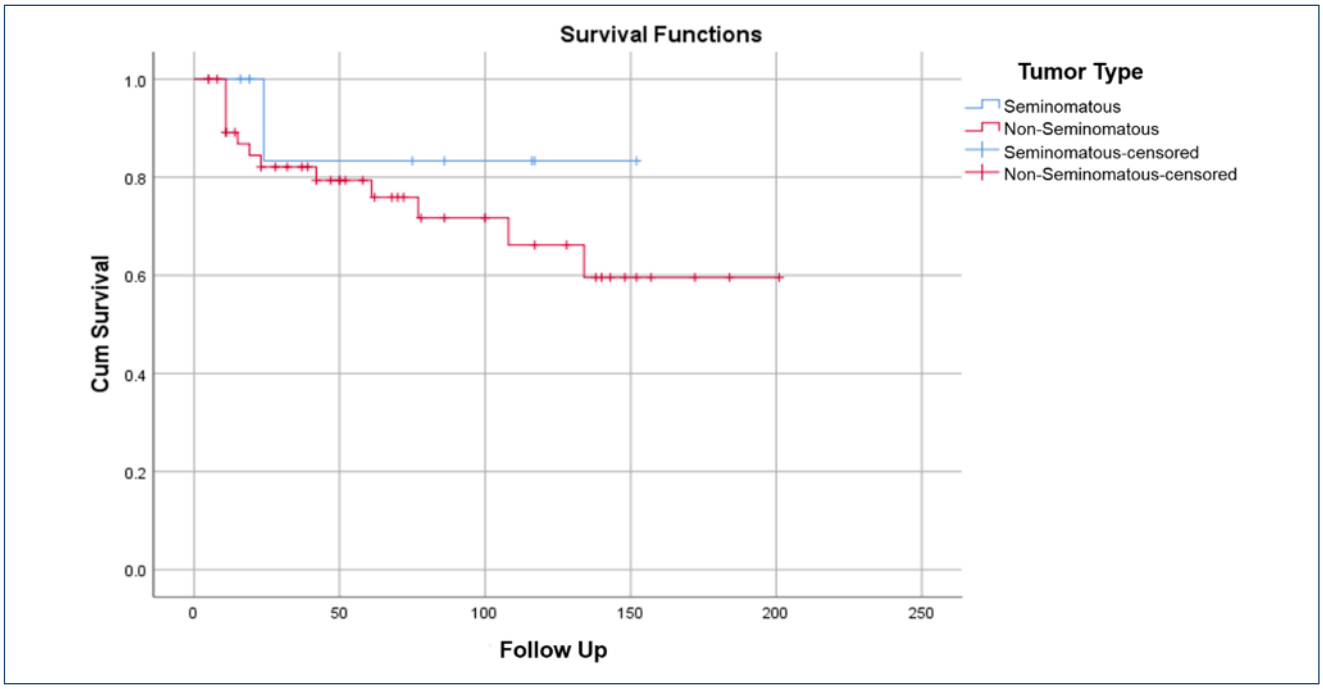

Figure 1. Kaplan-Meier survival curve for cancer-specific survival stratified by tumor type.

a high level of competence and may cause many serious intraoperative and postoperative surgical complications. Therefore, these patients should be managed in centers with a high volume of testicular cancer patients.

The excision of postchemotherapy residual masses in the retroperitoneal area is a major procedure with several intraoperative and postoperative difficulties. In a recent population-based study, the incidence of intraoperative and postoperative complications was higher for bilateral PC-RPLND than for unilateral PC-RPLND and they found that lymphatic leakage was the most common complication[14]. In another study in which primary and PC-RPLND complications were compared, the authors stated that the risk and severity of intraoperative and postoperative complications were higher with PC-RPLND though no significant difference was found between the two groups in terms of complication rates[15]. In our PC-RPLND series, no intraoperative or perioperative death was observed. Although the complication rate was at an acceptable level in our study, none of the patients had Clavien-Dindo grade 4 or 5 complications. In one patient who had Clavien-Dindo grade 3b complication, surgical intervention was performed again under general anesthesia due to abdominal evisceration secondary to postoperative ileus. In a large study of 603 patients who underwent PC-RPLND for clinical stages II and III, there were 144 complications in 125 (20.7%) patients, and the mortality rate was 0.8%[16]. In a recent systematic review comparing outcomes of different PC-RPLND techniques, 100 (29%) of 347 patients undergoing modified unilateral PC-RPLND experienced complications, and 27(8%) patients experienced grade 3 and 4 complications[17]. In a study comparing primary and PC-RPLND surgeries, it was emphasized that intraoperative and postoperative complications were more common in the PC-RPLND group without statistically significant difference, and ileus constituted the majority of postoperative complications in both groups[15]. In our study, we found that the hospitalization period was prolonged in patients with ileus especially in the postoperative period and two patient needed adjuvant surgery due to abdominal evisceration and two patients due to coloileal anastomosis leakage and intestinal necrosis. Patients undergoing PC-RPLND are more likely to develop complications due to factors such as a large volume of disease, a postchemotherapy desmoplastic reaction, and aggressive/extensive retroperitoneal dissection. In addition, the decrease in pulmonary reserves of these patients after chemotherapy, especially in those receiving bleomycin therapy, adds an additional burden to the perioperative and postoperative morbidities of the patients.

Fibrosis/necrosis, teratoma, and viable GCT are the most common findings after PC-RPLND. In a single-institution series of 504 patients who underwent PC-RPLND, 51% of

cases had fibrosis/necrosis, 37% had teratoma, and 15% had viable GCT[7]. A similar rate was found in another series of 152 patients from two tertiary referral centers, 84 (55.2%) patients had necrosis/fibrosis, 45 (29.6%) had mature teratoma, and 23 (15.1%) had vital cancer in the surgical specimens[4]. Reviewing our series, teratoma contributed to 49.1% of histopathological findings of retroperitoneal masses, fibrosis/necrosis to 26.3%, and viable GCT to the remaining 24.5%. In a clinical model for analyzing residual masses after chemotherapy, authors demonstrated that models that predict patients with non-seminoma with either necrosis or viable cancer after is irrelevant and not reliable and highlighted PC-RPLND should not be performed in these patients, as residual seminoma was not detected in 97% of patients with seminoma who received adequate systemic chemotherapy[18]. In a recent study, the levels of a new serum biomarker micro-RNA 371 were found significantly associated with clinical stage, primary tumor size, and response to treatment, and all histologic subtypes, except teratoma, express this micro-RNA. Compared with classical serum tumor markers, it was found to have a higher sensitivity and specificity of over 90%. After further validation, this marker could be considered in the management of GCTs even in advanced stages[19]. In another study, it was underlined that the levels of this marker decreased significantly after chemotherapy in patients with advanced disease and confirmed that it was not expressed at all in teratoma[20]. This novel biomarker should be considered in cases where the use of the classical tumor markers is inconclusive, postchemotherapy residual masses in seminoma and non-seminoma.

In our series, 14 patients died from disease progression. Our overall survival rate was 75.5%, with a median follow-up of 47 months. In a study demonstrating the long-term data of 100 patients who underwent modified left or right unilateral PC-RPLND, they reported a 99% survival rate at a 10-year follow-up. Unlike our study, this study consisted of only patients with a limited retroperitoneal limited disease on the affected testis side and normal serum tumor markers after systematic chemotherapy[21]. In our study, residual mass resection pathology was reported as viable GCT in 10 of the patients who died due to advanced disease during follow-up. In a study with similar survival rates as ours, 60% of the patients who died during follow-up had a GCT in the final pathology[22]. In another study investigating the pathological data and clinical results of patients who underwent RPLND after multiple chemotherapy regimens, it was emphasized that the predictors of worse disease-specific survival were the detection of a retroperitoneal mass larger than 5 cm and GCT[23]. Furthermore, in this study, a 5-year disease-specific survival rate of 74% was reported, which is also consistent with our study.

This study has several limitations. First is the retrospective nature of this study. Second, since information about the retrograde ejaculation status of the patients in the postoperative period is not reported in the database, this detail was not included in the study. Third, we did not use the bilateral modified RPLND technique, which may have affected the oncological outcomes. Another limitation is that there is no mention of additional adjuvant therapy.

CONCLUSIONS

We present the results of a single-center PC-RPLND procedure for advanced testicular tumors. PC-RPLND has a complementary role in the management of advanced GCTs, particularly non-seminoma. After chemotherapy, the majority of patients achieve complete remission, although a significant number will still have postchemotherapy masses. We hypothesized that a modified unilateral PC-RPLND would be equally effective in managing the masses in the retroperitoneum oncologically. In optimally diagnosed and well-evaluated patients with residual masses following systemic chemotherapy for advanced testicular cancer, modified PC-RPLND can be regarded as a safe surgical procedure. In particular, PC-RPLND procedures should be performed in high-volume clinics with extensive experience in the treatment of advanced testicular cancer. Centralizing the treatment of these patients is important in terms of disease control and prevention of perioperative mortality. The prediction of viable GCTs in these patients with newly developed tumor markers seems promising.

AUTHORS' CONTRIBUTIONS

SK, FK: Conceptualization. **KEE, BA:** Data curation. **SK, KEE, AS:** Formal Analysis. **AS:** Funding acquisition. **FK, AS:** Investigation. **SK, KEE, FK, AS:** Methodology. **SK, KEE, AS:** Project administration. **BA, FK, AS:** Resources. **SK, KEE, FK:** Software. **SK, FK, AS:** Supervision. **KEE, FK, BA:** Validation. **SK, KEE:** Visualization. **SK, KEE, FK**: Writing – original draft. **SK, AS, FK:** Writing – review & editing.

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ORIGINAL ARTICLE

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Labor interventions in low- and high-risk parturients in a university hospital

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SUMMARY

OBJECTIVE: The main aim of this study was to evaluate the impact of using interventions in low- and high-risk parturients on maternal and perinatal adverse outcomes during labor.

METHODS: This is a prospective study. The analyzed variables were obtained through a questionnaire with puerperal women (between 1- and 48-h postpartum) and through medical record searches. The study population was divided into two groups as follows: Group I included parturients who underwent at least one type of obstetric intervention and Group II included parturients who did not undergo any type of obstetric intervention.

RESULTS: Most parturients (75.3%) underwent at least one type of intervention, with oxytocin being the most prevalent intervention (49.5%), followed by misoprostol use (28.7%), elective cesarean section at the request of the patient (23.0%), amniotomy (21.2%), and episiotomy (21.0%). Regarding the adverse perinatal outcomes related to low-risk pregnancies, the prevalence of the second- or third-degree perineal tears (17.8% vs. 36.7%, p=0.001) was lower in Group I than in Group II. Moreover, in high-risk pregnancies, the prevalence of hospitalization in the neonatal intensive care unit (2.8% vs. 16.7%, p<0.001), adult intensive care unit admission (0.8% vs. 3.9%, p=0.004), and the need for oxygen therapy (26.8% vs. 40.4%, p<0.001) was lower in Group I than in Group II.

CONCLUSIONS: In low-risk parturients, the interventions performed were associated with lower prevalence of second- or third-degree perineal tears. There was a lower prevalence of neonatal and adult intensive care unit admissions, the need for oxygen therapy, intracranial hemorrhage, and neonatal infection among high-risk parturients.

KEYWORDS: First stage labor. Episiotomy. High-risk pregnancies. Morbidity.

INTRODUCTION

The advances in obstetrics contribute to the improvement of maternal and perinatal morbidity and mortality indicators; however, excessive interventions during labor no longer consider the emotional, human, and cultural aspects involved in childbirth. Therefore, the experiences had by the parturient women may leave indelible, positive, or negative marks for the rest of their lives[1].

Examples of obstetric interventions are as follows: elective cesarean section at the patient's request, Kristeller maneuver, amniotomy, episiotomy, epidural analgesia/labor analgesia, oxytocin, and misoprostol use. Regarding the cesarean section, the international medical community considers that the ideal rate would be between 10% and 15%, and there is no evidence that elective cesarean sections provide benefits; however, this intervention is becoming increasingly frequent in both developed and developing countries[2,3].

The Kristeller maneuver is used to shorten the second stage of labor. Although the literature does not show any benefits of this technique, it is still commonly used and can lead to potential complications, such as perineal tears, uterine rupture, uterine inversion, and increase in maternal and perinatal morbidity rates[4,5]. Early amniotomy may be associated with potential complications, such as fetal heart rate decelerations and infections. Episiotomy is recommended for use between 15% and 30% of cases to achieve progress when the perineum is rigid or when there is evidence of fetal or maternal distress. Routine episiotomy increases the need for suturing the perineum and risk of complications on the seventh postpartum day, leading to unnecessary pain and discomfort[6,7].

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Oxytocin is the most commonly used medication in obstetrics for the stimulation of labor because it increases uterine activity. However, oxytocin administration may present complications, such as increased rates of cesarean sections, use of epidural analgesia, intrapartum maternal fever, low pH values of umbilical cord blood, and postpartum hemorrhage[8,9].

This study aimed to evaluate the impact of using interventions in low- and high-risk parturients on maternal and perinatal adverse outcomes.

METHODS

This is a prospective, observational, and descriptive study, developed in the period between August 2019 and July 2021 in the Gynecology and Obstetrics sector of Mário Palmério University Hospital, Uberaba — MG, Brazil. The population under study was classified into two groups as follows: Group I included parturients who underwent at least one type of intervention during the labor and Group II included parturients who did not undergo any type of intervention. Following this, the parturients were subdivided into high- and low-risk pregnancies to evaluate the influence of obstetric risk in parturients who underwent or did not undergo interventions on maternal and perinatal adverse outcomes. The study was approved by the Research Ethics Committee of the University of Uberaba (UNIUBE) (CAAE: 96383118.7.0000.5145), and the consent form was obtained from all participants.

Pregnant women with single live fetus, age above 18 years, no prenatal diagnosis of fetal malformation, spontaneous or induced labor, and vaginal delivery or cesarean section were included during the study period. Women who refused to participate in the study and who were unable to complete the data collection instruments due to the difficulty in understanding it were excluded. The analyzed variables were obtained through a questionnaire applied to puerperal women (between 1- and 48-h postpartum) and through medical record searches.

Parturient women with at least one of the conditions presented in Table S1 were considered at high risk, whereas those without any condition as mentioned in Table S1 were considered at low risk. At least one of the following interventions was considered during the childbirth: episiotomy, amniotomy, Kristeller maneuver, epidural analgesia, oxytocin, and misoprostol use and elective cesarean section at the patient's request.

The maternal and perinatal adverse outcomes evaluated were as follows: 1-min Apgar score < 7, admission to the neonatal intensive care unit (ICU), admission to adult ICU, neonatal death < 72 h, maternal death, the need for oxygen therapy, neonatal hypotonia, intracranial hemorrhage, gastrointestinal hemorrhage, neonatal infection, tocotrauma, dehiscence and/or infection of the maternal surgical scar, second- or third-degree perineal tears, and puerperal hemorrhage. We considered a composite maternal/perinatal adverse outcome when at least one adverse perinatal outcome was present.

Data were entered into Microsoft Excel 2010 spreadsheet (Microsoft Corp., Redmond, WA, USA) and analyzed using SPSS version 20.0 and Prisma GraphPad version 7.0 (SPSS Inc., Chicago, IL, USA). Quantitative variables were initially submitted to the normality test (Kolmogorov-Smirmov) and presented in the form of median, minimum, and maximum values. Categorical variables were described as absolute frequencies and percentages and represented in table format. To study the difference between categorical variables and their proportions, the chi-square test was used, and to evaluate the difference between continuous variables, the Mann-Whitney U test was used. The significance level for all tests was $p<0.05$.

RESULTS

During the study period, data from 1064 parturients were obtained and divided into Group I (n=801) and Group II (n=263).

Group I presented a prevalence of public service (72.5% vs. 81.4%, p=0.011), tabagism (5.6% vs. 10.6%, p=0.005), high-risk pregnancies (61.4% vs. 77.2%, p<0.001), and nonelective cesarean section (12.2% vs. 60.1%, p<0.001), which was significantly lower than that in Group II. However, Group I presented a prevalence of health insurance (25.1% vs. 17.9%, p=0.011), third-trimester ultrasound (91.3% vs. 87%, p=0.044), adequate serology (87.1% vs. 80.6%, p=0.010), vaginal delivery (47.7% vs. 39.9%, p<0.001), elective cesarean section at the patient's request (38.2% vs. 0%, p<0.001), and forceps (1.9% vs. 0%, p<0.001) , which was higher than that in Group II. The median gestational age at admission (39.1 weeks vs. 38.4 weeks, p<0.001), number of prenatal care visits (9.0 vs. 8.0, p<0.001), weight gain (12 kg vs. 11 kg, p=0.038), and birth weight (3220 g vs. 2990 g, p<0.001) were significantly higher in Group I than that in Group II (Table S2).

Regarding the prevalence of interventions during labor, 49.5% (330/666) of the parturients used oxytocin, 28.7% (191/475) used misoprostol, 23.0% (171/666) had elective cesarean section, 21.2% (141/666) underwent amniotomy, 21.0% (140/666) underwent episiotomy, 11.9% (79/666) received analgesia epidural/analgesia, and 4.5% (30/666) received Kristeller maneuver (Figure S1).

The prevalence of admission to the neonatal ICU (2.2% vs. 13.3%, p<0.001), adult ICU admission (0.6% vs. 3.0%,

p=0.002), the need for oxygen therapy (23.6% vs. 34.2%, p<0.001), intracranial hemorrhage (0.0% vs. 1.1%, p=0.002), and neonatal infection (0.5% vs. 2.7%, p=0.003) was lower in Group I than that in Group II (Table 1).

Regarding the adverse perinatal outcomes related to low-risk pregnancies, the prevalence of second- or third-degree perineal tears (17.8% vs. 36.7%, p=0.001) was significantly lower in Group I than that in Group II (Table 2).

Table 1. Comparison of the prevalence of adverse perinatal ooutcomes among parturients who underwent interventions (Group I) and parturients who did not undergo intervention (Group II) during labor.

	Group I (n=801)	Group II (n=263)	p
Apgar score < 7 at the first min	6.7% (54/801)	8.9% (23/258)	0.242 ∫
Admission to the neonatal ICU	2.2% (18/801)	13.3% (35/263)	<0.001 ∫
Admission to the adult ICU	0.6% (5/801)	3.0% (8/263)	0.002 ∫
Need for oxygen therapy	23.6% (189/801)	34.2% (90/263)	<0,001 ∫
Neonatal hypotony	13.9% (111/801)	14.8% (39/263)	0.695 ∫
Intracranial hemorrhage	0% (0/801)	1.1% (3/263)	0.002 ∫
Gastrointestinal hemorrhage	0.1% (1/801)	0% (0/263)	0.566 ∫
Neonatal infection	0.5% (4/801)	2.7% (7/263)	0.003 ∫
Tocotrauma	1.5% (12/801)	1.5 (4/263)	0.979 ∫
Neonatal death within the first 72 h	0.1% (1/801)	0.8% (2/263)	0.092 ∫
Surgical scar dehiscence and/or infection	1.9% (15/801)	0.4% (1/263)	0.084 ∫
Second- or third-degree perineal tears	18.2% (146/801)	16% (42/263)	0.405 ∫
Puerperal hemorrhage	9.6% (77/801)	7.6% (20/263)	0.326 ∫
Maternal death	0% (0/801)	0% (0/263)	*
Composite adverse perinatal outcome	51.1% (409/801)	57.8% (152/263)	0.058 ∫

ICU: intensive care unit. Chi-square ∫: Percentage (absolute number/total number), p<0.05. * Statistical test was not applied.

Table 2. Comparison of the prevalence of composite perinatal adverse outcomes among parturients who underwent interventions (Group I) and who did not undergo (Group II) interventions in low-risk pregnancies.

	Group I (n=309)	Group II (n=60)	p
Apgar score < 7 at the first min	4.2% (13/309)	7.0% (4/57)	0.354 ∫
Admission to the neonatal ICU	1.3% (4/309)	1.7% (1/60)	0.820 ∫
Admission to the adult ICU	0.3% (1/309)	0% (0/60)	0.659 ∫
Need for oxygen therapy	18.4% (57/309)	13.3% (8/60)	0.341 ∫
Neonatal hypotony	10.0% (31/309)	11.7% (7/60)	0.703 ∫
Intracranial hemorrhage	0% (0/309)	0% (0/60)	*
Gastrointestinal hemorrhage	0.3% (1/309)	0% (0/60)	0.659 ∫
Neonatal infection	0% (0/309)	0% (0/60)	*
Tocotrauma	1.0% (3/309)	0% (0/60)	0.443 ∫
Neonatal death within the first 72 h	0% (0/309)	0% (0/60)	*
Surgical scar dehiscence and/or infection	2.6% (8/309)	1.7% (1/60)	0.672 ∫
Second- or third-degree perineal tears	17.8% (55/309)	36.7% (22/60)	0.001 ∫
Puerperal hemorrhage	7.1% (22/309)	13.3% (8/60)	0.107 ∫
Maternal death	0% (0/309)	0% (0/60)	*
Composite adverse perinatal outcome	45.3% (140/309)	56.7% (34/60)	0.107 ∫

ICU: intensive care unit. Chi-square ∫: Percentage (absolute number/total number), p<0.05. * Statistical test was not applied.

Regarding adverse perinatal outcomes related to high-risk pregnancies, the prevalence of hospitalization in the neonatal ICU (2.8% vs. 16.7%, p<0.001), adult admission to ICU (0.8% vs. 3.9%, p=0.004), the need for oxygen therapy (26.8% vs. 40.4%, p<0.001), intracranial hemorrhage (0.0% vs. 1.5%, p=0.007), and neonatal infection (0.8% vs. 3.4%, p=0.011) was significantly lower in Group I than that in Group II. The prevalence of second- or third-degree perineal tears (16.5% vs. 9.8%, p=0.025) and puerperal hemorrhage (11.2% vs. 5.9%, p=0.032) was higher in Group I than that in Group II (Table 3).

DISCUSSION

When evaluating the impact of obstetric interventions in low- and high-risk parturients on maternal and perinatal outcomes, this study divided the parturients into groups that underwent some intervention during labor (Group I) and parturients who did not undergo any intervention (Group II). Practical interventions, such as elective cesarean section; the use of medications to induce or conduct labor, such as misoprostol and oxytocin; amniotomy; epidural analgesia; episiotomy; and Kristeller maneuver were considered.

Despite what is recommended by the most recognized national and international societies, the rates of cesarean sections are increasing and, in Brazil, this rate exceeds the recommended limit, thereby making Brazil one of the countries with the highest cesarean section rates in the world[10]. This practice, which is more prevalent in private services and often disassociated with precise obstetric indications, is responsible for the increase in maternal and neonatal obstetric complications in short- and long-term follow-ups[11].

In this study, elective cesarean delivery was considered an intervention regardless of obstetric indication; therefore, patients were allocated to Group I, which represented 23% of the interventions studied and a total of 38.2% of the types of delivery. In Group I, 11.9% of the patients received epidural analgesia, which is known to be more accessible in the private or insurance health system[12,13]. This fact can be observed because 27.5% of Group I were attended at these two health systems, with only 18.5% in Group II, which may represent an important selection bias.

Among the patients in Group II, who did not undergo interventions, nonelective cesarean section represented 60.1% compared to 12.2% of Group I, which could indicate an increase in the possibilities of vaginal delivery if interventions were performed because they were performed according to good obstetric practices. The obstetric history of cesarean section was not heterogeneous between the groups and was not statistically significant.

The most common interventions were the use of the misoprostol and oxytocin medications to stimulate labor. Such interventions are often indicated at more advanced gestational ages[14-16], which were confirmed with significance at the median of

Table 3. Comparison of the prevalence of composite perinatal adverse outcomes among parturients who underwent interventions (Group I) and who did not undergo interventions (Group II) in high-risk obstetric pregnancies.

	Group I (n=492)	Group II (n=203)	p
Apgar score < 7 at the first min	8.3% (41/492)	9.5% (19/201)	0.634 [f]
Admission to the neonatal ICU	2.8% (14/492)	16.7% (34/203)	<0.001 [f]
Admission to the adult ICU	0.8% (4/492)	3.9% (8/203)	0.004 [f]
Need for oxygen therapy	26.8% (132/492)	40.4% (82/203)	<0.001 [f]
Neonatal hypotony	16.3% (80/492)	15.8% (32/203)	0.871 [f]
Intracranial hemorrhage	0% (0/492)	1.5% (3/203)	0.007 [f]
Gastrointestinal hemorrhage	0% (0/492)	0% (0/203)	*
Neonatal infection	0.8% (4/492)	3.4% (7/203)	0.011 [f]
Tocotrauma	1.8% (9/492)	2.0% (4/203)	0.901 [f]
Neonatal death within the first 72 h	0.2% (1/492)	1.0% (2/203)	0.153 [f]
Surgical scar dehiscence and/or infection	1.4% (7/492)	0.0% (0/203)	0.088 [f]
Second- or third-degree perineal tears	16.5% (81/492)	9.8% (20/203)	0.025 [f]
Puerperal hemorrhage	11.2% (55/492)	5.9% (12/203)	0.032 [f]
Maternal death	0% (0/492)	0% (0/203)	*
Composite adverse perinatal outcome	54.7% (269/492)	58.1% (118/203)	0.405 [f]

ICU: intensive care unit. Chi-square [f]: Percentage (absolute number/total number), p<0.05. * Statistical test was not applied.

39.1 weeks in Group I compared to 38.4 weeks in Group II. Contrary to expectations, these patients made up to 77.2% of Group II and only 61.4% of Group I, owing to the high-risk pregnancy that frequently necessitates early pregnancy resolution and, as a result, the use of medications to induce labor.

Furthermore, prophylactic oxytocin during the third trimester of pregnancy and misoprostol medication are effective treatments for controlling postpartum hemorrhage, reducing rates of hospitalization in adult ICU (as seen in this study), and reducing maternal mortality, as well as are strongly recommended by international medical societies[17,18]. Such use corroborates and may eventually distort the datum that the use of these medications accounted for 78.2% of the interventions studied in this study.

Regarding perinatal outcomes analyzed among low- and high-risk patients, a significant reduction was also found in neonatal ICU admission rates, the need for oxygen therapy, rates of intracranial hemorrhage, and neonatal infection, facts that may be related to factors not studied in this article, such as the decrease in the total time of the active phase and second stage of labor.

Regarding episiotomy and Kristeller maneuver practices, it was impossible to establish a beneficial relationship between their performance and perinatal outcomes. Therefore, according to the recommendations of global obstetric societies, good obstetric practice prescribes the Kristeller maneuver due to maternal and fetal risks. Episiotomy would be reserved for some cases, in which there would be strong suspicion or evidence of fetal or maternal distress[19-21].

CONCLUSIONS

This study does not aim to encourage routine obstetric interventions during labor. Moreover, the limitation of this study is recognized, considering the fact that it was a single-center study done in Brazil's Southeast region, which has higher socioeconomic power as a country with significant regional inequities and difficulty in accessing health services. However, it is important to evaluate the profile of patients who received more obstetric interventions and highlight the need for further studies that individually correlate these interventions with maternal and perinatal outcomes.

AUTHORS' CONTRIBUTIONS

KMDR: Data curation, Visualization. **CBOS**: Data curation, Visualization. **CZ**: Investigation, Visualization. **LMO**: Methodology, Visualization. **EFMS**: Visualization, Writing – original draft. **MFMC**: Formal analysis, Visualization. **EAJ**: Validation, Visualization, Writing – review & editing. **ABP**: Conceptualization, Project administration, Supervision, Visualization.

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ORIGINAL ARTICLE

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Risk factors associated with infections in pregnant women with systemic lupus erythematosus

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SUMMARY

OBJECTIVE: The aim of this study was to analyze the occurrence and risk factors associated with infections during pregnancy in patients with systemic lupus erythematosus.

METHODS: This is a retrospective cohort study using the data of pregnant women who were followed up between 2011 and 2018 at a university hospital.

RESULTS: The data of 221 pregnant women with systemic lupus erythematosus were analyzed. The incidence of infections was 22.6% (50/221), with the urinary tract being the most frequent site of infection (32/221, 14.5%) followed by the respiratory tract (15/221, 6.8%). The bivariate analysis showed that active disease, hematological systemic lupus erythematosus, reduced complement, and use of prednisone ≥5 and ≥10 mg increased the chance of infection during early pregnancy (p=0.05, p=0.04, p=0.003, p=0.008, and p=0.02, respectively), while disease activity and anti-DNA positivity increased it at the end of pregnancy (p=0.03 and p=0.04, respectively). Prednisone at a dose ≥5 mg increased the chance of infection in the beginning (p=0.01) and at the end of pregnancy (p=0.008). Multivariate analysis showed that increasing the dose of prednisone from 5 to 10 mg tripled the chance of developing infections in pregnant women with lupus (p=0.02).

CONCLUSION: The study showed an increased chance of infections in pregnant women with systemic lupus erythematosus and it was associated with the use of prednisone.

KEYWORDS: Systemic lupus erythematosus. Infection. Pregnancy. Risk factors.

INTRODUCTION

Systemic lupus erythematosus (SLE) is common in women of reproductive age[1]. There is a higher incidence of infection in pregnant women with SLE than in those without SLE[2]. The relationship between disease activity and infection has already been described, suggesting that an unbalanced immune response increases the vulnerability to infections[2]. Together, these factors increase the incidence of infections, which are the cause of 30% of all deaths in patients with SLE and are the leading cause of mortality in this population[3]. Evidence suggests that in pregnant women with SLE, there is a higher risk of maternal death due to serious infections[1].

The main objective of this study was to evaluate the incidence of infections in pregnant women with SLE and to identify the sites of infections and the associated risk factors.

METHODS

This is an observational cohort study based on retrospective data. It included pregnant women with SLE according to the classification criteria proposed by the American College of Rheumatology[4]. These women were followed up at the prenatal clinic for autoimmune diseases at Pedro Ernesto University Hospital (HUPE), State University of Rio de Janeiro, from 2011 to 2018.

Data were obtained through the review of physical and electronic medical records. The data of all pregnant women from the first medical appointment until the time of delivery were collected.

Sociodemographic and reproductive factors, such as the age at diagnosis and delivery, ethnicity, number of pregnancies and live births, and risk factors related to infection in patients with SLE, such as disease activity, clinical and laboratory parameters,

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and medications used in the treatment of the underlying disease, were evaluated.

Disease activity in early and late pregnancy was established using the pregnancy-adapted SLE Disease Activity Index (SLEPDAI)[5]. SLEPDAI values ≥3[5] were considered indicative of disease activity, and their association with potential infection was analyzed. Disease activity was categorized following the SLEPDAI values: inactive disease=0, mild activity=1–2, moderate activity=3–5, and high activity ≥6. For the clinical analysis of the underlying disease, the clinical manifestations were categorized into cutaneous, articular, serous, hematological, renal, pulmonary, and neuropsychiatric involvement. Laboratory evaluation was performed to detect the presence of lymphopenia at diagnosis, consumption of C3 and/or C4, and the presence of positive anti-DNA at the beginning and end of pregnancy.

Lupus nephritis was defined as proteinuria ≥500 mg in a 24-h urine sample or protein/creatinine ratio ≥0.5 in a single urine sample, the presence of nephrotic syndrome or acute or chronic renal failure due to SLE, or confirmation on renal biopsy, according to the 2003 International Society of Nephrology/Renal Pathology Society classification.

The use of prednisone, hydroxychloroquine, and azathioprine in the beginning of and during pregnancy was analyzed. The intake of prednisone at the dosage levels of ≥5, ≥10, and ≥40 mg/day was also analyzed.

Data were descriptively evaluated, and the analysis of the normal distribution of variables, and the proportions, means, standard deviations, medians, and the respective 95% confidence intervals (CI) were estimated. Categorical variables were expressed as frequency (n) and proportion (%), and continuous variables were expressed as mean and standard deviation or median. The relative proportions were calculated for two distinct groups, with and without infection. Medians were used to define the cutoff value used to convert numerical to categorical variables. The association between infection during pregnancy and the clinical and sociodemographic variables was determined by the bivariate analysis and multiple logistic regression, and the performance of the adjusted model was evaluated using the receiver operating characteristic (ROC) curve. The response variable of the adjusted model was the presence of infection, and the explanatory variables were the initial SLEPDAI score and final dose of prednisone. Missing data were excluded from the statistical analysis.

The chi-square (χ^2) and Fisher's exact tests were used to compare categorical variables and outcomes (infection in pregnant women with SLE). Continuous variables were compared using Student's t-test. The level of associations was evaluated by calculating the measures of associations (odds ratio [ORs]) and their respective 95%CIs. Statistical analyses were performed using Epi-info version 3.5.2 and R-Projeto version 3.3.1. A hypothesis test was used to compare proportions, using a significance level of 5%.

This study was approved by the Research Ethics Committee of HUPE (CAAE: 00407518.8.0000.5259).

RESULTS

A total of 221 pregnancies were analyzed. The age of patients with SLE at the time of delivery ranged between 15 and 47 years (mean=28.5±6.2; median=29 years). The mean age at the time of diagnosis of SLE was 20.3±6.8 years (from 4 to 47 years, median=20 years). The duration of disease remission ranged from 0 to 108 months (9 years), with a median of 6 months. Of the 221 patients, 42.5% (94) were Caucasian.

The most frequent clinical manifestations of the underlying disease over the years in the 221 patients analyzed were cutaneous-mucosal manifestations in 87.3% (193), hematological manifestations in 57.9% (123), and renal manifestations in 44.3% (98), with a predominance of proliferative forms (classes III and IV) and class V, arthritis in 91.4% (202), and serositis in 35.8% (79).

Laboratory tests performed during pregnancy and the frequency of disease activity evaluated by a SLEPDAI value ≥3 were classified, as shown in Table 1.

All patients were on medication for the treatment and/or control of SLE during early pregnancy. Of these, 136 (61.5%) used prednisone, with a minimum dose of 2.5 mg/day and a maximum dose of 80 mg/day, with a mean of 8.7±13.3 mg and a median of 5 mg.

Hydroxychloroquine was used by 92.3% (205/221) of the patients. Azathioprine was used by 37.6% (83/221) of the patients.

Of the 221 patients, 22.6% (50) had pregnancy-related infections. Urinary tract infections were the most common and seen in 14.5% (32/221), and respiratory tract infections were the second most common and seen in 6.8% (15/221). Of these, 4.5% (10/221) had community-acquired pneumonia.

Other infections with a lower incidence included skin infections in 2.3% (5/221), with herpes zoster occurring in 1.8% (4/221) and being the most frequent infection, gastroenteritis in 0.9% (2/221), meningitis in 0.5% (1/221), bloodstream infections caused by a dialysis catheter in 0.5% (1/221), and reactivation of ocular toxoplasmosis in 0.5% (1/221) of the patients.

Clinical and laboratory variables, as well as individual medications (Table 1) and their relationship with infections, were evaluated.

Disease activity, in general, was not associated with infections. However, an increased risk of active disease at the end of pregnancy in women with a SLEPDAI value 3–5 was identified (p=0.03).

Table 1. Clinical/laboratory variables and medications potentially associated with infection.

Clinical/laboratory variables				
Variables		**Infection**	**Without infection**	**p-value**
		n (%)	**n (%)**	
Cutaneous mucous SLE	Present	44 (19.9)	149 (67.4)	0.87
	Absent	6 (2.71)	22 (9.95)	
Hematological SLE	Present	35 (15.8)	93 (43.0)	0.04*
	Absent	15 (67.9)	78 (35.3)	
Renal SLE	Present	21 (9.5)	77 (34.8)	0.7
	Absent	29 (13.1)	94 (42.5)	
Neuropsychiatric SLE	Present	13 (5.9)	31 (14.3)	0.22
	Absent	37 (16.7)	140 (63.3)	
Joint SLE	Present	48 (21.7)	154 (69.6)	0.19
	Absent	2 (0.9)	17 (7.7)	
Serositis	Present	19 (8.6)	60 (27.1)	0.7
	Absent	31 (14.0)	111 (50.2)	
Initial C3 and/or C4	Consumed	22 (9.9)	47 (21.3)	0.03*
	Normal	28 (12.7)	124 (56.1)	
Final C3 and/or C4	Consumed	15 (6.8)	43 (19.5)	0.49
	Normal	35 (15.8)	128 (57.9)	
Initial Anti-DNA	Positive	21 (9.5)	49 (22.2)	0.07
	Negative	29 (13.1)	122 (55.2)	
Final Anti-DNA	Positive	26 (11.8)	61 (27.6)	0.04*
	Negative	24 (10.9)	110 (49.8)	
Initial SLEPDAI	≥3	22 (9.9)	50 (22.6)	0.05
	<3	28 (12.7)	121 (54.8)	
Final SLEPDAI	≥3	23 (10.4)	55 (24.9)	0.44
	<3	27 (12.2)	116 (54.5)	
Medications:				
Prednisone	Present	38 (17.2)	98 (44.3)	0.02*
	Absent	12 (5.4)	73 (33.0)	
Final prednisone	Present	39 (17.6)	98 (4.4)	0.008*
	Absent	11 (5.0)	73 (33.0)	
Initial prednisone ≥5 mg/day	Present	37 (16.7)	93 (42.1)	0.01*
	Absent	13 (5.8)	78 (35.3)	
Final prednisone ≥5 mg/day	Present	37 (16.7)	91 (41.2)	0.008*
	Absent	13 (5.8)	80 (36.2)	
Initial prednisone ≥10 mg/day	Present	22 (9.9)	46 (20.8)	0.02*
	Absent	28 (12.7)	125 (56.6)	
Final prednisone ≥10 mg/day	Present	23 (10.4)	54 (24.4)	0.06
	Absent	27 (12.2)	117 (51.5)	
Initial prednisone ≥40 mg/day	Present	3 (1.4)	10 (4.5)	0.93
	Absent	47 (21.3)	161 (72.8)	
Final prednisone ≥40 mg/day	Present	3 (1.4)	15 (6.8)	0.56
	Absent	47 (21.3)	156 (70.6)	
Initial hydroxychloroquine	Present	46 (20.8)	159 (72)	0.79
	Absent	4 (1.8)	12 (5.4)	
Final hydroxychloroquine	Present	48 (21.7)	169 (76.5)	0.26
	Absent	2 (0.9)	2 (0.9)	
Initial azathioprine	Present	21 (9.5)	62 (28.1)	0.46
	Absent	29 (13.1)	109 (49.3)	
Azatioprina final	Present	27 (1.2)	71 (32.1)	0.12
	Absent	23 (10.4)	100 (45.2)	

SLE: systemic lupus erythematosus; SLEPDAI: Pregnancy-adapted SLE Disease Activity Index. *Systemic Lupus Erythematosus Prenancy Disease Activity Index.

Hematological involvement also increased the chance of infection (p=0.04) and was associated with a twofold increase in the chance. Reduced complement during early pregnancy contributed to an increased chance of infection (p=0.003).

The presence of anti-DNA antibodies at the end of pregnancy was associated with an increased chance of infection (p=0.04).

Increased chance of infection with the use of prednisone, regardless of the dose, was observed at the beginning and end of pregnancy (p=0.008 and p=0.03, respectively).

Prednisone at a dose ≥5 mg/day was associated with an increased chance of infection in general (p=0.01) at the beginning, as well as at the end of pregnancy (p=0.008). A high chance of infection (p=0.02) in those on a dose ≥10 mg/day of prednisone was observed in early pregnancy. Multivariate analysis showed that increasing the dose of prednisone from 5 to 10 mg tripled the chance of developing infections in pregnant women with lupus in the study group (adjusted OR=3.15 [1.22–8.12]; p=0.02) (Table 2).

The goodness-of-fit of the model was evaluated using the ROC curve. The ROC curve graph for the infection outcome showed an area below the curve of 68%. The point of greatest sensitivity (Ŝ) and specificity (Ê) of the model was Ŝ=70% and Ê=62% (Figure 1). These values indicate the moderate performance of the adjusted model in differentiating the presence and absence of infection in pregnant women with lupus.

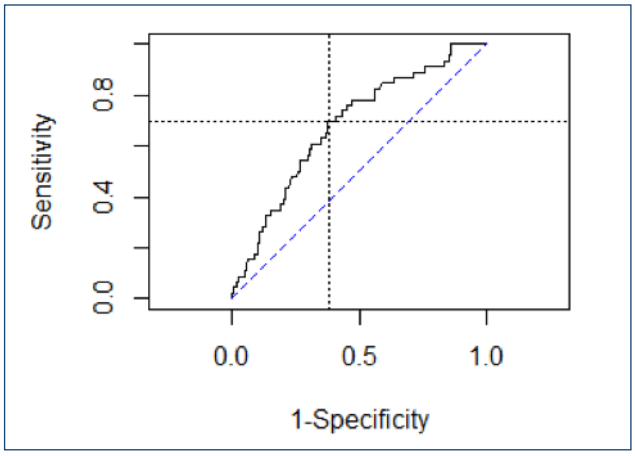

Figure 1. Performance of the model adjusted in the recevier operating characteristic curve for infection in general.

Table 2. Crude and adjusted odds ratio (OR) of infections in general in pregnant women with systemic lupus erythematosus.

	Crude OR (95%CI)	p-value	Adjusted OR	p-value
Initial SLEPDAI*	1.28 (0.88–1.81)	0.21	1.01 (0.65–1.57)	0.96
Initial SLEPDAI*	1.24 (0.88–1.75)	0.21		
Initial prednisone (mg)				
0	1			
5	2.32 (0.98–5.47)	0.05		
10	3.25 (1.39–7.62)	0.01		
40	2.18 (0.52–9.17)	0.29		
Final prednisone (mg)				
0	1			
5	2.82 (1.17–6.80)	0.02	3.05 (1.20–7.77)	0.02
10	3.27 (1.41–7.57)	0.01	3.15 (1.22–8.12)	0.02
40	1.49 (0.37–5.99)	0.57	1.57 (0.35–7.02)	0.56
Initial azathioprine (mg)				
0	1			
50	0.51 (0.06–4.24)	0.53		
100	2.09 (0.97–4.48)	0.06		
150	0.82 (0.28–2.36)	0.71		
200	3.44 (0.72–16.5)	0.12		
Final azathioprine (mg)				
0	1			
50	1.37 (0.41–4.58)	0.61		
100	1.19 (0.51–2.80)	0.68		
150	1.08 (0.37–3.16)	0.89		
200	2.06 (0.18–23.5)	0.56		

*Log transformation. Logistic regression models whose response variable was "infection in general." The explanatory variables of the multiple model were initial SLEPDAI and final prednisone. SLEPDAI; Pregnancy-adapted SLE Disease Activity Index.

DISCUSSION

Infections are a frequent concern in patients with SLE, being the major cause of mortality in this population[6]. They can increase morbidity, requiring the use of high doses of glucocorticoids (GCs) and the use of immunosuppressants in some situations. High disease activity, frequent reactivations, anti-DNA positivity, serum complement consumption, and renal impairment at diagnosis are also associated with a higher risk of infection[7].

The study population was homogeneous, showing no significant difference in terms of sociodemographic factors.

Our study found a higher rate of urinary tract infection than that reported in previous studies, which have reported the rate of urinary infection in pregnant women without SLE between 1% and 2%[8]. Our study showed a rate of pyelonephritis similar to that reported in the literature, with a frequency of 0.5–2%[8].

The presence of pneumonia was also frequent in this study. Lim et al. reported a rate of pneumonia of 0.01% in pregnant women[9].

Disease activity was not associated with infection in our study. Disease activity has been previously reported as a risk factor for infection. Jeong et al. found an association between infection and SLEPDAI value ≥12[7]. However, the authors reported that the infection was attributed to medications used in the treatment of reactivations and not directly related to the disease activity[2,10]. However, Pimentel-Quiroz et al. found an association between infection and SLEPDAI values ≥4[11].

In this study, the only clinical manifestation of SLE that increased risk of infection was hematological involvement of the disease. A similar result was reported in recent study[12], and the presence of hematological manifestations was considered a factor for poor prognosis in these patients[2].

Renal involvement was not a risk factor for infection in this study. It is known that the presence of kidney disease due to SLE is typically defined as a risk factor for infection[10]. Despite the significant number of patients having previous kidney disease (almost 50% of the study cohort), it was not associated with infection in the present study, which can be explained by the small number of patients using high doses of GCs, since only a small proportion of the patients had active kidney disease.

Serosal involvement was not associated with infection in the present study. However, Jung et al. found a higher frequency of serositis in patients with infection[2].

Complement consumption and positive anti-DNA are markers of disease activity and were also associated with infection in this study. This finding agrees with that reported in the previous study[7]. In the bivariate analysis in this study, both were significantly associated with infections in general; however, this was not seen in the multivariate analysis. However, these two markers of disease activity cannot always be easily dissociated. In some cases, an analysis of variation >25% is already predictive of reactivation[13], albeit mild and without clinical repercussions.

Glucocorticoids at variable doses are the first-line therapy to treat reactivations. In mild cases, prednisone is usually used at a maximum dose of 20 mg/day, with a dose between 5 and 10 mg considered safe during pregnancy[14]. Higher doses and pulse therapy with GCs are reserved for cases of moderate-to-severe activity[14]. In contrast to the safety of the 5–10 mg dose reported in the literature, we observed that the chance of infection was three times higher with an increase in the dose from 5 to 10 mg of prednisone. Jung et al. found an association between infection and the use of prednisone at a dose ≥7.5 mg/day[2]. The fact that our study did not find an association between high doses of GCs and the risk of infection can be explained by the small number of patients using doses ≥40 mg/day.

The present study found no association between the use of azathioprine and the risk of infection in pregnant women with lupus. Previous reports on this association are conflicting; hence, further studies are necessary. Feldman et al. also reported this association without defining the duration of use, while Jung et al. did not find this correlation[2,15].

A limitation of the present study is that some patients were followed up at the HUPE only during pregnancy, with data on the diagnosis of SLE being limited. Underdiagnosis of some infections during the period between the consultations that were not witnessed or reported at the time of the consultation might also have occurred. The infection was made by clinical diagnosis in some cases due to the limitations of performing the imaging tests during pregnancy. Some laboratory tests were not performed in the hospital laboratory, making it difficult to access information that was not in the medical records.

CONCLUSIONS

The present study clearly showed an association between some risk factors and infections. An increase in the number of infections was observed in the group of pregnant women with SLE. The most significant finding was the threefold increase in the risk of infection with an increase in the dose of prednisone from 5 to 10 mg. Therefore, conducting further studies and confirming the current evidence will help professionals who deal with pregnancy and SLE, thereby improving the quality of life and reducing the morbidity and mortality in this patient group. This study is relevant considering the scarce literature on this topic.

AUTHORS' CONTRIBUTIONS

DMJV: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, and Writing – review & editing. **DLMM**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, and Writing – review & editing. **NRJ**: Conceptualization, Data curation, and Writing – original draft. **GRRJ**: Conceptualization, Data curation, Formal Analysis, and Writing – original draft. **FCS**: Conceptualization, Data curation, and Writing – original draft. **MIL**: Conceptualization, Data curation, and Writing – original draft. **NCPR**: Conceptualization, Data curation, Formal Analysis, and Writing – original draft. **EMK**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, and Writing – review & editing.

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REVIEW ARTICLE

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Omentin-1 circulating levels as predictor of heart diseases: a systematic review and meta-analysis

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INTRODUCTION

Cardiovascular disorders (CVDs) are considered a significant global health problem. These disorders are the leading cause of mortality and disability, with more than three-quarters reported from developing countries[1,2]. Development of tools, guidelines, and designing models based on the prediction factors was conducted to prevent and control CVDs[3-5]. Several comorbidities and genetic and biochemical factors have been considered in the prediction systems of CVDs[6,7]. Some adipose tissue biomarkers including adipokines have been hypothesized as the predictive factors of the occurrence of CVDs[5,8,9]. Previous reports revealed the probable role of omentin-1 as the established adipokines of atherosclerosis in healthy men and in patients with type 2 diabetes mellitus (T2DM)[10-12].

There are conflicting studies about the role of omentin-1, the visceral adipose tissue adipokine[13], on the development of CVDs. A cross-sectional association of cardiometabolic parameters and omentin-1 showed that this adipokine is a cardioprotective factor. Inverse association of omentin-1 and carotid intima thickness in patients with metabolic syndromes[14] and a similar inverse correlation between this adipokine and cardiovascular events within individuals with T2DM were also investigated[14,15]. Given the previous conflicting reports, this systematic review and meta-analysis aimed to investigate the association between circulation omentin-1 and the occurrence of CVD.

METHODS

We conducted our meta-analysis based on observational studies, and it was reported according to MOOSE (Meta-analysis of Observational Studies in Epidemiology) checklist.

Search strategy

Online databases of MEDLINE/PubMed, Scopus, EMBASE, and Web of Science (ISI) were systematically searched for all observational studies until February 24, 2021. In addition to Google Scholar, the reference lists of previous all relevant review articles, including narrative or systematic reviews and selected studies, were manually checked to obtain further studies missed in online searches.

Study selection

After undertaking a comprehensive literature search, the selection process was conducted individually by two investigators (JW and XZ), and discrepancies were resolved by consensus or discussion with a third author (ZJ). Studies were selected if they met the following predefined inclusion criteria: those conducted on human with observational designs (cross-sectional, case-control, nested case-control, or cohort); those published in the English language without date restrictions; those that investigated the association of serum/plasma omentin-1 levels with heart diseases (e.g., heart failure, ischemic heart disease, coronary artery disease [CAD], acute coronary artery syndrome, slow coronary flow, and myocardial infarction), cardiomyopathies (e.g., dilated cardiomyopathy, obstructive hypertrophic cardiomyopathy, and atrial fibrillation); and those that reported sufficient data on mean [corresponding standard deviation (SD) or standard error (SE)] or median [interquartile range (IQR)] of these measurements in both case and control groups. We excluded studies that were animal studies, *in vivo*, *in vitro*, case reports, case series, meeting abstracts, reviews, letters, editorials, and studies that did not have a control group.

Data extraction and quality assessment

From the selected studies, we extracted the primary data. After extracting the data by two individual authors (JW and XZ), a third author (ZJ) checked for more accuracy. The Newcastle-Ottawa Scale (NOS) was used to assess each selected study (Table 1)[16].

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Table 1. Main characteristics of included studies.

First author	Publication year	Country	Type of study	Sample size (case/control)	Mean age (case/control)	Gender M/F (control vs. case)	Body fluid	Case population	Control population	Quality scores
Shang et al.[22]	2011	China	Case-control	107/46	62.56±9.35 64.41±8.14	26/20 65/42	Serum	CAD	Healthy subjects	6
ZHONG et al.[23]	2011	China	Case-control	127/26	59.81±9.88 61.85±12.05	38/14 90/37	Serum	ACS	Healthy subjects	6
Wang et al.[24]	2014	China	Case-control	59/31	75.85±50.05 73.90±64.44	20/11 36/23	Plasma	CHD	Healthy subjects	7
ZHONG et al.[23]	2011	China	Case-control	28/26	59.81±9.88 60.61±15.02	38/14 15/13	Serum	SAP	Healthy subjects	8
Huang et al.[25]	2016	China	Case-control	100/45	52 ± 12 54 ± 13	25/20 72/28	Plasma	Dilated cardiomyopathy	Healthy subjects	8
Tao et al.[26]	2016	China	Cross-sectional	220/115	58.43±9.55 59.75±10.03	61/54 113/107	Serum	AF	Individuals for routine checkup in hospital	7
Stejskal et al.[27]	2016	Czech Republic	Case-control	61/40	35.5±9.4 42.7±6.9	72.7% male 82.5% female	Serum	Premature CAD	Healthy subjects	7
Motawi et al.[20]	2017	Egypt	Case-control	45/15	54.6±3.1 53.7±7.6	14/16 23/22	Serum	CAD	Healthy subjects	6
Motawi et al.[20]	2017	Egypt	Case-control	45/15	54.6±3.1 55.3±6	14/16 11/34	Serum	CAD	Healthy subjects	7
Abd-Elbaky et al.[28]	2016	Egypt	Case-control	80/80	38.6±4.2 40.3±2.5	All men	Serum	CVD	Healthy, nonobese controls	5
El-Mesallamy et al.[29]	2011	Egypt	Case-control	22/15	52.6±11.61 59±9.38	14/1 18/4	Serum	IHD	Healthy subjects	7
Kadoglou et al.[30]	2015	Greece	Case-control	78/32	63.1± 9 66.2± 14.4	25/7 63/15	Serum	AMI	Healthy subjects	8
Biscetti et al.[31]	2020	Italy	Cohort	84/123	74.584 75.244	79/44 65/19	Serum	CAD	Patients without CAD	6
Shibata et al.[32]	2011	Japan	Case-control	78/61	61.3±39.83 63.6±72.42	All men	Plasma	CAD	Healthy subjects	6
Narumi et al.[33]	2014	Japan	Case-control	136/20	65±16 72±12	11/20 76/136	Serum	HF	Subjects without signs of significant heart disease	7
Nazar et al.[40]	2017	Pakistan	Case-control	250/100	49.7±6.4 51.3±6.38	71% male 63.2% male	Serum	CAD	Healthy subjects	6
Nazar et al.[34]	2020	Pakistan	Case-control	250/220	55.43±4.90 53.29±5	157/93	Serum	CAD	Healthy subjects	6
Baig et al.[35]	2020	Saudi Arabia	Case-control	122/52	53.38±5.99 54.94±9.08	105/17 34/18	Serum	AMI	Healthy subjects	6
Onur et al.[36]	2013	Turkey	Case-control	110/83	66.2+11.9 68.2+10.2	All women	Serum	CAD	Patients without CAD	6
Yıldız et al.[21]	2018	Turkey	Cohort	50/25	37.7±9.6 36.6±13	24/26 25/25	Serum	Nonobstructive hypertrophic cardiomyopathy	Without HCM	7
Yıldız et al.[21]	2018	Turkey	Cohort	37/25	37.7±9.6 40.9±12.1	24/26 20/17	Serum	Obstructive hypertrophic cardiomyopathy	Without HCM	7

Statistical analysis

Mean differences and their SDs in blood omentin-1 levels between cases and controls were considered ESs, and these were expressed as weighted mean difference (WMD) and corresponding 95% confidence interval (CI) in our meta-analysis. A random-effects model was used to pool ESs with DerSimonian and Laird method. Between-study heterogeneity was quantified by chi-square test and inconsistency index (I^2) statistic. A $p<0.1$ with $I^2>50\%$ represented significant heterogeneity across included studies[17]. Furthermore, to detect the source of heterogeneity, additional analyses, including subgroup and sensitivity analyses, were conducted. Egger's regression asymmetry test[18,19] and visual-filled funnel plot were used to assess the evidence of potential publication bias. The data were analyzed using STATA version 16.0 software (STATA Corp, College Station, TX, USA).

RESULTS

Literature search and study characteristics

A total of 873 records were identified using our search strategies. As shown in Figure 1, 855 records were excluded step by step due to the aforementioned reasons. Finally, the remaining 18 articles (or 21 studies) were investigated for our meta-analysis[20-36]. It should be noted, however, that out of all these 18 articles, 9 studies reported data on CAD, and the rest addressed other heart diseases. Overall, the included studies, published from 2011 to 2020, contained 2089 cases and 1195 controls. Seventeen studies were conducted using a case-control design, three studies employed a cohort design, and one studies had a cross-sectional design. Six studies were performed in China, four in Egypt, three in Turkey, two studies in Japan and Pakistan, and finally one in Czech Republic, Greece, Italy, and Saudi Arabic. However, the main characteristics of each included study are summarized in Table 1.

Meta-analysis results for omentin-1 levels

Figure 2 is a forest plot that indicated the individual and pooled WMD of blood omentin-1 levels between cases with heart diseases and controls. Based on 21 qualified studies, the pooled result using the random-effects model showed that the omentin-1 levels among cases were significantly lower than controls (WMD: -15.20 ng/mL; 95%CI -16.38, -14.01; $p<0.001$).

Due to considerable heterogeneity across included studies ($I^2=99.73\%$, $p<0.001$), we conducted additional analyses. The subgroup findings based on potential modifying variables, including continent, study design, body fluid, type of heart disease, and quality status. The pooled WMDs were remained significant in the different strata of all subgroup analyses. Moreover, publication year ($\beta=-10.39$, $p=0.431$) and total sample size ($\beta=-0.12$, $p=0.741$) had no statistically significant effects on omentin-1 levels in circulating between cases with heart diseases and controls.

Sensitivity analysis was conducted based on the heterogeneity statistic (I^2: 25%) and there was no significant change in pooled ES (WMD: -0.25 ng/mL; 95%CI -0.30, -0.19). Furthermore, in sensitivity analysis, after excluding each study using the leave-one-out method, the pooled WMD remained stable.

Publication bias

We found evidence of potential publication bias across included studies using visual-filled funnel plots and the results of Egger's asymmetry test for omentin-1 ($p<0.001$). Therefore, after considering censure studies using nonparametric trim-and-fill analysis, we found that the pooled WMD for omentin-1 (WMD: -15.19 ng/mL; 95%CI -16.38, -14.01) was still significant.

DISCUSSION

Findings demonstrated a significantly lower circulating level of omentin-1 in patients with CVDs. This outcome was also confirmed by subgroup analysis due to the significant heterogeneity among included studies after modifying the continent, study design, body fluid, type of heart disease, and quality status.

Omentin-1 has a modulator and vasodilator effect on endothelium. Its probable association with chronic inflammatory disease, obesity, insulin resistance, and CADs was reported in several studies. The results obtained from a meta-analysis on six studies conducted by Agasthi et al. were in consistent with the present study, indicating a significant negative association between serum level of omentin-1 and risk of CAD with a standard mean difference (SMD) of -2.27[37]. Epicardial fat tissue, which was described as distributed visceral fat in coronary arteries in a meta-analysis study by Li et al., was considerably higher in patients with diabetes[38]. Following these results, in a meta-analysis study by Ashabi et al., omentin-1 had a significantly lower serum level in patients with T2DM and IGT after pooled analysis of 28 included case-control studies[39]. Summarizing these two studies' results might reveal the probable negative association between omentin-1 and epicardial fat tissue, which

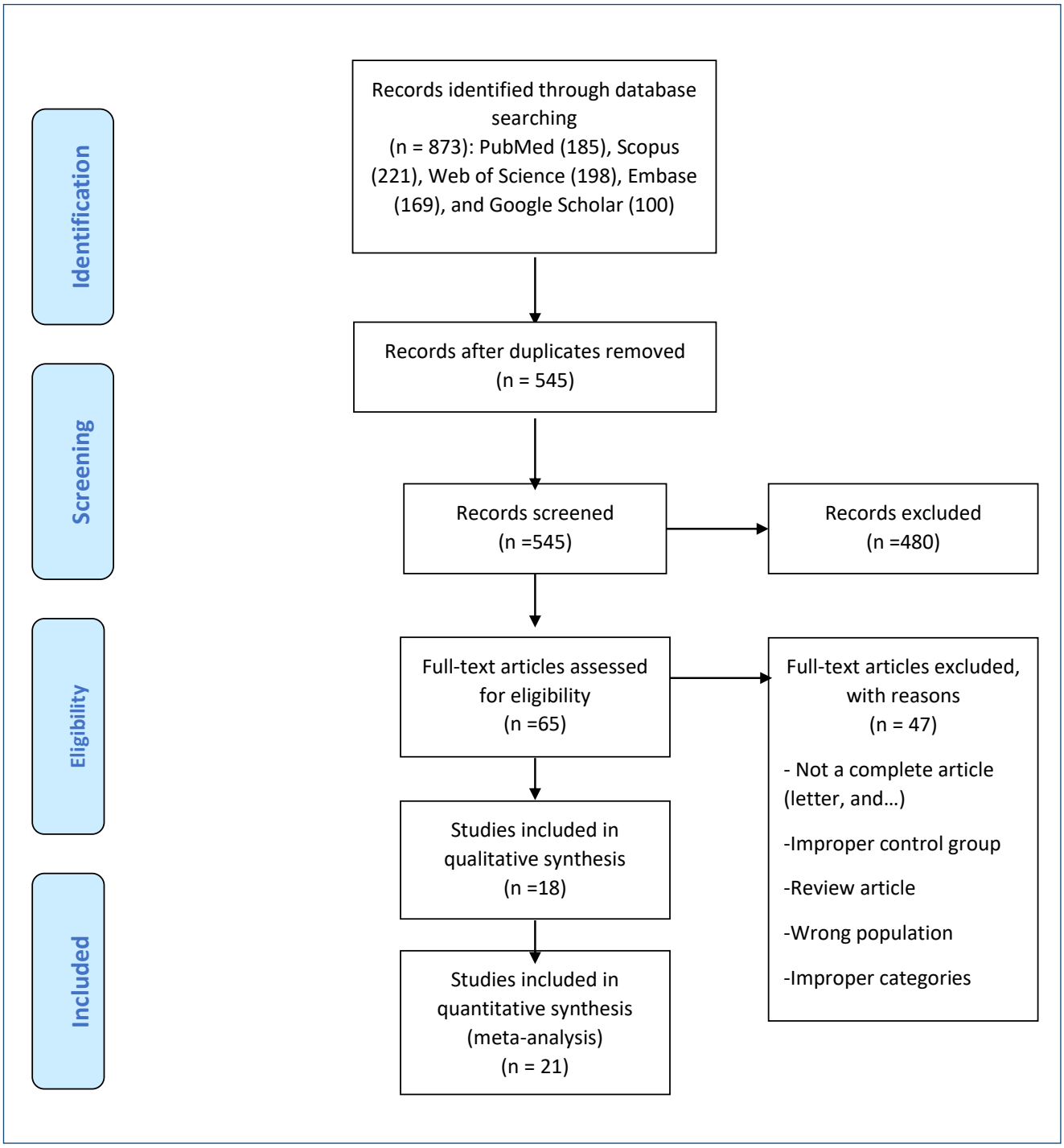

Figure 1. Flowchart of study identification and selection process.

could involve coronary arteries as atherosclerosis and could result in CADs in patients with diabetes.

This study has several limitations. There was significant heterogeneity by evaluating the role of omentin-1 in predicting CVDs. In this study, we did not evaluate the effect of duration and the role of therapeutic management of underlying CVDs on omentin-1 levels. Moreover, the role of this adipokine should be assessed for predicting more arrhythmias than atrial fibrillation. The results of this meta-analysis should be interpreted by considering these limitations.

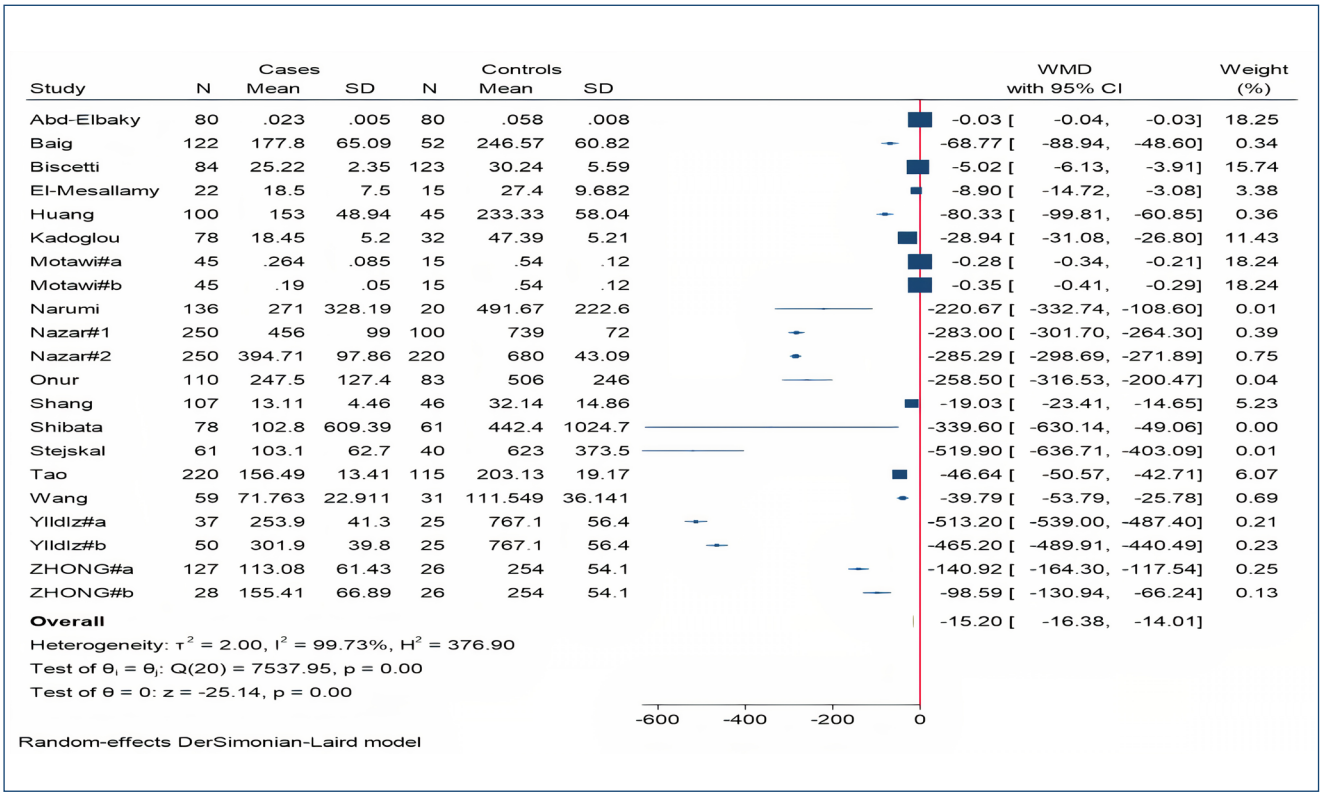

Figure 2. Forest plot of pooled estimates of the weighted mean differences of circulating omentin-1 levels between cases with heart diseases and controls.

CONCLUSIONS

The findings of this study about the effect of diabetes on omentin-1 circulating levels coincided with their CVD changes. Therefore, this adipokine could be the independent prediction factors of CVDs in a population with or without diabetes/MetS. Nonetheless, omentin-1 was shown to have a negative association with prediction of CVDs.

AUTHORS' CONTRIBUTIONS

JW: Conceptualization, Data curation, Formal Analysis, Writing – original draft, and Writing – review & editing. **XZ**: Conceptualization, Data curation, Formal Analysis, and Writing – original draft. **ZJ**: Conceptualization, Data curation, Formal Analysis, Investigation, Supervision, Writing – original draft, and Writing – review & editing.

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COMMENTARY

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Comment on "The impact of visceral fat and levels of vitamin D on coronary artery calcification"

Jinyu Sheng[1], Wei Shen[1], Lianping He[1*]

Dear Editor,

We were glad to read the interesting article entitled "The impact of visceral fat and levels of vitamin D on coronary artery calcification" written by the Rodrigues Isa Galvão[1] and his study team. The authors found that excess visceral fat was associated with subclinical atherosclerosis, regardless of other risk factors for cardiovascular disease, and that serum levels of 25-hydroxyvitamin D3 were not associated with coronary artery calcification in its early stages. Although the findings of their study offer innovative ideas and are supported by useful arguments, we consider some issues should be further discussed.

According to the categories of the World Health Organization, the definitions of overweight (body mass index [BMI]: 25–<30 kg/m²) and obese (BMI ≥30 kg/m²) are different. However, it is given in this article that overweight refers to the BMI ≥30 kg/m². In consideration of scientific rigor, this definition can be slightly modified.

In this study, logistic regression analysis was conducted to assess for confounding factor such as visceral adipose tissue, age, and hypertension. Apart from this, smoking[2] and drinking[3] are considered high-risk factors of coronary artery calcification. In addition, tobacco nicotine and alcohol can also cause significant damage in coronary artery. Therefore, smoking and drinking should be treated as confounding variables. The age of the sample ranges from 43.5 to 68.3 years, which contains a large span. It is found that there is a certain connection between age and coronary artery calcification. Thus, it is a good idea to shorten the age span. Moreover, we cannot find references about VRT, CAD, and TAV in the whole article. We recommend that they can be explained, but if they are misspelled, they can be corrected.

AUTHORS' CONTRIBUTIONS

JS: Writing – original draft. **WS, LH:** Conceptualization, Writing – review & editing.

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COMMENTARY

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Comment on "The efficiency of a mixed exercise program on quality of life and fatigue levels in patients with breast cancer"

Siyun Zhou[1], Juan Zhang[1*]

Dear Editor,

We are glad to read the interesting study by Köse[1] and his team. The study found that a mixed (i.e., fitness center and home) 12-week exercise program provides an improvement in general health and reduces the side effects of the treatments and fatigue in patients with breast cancer. This is a significant study that can help breast cancer patients. However, from our point of view, there are some issues worthy of discussion.

To begin with, in the "Method" section of the article, the concepts of "educated," "not exercise regularly," and "exercise regularly" are ambiguous. We need a clear definition of education level and exercise frequency. Also, it is mentioned that 62 women were included with convenience sampling in the "Method" section of the study. Convenience sampling is a non-probabilistic sampling and the sample size is slightly small; therefore, the sampling has a deviation and is not representative.

In addition, we find that the premise of the study group is that they have already completed the treatment for breast cancer. However, there are several treatments of breast cancer, such as surgery, radiotherapy, and chemotherapy. The different treatments of breast cancer also vary; from our point of view, whether they have a certain influence on the results should be considered.

AUTHORS' CONTRIBUTIONS

SZ: Data curation, Formal Analysis, Writing – original draft.
JZ: Conceptualization, Writing – review & editing.

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