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Brasil has the world's largest public kidney transplant program

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Brasil started its kidney transplant program in 1964, 10 years after the historic successful kidney transplant in Boston[1,2]. In the 1980s, the foundations for a stronger national kidney transplant program were laid with the creation of the Brazilian Association for Organ and Tissue Transplantation (ABTO) and of a universal public health program (Unified Health System). Since then, many efforts have been made to develop the Brazilian kidney transplant program, such as detailed logistics for organ allocation, adequate reimbursements, and regular revisions in regulatory and legislative policies[3,4].

The Brazilian transplant program reimbursement and organization promoted it as the largest public kidney transplant program worldwide, with righteous organ allocation logistics based on HLA compatibility and no cultural or social biases. This scenario allowed more than 95% of the transplants to be performed by the public system in Brasil, including outpatient and hospital care, as well as free of charge provision of every medication used in the long transplant follow-up[5]. The other 5% of transplants are performed in private institutions, which are also regulated and monitored by the government.

The national transplant system coordinates organ procurement/allocation and regulates transplant centers. Organ procurement organizations and hospital commissions are strategically spread throughout the national territory, although wide regional disparities still exist in Brasil[4]. The allocation system is primarily based on HLA compatibility within a regional waiting list. Brazilian legislation allows only brain-death donation after family consent for a deceased-donor kidney transplant; donation after circulatory death is still prohibited. For living donation, parents and spouses are allowed, and unrelated donors need ethical and judicial authorization[3].

The impact of transplant legislation and investments was remarkable soon after the Unified Health System foundation in 1988. In the first period of the Brazilian kidney transplant program (1964-1978), 6,808 kidney transplants were performed, and within the following period of just five years (1989-1993), 6,578 patients received a kidney allograft, reaching 28,629 in the last period (2014-2018), with an accumulated number of 107,989 kidney transplants performed in Brasil. The use of kidneys from deceased donors was uncommon in the first 25 years (around 20%), but the increase of effective deceased donors and tighter regulation for living donors helped deceased-donor grafts be responsible for up to 79.2% of transplants (Table 1). With 5,920 kidney transplants performed in 2018, the Brazilian transplant program is considered the world's second-largest, behind only the US, with more

TABLE 1. HISTORICAL NUMBER OF LIVING- AND DECEASED-DONOR KIDNEY TRANSPLANTS IN BRASIL (1964-2018)

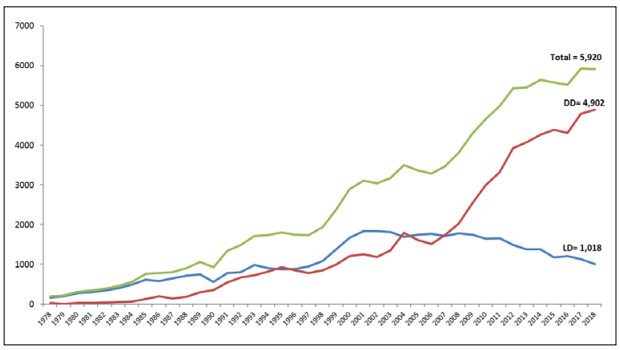
Period	LD, n (%)	DD, n (%)	Total	Accumulated
1964-1978	913 (78.1%)	256 (21.9%)	-	1,169
1979-1983	1,569 (88.6%)	202 (11.4%)	1,771	2,940
1984-1988	3,095 (80.0%)	773 (20.0%)	3,868	6,808
1989-1993	3,922 (59.6%)	2,656 (40.4%)	6,578	13,386
1994-1998	4,742 (52.6%)	4,271 (47.4%)	9,013	22,399
1999-2003	8,600 (58.7%)	6,037 (41.3%)	14,637	37,036
2004-2008	8,744 (50.0%)	8,728 (50.0%)	17,472	54,508
2009-2013	7,960 (32.0%)	16,892 (68.0%)	24,852	79,360
2014-2018	5,947 (20.8%)	22,682 (79.2%)	28,629	107,989

LD: living donor; DD: deceased donor; n: number.

than 21,000 kidney transplants performed annually, most of them with private funding[6]. The success of the Brazilian transplant program is a result of many factors, but efforts were focused on the use of deceased donor grafts in the last years (Figure 1). Moreover, both patient and graft survival have also been steadily improving as a result of a consolidated national program, improved surgical technique, advances in immunosuppressive therapy, and transplant center efforts. According to the Brazilian Transplant Registry, patient survival is 97% and 93% at 1 and 5 years, living-donor graft survival is 93% and 85%, and deceased-donor graft survival is 84% and 72%, respectively[6].

Despite the satisfactory results achieved, Brasil has a great challenge to face regarding its regional and economic disparities. Brasil has 131 active centers distributed in 22 of its 27 states. However, the Southern region reaches 35.9 pmp, not far from Spain with 46.1 pmp, whereas the Northern region has the lowest performance, with 3.6 pmp. Some Northern states do not have an active transplant program, which forces patients living in those states to travel long distances to access the transplant program. The national estimated need for kidney transplantation is still at least two times higher than its annual performance (60 pmp versus 28.5 pmp performed in 2018)[6]. To improve results, benefits of transplantation are widespread among the population, as well as the safety and suitability of the Brazilian program, in which there is no organ trafficking. Strategic measures in health professional training, adequate reimbursements of transplant centers, and clinical research funding are essential to continue expanding the number and quality of transplants in Brasil.

FIGURE 1. EVOLUTION OF KIDNEY TRANSPLANTS IN BRASIL (1978-2018)



LD: living donor; DD: deceased donor.

Contributions

RDF, JOMP, HTSJ (Writing and proofreading)

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Systemic Treatment and Surgery versus Systemic Treatment Alone for Metastatic Breast Cancer

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

INTRODUCTION

Breast cancer is the leading cause of cancer-related mortality among women worldwide[1]. In Brasil, and according to the National Institute of Cancer (INCA), breast cancer is also the type of cancer that mostly affects women in the country (except for non-melanoma skin cancer). For 2019, there were an estimated 59,700 new cases, which represents an incidence rate of 51.29 cases per 100,000 women[2].

From all new cases of breast cancer diagnosed worldwide every year, approximately 60% to 65% are hormone receptor (HR) positive, 20% to 25% are human epidermal growth factor receptor 2 (HER2) positive, and 15% to 18% are triple-negative (estrogen receptor-negative, progesterone receptor-negative, HER2-negative). The expression of these biological markers is correlated with the prognosis and response to treatment and, therefore, plays an important role in treatment decisions[3].

The prognosis of a patient is determined by the anatomic extension and pathobiological characteristics of their cancer, established during the staging[4]. As for metastatic breast cancer, in its initial presentation, the mean survival remains at around 18 to 24 months, and this variation may be extended to many years. This group of patients is usually treated with palliative intent; therefore, surgery is performed for the relief of symptoms. However, this approach was defined before modern advances in systemic treatments and supportive care[5,6].

Mammography screening and other exams with improved technologies have resulted in fewer patients with inoperable presentations, and the introduction of new systemic treatments and targeted therapies, in particular, has brought a significant improvement in survival among patients with metastatic breast cancer over the past decade[5]. Having said this, it is important

to emphasize that the role of surgery in the context of metastatic breast cancer, in its initial presentation, remains controversial[5,6].

Some researchers have postulated that the physiological stress from surgery under general anesthesia promotes metastatic proliferation. In addition, the primary tumor was thought to inhibit angiogenesis in metastatic lesions. However, these theories against the surgical resection of the primary tumor in the context of metastatic breast cancer were based on studies with animals, without translational clinical parameters to determine to what extent they affected survival[6,7].

Currently, the common practice is still to reserve the surgical resection of the primary metastatic tumor to patients with bleeding, ulceration, or resistant pain. In this context, the evidence of survival benefits from surgery of the primary tumor has been conflicting[8,9,10,11].

OBJECTIVE

The objective of this assessment is to identify the benefits and harms of systemic therapy with surgery in the treatment of patients with metastatic breast cancer, compared with systemic therapy alone.

METHODS

The clinical question is: What is the impact of systemic therapy with surgery in the treatment of patients with metastatic breast cancer on overall mortality outcomes (death from any cause) and quality of life, compared to systemic therapy alone?

The eligibility criteria for the studies are:

- Adult patients with metastatic breast cancer;
- Treatment by systemic therapy with surgery compared with systemic therapy alone;
- Outcomes - death (any cause); recurrence and quality of life;
- Excluded intermediate outcomes;
- Randomized clinical trial;
- No time or language restrictions;
- Full text available for access.

The search for evidence will be conducted on the virtual database Medline using the following search strategy - (Breast Neoplasm OR Breast Neoplasms OR Breast Tumor OR Breast Tumors OR Breast Cancer OR Breast Carcinoma OR Breast Carcinomas) AND (stage IV OR metastatic) AND (primary surgery OR Primary Tumor Resection OR Primary Tumor Surgery OR primary site treatment OR surgical resection) AND Random*; and on CENTRAL / Cochrane with the search strategy - (breast cancer) AND (stage IV OR metastatic) AND (primary surgery OR surgery OR resection). The search on these databases was performed by April 2020, along with a systematic review as recommended by the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISM)[12].

We will extract the following data from the studies: name of the author and year of publication, study population, intervention and comparison methods, the absolute number of deaths and adverse events (if any), variations in the quality of life between the groups, and follow-up time.

Randomized clinical trials will have their risk of biases analyzed according to the following criteria: randomization, blinded allocation, double-blinding, losses, prognostic characteristics, presence of relevant outcome, time for the outcome, the method for outcome measurement, sample size calculation, early interruption, presence of other biases.

The results will be expressed by the difference of the mean (MD) or SMD for continuous results. We will use hazard ratio (HR) for the results of time for the event and difference in risk for dichotomous outcomes. The confidence level adopted was 95%.

The results of the studies included will be meta-analyzed by RevMan 5.3[13], and HR will be the final measure used to support the synthesis of evidence that will answer the clinical question (survival analysis) of this review. We will use the inverse variance method to estimate the size of the combined effect for the results and the random-effects model by default, since we expect clinical or methodological heterogeneity, or both, in the studies included.

The heterogeneity was inspected graphically using forest plots that exhibit the effects of individual studies with confidence intervals (CIs) of 95%. When appropriate, we will evaluate the heterogeneity among the studies using the Chi^2 statistics (considering a value of $p < 0.10$ as significant). We will also use I^2 statistics as an approximate guide to interpret the magnitude of heterogeneity: a value of R^2 between 30% and 60% is indicative of moderate heterogeneity, while values greater than 50% are considered substantial heterogeneity[14].

The quality of evidence will be graded as high, moderate, low, or very low using the GRADE (*Grades*

of Recommendation, Assessment, Development, and Evaluation)[15] instrument, taking into account the risk of bias, the presence of inconsistency, vagueness or indirect evidence in the meta-analysis of the outcomes of death and adverse events, and the presence of publication bias.

RESULTS

The search for evidence retrieved 1044 papers, of which 3 (RCTs) were selected based on their title and abstract[16 -18] on systemic treatment with surgery in patients with metastatic breast cancer, in comparison with systemic treatment alone. The 3 studies that met the eligibility criteria were then were accessed for analysis of their full text. The 3 studies were selected to support this assessment; the grounds for exclusion are available in the references, Figure 1 under ANNEXES.

The population included comprises 714 metastatic breast cancer patients who underwent breast surgery associated to systemic treatment (N = 356) compared with systemic therapy alone (N = 358), and followed-up to measure the outcomes of death, recurrence, and quality of life for an average of 23 to 40 months (Table 1).

Regarding the risks of bias in the 3 studies included[16-18], all had differences in prognostic characteristics between the intervention and control groups that could interfere with the results obtained (e.g. number of women aged less than 55 years, SR-positive and HER2-negative tumors, cT3, single bone metastases), and one study presented early interruption (5 years) due to poor recruitment; thus, the overall risk of the studies can be considered moderate (Table 2).

TABLE 1. CHARACTERISTICS OF THE STUDIES INCLUDED

Study	Population (N)	Intervention (N)	Comparison (N)	Outcomes	Time (median, months)
Badwe, 2015	(N=350) Women (mean age of 48 years) in India, with *de novo* metastatic breast cancer stage IV and response to initial chemotherapy (96%) or who received initial endocrine therapy (4%). Only 9 of 107 HER2+ received targeted therapy.	(N = 173) Mastectomy or conservative breast surgery with complete dissection of the axillary lymph nodes (locoregional treatment), followed by standard postoperative treatment of RT in the thoracic wall or remaining breast. Pre-menopausal women with menstruation after chemotherapy had ovarian ablation (not received by 22% in this group and 33% in the control group). Hormone receptors positive tumors received endocrine therapy after locoregional treatment or initial chemotherapy until their progression.	(N = 177) ST without locoregional treatment	Overall survival (OS) Local progression-free survival (local PFS) Distant progression-free survival (distant PFS)	Mean of 23 months
Soran, 2016	(N=274) Women in Turkey with *de novo* breast cancer stage IV previously untreated. Targeted therapy for all HER2+	(N = 138) Mastectomy or conservative breast surgery with tumor-free margins. SLN biopsy for patients with clinically negative lymph nodes. Axillary drainage levels I and II required for SLN-positive patients, patients with clinically positive lymph nodes, and SNL not identified during surgery. All women treated with conservative breast surgery underwent whole-breast RT 3-6 months after surgery; RT for the breast, local lymph node chains, chest wall, and metastatic site by choice of the doctor.	(N = 136) ST without locoregional treatment	OS Local PFS	Mean of 40 months
Fitzal, 2018 Prematurely interrupted after 5 years due to poor recruitment.	(N=90) Women in Austria with breast cancer stage IV previously untreated. Targeted therapy applied in HER2 +	(N=45) Lumpectomy or mastectomy without tumors in the margins, in addition to axillary dissection level I and II or sentinel lymph node biopsy. RT performed at the discretion of the researcher, initiated within 6 months after the surgery, but not concurrent to chemotherapy ST included chemotherapy, anti-HER2 therapy, or anti-hormonal therapy, at the discretion of the investigator. T3 cancer in 22%.	(N = 45) ST In this group without surgery, local surgery was performed in cases of local progression, uncontrolled bleeding, or wound problems. T3 cancer in 7%.	OS Local PFS Distant PFS Quality of life	Median 37.5 months

ST = systemic therapy, SLN = Sentinel lymph node, RT = Radiotherapy

TABLE 2. DESCRIPTION OF THE BIASES OF THE STUDIES INCLUDED. METASTATIC CANCER THERAPY - ST + SURGERY VERSUS TS RISK OF BIASES OF THE STUDIES INCLUDED

Study	Random	Allocation Blinded	Double Blind	Losses	Character-istics (prognostic)	Outcomes	Sample calcula-tion	ITT	Early termina-tion
Badwe, 2015			NA						
Soran, 2016			NA						
Fitzal, 2018			NA						

Description of the biases of the studies included (orange = presence; blue = absence; Yellow = unclear risk of bias) ITT = analysis by intention to treat. NA = not applicable

All studies assessed the outcomes of overall survival and local progression-free survival, as well as carried out an analysis of subgroups [e.g., site and number of metastases, the status of the estrogen or progesterone receptor and status of the human epidermal growth factor receptor 2 (HER2)].

Overall Survival

Three RCTs[16-18] compared locoregional therapy with ST (N = 356) versus ST alone (N = 358) and found no significant difference regarding the OS (HR: 0.93; 95% CI 0.63 to 1.40; R^2 = 73%; evidence of very low quality, demoted due to limitations of the studies, inconsistency, and inaccuracy, Figure 1 and Tables 3 and 4.

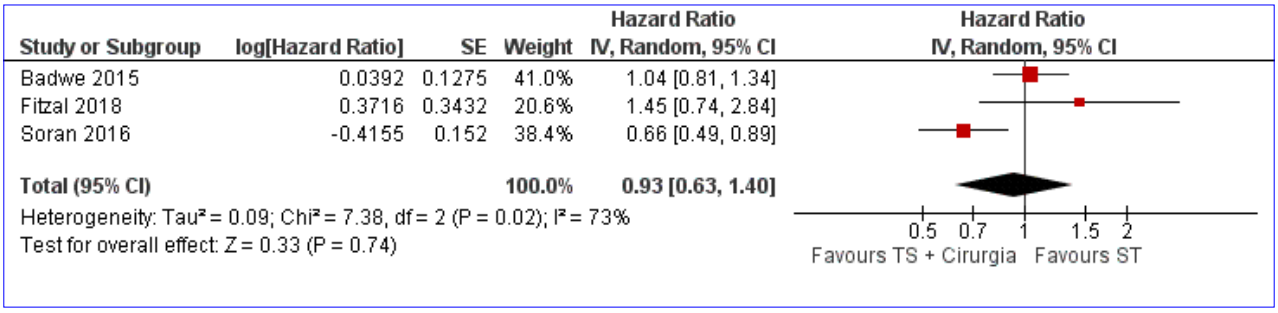


FIGURE 1. COMPARISON FOREST PLOT: 1 SYSTEMIC THERAPY WITH SURGERY VERSUS SYSTEMIC THERAPY, OUTCOME: 1.1 OVERALL SURVIVAL.

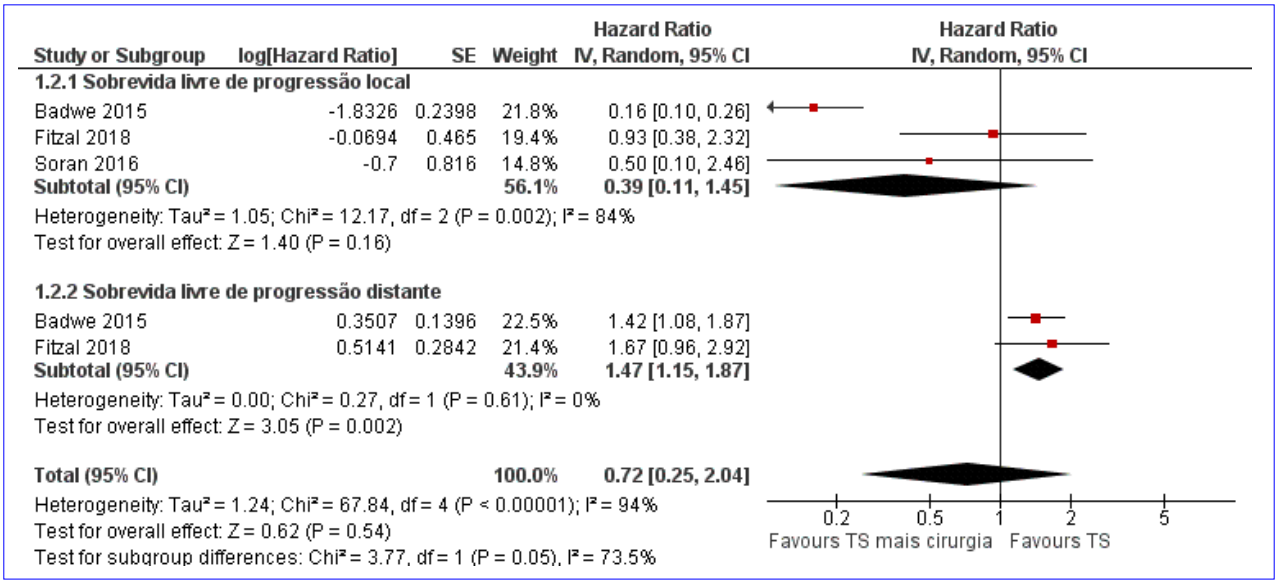


FIGURE 2. COMPARISON FOREST PLOT: 1 SYSTEMIC THERAPY WITH SURGERY VERSUS SYSTEMIC THERAPY, OUTCOME: 1.2 PROGRESSION-FREE SURVIVAL.

TABLE 3. SUMMARY OF THE RESULTS FOR THE OUTCOMES OF OS, PFS, AND SUBGROUP ANALYSIS

Outcome or Subgroup	Studies	Statistical Method	Estimated Effect
1.1 Overall survival	3	Hazard Ratio (IV, Random, 95% CI)	0.93 [0.63, 1.40]
1.2 Progression-free survival	3	Hazard Ratio (IV, Random, 95% CI)	0.72 [0.25, 2.04]
1.2.1 Local progression-free survival	3	Hazard Ratio (IV, Random, 95% CI)	0.39 [0.11, 1.45]
1.3 Overall survival - HER2 status	3	Hazard Ratio (IV, Random, 95% CI)	0.94 [0.70, 1.24]
1.3.1 HER2-positive	3	Hazard Ratio (IV, Random, 95% CI)	0.91 [0.64, 1.30]
1.3.2 HER2-negative	3	Hazard Ratio (IV, Random, 95% CI)	0.99 [0.60, 1.62]
1.4 Overall survival - SR Status	3	Hazard Ratio (IV, Random, 95% CI)	0.97 [0.73, 1.29]
1.4.1 SR-positive	3	Hazard Ratio (IV, Random, 95% CI)	1.01 [0.59, 1.72]
1.4.2 SR-negative	3	Hazard Ratio (IV, Random, 95% CI)	0.98 [0.72, 1.33]
1.5 Overall Survival - Bone metastasis alone	3	Hazard Ratio (IV, Random, 95% CI)	0.97 [0.58, 1.62]
1.6 Overall Survival - Number of metastases	1	Hazard Ratio (IV, Random, 95% CI)	1.02 [0.79, 1.32]
1.6.1 ≤3	1	Hazard Ratio (IV, Random, 95% CI)	1.16 [0.69, 1.95]
1.6.2 >3	1	Hazard Ratio (IV, Random, 95% CI)	0.98 [0.73, 1.32]
1.7 Overall Survival - Molecular subtypes	1	Hazard Ratio (IV, Random, 95% CI)	1.06 [0.08, 13.72]
1.7.1 Luminal A	1	Hazard Ratio (IV, Random, 95% CI)	3.62 [1.25, 10.50]
1.7.2 Luminal B	1	Hazard Ratio (IV, Random, 95% CI)	0.26 [0.05, 1.39]

TABLE 4. QUALITY OF EVIDENCE FOR THE OUTCOMES OF OVERALL AND PROGRESSION-FREE SURVIVAL (GRADE)

Outcomes	Relative Effect (95% CI)	Nº of participants (studies)	Certainty of the evidence (GRADE)	Comments
Overall survival (OS) up to 2 years (23 - 40 months)	HR 0.93 (0.63 to 1.40)	714 (3 RCTs)	⊕○○○ VERY LOW [a,b,c]	none
Local progression-free survival up to 2 years (23-40 months)	HR 0.39 (0.11 to 1.45)	714 (3 RCTs)	⊕○○○ VERY LOW [a,b,d]	none
Distant progression-free survival up to 2 years (23- 40 months)	HR 1.47 (1.15 to 1.87)	440 (2 RCTs)	⊕⊕⊕○ MODERATE [a]	none

CI: confidence interval; HR: hazard ratio; RCT: randomized controlled trial.

GRADE Working Group grades of evidence

High quality: We are very confident that the true effect lies close to that of the estimate of the effect.

Moderate quality: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low quality: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low quality: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Progression-Free Survival

These three RCTs[16-18] also evaluated local progression-free survival (PFS), including a total of 714 patients, and found no significant difference in the comparison between locoregional therapy versus ST alone (HR: 0.39; 95% CI 0.11 to 1.45; R^2 = 84%; evidence of very low quality, demoted due to limitations of the studies, inconsistency, and inaccuracy Figure 2 and Tables 3 and 4.

Two studies[16,18] including a total of 440 patients evaluated distant progression-free survival, and the group that received breast surgery with systemic treatment had a shorter time for distant PFS than the group that received the systemic treatment alone (HR: 1.47; 95% CI 1.15 to 1.87; R^2 = 0%; evidence of Moderate quality, demoted due to limitations of the studies, inconsistency, and inaccuracy, Figure 2 and Tables 3 and 4.

Overall survival - HER2 status (Subgroup Analysis)

There was no difference in overall survival between the subgroups of HER2 positive and negative (Chi^2 = 0.07, df = 1 (P = 0.79), R^2 = 0%), and the results for HER2 positive and negative were consistent with the primary analysis: HER2- positive HR 0.91, 95% CI 0.64 to 1.30, I^2 0%, 3 studies, 211 patients; HER2- negative HR 0.99, 95% CI 0.60 to 1.62, I^2 75%, 3 studies, 490 patients; Figure 3.

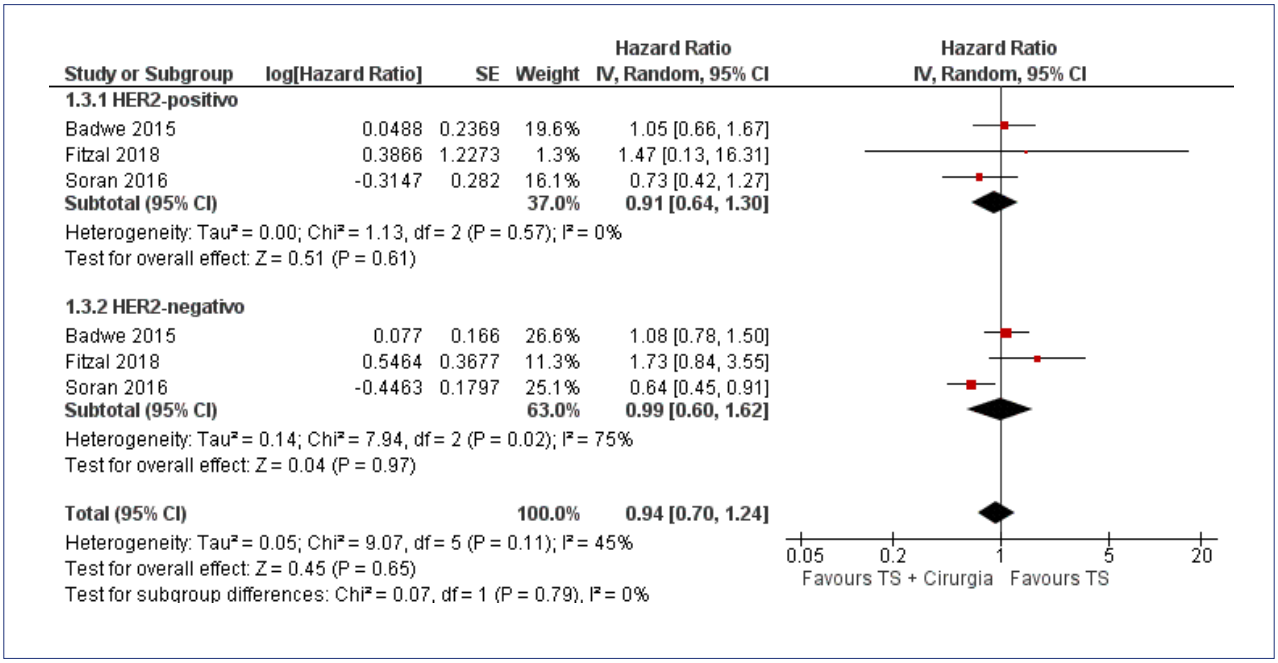


FIGURE 3. COMPARISON FOREST PLOT: 1 SYSTEMIC THERAPY WITH SURGERY VERSUS SYSTEMIC THERAPY, OUTCOME: 1.3 OVERALL SURVIVAL - HER2 STATUS.

Overall survival - SR status (Subgroup Analysis)

There was no difference in overall survival between the SR-positive and negative subgroups (Chi^2 = 0.01, df = 1 (P = 0.94), I^2 = 0%), and the results for SR-positive and negative were consistent with the primary analysis: SR-positive HR 1.01, 95% CI 0.59 to 1.72, I^2 76%, 3 studies, 496 patients; SR- negative HR 0.98, 95% CI 0.72 to 1.33, I^2 0%, 3 studies, 233 patients; Figure 4.

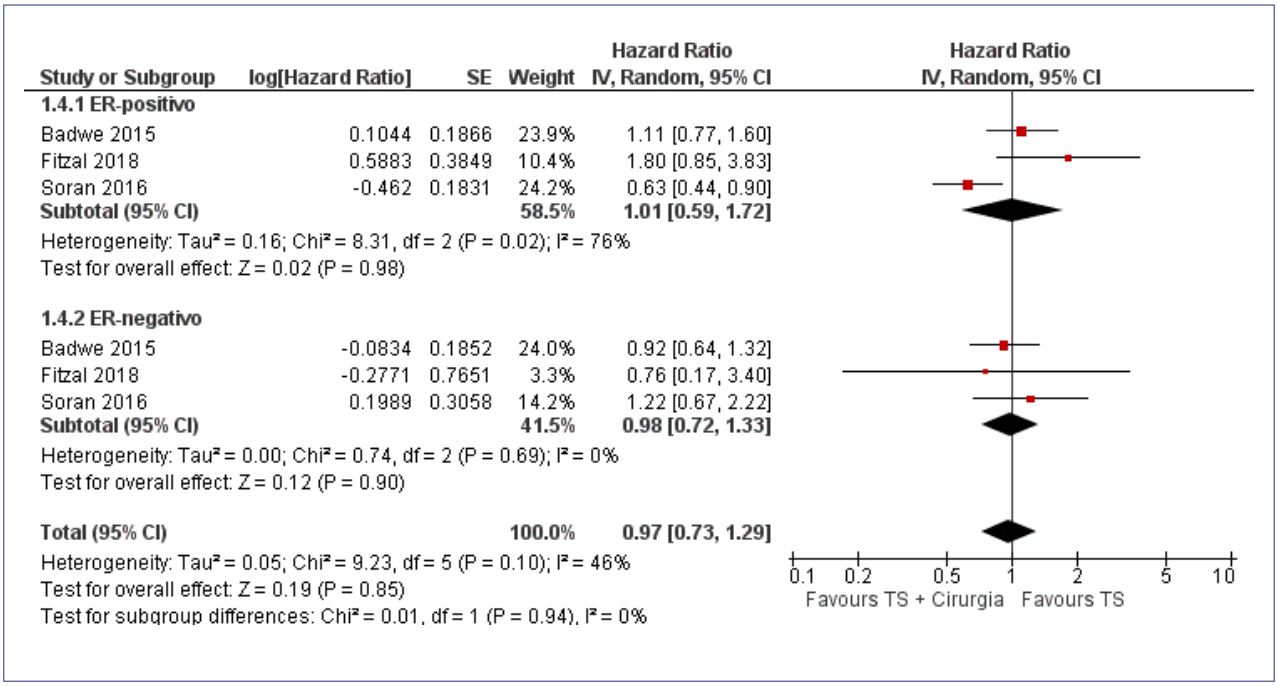


FIGURE 4. COMPARISON FOREST PLOT: 1 SYSTEMIC THERAPY WITH SURGERY VERSUS SYSTEMIC THERAPY, OUTCOME: 1.4 OVERALL SURVIVAL - SR STATUS.

Overall survival - Bone metastasis alone (Subgroup Analysis)

For the subgroup of women with bone metastasis alone, there was no difference in overall survival (OS) when analyzing those who underwent surgery for the primary breast tumor or not: HR 0.97 (CI 95% 0.58 to 1.62; 3 studies; 260 women; I^2 = 51%), Figure 5

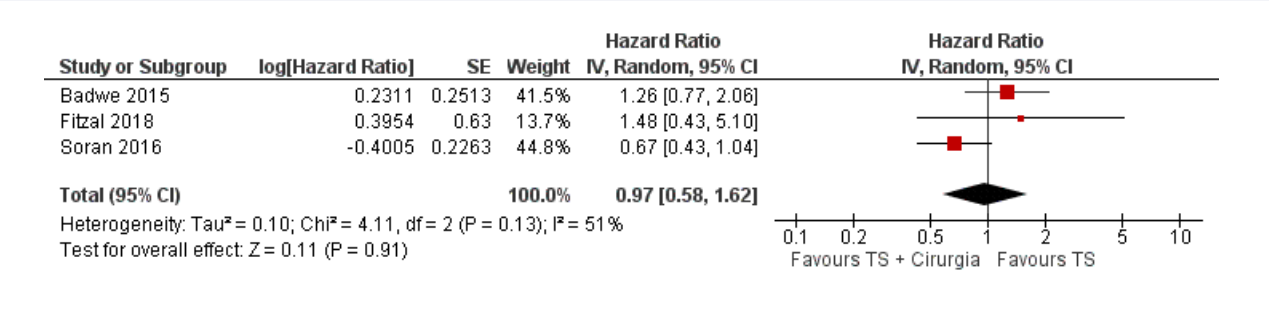


FIGURE 5. COMPARISON FOREST PLOT: 1 SYSTEMIC THERAPY WITH SURGERY VERSUS SYSTEMIC THERAPY, OUTCOME: 1.5 OVERALL SURVIVAL - BONE METASTASIS ALONE.

Overall Survival - Number of metastases (Subgroup Analysis)

Only one RCT[16] assessed the possible relationship between the number of metastases (≤3 and >3) and overall survival. There was no difference in overall survival between the ≤ 3 and > 3 metastases subgroups (Chi^2 = 0.07, df = 1 (P = 0.79)), and the results for ≤ 3 and > 3 metastases were consistent with the primary analysis: ≤3 HR 1.16, 95% CI 0.69 to 1.95, 89 patients; >3 HR 0.98, 95% CI 0.73 to 1.32, 261 patients; Figure 6.

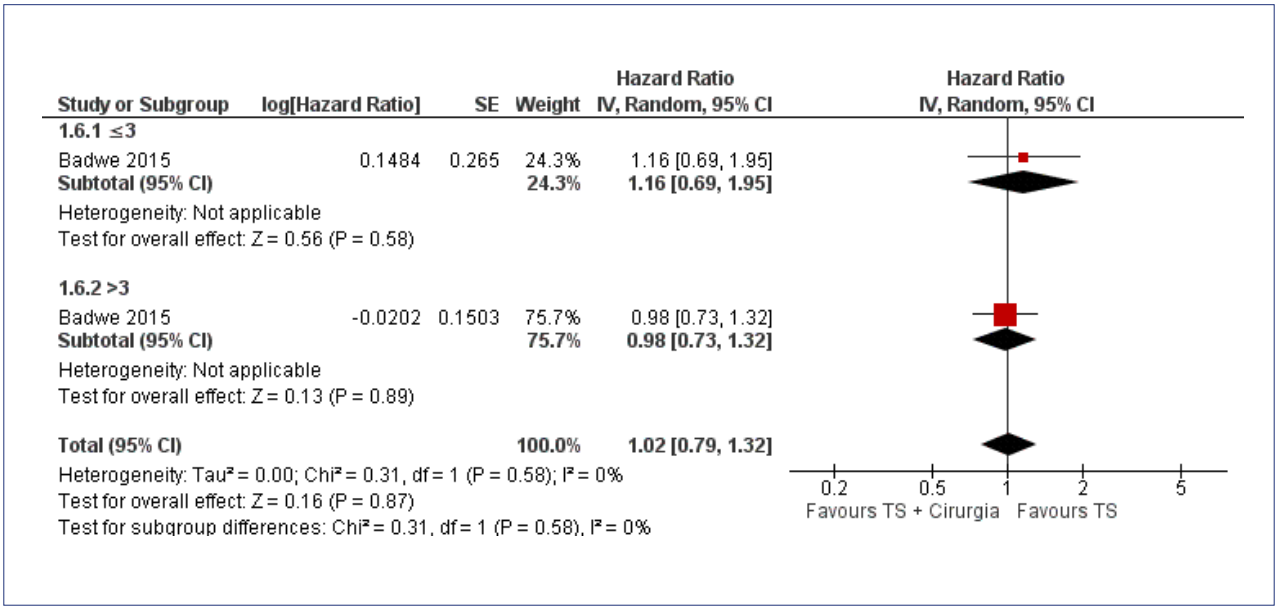


FIGURE 6. COMPARISON FOREST PLOT: 1 SYSTEMIC THERAPY WITH SURGERY VERSUS SYSTEMIC THERAPY, OUTCOME: 1.6 OVERALL SURVIVAL - NUMBER OF METASTASES.

Overall survival - Molecular subtypes (Subgroup Analysis)

One study[18] evaluated the overall survival in the luminal A and B molecular subgroups comparing surgery with ST versus systemic therapy alone. Patients with luminal A breast cancer showed a worse performance after the initial surgery (HR 3.62, 95% CI 1.25 to 10.50, 46 patients) and luminal B ones showed no significant difference in favor of surgery (HR 0.26, 95% CI 0.05 to 1.39, 12 patients), Figure 7.

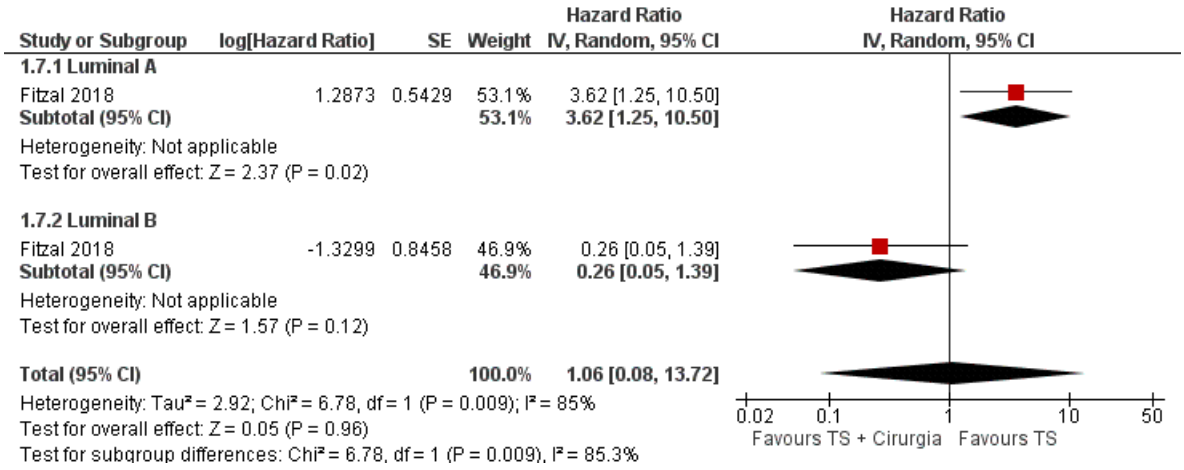


FIGURE 7. COMPARISON FOREST PLOT: 1 SYSTEMIC THERAPY WITH SURGERY VERSUS SYSTEMIC THERAPY, OUTCOME: 1.7 OVERALL SURVIVAL - MOLECULAR SUBTYPES.

QUALITY OF LIFE

One study[18] aimed to evaluate if surgery leads to an improvement of quality of life (QOL) when compared to systemic therapy alone, based on the QOL questionnaire by the European Organization for Research and Treatment of Cancer (EORTC), i.e., the QoL questionnaire (QLQ C30, and the EORTC QLQ-BC23, a questionnaire for breast cancer patients.

Thirty-four (76%) patients in the surgical branch and 41 (91%) in the non-surgical branch were included in the QOL analyses.

EORTC-QLQ C30 - With time (up to 24 months of follow-up), the patients in both branches experienced clinically relevant and statistically significant improvements on the Global Health Status scale (p = 0.003), as well as in the emotional functioning scale. There was statistically significant worsening in the scale of dyspnea symptoms (p = 0.025) in both branches, but without clinical relevance.

EORTC-QLQ BR23 - In both branches, there was a statistically significant and clinically relevant improvement over time on the scale of future perspective (p = 0.009) and on the scale of breast symptoms (p = 0.006). There was a worsening of symptoms in the scales of body image (p = 0.017), symptoms of systemic therapy (p <0.001), and hair loss (p <0.001), but these differences were not clinically relevant in both branches.

Therefore, primary tumor surgery does not improve or alter the QOL of patients with *de novo* breast cancer stage IV.

Explanations

a. Different prognostic characteristics among the studies, which can interfere with the results.

Fitzal, 2018 prematurely interrupted after 5 years due to poor recruitment.

b. Statistical heterogeneity.

c. 95% CI (0.63, 1.40), including the null effect.

d. 95% CI (0.11 to 1.45), including the null effect.

SYNTHESIS OF EVIDENCE GRADE QUALITY OF EVIDENCE

In women with metastatic breast cancer, breast surgery (mastectomy: removing the whole breast, including the nipple and areola, or Lumpectomy: removing the tumor and breast tissue around it, preserving the nipple and the areola) combined with medical treatment (such as chemotherapy and hormone therapy) compared with medical treatment alone:

- Does not improve the overall survival. The quality of the evidence is very low.
- Does not improve local progression-free survival. The quality of the evidence is very low.
- Abbreviates distant progression-free survival. Moderate quality of evidence.
- Does not improve or alter the quality of life. The quality of the evidence is very low.

DISCUSSION

Metastatic breast cancer continues to be an incurable disease despite the improvement in survival

in recent decades, attributed mostly to advances in systemic treatment options, while the role of primary tumor resection (PTR) in this scenario remains controversial.

Based on evidence from three randomized clinical trials[16-18], it was not possible to draw any definitive conclusions about the benefits and risks of breast surgery associated with systemic treatment for women diagnosed with metastatic breast cancer.

A RCT[16] conducted at the Tata Memorial Hospital, in India, with 350 patients randomized to surgery versus no surgery after chemotherapy found the median overall survival was 19.2 months in the surgery group versus 20.5 months in the group without surgery. However, the patients did not receive systemic therapies according to the subtypes of breast cancer. Therapies aimed at anti-HER2 were used in only 9% of patients with the HER2-positive subtype, and very few patients with SR-positive tumors received hormone therapy.

The MF07-01 study from Turkey[17] evaluated the prognostic effect of breast surgery as the primary treatment and observed that breast surgery can prolong the OS. However, it was not possible to confirm that surgery provides an improvement of 18% in the survival rate after three years, based on the preplanned analysis.

The phase-III randomized study ABCSG-28 (Austria)[18] is the third to prospectively study the effectiveness of breast surgery in patients with metastases. It evaluated breast surgery for patients with *de novo* breast cancer stage IV, without a history of systemic therapy. The patients were allocated to surgery (standard conservative breast surgery or mastectomy, including axillary staging) with systemic therapy or systemic therapy without surgery. The patients were stratified, according to their classification, receptor status, HER2 status, site of metastases (visceral metastases versus bone alone), and first-line therapy planned. As a systemic therapy, chemotherapy, anti-HER2 therapy, or anti-hormonal therapy were administered according to local standards, with schemes including modern and effective drugs. The primary outcome was the OS, and no benefit from PTR was demonstrated. In addition, there was a worsening of the results of patients with distant metastases. Due to poor recruitment, this study was prematurely interrupted after 5 years, when only 90 patients were enrolled, 45 in each branch.

A meta-analysis of these three RCTs[16-18] showed no benefit regarding an improvement in OS, as well as in local progression-free survival, with the resection of the primary tumor (the quality of the evidence was too low); however, it indicates a shorter progression-free survival with surgery (the quality of the evidence was moderate)

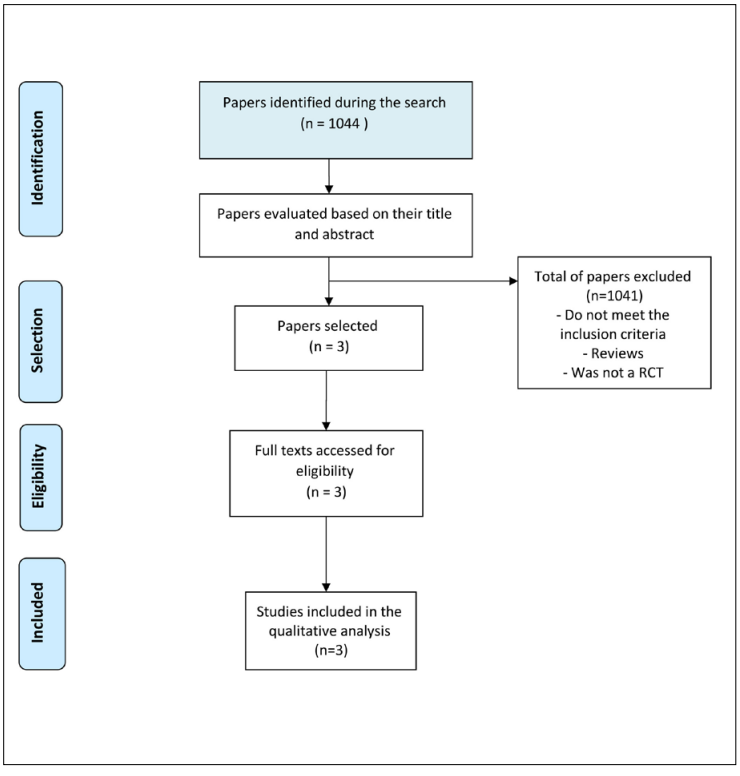
We did not consider the results of subgroup analyses of the studies in this review conclusive due to the risk of false-positive results, avoiding the following question: is there indeed a significant difference in the treatment effect or it is merely a random occurrence (considering the absence of prior sample size definition and subsequent statistical power for this difference)?

STUDIES IN PROGRESS

NCT01242800. NCT00941759. NCT01242800 (ECOG2108). UMIN000005586 (JCOG1017)

ANNEXES

FLOWCHART .THE SELECTION OF RETRIEVED FROM THE VIRTUAL DATABASES OF SCIENTIFIC INFORMATION IS DETAILED IN THE FLOWCHART BELOW:



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Is the use of microwave ablation more effective and/or safe that radiofrequency ablation in the treatment of hepatocellular carcinoma?

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QUESTION: Is the treatment of hepatocellular carcinoma more effective and/or safe with the use of microwave ablation than with radiofrequency ablation?1

Answer: There are no differences between the use of microwave ablation in comparison with radiofrequency ablation in the treatment of hepatocellular carcinoma lesions ≤ 5.0 cm regarding the outcomes: local recurrence, mortality, complications, and progression of the disease. This means that it is not known whether the efficacy or safety is greater or SMALLER than those of the treatment already in use (radiofrequency). The quality of the evidence is very low.

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Putting the pieces together: Castleman disease in a patient with HIV

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KEYWORDS: *Castleman disease. HIV. Sarcoma, Kaposi.*

INTRODUCTION

Castleman disease (CD) is a nonclonal lymphoproliferative disorder that can present as localized lymphadenopathy or disseminated disease. The simultaneous occurrence of Kaposi Sarcoma (KS) and multicentric CD was first described by *Lachant* in 1985[1,2]. Human herpesvirus 8 (HHV-8) infection plays a central role in the pathogenesis of its disseminated form and in Human immunodeficiency virus (HIV) associated CD[3].

We present the case of a 43-year-old man with HIV stage C3 infection with mucocutaneous Kaposi Sarcoma and multicentric Castleman Disease.

CASE DESCRIPTION

A 43-year-old man with HIV stage C3 infection (poor compliance with anti-retroviral therapy) and mucocutaneous Kaposi Sarcoma (undergoing treatment with liposomal B Doxorubicin every other week) was admitted to the infectious diseases ward with a six-day history of asthenia, high fever (39.5ºC), diarrhea, dyspnea, and cough. Amoxicillin/clavulanate and Ciprofloxacin was prescribed four days earlier without clinical improvement. On admission, he was hypotensive and febrile, with no remarkable finds on physical examination other than the cutaneous lesions related to his Kaposi Sarcoma. The blood work showed Hb 12.1g/dL (normal range (NR) 13-16g/dL), platelets 42000/mcL (NR 150-450000/mcL), leukocytes 5000/mcL (NR 10-15000/mcL), C-reactive protein 30.8mg/dL (NR < 0.5mg/dL), creatinine 1.98mg/dL (NR 0,7 - 1.2mg/dL), urea 79mg/dL (NR 13 – 43 mg/dL), AST 69U/L (NR < 74U/L), ALT 101U/L (NR < 30U/L), FA 177U/L (NR 35 – 105U/L), GGT 95U/L (NR 6 – 42U/L). Cytomegalovirus viral load was negative, as was Cryptococcus neoformans antigen and fecal examination (including Clostridium difficile antigen). The urine culture grew Enterococcus faecalis, and he was started on

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Piperacillin/tazobactam admitting urinary sepsis. After one week, the patient was transferred to the Intermediate-Care Unit due to clinical degradation: asthenia, dyspnea, and blood loss through the rectum were noticed. On examination, the patient was jaundiced and with peripheral saturation of 88% on 5L/min oxygen mask, with no pain, and mobile axillary and inguinal adenopathies were noticed as well as tender hepatosplenomegaly. The blood work was redone and it showed anemia (Hb 7.1g/dL), thrombocytopenia (Platelets 33000/mcL), hyperbilirubinemia (total bilirubin 21.5mg/dL, conjugated bilirubin 16.3mg/dL) with cytocholestasis (AST 112U/L, ALT 50U/L, FA 1145U/L, GGT 493U/L) and C-reactive protein 31.5mg/dL. The arterial blood gas showed hypoxemia, and chest CT revealed bilateral pleural effusion and abdominal CT ascites and intraabdominal adenopathies. Abdominal ultrasonography showed no biliary duct dilatation. Pulmonary sepsis was admitted, and the patient was started on Meropenem and Atovaquone, which he maintained for fourteen days. A colonoscopy was done, which excluded involvement by Kaposi Sarcoma. Pneumocystis jiroveccii infection was confirmed by a positive polymerase chain reaction in the bronchoalveolar lavage product. After four days of therapy, there was significant clinical and analytical improvement. Inguinal adenopathy was biopsied, and the histology confirmed hyaline vascular Castleman disease. The patient was discharged and chemotherapy with Doxorubicin and Rituximab was planned.

DISCUSSION

First described by Benjamin Castleman in 1954, Castleman Disease describes a rare heterogeneous group of disorders that share lymph node enlargement and similar pathologic findings (abnormal vascularization, plasmacytosis, or both)[4]. Regarding the latter, three distinct subtypes can be distinguished: hyaline vascular, plasmacytic, and mixed[1].

Unicentric CD (UCD) affects one lymph node station, and patients are rather asymptomatic, with the diagnosis being incidental, or with symptoms due to compression of neurovascular or other vital structures[1,4,5]. On the other hand, *Multicentric CD* (MCD) is characterized by the involvement of more than one lymph node station, presence of systemic symptoms (fever, night sweats, asthenia, anasarca, and pleural effusion), hepatosplenomegaly and an increase of inflammation markers and acute phase proteins (elevated C-reactive protein, hypergammaglobulinemia, hypoalbuminemia). Multicentric CD comprises two subgroups: HHV-8-related MCD and idiopathic MCD (HIV and HHV-8 negative and with autoimmune associated phenomena)[4].

HHV-8 and HIV positive MCD is the most frequent form of MCD. Its incidence has increased over time since antiretroviral therapy does not prevent disease development[1,4]. Kaposi Sarcoma (another clinical entity HHV-8 and HIV related) can coexist with CD in up to 40% of the cases[4].

Interleukin-6 (IL-6) plays a central role in CD pathophysiology. Excess IL-6 in these patients induces a pro-inflammatory syndrome with severe systemic symptoms and elevation of acute phase reactants[1,5]. Anemia, typically present in MCD patients, is related to IL-6 mediated hepcidin overproduction[5].

Given its heterogeneity and nonspecific clinical presentation, diagnosis depends on a high suspicion[1]. Excisional lymph node biopsy and examination by an experienced pathologist are essential[4].

The authors report the case of an HIV patient with known KS in whom disease progression was first suspected with pulmonary and gastrointestinal involvement. After the exclusion of pulmonary and gastrointestinal involvement, and given the clinical presentation with systemic symptoms, hepatosplenomegaly, and an inflammatory syndrome, the hypothesis of MCD was considered and later confirmed by lymph node biopsy. The *Pneumocystis jiroveccii* infection was related to the patient's immunosuppression status.

CD disease treatment differs upon presentation and association with KS. The preferred management of UCD is surgical excision or local radiotherapy. MCD is treated with single or combination chemotherapy with Rituximab being used mainly in HIV patients and idiopathic forms[5].

MCD in HIV patients behaves aggressively, with poor prognosis. The median survival does not exceed 25 months and it has a high mortality rate[1]. With this case, the authors aim to raise awareness of this rare but severe entity, especially in patients with HIV.

PALAVRAS-CHAVE: *Hiperplasia do linfonodo gigante. HIV. Sarcoma de Kaposi.*

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Dobutamine-induced Takotsubo syndrome during stress echocardiogram – An unusual but potentially severe association

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KEYWORDS: *Takotsubo cardiomyopathy. Dobutamine. Echocardiography.*

INTRODUCTION

Takotsubo syndrome is a clinical condition characterized by transient left ventricular apical dysfunction, associated with electrocardiographic alterations and increased markers of myocardial necrosis, in the absence of obstructive coronary lesion. This condition represents a differential diagnosis in patients with a scenario suggestive of acute coronary syndrome. Despite its association with situations of physical or emotional stress, there is very limited literature on the association of this condition with cardiac stress testing, methods widely employed in clinical practice. In this report, we describe a case of Takotsubo syndrome documented during an examination of stress echocardiography with dobutamine.[1]

Case

A 75-year-old female patient, undergoing outpatient follow-up due to atypical chest pain, was referred for stress echocardiography with dobutamine. She reported a history of hypertension, dyslipidemia, chronic kidney disease, obesity, and hyperthyroidism and made prior use of carvedilol 25 mg/day, enalapril 5 mg/day, and simvastatin 20 mg/day.

During the resting echocardiogram, she showed preserved systolic function (56% left ventricle ejection fraction), without changes in segmental mobility. After dobutamine 20 mcg/kg/min and atropine 0.25 mg infusion, she reached a heart rate of 148 bpm (102% of the maximum expected for her age). At peak stress, she

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began to report nausea and presented hypotension associated with left ventricular systolic dysfunction (ejection fraction estimated at 30%), at the expense of apical akinesia, and hypokinesia of the other walls. An electrocardiogram showed ST-segment depression of the inferior wall, and the examination was interrupted (Figure 1). During the recovery stage, there was an improvement of symptoms with partial recovery of systolic function (40% ejection fraction), but apical hypokinesia and a discreet ST-segment elevation in the inferior wall remained.

Thus, the patient was referred to the emergency department. At admission, she presented a blood pressure of 100 x 50 mmHg, heart rate of 80 bpm, and respiratory and heart auscultation without changes. The electrocardiogram at admission in the emergency department showed no acute ischemic changes in the sinus rhythm. The markers of myocardial necrosis were positive with 1.19 ng/mL troponin I.

On the same day, the patient underwent cardiac catheterization, which showed coronary arteries without obstructive lesions, and ventriculography showing apical ballooning akinesis, with a diagnosis of the typical pattern of Takotsubo syndrome (Figure 2).

The patient was maintained on clinical therapy with carvedilol and enalapril and underwent cardiovascular

FIGURE 1

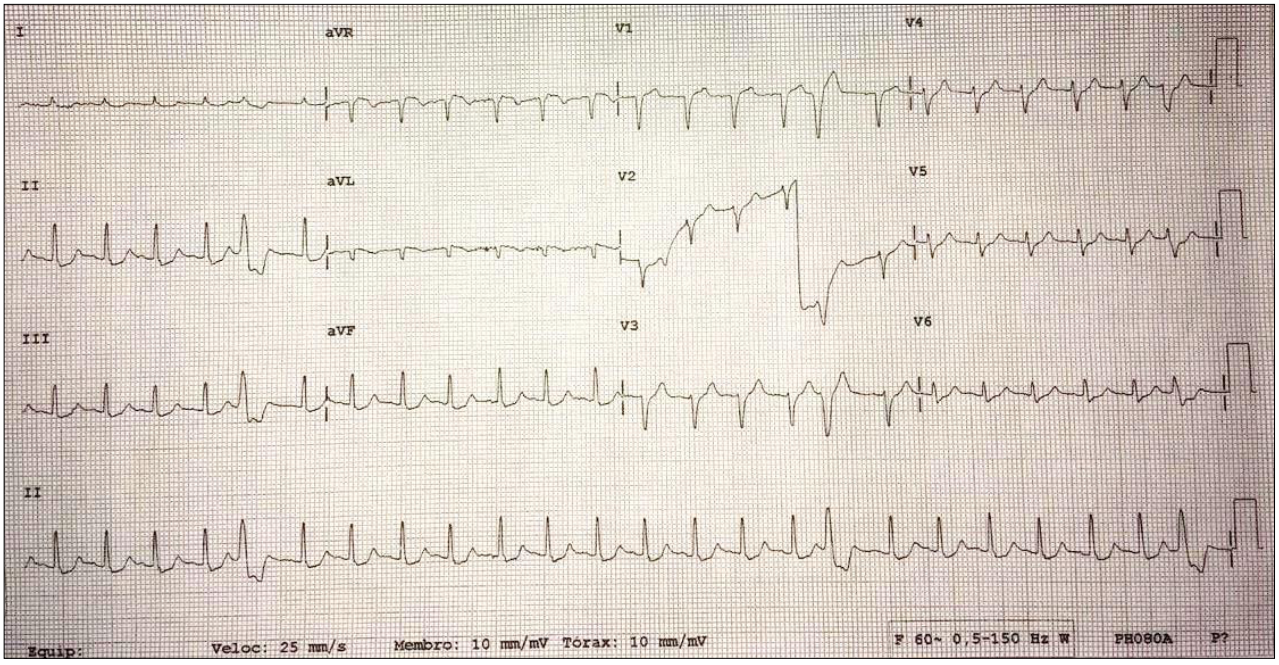
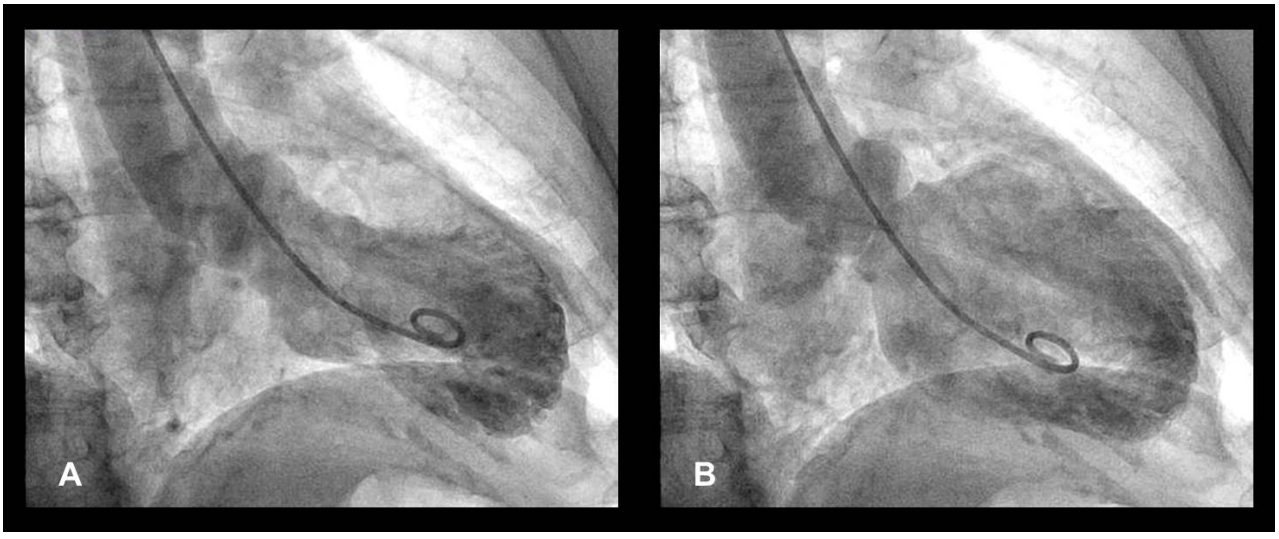


FIGURE 2



magnetic resonance, which showed a slightly reduced global systolic function of the left ventricle (46% ejection fraction) at the expense of diffuse hypokinesia, more accentuated in the apical portion. The result corroborated previous examinations, demonstrating findings compatible with Takotsubo syndrome.

The patient progressed uneventfully and was discharged after 8 days of hospitalization.

DISCUSSION

Takotsubo Syndrome - also known as "broken heart syndrome" - was described for the first time around 1990 and is currently recognized as a differential diagnosis of chest pain. It is an unusual condition. Although its exact prevalence is not well established, it is estimated that it is responsible for about 2% of all cases treated as acute myocardial infarction with ST-segment elevation.[1] This proportion rises to almost 6% when only women are studied. The estimated rate of recurrence of cases is around 1.8% per year.[2]

Its diagnosis is often challenging due to its clinical similarity with acute myocardial infarction, changes in electrocardiogram and biomarkers. Currently, cardiac catheterization with ventriculography is considered the gold-standard examination, along with the international diagnostic criteria for Takotsubo syndrome.[3]

Although its etiology is not fully known, there is evidence that it involves an intense sympathetic stimulation. Some of the proposed mechanisms by which the excess of catecholamines triggers the condition include: plaque rupture, epicardial vessel spasms, microcirculatory dysfunction, and direct toxicity in the cardiomyocytes.[2] There is still no consensus regarding the treatment of Takotsubo syndrome, in both its acute or long-term presentation. Despite this, the evolution of the Takotsubo syndrome tends to be benign, with ventricular dysfunction being solved over a period of days or weeks.[4]

However, the description of the syndrome after stress echocardiography with dobutamine is unusual.

In 2008, Silberbauer et al.[5] described one of the first cases of a 75-year-old woman who presented Takotusubo syndrome during stress echocardiography with dobutamine. The patient presented ST-segment elevation and received antithrombotic medication before cardiac catheterization. She had full recovery of ventricular function during clinical follow-up.[5] Similarly, another case reported in 2009 by Margey et al.[6], also in a woman, occurred during dobutamine infusion, and the left ventricle ejection fraction was fully recovered in 72 hours.

In Brasil, there is only one case, reported in 2009, associated with stress echocardiography in a 76-year-old patient who presented the condition during the infusion of the medication, showing an elevation of the sidewall all and taking 21 days to fully recover ventricular motility.[7]

In 2011, there was the first description of a case of Takotsubo syndrome related to the infusion of dobutamine in stress echocardiogram during the recovery stage in a 85-year-old patient. Back then, the authors called attention to the possibility of the condition happening not only at peak effort but also during the recovery from the exam.[8]

A survey of 22 cases of dobutamine-induced Takotsubo syndrome in imaging exams found that most reports were in females (86%), with a mean age of 65 years[9]. The most frequent clinical presentations were chest pain (56%), followed by asymptomatic patients. A dobutamine dose of 30 - 40 mcg/kg/min was involved in a greater number of the reported cases (n=16) compared with lower doses. In addition, in 6 of these cases, the syndrome occurred during the period of recovery from the stress test. In the vast majority of cases, the evolution was benign (90%), although one death was reported in the 22 documented patients.[9]

CONCLUSION

Takotusubo syndrome can be triggered by pharmacological stress and is mainly associated with dobutamine. Although rare, its risk should not be overlooked in exams with pharmacological adrenergic stress, and the indication for these exams should involve caution and knowledge of its complications.

PALAVRAS-CHAVE: *Cardiomiopatia de Takotsubo. Dobutamina. Ecocardiografia.*

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Lattice radiation therapy – its concept and impact in the immunomodulation cancer treatment era

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SUMMARY

Voluminous tumors represent a challenge in radiation oncology, particularly when surgical resection is not possible. Lattice radiotherapy (LTR) is a technique that may provide equivalent or superior clinical response in the management of large tumors while limiting toxicity to adjacent normal tissues. LRT can precisely deliver inhomogeneous high doses of radiation to different areas within the gross tumor volumes (GTV). The dosimetric characteristic of LTR is defined by the ratio of the valley dose (lower doses – cold spots) and the peak doses, also called vertex (higher doses - hot spots), or the valley-to-peak dose ratio. The valley-to-peak ratio thereby quantifies the degree of spatial fractionation. LRT delivers high doses of radiation without exceeding the tolerance of adjacent critical structures. Radiobiological experiments support the role of radiation-induced bystander effects, vascular alterations, and immunologic interactions in areas subject to low dose radiation. The technological advancements continue to expand in Radiation Oncology, bringing new safety opportunities of treatment for bulky lesions.

KEYWORDS: *Radiotherapy. Immunology. Dose fractionation, radiation. Immunomodulation. Neoplasms.*

INTRODUCTION

Voluminous tumors represent a challenge in radiation oncology, particularly when surgical resection is not possible. In many situations, conventional-dose radiation with or without chemotherapy has limited efficacy for local control. Lattice radiotherapy (LTR) is a technique based on 3-dimensional plans derived from grid radiation therapy. Grid was used to deliver tumoricidal doses of radiation to deep-seated tumors in the orthovoltage era, due to skin toxicity and difficulties in dose distribution[1]. Lattice may provide equivalent or superior clinical response in the management of large tumors while limiting toxicity to adjacent normal tissues. LRT can precisely deliver inhomogeneous high doses of radiation to different areas within the gross tumor volumes (GTV). Recently, tridimensional plans (3D) started to be used for LTR planning and delivery[2].

LTR DOSIMETRIC CHARACTERISTICS

The principle of LTR is based on the use of volumes of high-dose areas, called vertices, distributed within the central areas of the GTV and lower dose areas, also within the GTV, called valleys. This high-dose of radiation is delivered to the bulk of the tumor, sparing the peripheral areas and normal tissues[3].

The dosimetric characteristic of LTR is defined by the ratio of the valley dose (lower doses – cold spots) and the peak doses, also called vertex (higher doses - hot spots), or the valley-to-peak dose ratio. The valley-to-peak ratio thereby quantifies the degree of spatial fractionation[2].

Developments in 3-dimensional planning are important in LTR. For LTR planning, important parameters include the vertex volume diameter and

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center-to-center spacing. By Monte Carlo simulations, Gholami et al.[4] found that hot spots diameters of 1 to 1.25 cm and separated by 1.7 to 1.8 cm are key to optimize therapeutic ratio and normal tissue sparing.

RADIOBIOLOGICAL MECHANISMS

The main radiobiological mechanisms related to LTR involve radiation-induced bystander effects, microvascular alterations, and immunomodulation[2].

A classification of more general radiation-induced signaling effects based on human radiation exposure scenarios divided the effects into three categories: bystander, cohort, and abscopal effects. The bystander effects are defined for human exposure scenarios as radiation-induced, signal mediated effects in unirradiated cells adjacent to a target volume that are exposed to only very low levels of scatter radiation. The second classification of effects, termed cohort effects, describes the overall radiobiological response in irradiated cells that is not a consequence of direct energy deposition in the target cell, but due to the communication between cells within an irradiated volume. Cohort effects are relevant for any exposures in which most of a cell population is exposed to a significant dose. This effect is based on intercellular signaling[5,6]. The abscopal effect refers to an immune-mediated response of distant lesions to irradiation of other lesions[7].

Large tumor volumes, in general, have a poor blood supply that leads to hypoxic volumes within the tumor that stimulates the production of factors such as hypoxia-inducible factor 2 alpha (HIF-2α) thus reducing apoptosis[8]. High doses of radiation can increase apoptosis due to the bystander effects via secondary cellular signaling either by direct physical contact or by cell-released signaling molecules - cytokines, nitric oxide, or reactive oxygen species. For LTR, most of the benefits would come from the destruction of volumes of the tumor receiving the highest dose of radiation while inducing bystander effects in those peripheral cells that are well within the target but farther away from healthy adjacent structures[9].

High doses of radiation have been shown to cause endothelial apoptosis through the production of soluble factors, consequently altering tumor microvasculature, which is vital for tumor growth and metastatic evolution[10].

IMMUNOMODULATION

Radiation is known to be a potent immune-modulator that elicits cell death upon the tumor, stromal and angiogenic compartments of the tumor microenvironment. The use of ablative radiation doses can impact local and metastatic or distant tumor control through the modulation of tumor-immune micro-environment and activating the host immune system[10].

LRT, through its high-dose component, also induces immune responses. It can modify the immunosuppressive tumor environment, potentially enhancing antigen-specific immunotherapy. Radiation kills not only cancer cells, but other cells within the tumor stroma, including endothelial cells and intra-tumoral lymphocytes, leading to an induced upregulation and activation of macrophages genes. The macrophage activation is also induced by signals from apoptotic cells. Furthermore, this stimulation of the host immune response could be responsible for the abscopal effect[11]. Other possible mechanisms of synergic effects of irradiation combined with immunotherapy include enhancement of T cell infiltration and inhibition of myeloid-derived suppressor cells (MDSCs) and regulatory T cells[12]. Local irradiation also enhances effector T lymphocytes, E7-specific antibodies, and decreases regulatory T cells[13].

It is important to note that greater irradiation doses above the threshold of ablative doses do not enhance the potency of antigen-specific immunotherapy[14]. Chang et al.[13], in an experiment, observed that a biweekly moderate radiation regimen (6 Gy twice a week) combined with the DNA vaccine generated more potent antigen-specific immunologic responses and anti-tumor effects when compared with groups treated with local irradiation or immunotherapy alone, or other radiation fractions (3 Gy four times per week or 12 Gy once per week).

The lymphocytes are considered very vulnerable to radiation, and therefore radiation therapy is taken to be immunosuppressive; however, some studies have already shown an increment in tumor-infiltrating lymphocytes after irradiation. This also stimulates the activity of antigen-presenting cells, which, in turn, primed CD8+ T cells in draining lymph nodes. The number of cytotoxic T cells infiltrating the irradiated tumor increases after radiation therapy, and, in a study by Lugade et al.[15] it was noted that a single fraction of 15 Gy was more effective in this setting than 15 Gy over 3 fractions.

Radiation therapy was also shown to upregulate Type 1 interferon, which is necessary for T cell priming by dendritic cells. The presentation of exogenous antigen molecules, known as cross-presentation, is essential for the initiation of CD8(+) T cell responses. In vivo, cross-presentation is mainly carried out by specific dendritic cell subsets through an adaptation of their endocytic and phagocytic pathways[16,17]. Dendritic cells are the main antigen-presenting cells for the induction of T-cell adaptive responses. Cancer cells express tumor antigens, including neo-antigens generated by non-synonymous mutations, but are poor for antigen presentation and for providing co-stimulatory signals for T-cell priming. Evidence suggests that antigen transfer to dendritic cells and their presentation to histocompatibility complex molecules plus co-stimulatory signals is paramount for the induction of cancer immunity[18].

CURRENT INDICATIONS

Currently, the use of LTR has been reported for tumors > 45cc, primary or secondary, arising in the lung, pancreas, cervix, rectum, brain, retroperitoneal, and extremity sarcomas, kidney, melanoma, recurrent and metastatic tumors[3].

Current indications include bulky or locally advanced disease that would not be tractable by conventional radiation or that has been proven to be refractory to chemoradiation. Early-phase clinical trials have shown remarkable success, with some response rates >60% and minimal toxicity[14].

TOXICITY

One important thing is that applying LRT to large volume tumors would not incur additional toxicity to the surrounding normal tissues since the high-dose volumes are situated within the GTV. The presence of low dose volumes also inside the GTV preserve the vasculature and lymphatic drainage, allowing immunogenic-activated cells to circulate and, subsequently, maximize bystander and abscopal effects[10].

CONCLUSION

LRT delivers high doses of radiation without exceeding the tolerance of critical structures. Radiobiological experiments support the role of radiation-induced bystander effects, vascular alterations, and immunologic interactions.

Technological advancements continue to expand in Radiation Oncology and, despite the fact that more clinical and biological data are needed to specify the ideal dosimetric parameters and to formulate robust clinical indications, LTR is an additional treatment option for bulky tumors unsuitable for surgery.

Conflict of interest statement

I have nothing to disclose.

RESUMO

Tumores volumosos representam um desafio para a radio-oncologia, em especial quando a ressecção cirúrgica não é possível. A radioterapia com técnica Latisse (LTR) pode gerar resposta clínica equivalente ou superior ao tratamento convencional de grandes tumores, limitando a toxicidade nos tecidos normais adjacentes. A LRT pode fornecer com precisão altas doses não homogêneas de radiação em diferentes áreas do volume tumoral (GTV). A característica dosimétrica da LTR é definida pela razão entre a dose na região do vale (doses mais baixas – pontos frios) e as doses de pico, também chamadas de vértice (doses mais altas – pontos quentes) ou a razão da dose vale/pico. Dessa forma, a razão vale/pico quantifica o grau de fracionamento espacial da entrega de dose. A LRT entrega, dessa forma, altas doses de radiação sem exceder a tolerância de estruturas críticas adjacentes. Experimentos radiobiológicos suportam o chamado "efeito espectador" induzido por radiação, o qual promove alterações vasculares e interações imunológicas, levando à resposta tumoral mesmo em áreas expostas a baixas doses de radiação. Os avanços tecnológicos continuam a se expandir na radio-oncologia, trazendo, por meio da LTR, uma nova oportunidade segura de tratamento para lesões volumosas.

PALAVRAS-CHAVE: *Radioterapia. Imunologia. Fracionamento da dose de radiação. Imunomodulação. Neoplasias.*

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ORIGINAL ARTICLE

Dysregulation of serum miR-1204 and its potential as a biomarker for the diagnosis and prognosis of breast cancer

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SUMMARY

OBJECTIVE: *A previous study has reported that miR-1204 exerted oncogenic effects in breast cancer (BC). The purpose of our paper was to evaluate the expressions of tissue and serum miR-1204 in patients with BC and further investigate its biomarker potential.*

METHODS: *The expressions of tissue and serum miR-1204 were investigated by qRT-PCR in 144 BC patients and 38 healthy controls. Chi-square tests were conducted to examine the associations between miR-1204 expressions and clinicopathological factors. Then, the associations of miR-1204s level with the survival of BC patients were determined by performing the Kaplan-Meier and multivariate analysis. The receiver operating characteristics (ROC) and area under the OC curve (AUC) were obtained to validate the diagnostic values of miR-1204.*

RESULTS: *We found that the expressions of miR-1204 were increased in both tissue and serum samples from BC patients. Multivariate assays identified tissue and serum miR-1204 overexpression as an independent poor prognostic factor. In addition, ROC curve assays indicated that tissue and serum miR-1204 are potential diagnostic markers of BC.*

CONCLUSIONS: *Detection of tissue and serum miR-1204 levels could have clinical potential as a novel prognostic/diagnostic biomarker for BC patients.*

KEYWORDS: *MicroRNAs. Breast neoplasms/diagnosis. Prognosis, Biomarkers. Serum.*

INTRODUCTION

Breast cancer (BC) is one of the frequently diagnosed malignant tumors and the second leading cause of neoplasm mortality for women. Moreover, its incident rate has increased in recent years[1]. BC is a complex disease, which involves multiple risk factors[2]. During recent decades, remarkable advancement has been made, and this malignancy can now be treated by surgery, endocrine, cytotoxic, or targeted therapies[3]. However, the overall prognosis of BC is not satisfactory.

MicroRNAs (miRNAs) are highly conserved, single-stranded, and non-coding RNAs whose lengths are approximately twenty-two nucleotides (nt) and that are involved in the post-transcriptional modulation of genes expression[4]. MiR-1204 is a recently identified miRNA that has also been shown to be upregulated in

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several types of human malignancy, and its dysregulations played an essential role in tumor progression by affecting the tumor cellular function[5]. Recently, Liu et al.[6] firstly reported that miR-1204 was highly expressed in BC and served as a tumor promoter *in vitro*. However, the clinical importance of miR-1204 in BC remained largely unclear.

In this study, we aimed to investigate the possibility that miR-1204 could serve as a diagnostic and prognostic biomarker in BC.

METHODS

Patients and Tissue Samples

Serum samples and BC samples were obtained from patients who underwent radical mastectomy or modified radical mastectomy at the Jilin Cancer Hospital from 2010 to 2013. Meanwhile, 38 healthy individuals' sera were included as normal controls. All the patients with BC were diagnosed by two skilled pathologists. All patients had not received radiotherapy or chemotherapy prior to mastectomy. Tumors were staged based on the Seventh Edition of the Cancer Staging Manual. The clinicopathological information, collected from medical records. Prior to enrollment, written informed consent was obtained from every contributor. The study was approved by the Ethical Committee of the Jilin Cancer Hospital.

Quantitative real-time RT-PCR

Total RNA was extracted from 500 μL of blood samples using a miRVana PARIS Kit (Ambion, Kunmin, Yunnan, China) and washed out into 150 μL of pre-heated Elution Solution. Total RNA was extracted from all tissues from every contribution with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized with the PrimeScript II First Strand cDNA Synthesis kit (TaKaRa, Otsu, Shiga, Japan). RT-PCR was then conducted via miRNA-specific TaqMan MiRNA Assay Kit (ABI, Xuhui, Shanghai, China) on the useful Biosystems 7500 (ThermoFisher Scientific, Waltham, MA, USA). The levels of miRNA were determined based on the threshold cycle (Ct), and relative expressions were estimated employing the $2^{-\Delta\Delta Ct}$ methods, using the expression level of the glyceraldehyde phosphate dehydrogenase (GAPDH) as a reference gene. The following primers were used: miR-1204 sense primer 5'- GGCTCGTGGCCTGGTCTC-3′ and antisense primer 5'- CTCAACTGGTGTCGTGGA -3' and GAPDH sense primer 5'-GCTGGCGCTGAGTACGTCGT-3' and antisense primer 5'-ACG TTGGCAGTGGGGACACG-3'.

Statistical analysis

Statistical analyses were performed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The possible significances of differences between groups were assessed by the Student's *t*-test, Wilcoxon test, or Chi-square test. The cumulative survival probabilities were measured using the Kaplan-Meier method, and differences were determined by the use of the log-rank tests. Hazard ratios were determined by the Cox regression model. ROC curve assays were performed to complete the diagnostic performances of miR-1204 expressions by originally identifying patients with BC from the healthy controls. A p value<0.05 was considered statistically significant.

RESULTS

The miR-1204 expression was upregulated in the tissue and serum of BC patients

In order to explore the role of miR-1204 in BC, we performed RT-PCR to study whether miR-1204 was abnormally expressed in BC tissues and serum. As presented in Figure 1A, we found that miR-1204 expressions were significantly up-regulated in BC tissues compared to matched normal breast tissues (p<0.01). In addition, we also observed that serum miR-1204 levels were obviously higher in BC patients compared with normal controls with $p < 0.01$ (Figure 1B). Then, we wondered whether the expressions of tissue miR-1204 were associated with the levels of serum miR-1204. As shown in Figure 1C, Spearman order correlations assays were performed, and linear correlations between the miR-1204 expressions in the BC tissues and the serum ones were validated ($p < 0.001$).

Associations between miR-1204 expressions and the clinicopathological features of BC

For a superior understanding of the clinical significance of miR-1204 expressions in BC, our group used the median levels of miR-1204 in tissues and serum of BC for the division of all 144 BC patients into a high-expression group and a low-expression group. As shown in **Table I**, we found that high miR-1204 levels distinctly correlated with differentiation grade (p = 0.008), advanced TNM stage (p = 0.013), and lymph nodes metastasis (p=0.002). Moreover, similar results were also observed in serum samples.

However, no distinct alteration was found between miR-1204 expressions and patients' age, tumor size, ER, PR, HER-2 ($p > 0.05$).

Prognostic value of tissue and serum miR-1204 in BC

In order to scrutinize the associations between miR-1204 expressions and clinical overall survival, our group reexamined the detailed clinical data of 144 BC patients and performed Kaplan-Meier analysis. As shown in Figure 2A, we found that patients with high tissue miR-1204 expressions lived shorter than those with low tissue miR-1204 expressions ($p = 0.0082$). Similar results were also observed in patients with high serum miR-1204 expression ($p = 0.006$, Figure 2B). Furthermore, we also found that patients with high tissue and serum miR-1204 expression levels tended to have shorter disease-free survival times (both $p < 0.05$, Figure 2C, and 2D). In addition, univariate analysis showed that differentiation grade, TNM stage and lymph nodes metastasis, tissue miR-1204 expression, and serum miR-1204 expressions were powerfully correlated with worse overall survival ($p < 0.05$,). Further multivariate assays confirmed that high tissue miR-1204 expression level (HR=3.895, 95% CI: 1.213-6.014; $p = 0.001$) and high serum miR-1204 expression (HR=3.243, 95% CI: 1.178-4.468; $p = 0.009$) were independent prognostic biomarkers for suggesting unfavorable overall survival in BC patients.

Diagnostic value of tissue and serum miR-1204 marker

As shown in Figure 3A, the AUC of 0.854 was obtained according to ROC assay. Furthermore, the specificity was 82.3% and the sensitivity was 94.8% with an optimal cut-off value of 4.82. Moreover, we evaluated the diagnostic capabilities of serum miR-1204 for BC; the result of ROC indicated a optimal cutoff value of 4.77 for serum miR-1204 levels, with sensitivity at 79.6.1 % and specificity at 92.5 %, with an AUC of 0.823 in differentiating BC patients from healthy controls (Figure 3B).

DISCUSSION

In this study, in line with the previous one, our group also revealed, using RT-PCR, that tissue miR-1204 expressions were up-regulated in BC tissues. Those results revealed that the dysregulation of miR-1204 may be involved in the progression of BC. Recently, emerging studies have highlighted the novel application of miRNAs as potential biomarkers for the detection of BC[7-9]. Our findings provided evidence that miR-1204 could be a candidate.

In this study, we analyzed the association between tissue and serum miR-1204 and clinicopathological characteristics in BC patients, finding that high miR-1204 expression was associated with TNM stage, differentiation grade, and lymph nodes metastasis. In addition, the result of the Kaplan-Meier survival was that BC patients with higher tissue and serum miR-1204 expression had shorter overall survival and disease-free survival time. More importantly, multivariate analysis indicated that tissue and serum miR-1204 expression was an independent prognostic factor for poor OS and DFS rates of BC patients.

In this study, ROC curve assays revealed that specimens of miR-1204 were useful markers for discriminating BC tissues from matched normal breast tissues, with an AUC of 0.854. Moreover, our results

FIGURE 1. MIR-1204 EXPRESSION IS UPREGULATED IN BOTH BC TISSUES AND PATIENTS' SERA BY RT-PCR. (A) THE EXPRESSION LEVELS OF MIR-1204 IN BC TISSUES WERE SIGNIFICANTLY HIGHER THAN THOSE IN NONCANCEROUS BREAST TISSUES (P < 0.01). (B) THE SERUM LEVELS OF MIR-1204 WERE ALSO MARKEDLY UPREGULATED IN PATIENTS WITH BC COMPARED WITH HEALTHY CONTROLS (P < 0.01). (C) INVERSE CORRELATION BETWEEN TISSUES MIR-1204 AND SERUM MIR-1204 EXPRESSION USING PEARSON'S CORRELATION COEFFICIENT.

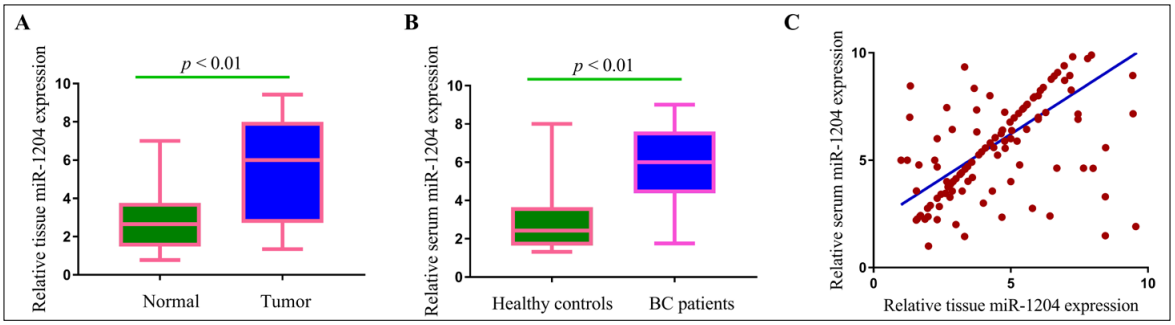


FIGURE 2. KAPLAN-MEIER CURVES FOR OVERALL SURVIVAL AND DISEASE-FREE SURVIVAL OF 144 BC PATIENTS, DIVIDED ACCORDING TO MIR-1204 EXPRESSION LEVELS. (A, B) HIGH TISSUE AND SERUM MIR-1204 EXPRESSION WAS SIGNIFICANTLY ASSOCIATED WITH POOR OVERALL SURVIVAL (BOTH P < 0.05). (C, D) HIGH TISSUE AND SERUM MIR-1204 EXPRESSION WAS SIGNIFICANTLY ASSOCIATED WITH POOR DISEASE-FREE SURVIVAL (BOTH P < 0.05).

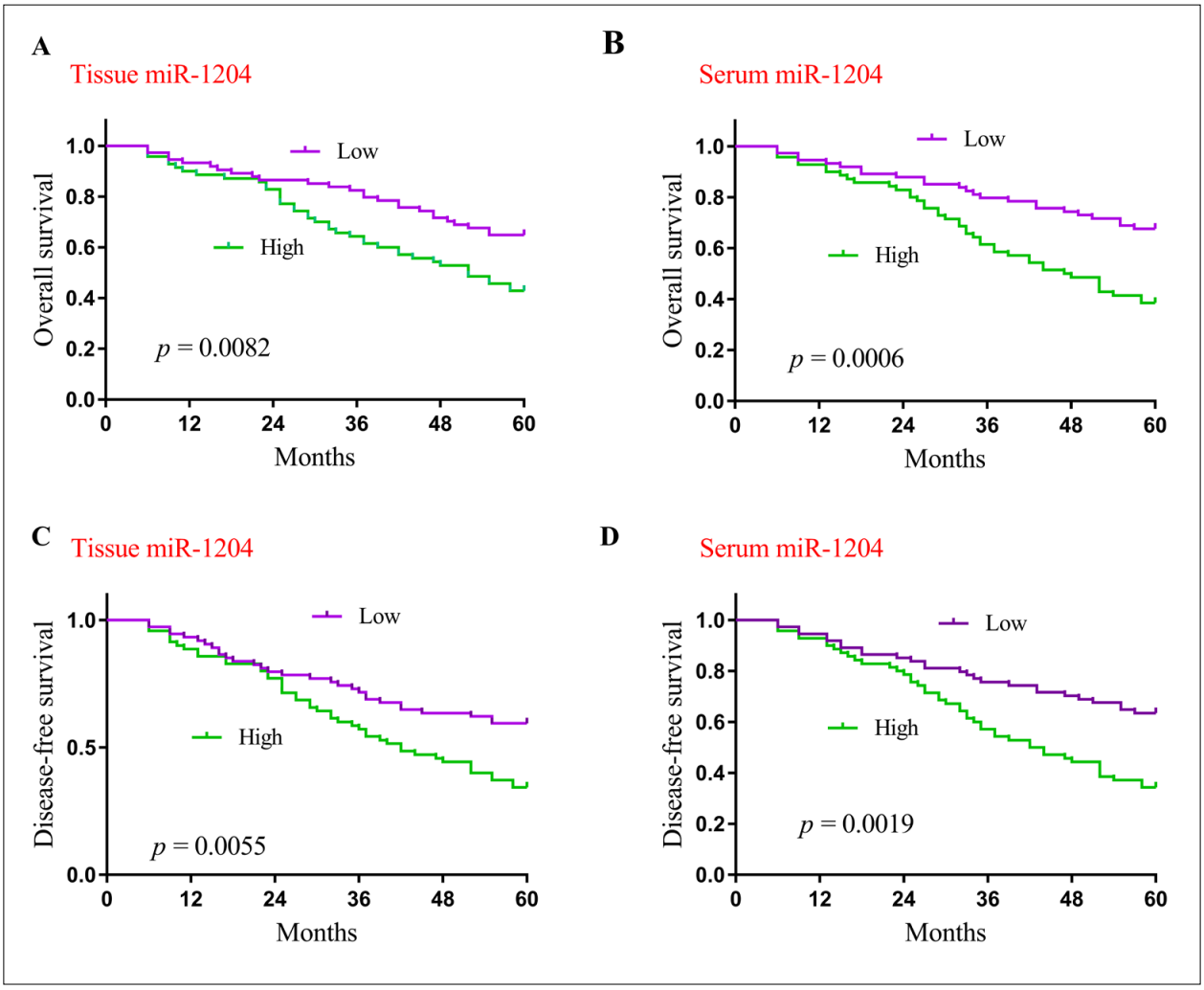
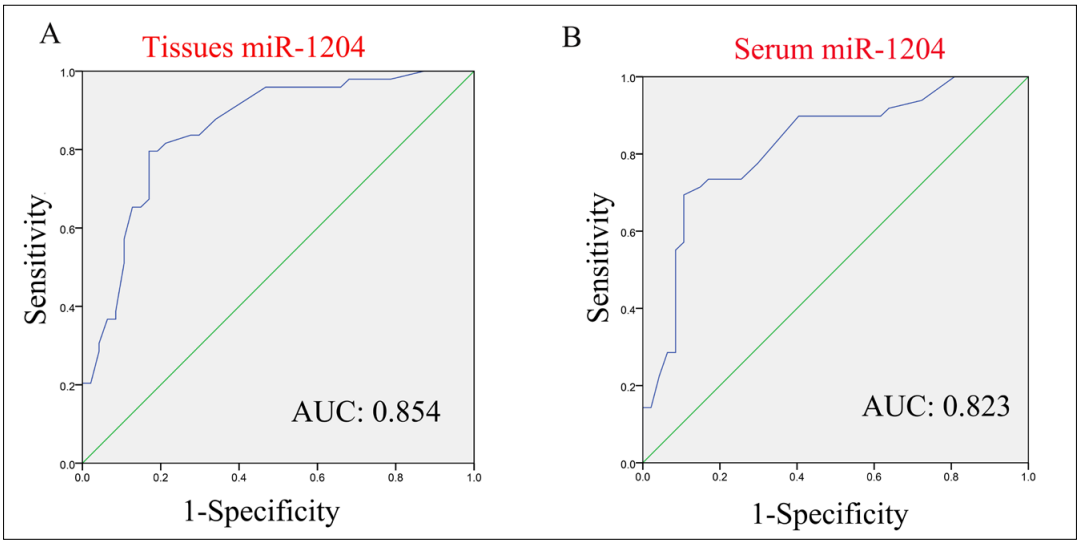


FIGURE 3. ROC CURVE ASSAYS WERE CONDUCTED TO DETERMINE THE VALUE OF DIAGNOSIS OF TISSUE MIR-1204(A) AND SERUM MIR-1204 (B). THE AUC WAS 0.854 (95% CI: 0.777-0.931) AND 0.823 (95% CI: 0.737-0.908), RESPECTIVELY.



also confirmed that serum miR-1204 could be served as a hopeful noninvasive marker in early detections of BC. To the best of our knowledge, our current research is the first to provide important evidence showing the potential role of serum miR-1204 in the screening of BC.

CONCLUSIONS

This study indicated that tissue and serum miR-1204 might potentially serve as a novel biomarker for BC patients.

Conflict of Interest

The authors declare that they have no conflict of interests.

Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

RESUMO

OBJETIVO: *Um estudo anterior indicou que o miR-1204 exerce efeitos oncogênicos no Câncer de Mama (CM). O objetivo deste trabalho foi avaliar as expressões de miR-1204 sérico e em tecidos em pacientes com CM e investigar o seu potencial biomarcador.*

METODOLOGIA: *As expressões de miR-1204 sérico e em tecidos foram investigadas por qRT-PCR em 144 pacientes com CM e 38 controles saudáveis. Testes qui-quadrados foram realizados para examinar as associações entre as expressões de miR-1204 e os fatores clinicopatológicos. Em seguida, as associações entre nível de miR-1204s e sobrevida de pacientes de CM foram determinadas através de análises de Kaplan-Meier e multivariadas. A Curva Característica de Operação do Receptor (ROC) e área sob a curva (AUC) foram obtidas para validar o valor diagnóstico do MIR-1204.*

RESULTADOS: *Descobrimos que as expressões do MIR-1204 estavam aumentadas em amostras de tecido e séricas de pacientes com CM. Análises multivariadas identificaram a superexpressão de miR-1204 sérico e em tecidos como um fator independente de prognóstico ruim. Além disso, as curvas ROC indicaram que o miR-1204 sérico e em tecidos é um possível marcador de diagnóstico de CM.*

CONCLUSÃO: *A detecção dos níveis MIR-1204 em tecidos e séricos pode ter potencial clínico como um novo biomarcador de prognóstico/diagnóstico para pacientes de CM.*

PALAVRAS-CHAVE: *MicroRNAs. Neoplasias/diagnóstico da mama. Prognóstico. Biomarcadores. Soro.*

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ORIGINAL ARTICLE

The relationship between low-intensity exercise and psychological distress among college students

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SUMMARY

OBJECTIVE: *The purpose of our study was to confirm the relationship between low-intensity exercise and physical and mental health status among college students in China.*

METHODS: *This was a school-based cross-sectional study. The physical and mental health status was measured using a 12-item general health questionnaire(GHQ12) and low-intensity exercise was recorded by a self-reporting questionnaire.*

RESULTS: *The results revealed that the score of the GHQ12 was inversely associated with a higher frequency of low-intensity exercise (r=-0.38,p=0.001).*

CONCLUSIONS: *Our study suggests that low-intensity exercise may be a proper mean for improving the physical and mental health status of college students. School departments should take measures to push students to take part in physical activity.*

KEYWORDS: *Cross-sectional studies. Mental health. Motor activity. Exercise. Students.*

INTRODUCTION

In recent decades, the number of adolescents and young adults with poor mental health has increased, especially among college students[1]. In the meantime, the prevalence of medical and nonmedical uses of psychiatric medication among undergraduate students is still high. A study found that individuals who engage in regular physical activity have lower levels of anxiety[2] and higher quality of life than patients with advanced chronic kidney disease[3]. Engagement in physical activity can be an important contributing factor in the mental health of undergraduate students[4].

We hypothesize that the relationship between low-intensity exercise and physical and mental health status among college students in China still exists. However, little is known on whether low-intensity exercise has a positive effect on the mental health status of college students in China.

The aim of this study was to confirm the relationship between low-intensity exercise and the physical and mental health status among college students in China to offer a basis for the prevention of poor physical and mental health status among college students.

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METHODS

Subjects

Data collection was performed in a school to obtain information on low-intensity exercise and the physical and mental health status of college students. A total of 1287 students (452 males and 832 females) aged from 18 to 20 years old were included in the study. Personal information and written informed consent were obtained from all the subjects.

Measurement of physical and mental health status(psychological distress)

The physical and mental health status was measured using a 12-item general health questionnaire (GHQ-12)[5]. The GHQ-12 questionnaire contained twelve items, with four possible answers each: "Better than usual=0", "Same as usual=1", "Worse than usual=2", and "Much worse than usual=3". The GHQ-12 score was calculated based on the score of each item. The higher the total score of the GHQ-12, the worse the physical and mental health status of students.

Measurement of low-intensity exercise

Low-intensity exercise was defined as at least 20 minutes, once a day, of activities such as walking or shadowbox. Then the frequency of low-intensity exercise in a week was calculated.

Statistics analysis

Data were analyzed using the SPSS20.0 software (SPSS Inc, II, Chicago, IL, USA). Description statistics were used for the frequency of gender. The distribution of the GHQ-12 score and the frequency of daily low-intensity exercise and line chart was drawn. The Pearson correlation analysis was performed to explore the relationship between the GHQ-12 score and the frequency of daily low-intensity exercise. A P-value of less than 0.05 was considered as statistically significant.

RESULTS

A total of 1287 students (452 for males and 832 for females) aged from 18 to 20 years old were included in the study. We first explored the distribution of the GHQ-12 score and the frequency of daily low-intensity exercise. The Normal distribution of the GHQ-12 score and the frequency of daily low-intensity exercise were observed in the present study(Figure 1 and Figure 2).

Then, we explored whether the frequency of daily low-intensity exercise was associated with the GHQ-12 score. Figure 3 shows that the GHQ-12 score decreases with a higher frequency of daily low-intensity exercise. The Pearson correlation analysis also found that the score of GHQ12 was inversely associated with a higher frequency of low-intensity exercise (r=-0.38,p=0.001).

DISCUSSION

The main finding of this study was that the GHQ-12 score decreased with a higher frequency of daily low-intensity exercise. The results of the present study further confirmed the results of a previous study and were higher than the results from a meta-analysis in China[6]. It has also been found that physical activity

FIGURE 1. DISTRIBUTION OF THE GHQ12 SCORE IN COLLEGE STUDENTS

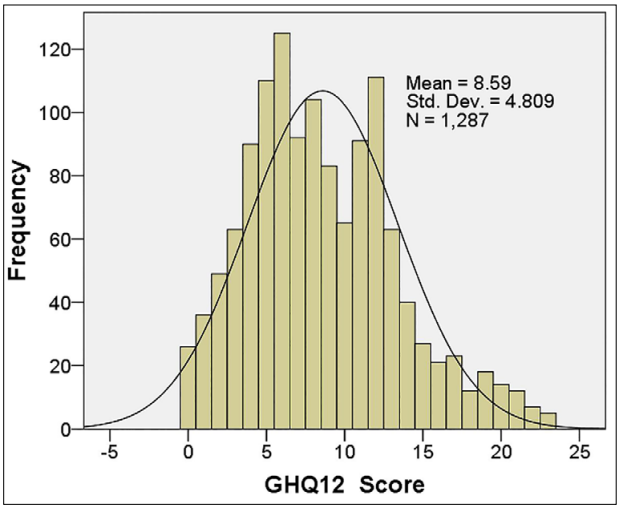


FIGURE 2. DISTRIBUTION OF THE TIMES OF LOW-INTENSITY EXERCISE EVERY WEEK IN COLLEGE STUDENTS

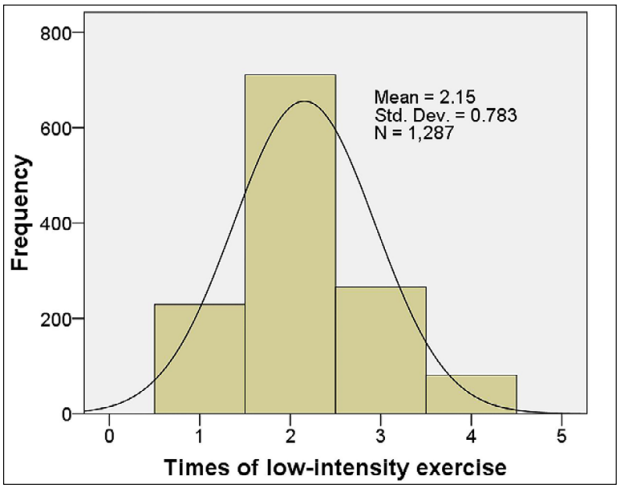
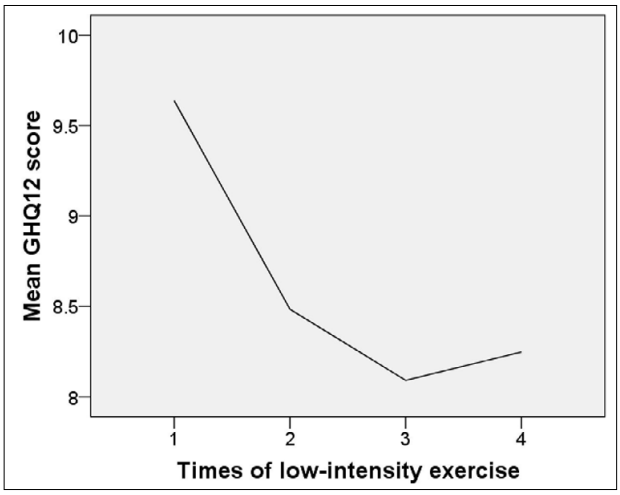


FIGURE 3. LINE TREND OF THE GHQ12 MEAN SCORE WITH THE TIMES OF LOW-INTENSITY EXERCISE



can improve the mental health of older populations[7].

Some limitations should be addressed in this work. The small sample size of the study is limited are requires further exploration of the risks of a poor physical and mental health status among college students. Additionally, a cross-section study cannot explain the cause-results relationship between low-intensity exercise and physical and mental health status. Although some shortcomings exist, the results of our work offer a direction for future studies.

CONCLUSIONS

Our study suggests that low-intensity exercise may be a proper mean for improving the physical and mental health status of college students. School departments should take measures to push students to take part in physical activity.

Acknowledgment

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Conflict of interest

The authors declare no conflicts of interest.

Informed Consent

Informed Consent was obtained from all participants included in the study.

Author Contributions

Conceptualization, Ergang Zhu; formal analysis, Ergang Zhu; writing—original draft preparation, Ergang Zhu; writing—review and editing, Tianhua Du; supervision, Tianhua Du; funding acquisition, Tianhua Du.

RESUMO

OBJETIVO: *O objetivo do nosso estudo foi confirmar a relação entre o exercício de baixa intensidade e o estado de saúde física e mental entre estudantes universitários na China.*

MÉTODOS: *Estudo transversal com base na escola foi realizado neste estudo. O estado de saúde física e mental foi medido recorrendo-se a questionários gerais de saúde (GHQ12); exercícios de baixa intensidade foram coletados por questionários de autorrelato.*

RESULTADOS: *Os resultados revelaram que a pontuação do GHQ12 foi inversamente associada com maior frequência de exercícios de baixa intensidade (r=−0,38, p=0,001).*

CONCLUSÕES: *Nosso estudo sugeriu que o exercício de baixa intensidade pode ser um meio adequado para melhorar o estado físico e mental dos estudantes universitários. O departamento relacionado à escola deve tomar alguma medida para forçar os alunos a participar da atividade física.*

PALAVRAS-CHAVE: *Estudos transversais. Saúde mental. Atividade motora. Exercício. Estudantes.*

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ORIGINAL ARTICLE

COL6A3 promotes cellular malignancy of osteosarcoma by activating the PI3K/AKT pathway

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SUMMARY

OBJECTIVE: *In this study, we aimed to investigate the role of COL6A3 on cell motility and the PI3K/AKT signaling pathway in osteosarcoma.*

METHODS: *The relative expression of COL6A3 was achieved from a GEO dataset in osteosarcoma tissue. siRNA technology was applied to decrease the COL6A3 expression in cells, and cell counting kit-8 (CCK-8) assay and colony formation analysis were used to examine the cell proliferation potential. Knockdown COL6A3 made the proliferation and colony formation abilities worse than the COL6A3 without interference. Likewise, in contrast to the si-con group, cell invasion and migration were inhibited in the si-COL6A3 group. Moreover, the western blot results suggested that the PI3K/AKT signaling pathway was manipulated by measuring the protein expression of the PI3K/AKT pathway-related markers, due to the COL6A3 inhibition.*

CONCLUSION: *COL6A3 plays a crucial role in modulating various aspects of the progression of osteosarcoma, which would provide a potentially effective treatment for osteosarcoma.*

KEYWORDS: *Osteosarcoma. Neoplasms, bone tissue. Molecular targeted therapy. Collagen type VI.*

INTRODUCTION

Osteosarcoma is a prevalent and aggressive tumor in the bones that has received widespread attention because of its particular features[1]. Up to now, radical surgery, chemotherapy, and radiotherapy are the most commonly used treatments in osteosarcoma, but the cure rate is not significant[2]. It is well-known that targeted therapy is a major and effective modality for cancer, and numerous genes have been proved to be associated with the progression of tumors[3,4]. Hence, novel biomarkers are urgently needed for the diagnosis and treatment of osteosarcoma.

Collagen VI (COL6) is a extracellular-matrix protein, which is related to the basement membrane and made up of three chains: alpha 1, alpha 2, and alpha 3[5]. Previous reports have illustrated that COL6 is involved in muscle regularity and cell membrane

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integrity[6]. There is evidence that the loss of COL6 can cause serious Bethlem myopathy and Ullrich congenital muscular dystrophy[7].

However, there is no literature to interpret the functional characteristics of COL6A3 in osteosarcoma. The purpose of the study was to investigate the role of COL6A3 on cell motility and the PI3K/AKT signaling pathway in osteosarcoma.

ETHODSCELL LINES AND CULTURE CONDITION

Human osteosarcoma cell lines (U2OS, HOS, and MG-63) and normal cell lines hFOB1.19 were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China), which were then incubated in RPMI-1640 medium. To maintain the normal viability of cells, fetal bovine serum (FBS; 10%), penicillin (100 U/mL), and streptomycin (100 U/mL) were used. All the cells were stored at 37˚C and 5% CO2.

TRANSFECTION

To knockdown COL6A3, small interfering RNAs (siRNAs) including si-COL6A3 and si-con were designed by GenePharma (Shanghai, China). The sequences of two siRNAs are as follow: COL6A3 siRNA: 5'-GCTTTGCACATATTCGAGATT-3'; si-con: 5'-AATTCTCCGAACGGTCACGT-3'. The si-con was used as a negative control. For transfection, cells were treated with siRNAs using Lipofectamine2000 per the manufacture protocol. After 24 h transfection, the knockdown efficiency of COL6A3 can be observed for further detection.

Reverse transcription-quantitative polymerase chain reaction (qPCR)

To detect the mRNA expression level of a specific gene, TRIzol solution (Invitrogen, Carlsbad, CA, USA) was used to isolate total RNA, and then qRT-PCR analysis was carried out. Then RNA was converted into cDNA using the Fast Quant RT Kit (TaKaRa, Otsu, Shiga, Japan). qPCR was performed with the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and, in the meantime, SYBR Green Master Mix was also utilized as a dyeing probe. The reaction procedure was the following: pre-heated at 95º for 5 min, denatured at 95˚C for 30 s and 60º for 45 s with 40 cycles, 72º for 30 min. The forward primer of COL6A3 was 5'-AACATCCTGGTCAGCTCTGC-3' and the reverse primer was 5'-TCCGGGATGAAGGAGATGGT-3'. In addition, the forward primer of GAPDH was 5'-TCCAAAATCAAGTGGGGCGA-3', and its reverse primer was 5'-TGATGACCCTTTTGGCTCCC-3'. GAPDH was assessed as internal control and all detections were conducted three times. The $2^{-\Delta\Delta CT}$ method was used to examine relative expression.

Western blotting

Transfected cells for 24 h were placed on ice and lysed by RIPA reagent (Beyotime, Shanghai, China) supplemented with a protease inhibitor to extract proteins. Then, the isolated proteins were concentrated by the BCA method and boiled at 95º for 5 min. In the vertical electrophoresis tank, 20 μg protein was added into each well, separating by 12% SDS-PAGE at 110v for 1 h and transferred onto the PVDF membrane. Subsequently, the PVDF membrane was blocked using 5% skim milk powder for 1 h and antibodies. Primary antibodies (1:1,000; Abcam, Cambridge, MA, USA) were used to incubate the membrane at 4°C overnight, and a secondary antibody (1:2,000; Abcam, Cambridge, MA, USA) at room temperature for 1 h. Finally, ECL was added for development and the gray value of protein bands was scanned through the QUANTITY ONE software.

Cell proliferation and colony formation assays

Cell counting kit 8 (CCK-8) analysis and colony formation exploration were applied to evaluate the proliferative and clonal abilities. For the CCK-8 assay, the single-cell suspensions generated by the transfected cells were seeded into a 96-well plate with a density of 1, 000 cells per well, at 37°C for 0-72 h. Every 24 hours were considered as a detection time point and the cells should go through an additional culture 1.5 h after supplementing with 10 μL CCK-8 reagent. At last, the OD value was measured at 450 nm using a microplate reader. For colony formation exploration, transfected cells were inoculated in a 60 mm-depth dish, which was pre-filled with 5 mL warm medium; the density was 400 cells/dish. The culture was completed when macroscopic clones appeared in the culture dish (about two weeks), fixed then stained by 4% paraformaldehyde and 0.1% crystal violet, respectively, for 30 min. Finally, the clone was counted and captured under a microscope.

Transwell assay

Transwell assay was carried out to assess cell migration and invasion. Cells after 24 h transfection were

turned into a cell suspension and put into the upper chamber. Meanwhile, the lower chamber was filled with 500 µL complete medium. After waiting overnight, the residual cells on the surface of the upper chamber were wiped out and the chamber was washed using PBS for three times. The cells on the surface of the lower chamber were fixed using 4% paraformaldehyde, dyed via 0.1% crystal violet, and imaged through the microscope. Attentively, the upper chamber was pre-coated with Matrigel (BD Sciences, Franklin Lakes, NJ, USA) for invasion while migratory detection was not. Furthermore, the inoculated density was different: 5, 000 cells for migration and 1×10^5 cells for invasion.

Statistical analysis

Based on the SPSS 22.0 statistics software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA), all data were analyzed and described as mean ± standard deviation (SD). Differential analysis of the two groups was conducted with the student's t-test; the comparison of multiple groups was conducted by one-way ANOVA and Dunnett's post hoc test. The significance of this test was $p < 0.05$.

RESULTS

COL6A3 was associated with metastasis of osteosarcoma

Initially, we collected two arrays from the GEO dataset to examine the expression level of COL6A3 in osteosarcoma tissues and discovered that the expression level of COL6A3 was significantly increased in both GSE16088 (P = 0.0324) involving 14 osteosarcoma cases and 3 normal cases and GSE49003 (P = 0.0022) containing 6 non-metastasis cases and 6 metastasis cases. These findings indicate that the development of osteosarcoma was closely associated with the COL6A3

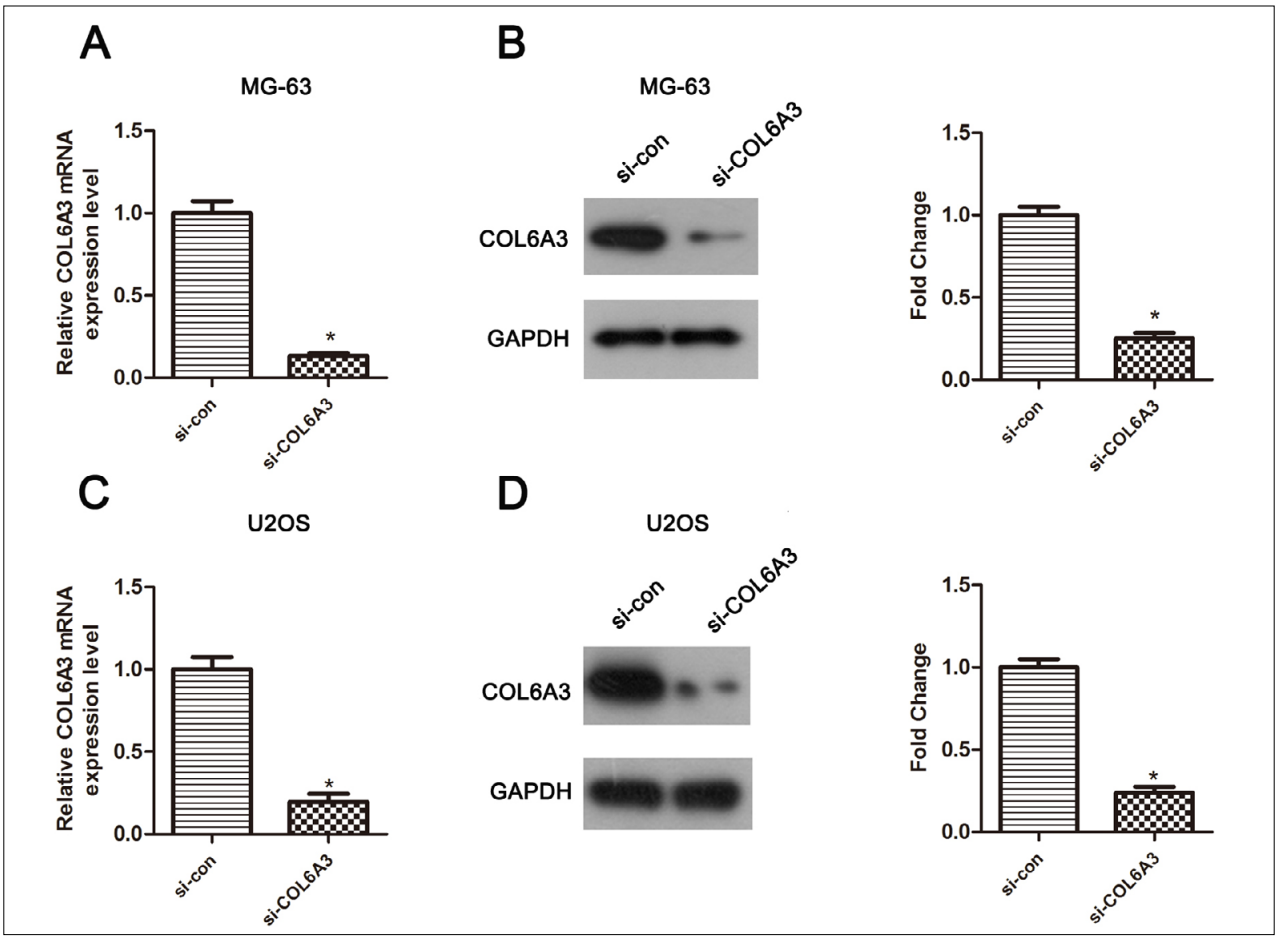

FIGURE 1. HIGH EXPRESSION OF COL6A3 WAS ASSOCIATED WITH METASTASIS IN OSTEOSARCOMA.

A) Relative expression of COL6A3 between normal tissue and osteosarcoma tissue, which was obtained from the GEO database, P = 0.0324. B) Relative expression of COL6A3 between non-metastasis tissue and metastasis tissue from the GEO database, P = 0.0022. C) The qRT-PCR analysis revealed a multiple expression tendency in three osteosarcoma cell lines (U2OS, HOS, and MG-63) and one in a normal cell line hFOB1.19, ***P < 0.001.

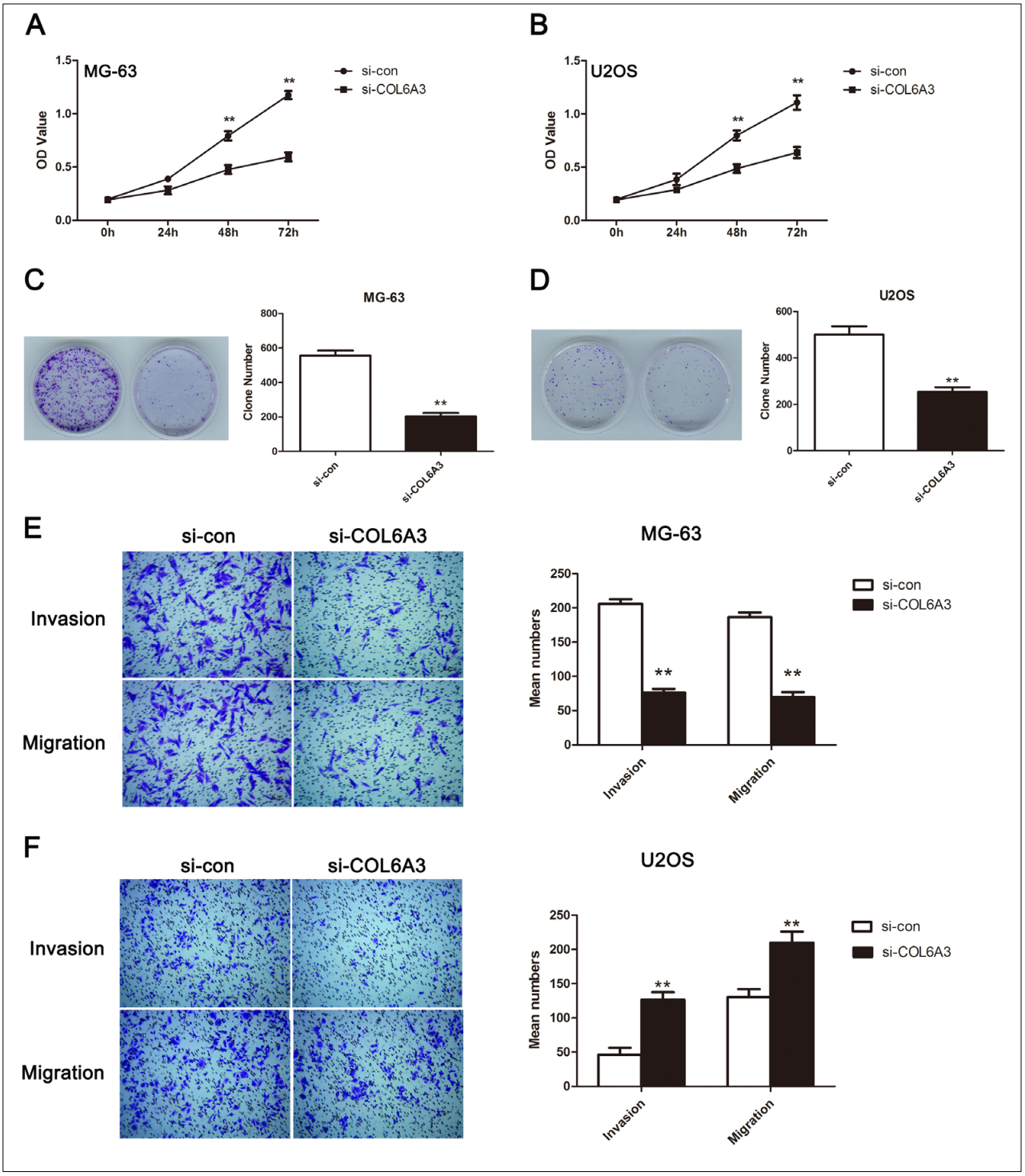

FIGURE 2. REDUCTION OF COL6A3 EXERTED NEGATIVE EFFECTS ON CELL MALIGNANT BEHAVIORS IN MG-63 AND U2OS CELLS. (A AND B) THE CELL VIABILITY OF OSTEOSARCOMA CELLS TRANSFECTED WITH SI-COL6A3 WAS HINDERED USING CCK-8 ASSAY, **P < 0.01. (C AND D), COLONY ACTIVITY WAS WEAKENED BY COLONY FORMATION ASSAY AFTER KNOCKDOWN OF COL6A3, **P < 0.01. (E AND F) SILENCING THE COL6A3 HAD NEGATIVE IMPACTS ON MIGRATORY AND INVASIVE ABILITIES IN OSTEOSARCOMA, **P < 0.01.

expression. Subsequently, we detected the COL6A3 expression in three osteosarcoma cell lines (U2OS, HOS, and MG-63) and one normal human cell line hFOB1.19. Compared with the normal human cell line hFOB1.19, COL6A3 showed diverse expression patterns (Fig.1C, P < 0.001). Based on the expression level of COL6A3, we selected the two cell lines U2OS and MG-63 for future experiments.

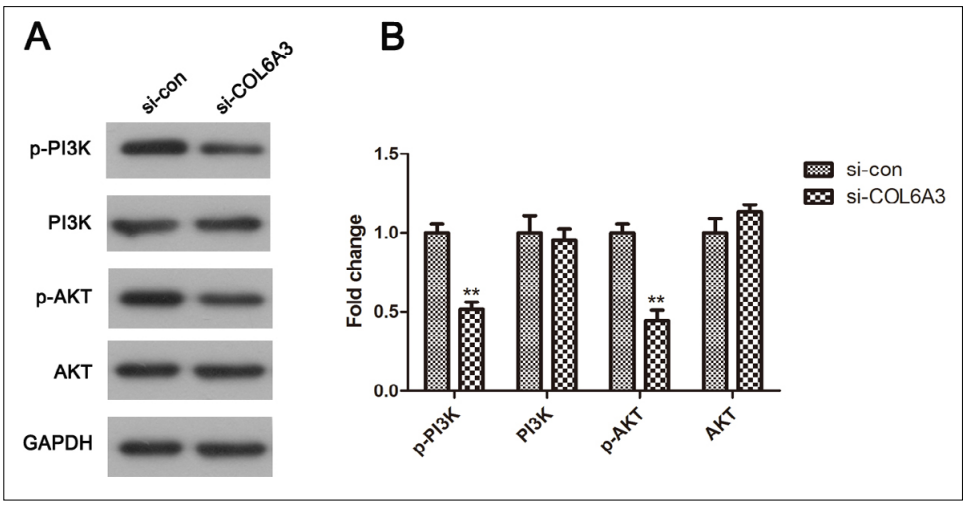

FIGURE 3. COL6A3 KNOCKDOWN WAS ASSOCIATED WITH THE INACTIVATION OF THE PI3K/AKT SIGNALING PATHWAY.

COL6A3 expression was down-regulated in U2OS and MG-63 cells

To verify the following results correctly, we used siRNA technology to knockdown the expression of COL6A3 and evaluate the silencing efficiency by qRT-PCR and western blot. As shown in Figure 1A and 2C, we observed that the interference of si-COL6A3 successfully decreased COL6A3 mRNA expression by qRT-PCR ($P<0.05$). Consistently, the western blotting results indicated that the COL6A3 protein expression level was inhibited in U2OS and MG-63 cells (Figure 1B and 2D, $P < 0.05$).

Depletion of COL6A3 inhibited cell proliferation, invasion, and migration in U2OS and MG-63

Next, we assessed the effects of knockdown COL6A3 on cell aggressive behaviors. The results of the CCK-8 assay revealed that the down-regulation of COL6A3 suppressed cell growth and an evident difference was observed at 48 h, 72 h between the si-COL6A3 group and si-con group in both MG-63 and U2OS cells (Figure2A and 2B, $P < 0.01$). In the same way, the clonogenic ability of MG-63 and U2OS cells transfected with si-COL6A3 also showed a downward trend (Figure2C and 2D, $P < 0.01$). To explore whether si-COL6A3 affected the capabilities of migration and invasion in osteosarcoma cells, we then performed a transwell assay. The representative images indicated that migratory and invasive cells in the si-COL6A3 group were fewer than in the si-con group in osteosarcoma cells (Figure2E and 2F, $P < 0.01$).

COL6A3 knockdown was associated with the inactivation of PI3K/AKT signaling pathway

We determined the protein expression level of the PI3K/AKT pathway-related markers, including p-PI3K/PI3K and p-AKT/AKT, in osteosarcoma cells. As illustrated in Figure 3A, U2OS and MG-63 cells transfected with si-COL6A3 exhibited a more down-regulated expression of p-PI3K/p-AKT than cells in the si-con group. The quantified analysis of gray value encouraged the above-mentioned results (Figure 3B, $P < 0.01$).

DISCUSSION

We found that COL6A3 was increased in osteosarcoma tissues and cells when compared with normal control. COL6 is expected to generate a flourishing condition for the development of tumors by cooperating with the extracellular matrix (ECM) rebuilding. Cell anchoring has also been proven to correlate with COL6[8-10]. Considering the mentioned researches, we predicted that COL6A3, which acts as a component of COL6, would participate in tumor progression. Furthermore, its function has been evaluated in several cancers, such as pancreatic[11], giant cell tumors[12], and prostate[13]. However, the effects of COL6A3 on osteosarcoma are still unclear. Hence, we performed this study to determine the biological potential of COL6A3 in osteosarcoma.

Next, we paid much attention to the molecular mechanism of how COL6A3 manipulates cell activities. It is well-known that the PI3K/AKT signaling pathway is one of the most important cancer-related

pathways[14]. The activation of the PI3K/AKT signaling pathway is recognized to exert a pivotal role in cell malignant motility in cancer progression[15,16]. In addition to that, COL6A3 has been reported to be involved in the progression of gastric cancer by regulating the PI3K/AKT signaling pathway[17]. The same function has also been indicated in esophageal cancer in the Chinese population[18]. Therefore, we conducted western blotting to demonstrate the expression level of the PI3K/AKT signaling pathway-related markers after knockdown of the COL6A3.

CONCLUSION

These findings propose a new perspective in the diagnosis and treatment of osteosarcoma, manifesting important clinical implications against osteosarcoma.

Conflicts of interest

They have no conflicts of interest.

Author Contributions

Conceptualization, Jia Sun; formal analysis, Ze-Long Song; writing (original draft preparation), Hong-Li Guo; writing (review and editing), Gang Chen; supervision, Xi-Hai Gao; funding acquisition, Yu-Xia Han

RESUMO

OBJETIVO: *Neste estudo, investigamos a função do COL6A3 na mobilidade celular e na via PI3K/AKT em osteossarcomas.*

METODOLOGIA: *A expressão relativa do COL6A3 foi obtida a partir de dados GEO em tecidos de osteossarcoma. O RNA de interferência (siRNA) foi utilizado para reduzir a expressão do COL6A3 nas células, e o teste de contagem de células kit-8 (CCK-8) e a análise de formação de colônias foram realizados para examinar o potencial de proliferação celular. Além disso, o Transwell comprovou os efeitos do si-COL6A3 na invasão celular e migração em células de osteossarcoma. Para medir os níveis de expressão das proteínas e mRNAs, utilizamos transcriptase reversa quantitativa (qRT-PCR) e western blot.*

RESULTADOS: *O COL6A3 foi regulado nos tecidos e células do osteossarcoma quando comparado com o controle normal. A redução de COL6A3 reduziu a proliferação e a capacidades de formação de colônias em relação ao COL6A3 sem interferência. Do Mesmo modo, ao contrário do observado no grupo si-con, a invasão e migração celular foram inibidas no grupo si-COL6A3. Além disso, o resultado do western blot sugere que a via PI3K/AKT foi manipulada, medindo a expressão proteica dos marcadores relacionados à PI3K/AKT, devido à inibição do COL6A3.*

CONCLUSÃO: *O COL6A3 desempenha um papel crucial na modulação de vários aspectos da progressão do osteossarcoma, o que pode representar um possível tratamento eficaz para a doença.*

PALAVRAS-CHAVE: *Osteossarcoma. Neoplasias de tecido ósseo. Terapia de alvo molecular. Colágeno tipo VI.*

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ORIGINAL ARTICLE

Can the neutrophil/lymphocyte ratio (NLR) have a role in the diagnosis of coronavirus 2019 disease (COVID-19)?

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SUMMARY

OBJECTIVE: *The present study aimed to investigate the role of neutrophil/lymphocyte ratio (NLR), an inflammation marker, complete blood count, and biochemical parameters in the diagnosis of COVID-19.*

METHODS: *A total of 80 patients who had been hospitalized in the internal medicine clinic were enrolled in the study. The cases were allocated into two groups, i.e., COVID (+) and (-), based on real-time reverse transcription-polymerase chain reaction. The demographic, clinical, and laboratory [NLR, platelet/lymphocyte ratio (PLR), complete blood count, biochemistry, and serology] data of the patients were retrospectively obtained from the hospital data management system.*

RESULTS: *NLR and fever levels were found to be higher in COVID-19 (+) cases (P=0.021, P=0.001, respectively). There was no difference between males and females with regard to COVID-19 positivity (P=0.527). Total bilirubin levels were found to be lower in COVID-19 (+) cases (P=0.040). When the ROC analysis was carried out for NLR in COVID-19 (+) cases, the AUC value was found to be 0.660 (P=0.021), sensitivity as 69.01 %, specificity as 65.40 %, LR+: 1.98 and LR- : 0.48, PPV: 80.43, and NPV: 50.00, when the NLR was ≥2.4. The risk of COVID-19 was found to be 20.3-fold greater when NLR was ≥ 2.4 in the logistic regression (P=0.007).*

CONCLUSION: *NLR is an independent predictor for the diagnosis of COVID-19. We also found that fever and total bilirubin measurements could be useful for the diagnosis of COVID-19 in this population.*

KEYWORDS: *Coronavirus Infections. Coronavirus. Fever. Lymphocytes. Neutrophils.3+q*

INTRODUCTION

Coronaviruses (CoVs) are single-chain, enveloped RNA viruses. They do not contain the RNA polymerase enzyme; however, they encode this enzyme in their genome. They are defined as CoV due to the protrusions on their surface (Latin: corona=crown)[1]. Coronaviruses belong to the *Orthocorona-virinae* sub-family and are classified as four types (alpha, beta, gamma, and delta CoVs) and multiple subspecies. Coronavirus 2019 is within the beta-coronavirus 2b strain. The genomes of the *beta-coronaviridae* were

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shown to be closely related to the bat SARS-like coronavirus[2]. This type of virus may be found in humans, bats, pigs, cats, dogs, remnants, and winged animals[3]. Coronaviruses are a large virus family that may lead to self-limited, mild, and common infections like the common cold, and also more severe infections like Severe Acute Respiratory Syndrome (SARS)[4] and Middle East Respiratory Syndrome (MERS)[5]. These viruses may lead to clinical conditions with various degrees of respiratory, enteric, hepatic, nephritic, and neurological involvement.

Pneumonia cases with unknown etiology and of suggested viral origin were reported in Wuhan, Hubei, China on 31 December 2019. The virus was shown in workers of the seafood wholesale market where different animal types are sold. The patients exhibited fever, dyspnea, cough, and, radiologically, bilateral pneumonic infiltrations[6]. Death usually occurred in individuals who were elderly or who had comorbid systemic diseases (hypertension, diabetes mellitus, cardiovascular diseases, cancer, chronic pulmonary diseases, and other immune-suppressive conditions)[7,8].

The pathophysiology of the high pathogenicity of this unusual highly contagious SARS-CoV2 could not be fully understood yet. Inflammation plays an important role in infectious diseases. Accumulating evidence has shown the importance of inflammation in the progression of viral pneumonia, including in coronavirus disease 2019 (COVID-19) cases[8]. Pro-inflammatory cytokines have been shown to increase in sera of patients with pulmonary inflammation[9]. The white blood cell (WBC) count, neutrophil, lymphocyte count, neutrophil/lymphocyte ratio (NLR), and platelet/lymphocyte ratio (PLR) are markers of systemic inflammation[10,11]. These markers are useful predictors for the prognosis and follow-up of patients with viral pneumonia. NLR is a very useful, rapid, and inexpensive indicator, the significance of which has been shown in bacterial pneumonia[12] and viral infections[13].

This retrospective, single-center study was conducted to investigate the complete blood count parameters, NLR, PLR, C-reactive protein (CRP), and the other infection bio-markers and biochemical data of a total of 80 COVID-19 positive and negative cases.

METHODS

A total of 80 patients who had been hospitalized at the medical clinic between 01 April 2020 and 25 April 2020 and tested for COVID-19 with real-time reverse transcription-polymerase chain reaction (rRT-PCR) were enrolled in the study. The nasal and pharyngeal swabs of all patients were obtained. Isolated patient samples that were obtained with VNAT viral transport and brought to the molecular virology laboratory were examined using the Biospedy (Bioeksen, Turkey) rRT-PCR kit provided by the Ministry of Health of Turkey. The patients whose rRT-PCR results were positive were regarded as COVID-19 (+), and those whose rRT-PCR results were negative twice with a 48-hour interval were regarded as COVID-19 (-). Hospital records (demographic, clinical, and laboratory data) of the cases above 18 years were analyzed retrospectively. The patients were divided into two groups, i.e., COVID-19 (+) and COVID-19 (-). Neutrophil, lymphocyte, platelets, MPV, hemoglobin, and CRP values of all patients were recorded, and the NLR and PLR values were calculated. The reports from the thoracic computed tomographies were obtained from the data management system. Serum urea, creatinine, total cholesterol, triglyceride, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin were analyzed using the kinetic alkaline picrate method with the Architect C 16000 (Abbott) device at the biochemistry laboratory of the hospital. The complete blood count parameters were examined with the Celldyn 3700 device. Ethics committee approval was obtained from the Ministry of Health of the Turkish Republic and the Sakarya University Medical School (No: 715224737050.01.04/131; April 04, 2020).

STATISTICAL ANALYSIS

Data analysis was performed by using statistical software (SPSS, version 10.0 [SPSS Inc, Chicago, IL]. Normally distributed data were compared by one-way analysis of variance, and non–normally distributed data were compared via the Mann-Whitney U test. Categorical associations were evaluated by using the $\chi 2$ test and multiple logistic regression. The goodness of fit was determined by using the Nagelkerke R^2 and Hosmer-Lemeshow goodness-of-fit test. The performance of NLR was assessed using receiver operating characteristic (ROC) curve analysis and by calculating the area under the curve (AUC) of the ROC curves. Statistical significance was defined as $P \leq 0.05$.

RESULTS

Of the total 80 patients, 39 (49%) were females and 41 (51%) were males. COVID-19 was determined to be positive in 54 out of the 80 cases (67.5%). The mean age (SD) was 53 (18) years for COVID-19 (+) patients and 60 (14) for COVID-19 (-) patients, and the difference was not statistically significant (F=3.029; P=0.086). Similarly, there was no difference between the groups concerning gender ($\chi 2$ =0.400 P=0.527). Fever was present in 41% of COVID-19 (+) cases. There was a significant difference between the groups concerning HDL-cholesterol values (F=4.984; P=0.031). The rates of fever, lactate, and ferritin levels were significantly higher in COVID-19 (+) cases compared to COVID-19 (-) cases (Mann-Whitney U=390.0, P<0.001; 152.0, P=0.040; 202.5, P=0.046; 396.0, P=0.008, respectively). The rates of total bilirubin level were significantly lower in COVID-19 (+) cases (Mann-Whitney U=152.0, P=0.040). While the NLR, PLR, and CRP values were significantly higher (Mann-Whitney U 477.5, P=0.021; 508.0, P=0.046; 448.5, P=0.012, respectively), the lymphocyte count was significantly lower (Mann-Whitney U 419.0, P=0.004) in COVID-19 (+) cases compared to COVID-19 (-) cases. There was no difference between COVID-19 (+) and (-) cases concerning WBC, neutrophil, platelet count, MPV, and procalcitonin. The demographic and laboratory characteristics of patients infected with and without COVİD-19 are shown in Table - I. The mean neutrophil/lymphocyte ratio and fever in COVID-19 (+) and (-) cases are displayed in Figure 1. The effect of NLR on the diagnosis of COVİD-19 was analyzed by ROC curve and AUC and was found to be significant (AUC:0.660; P=0.021, 95% CI 0.538 to 0.783) (Fig.2). Sensitivity, specificity, positive predictive value, negative NPV, LR+, LR- values, and the disease prevalence for NLR ≥ 2.4 were 69.01%, 65.40%, 80%, 50%, 1.98, 0.48 and 67.5%, respectively. The effect of fever on the diagnosis of COVİD-19 was analyzed by

TABLE 1. DEMOGRAPHIC AND LABORATORY CHARACTERISTICS OF PATIENTS INFECTED WITH AND WITHOUT COVİD-19.

Indicators	COVİD-19 (+) n=54	COVİD -19 (-) n=26	P-value
Mean age (SD), year	53(18)	60(14)	0.086
Men	29	12	0.635
Women	25	14	0.347
Lymphocyte (IR),K/uL	1.3(0.7)	2.0(1.0)	0.004
NLR median (IR)	4.7(2.8)	2.9(1.7)	0.021
Platelet (IR), K/uL	183(21)	221(43)	0.681
MPV (SD), fl	9(1.3)	9.2(1.1)	0.987
Hemoglobin (SD), gr/dL	12.4(1.7)	11.5(1.8)	0.033
PLR median (IR)	141(22)	104(14)	0.046
Kreatinin mg/dL	0.8(0.6)	0.6(0.4)	0.703
Total Cholesterol (SD), mg/dL	151(34)	158(49)	0.197
Triglycerides (IR), mg/dL	115(45)	80(23)	0.120
Low-density lipoprotein (SD), mg/dL	95(24)	109(29)	0.099
High-density lipoprotein (SD), mg/dL	30(9)	38(14)	0.031
Alanine aminotransferase (IR), U/L	33(27)	25(22)	0.170
Aspartate (IR), U/L	33(9)	28(8)	0.015
Albumin (SD), mg/dL	3.4(0.5)	3.4(0.6)	0.934
Protrombin zamanı (SD), s	12.7(1.4)	12.3(1.2)	0.304
INR (IR)	1.2(0.3)	1.0(0.2	0.016
Activated partial thromboplastin time (SD) , s	25(2.8)	26(3.4)	0.567
LDH (IR), U/L	322(17)	211(15)	0.016
Creatine kinase (IR), U/L	64(14)	71(18)	0.039
Ferritin(IR) µg/L	503(131)	108(51)	0.008
Total bilirubin (IR)mg/dL	0.64(0.1)	0.9(0.2)	0.040
d-Dimer (IR), ugFEU/L	633(176)	570(150)	0.934
CRP (IR) mg/L	89(88)	3.1(1.1)	0.012
Procalcitonin (IR)	0.09(0.2)	0.05(0.1)	0.945
Laktat (IR) mmol/L	1.7(1.5)	1.4(1.1)	0.046
Thorax computorize tomography (typical viral pneumonia sign)	10	2	0.204

ROC curve and AUC and was found to be significant (AUC:0.722; P=0.001, 95% CI 0.606 to 0.838). Sensitivity, specificity, positive predictive value, negative NPV, LR+, LR- values, and the disease prevalence for fever ≥ 36.8 were 66.67%,76.92%, 86%, 43%, 2.98, 0.43 and 67.5%, respectively.

We built a logistic regression model including NLR ≥ 2.4, temperature≥ 36.8, and serum total bilirubin as free predictors of a Covid-19 positive diagnosis. According to our model, the odds ratio for a covid-19-positive result was 20.3 and 10.5 when NLR was >2.4 and temperature was >36.8 (B=3.011, Standart Error=1.324, Wald=5.170, Odds ratio=20.3, P=0.023 for NLR; B=2.356, Standart Error=1.079, Wald=4.768, Odds ratio=10.5, P=0.029 for fever and B=-7.726, Standart Error=3.141, Wald=6.049, Odds ratio=0.0, P=0.014 for serum total bilirubin). The decrease of total serum bilirubin was significant for a covid-19-positive result, but without affecting the odds ratio. Nagelkerke R^2 was 65%.

DISCUSSION

In the present study, we reported the cohort of 54 COVID-19 (+) cases and 26 COVID-19 (-) cases confirmed with laboratory tests. NLR, fever, and total bilirubin levels were found to be significantly higher in COVID-19 (+) cases. There was no difference between COVID-10 (+) and (-) cases concerning age and gender.

Fever is among the most important clinical manifestations of CoV infections. In a study reported in the Lancet, fever was detected in 83% of the cases with COVID-19 pneumonia[14]; this rate was found to be 43.8% in another study[7]. The rate of fever was found to be 41% in our study. If the definition of case surveillance is mainly focused on the detection of fever, patients may be overlooked in the absence of fever, since fever was not detected in about half of the patients at the beginning. We determined a significant difference between COVID-19 (+) and (-) cases concerning fever. The risk of COVID-19 was found to be 10.5-fold greater when the fever was ≥36.8 degrees. Fever and CRP are not only systemic markers of inflammation but also mediators of inflammatory factors[15]. CRP was found to be high in COVID-19 patients in a previous study[16]. We also found CRP to be significantly high in our study.

The decrease of total serum bilirubin was significant for covid-19-positive results but without affecting the odds ratio. Also, AST levels were significantly higher in COVİD-19 cases; however, this increase was not observed for ALT. This was compatible with a study in China[17,18]. This elevation may be related to viral load and changes in the liver synthesis capacity. Other causes of changes in liver function include ACE2-mediated direct viral infection of hepatocytes or critically-ill status and immune-mediated injury.

Thrombocytopenia is another pathological finding that could be detected in a complete blood count[19].

FIGURE 1. MEAN NEUTROPHIL TO LYMPHOCYTE RATIO TEMPERATURE IN PATIENTS WITH AND WITHOUT COVID-19.

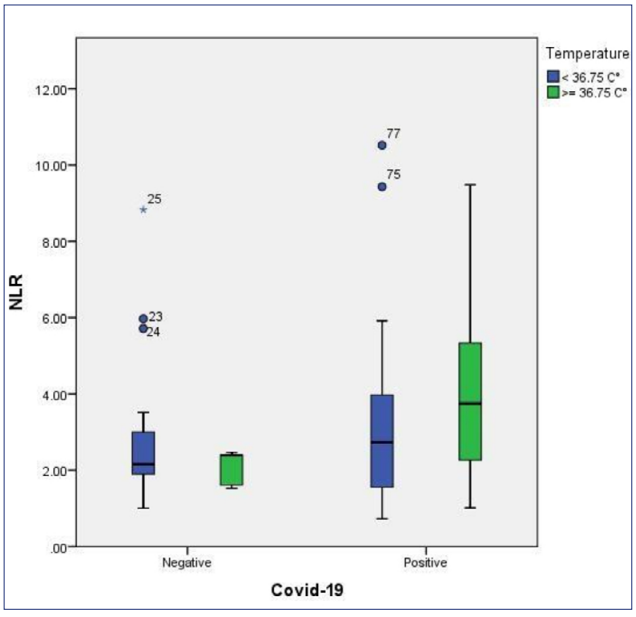
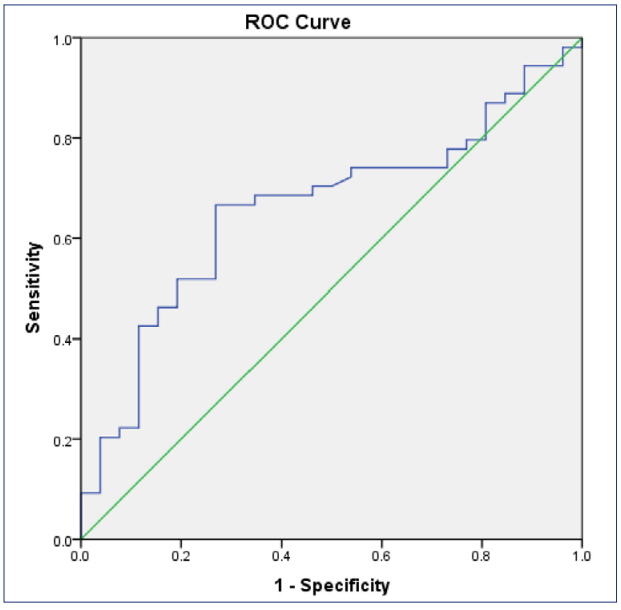


FIGURE 2. RECEIVER OPERATING CHARACTERISTIC CURVE FOR NEUTROPHIL TO LYMPHOCYTE RATIO IN PATIENTS WITH OR WITHOUT COVID-19.



Thrombocytopenia was detected in our study, consistent with the previous study; furthermore, PLR was significantly high. The platelet count, dynamic changes during treatment, and PLR were a source of concern in severe COVID-19 pneumonia cases. It was interpreted that PLR could serve as a novel indicator of the degree of cytokine storm[20].

In a study conducted in China, no difference was found between severe and moderate cases concerning the WBC count in the correlation analysis, and lymphopenia was reported to develop when the WBC count was normal[21]. Consistent with the previous study, we detected lymphopenia when the WBC and the neutrophil count were normal in hospitalized COVID-19 cases.

A decrease was determined in the peripheral blood lymphocyte count of critically ill COVID-19 patients[9,14,16]. Immune cells infiltrate the lungs and lead to unexplained severe lung infections[4]. In a study, the lymphocyte count was found to be <1.0x10^9/L (9). We found the lymphocyte value as 1.3x10^9/L, consistent with the previous studies.

The human immune response is created by lymphocytes triggered by viral infections[22]. Systemic infections suppress cellular immunity. The novel coronavirus may mainly act on lymphocytes, especially T lymphocytes[23]. The total lymphocytes, CD4+ T cells, CD8+ T cells, B cells, and NK cells decreased in COVID-19 patients, and severe cases had lower levels of these cells than mild cases[22,24]. Therefore, CoV-induced inflammation-related lymphopenia increased NLR. In the only study conducted before ours, the optimal threshold of 3.3 for NLR showed a superior prognostic possibility of clinical symptoms for change from mild to severe[7]. Our study was among the first studies in the literature. We found NLR to be high and the likelihood of COVID-19 was 20-fold greater when NLR was ≥2.4.

The results of rRT-PCR can be obtained in hours; hence the diagnosis and treatment may be delayed. The shortcomings of the PCR method due to false positive/false negative results from insufficient sampling, insufficient laboratory facilities due to the pandemic, samples collected too early or too late, and the binding sites of primer/probe couples used in the rRT-PCR lead to some difficulties in the diagnosis[25]. However, NLR is a rapid, inexpensive, and useful indicator that could be estimated via the complete blood count. The clinical use of NLR has been shown in bacterial pneumonia[12] and viral infections[13]. The surveillance of NLR and lymphocyte subsets is helpful in the early screening of critical illness, diagnosis, and treatment of COVID-19[17].

The COVID-19 pandemic may spread rapidly from human-to-human. The clinical characteristics of the disease vary among patients. The severity of the condition may be related to the number of immune cells.

CONCLUSION

In conclusion, we found that NLR was significantly elevated in COVID-19 patients. We also provided a cut-off for this readily available test and showed that patients with NLR ≥2.4 were 20.5 times more likely to have COVID-19 compared to patients whose NLR was ≤2.4. Similarly, the likelihood of COVID-19 was 10.5-fold greater when fever was ≥36.8 °C. This study indicates that high fever and NLR are independent biomarkers for COVID-19 patients. Our findings may also help in the early diagnosis of COVID-19.

Authors contribution

AN, CV & SY did data collection and manuscript writing; AN, TK & HC conceived, designed, and did the statistical analysis & editing of the manuscript; AT did the review and final approval of the manuscript; AN takes responsibility and is accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

RESUMO

OBJETIVO: *O objetivo do presente estudo foi investigar o papel da razão neutrófilos/linfócitos (RNL), um marcador de inflamação, hemograma completo e parâmetros bioquímicos no diagnóstico de COVID-19.*

MÉTODOS: *Um total de 80 pacientes internados na clínica médica foram incluídos no estudo. Os casos foram alocados em dois grupos, COVID (+) e (-), de acordo com a reação em cadeia da polimerase com transcrição reversa em tempo real. Os dados demográficos, clínicos e laboratoriais [NLR, relação plaquetas / linfócitos (PLR), hemograma completo, bioquímica e sorologia]) dos pacientes foram obtidos retrospectivamente no sistema de gerenciamento de dados hospitalares.*

RESULTADOS: *Os níveis de NLR e febre foram maiores nos casos de COVID-19 (+) (P = 0,021, P = 0,001,respectivamente). Não houve diferença entre homens e mulheres em relação à positividade para COVID-19 (P = 0,527). Os níveis totais de bilirrubina foram menores nos casos de COVID-19 (+) (P = 0,040). Quando a análise ROC foi realizada para NLR nos casos COVID-19 (+), o valor da AUC foi de 0,660 (P = 0,021), sensibilidade 69,01%, especificidade 65,40%, LR +: 1,98 e LR-: 0,48 , PPV: 80,43 e NPV: 50,00 quando o NLR era> 2,4. The risk of COVID-19 was found to be 20.3-fold greater when NLR was ≥ 2.4 in the logistic regression (P=0.007).*

CONCLUSÃO: *NLR é um preditor independente para o diagnóstico de COVID-19. Também concluímos que aferições de febre e bilirrubina total podem ser úteis para o diagnóstico de COVID-19 nesta população.*

PALAVRAS-CHAVE: *Infecções por Coronavirus. Coronavirus. Febre. Linfócitos. Neutrófilos.*

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ORIGINAL ARTICLE

Serum immunoglobulin a deficiency and autoimmune comorbidities: a crossectional study in 281 patients with systemic lupus erythematosus

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SUMMARY

OBJECTIVE: *To study the profile of associated autoimmune diseases in a series of patients with systemic lupus erythematosus (SLE) and see if such associations are linked to IgA deficiency.*

METHODS: *Two hundred eighty-one patients with SLE were studied for Ig A levels by nephelometry. Levels equal to or under 0.05g/dL were considered as IgA deficiency. Epidemiological and clinical data, including the presence of associated autoimmune diseases, were extracted from the patient's charts.*

RESULTS: *Ig A deficiency was found in 6% of the patients. In 30.2% of SLE patients, there was at least one more autoimmune disease; Hashimoto thyroiditis and Sjögren's syndrome were the most common. No association between the occurrence of associated autoimmune disease with IgA deficiency was found.*

CONCLUSIONS: *There is a high prevalence of autoimmune diseases associated with SLE. IgA deficiency does not affect the presence of these associations.*

KEYWORDS: *Lupus erythematosus, systemic. Immunoglobulin A. IgA deficiency. Autoimmune diseases. Hashimoto disease. Sjögren's syndrome.*

INTRODUCTION

The co-existence of more than one autoimmune disease in the same patient is a well-known clinical situation, although the reasons for this association are not fully explained. The existence of a shared genetic background that favors the rupture of immune tolerance is one of the hypotheses for this association[1]. Another reason proposed is the exposure to a common environmental trigger such as infections, drugs, pollutants[2,3], and even birth by cesarean section[4].

Autoimmune diseases (AID) are classified as systemic when the clinical profile reaches several tissues such as connective tissue diseases or organ-specific when a unique structure is affected[5]. Different combinations of these two types of situations are seen,

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generating an excessive burden on the patient. Chambers et al.[6] analyzed the association of systemic lupus with other AIDs and found that those with this association had more cumulative damage and higher mortality.

AIDs are considered to be more common in patients with immunoglobulin (Ig) A deficiency[7]. Ig A constitutes 15 to 20% of the total immunoglobulin pool in the body and it is primarily responsible for mucosal defense[8,9]. Ig A deficiency is one of the most common immune deficiencies, with a prevalence that shows variance according to the studied geographical area[9,10]. It is found in 1.96% of the general population in our region[11]. Autoimmune diseases linked to IgA deficiency are systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), thyroiditis, and celiac disease, among others[8,12].

In the present study, we aimed to know the autoimmune diseases that co-occur in a cohort of SLE patients and if this association was higher in those with IgA deficiency.

METHODS

This study was approved by the local Research Ethics Committee, and the participants signed informed consent. We included 281 individuals with a diagnosis of SLE, older than 18 years, fulfilling at least 4 criteria of SLE classification from the American College of Rheumatology (ACR)[12,13]. This is a convenience sample that includes all patients from a single Rheumatology Unit that came for regular consultation for one year and agreed to participate in the study. Patients charts were reviewed for epidemiological, serological, and clinical data as well as diagnosis of associated autoimmune diseases.

The diagnosis of associated RA was done when the patient met six or more 2010 Classification Criteria for RA from the ACR/ EULAR (European League against Rheumatism)[13]; the diagnosis of scleroderma was considered when 9 or more points of 2013 ACR/ EULAR classification criteria for scleroderma were completed[14]. The American/European Classification Criteria for Sjogren's syndrome were used to perform the diagnosis of Sjögren's syndrome[15]; the Bohan and Peter[16] criteria for myositis and the 2006 Sydney criteria for antiphospholipid antibody (APS) syndrome[17]. The diagnosis of morphea, cutaneous polyarteritis nodosa (PAN), vitiligo, alopecia areata, and psoriasis was done by a dermatologist and/or skin biopsy. Hashimoto thyroiditis (HT) was diagnosed when the patient had hypothyroidism or goiter and the presence of anti-thyroperoxidase antibodies[18]. Celiac and Chron's disease required a compatible intestinal biopsy to be considered present. The diagnosis of type 1 diabetes mellitus (DM) was established by an endocrinologist, and of autoimmune hepatitis by a hepatologist. To perform the diagnosis of pernicious anemia, the patient needed to have histologically proven atrophic gastritis, megaloblastic anemia, cobalamin deficiency, and antibodies for intrinsic factor or anti-parietal cell[19]. Neuromyelitis Optica or Devic's disease was diagnosed when the patient had characteristic clinical manifestations in the presence of serum aquaporin (AQP)4-IgG positivity and/or specific neuroimaging findings[20].

Ig A measurement was done in venous blood by nephelometry; patients with 50 mg/dL or lower were considered IgA deficient[21]. At the moment of blood collection, none of the participants was using gold salts, sulphasalazine, D-penicillamine, or phenytoin nor had HIV or hepatitis C infection, which are situations known to be associated with acquired IgA deficiency[7].

Data were collected in frequency and contingency tables. Autoimmune disease frequency was expressed in percentage. Patients with and without IgA deficiency had their number of autoimmune diseases compared between themselves by Fisher and chi-squared tests. Numeric data (age at disease diagnosis) were compared by the Mann Whitney test. The adopted significance was 5%.

RESULTS

In the 281 studied patients, 262/281 (93.2%) were females; the median age was 43 years (Interquartile rate or IQR= 34-53 years), and the median disease duration was 36 months (IQR=12-72 months); 100/255 (39.2%) were auto-declared afro descendants and 155/255 (60.7%) were auto-declared Caucasians.

The co-occurrence of autoimmune diseases was seen in 85/281 (30.2%) of the cohort: 61/85 (72%) had an additional organ-specific AID, and 54/85 (64%) had an additional systemic AID. Twenty patients (24%) had additional organ-specific and systemic AIDs.

In 58/281 (20.6%) of SLE patients there was one more autoimmune disease; in 21/281 (7.4%) there were two others, and in 6/281 (2.1%) there were 3 or more associated diseases. The frequency of associated diseases is presented in Table 1.

In this cohort, 17/281 (6.0%) had IgA deficiency. The comparison of the epidemiological profile and prevalence of autoimmune diseases in patients with and without IgA deficiency is shown in Table 2.

DISCUSSION

We observed a high number of associations of AIDs, since almost 1/3 (30.2%) of the studied cohort had, at least, one more autoimmunity. Our results are very similar to those of Chambers et al.[6], who found a prevalence of 33% of additional autoimmune disease in a multiethnic cohort of SLE patients. The presence of two or more autoimmune diseases in the same individual is known as polyautoimmunity, while the coexistence of three or more is called multiple autoimmune syndrome (MAS)[22]. This aggregation of autoimmunity has been described as the kaleidoscope of autoimmunity by Weiss and Shoenfeld[23], who also observed familial aggregation of this phenomenon. Family history of autoimmune disease and female gender are considered to be risk factors of polyautoimmunity in general populations[24]. MAS has been detected in 8%-12% of cases of SLE according to a review by Matusiewicz et al.[22]. We found that SLE plus at least 2 more associated DAI, characterizing MAS, in 27/281 (9.6%) of our cohort.

In the present study, the most common association seen was with Hashimoto thyroiditis and Sjogren's syndrome, which appeared in 11.7% and 7.1%, respectively. The same profile was also observed by Rojas-Villarraga et al.[24], who studied 1,083 individuals with connective tissue diseases in general, including 335 with SLE.

Our studied series of patients with SLE had a high proportion of IgA deficiency (6.0%) when compared to a population of blood donors from our regions (1.96%). The hypotheses that have been used to explain this association are: (1)- since IgA is responsible for mucosal defense, an increased number of infections at these sites may offer antigens that can cross-react to auto-antigens by molecular mimicry[25,26]; (2)- a

TABLE 1. FREQUENCY OF ASSOCIATED AUTOIMMUNE DISEASES IN A COHORT OF 281 SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS.

Organ-specific autoimmune diseases		
	Number	%
Hashimoto thyroiditis	33/281	11.7%
Vitiligo	8/281	2.8%
Pernicious anemia	4/281	1.4%
Autoimmune hepatitis	3/281	1%
Morphea	3/281	1.0%
Psoriasis	3/281	1.0%
Neuromyelitis Optica (Devic's)	2/281	0.7%
Celiac disease	1/281	0.3%
Crohn disease	1/281	0.3%
Alopecia areata	1/281	0.3%
Diabetes mellitus I	1/281	0.3%
Cutaneous polyarteritis nodosa	1/281	0.3%
Systemic autoimmune diseases		
Sjogren's syndrome	20/281	7.1%
Antiphospholipid antibody syndrome	18/281	6.4%
Scleroderma	9/281	3.2%
Rheumatoid arthritis	6/281	2.1%
Dermatomyositis	1/281	0.3%

TABLE 2. COMPARISON OF EPIDEMIOLOGICAL PROFILE AND ASSOCIATED AUTOIMMUNE DISEASE IN PATIENTS WITH AND WITHOUT AUTOIMMUNE DISEASES IN A COHORT OF 281 SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS.

	With IgA deficiency N=17	Without IgA deficiency N=264	P
Median age at diagnosis (IQR)	25 (18-41)	29.0 (21.0-39.0)	0.76
Females (n)	17/17 – 100%	245/264 – 92.8%	0.61
Afrodescendants ethnic	6/15 – 40%	94/238 – 39.4%	0.96
Patients with associated DAI	7/17 – 41.1%	78/264 – 29.5%	0.31
Systemic DAI (n)	2/7 – 28.5%	48/78 – 61.5%	0.11
Organ-specific DAI (n)	7/7 – 100%	47/78 – 60.2%	0.12
Systemic + organ-specific DAI (n)	2/7 – 28.5%	17/78 = 21.7%	0.65
Hashimoto thyroiditis	2/17 – 11.7%	31/264 – 11.7%	1.0
Sjogren's syndrome	1/17 -5.8%	19/264 – 7.1%	1.0
Scleroderma	0	9/264 – 3.4%	0.61
Antiphospholipid antibody syndrome	1/17- 5.8%	17/264 – 6.4%	1.0
Vitiligo	1/17 – 5.8%	7/264 – 2.6%	0.39

DAI= Autoimmune disease; IQR= interquartile rate; n= number.

common genetic background, such as the presence of HLA A1-B8-DR3, may predispose to autoimmunity and immune deficiency[27]; (3)- an abnormal T cell regulation in individuals with IgA deficiency, mainly T regulatory cells, that also favor autoimmunity[25].

Although we found a high prevalence of SLE in IgA deficiency, we could not prove that IgA deficiency favored the association of autoimmune diseases in general nor facilitates a particular combination of them. Even after finding a high number of associations, it is necessary to observe that, in our study, the absolute number of a particular AID was low and the size of our cohort of individuals with lupus was relatively small (281 patients); it may not have had enough strength to prove such associations. So, the value of our data is primarily descriptive rather than comparative. This is a limitation of the present study. Studies with larger samples are necessary to clarify the role of Ig A deficiency in this context. Another limitation is not studying the SLE cumulative damage for comparison between those with and without association with DAIs. However, this study does highlight the high prevalence of associated DAI on SLE, warning clinicians to look for them in order to provide good care for the patient.

CONCLUSIONS

There was a high prevalence of associated AID in our cohort of SLE, with MAS appearing in 9.6% of patients. Hashimoto thyroiditis and Sjogren's syndrome were the most commonly seen. The presence of IgA deficiency did not favor the appearance of associations.

Author' contribution

Gustavo Felício Alexandroni Linzmeyer - Project conception, data collection, bibliographic review, draft; Fabiane Karen Miyake - Data collection, bibliographic review, draft; Thiago Alberto F. C. Gomes Dos Santos - Project conception, draft, review; Thelma L Skare - Project conception, statistical analysis, review.

Conflict of interest

None

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RESUMO

OBJETIVO: *Estudar o perfil de doenças autoimunes associadas em uma série de pacientes com lúpus eritematoso sistêmico (LES) e verificar se tais associações estão ligadas à deficiência de imunoglobulina (Ig) A.*

MÉTODOS: *Foram estudados 281 pacientes com LES para os níveis de IgA por nefelometria. Níveis iguais ou menores que 0,05 g/dL foram considerados como deficiência dessa imunoglobulina. Dados epidemiológicos e clínicos, incluindo a presença de doenças autoimunes associadas, foram extraídos dos prontuários dos pacientes.*

RESULTADOS: *A deficiência de IgA foi encontrada em 6% dos pacientes. Em 30,2% dos pacientes com LES encontrou-se a presença de, pelo menos, mais uma doença autoimune. Tireoidite de Hashimoto e síndrome de Sjögren foram as mais comuns. Não foi possível ligar a ocorrência de uma doença autoimune associada ao LES com deficiência de IgA.*

CONCLUSÕES: *Existe uma alta prevalência de doenças autoimunes associadas ao LES. A deficiência de IgA não afeta a presença dessas associações.*

PALAVRAS-CHAVE: *Lúpus eritematoso sistêmico. Imunoglobulina A. Deficiência de IgA. Doenças autoimunes. Doença de Hashimoto. Síndrome de Sjögren.*

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ORIGINAL ARTICLE

Perception of risk factors for cancer in the ABC population

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SUMMARY

OBJECTIVE: *To evaluate the knowledge about risk factors for cancer in patients treated at the ABC Medical School (FMABC).*

METHODS: *Cross-sectional observational study conducted in 2019. The American Cancer Institute's Cancer Risk Awareness Survey questionnaire was used with 29 cancer risk factors, 14 of which were proven to cause cancer and 15 without consensus or scientific evidence of causality with cancer but that are often reminded by most of the population. Qualitative variables were described by frequency and percentage, and quantitative variables by mean and standard deviation or median and range depending on normality, assessed by the Shapiro-Wilk test. The study was conducted in accordance with the Helsinki Declaration for Research and approved by the Research Ethics Committee.*

RESULTS: *191 patients were included. Median age 54 (20 to 90), 64% female. 35.6% reported current or previous smoking. 3.1% consumed alcohol more than 5 drinks/week. 56% reported sedentary lifestyle. 44% had at least 1 case of cancer in relatives up to 2nd degree. The average of correct answers in the analyzed population was 12.83 ± 3.06. A weak positive correlation was observed between income and number of cases (rho = 0.177, p = 0.02). No relationship was observed between the number of correct answers and level of education, age, sex, marital status, race or patients with a positive family history for cancer.*

CONCLUSION: *The knowledge about risk factors for cancer in the ABC population is low, which may contribute to the adoption of risk behaviors for the disease.*

KEYWORDS: *Neoplasms. Perception. Risk factors.*

INTRODUCTION

Neoplasms, according to estimates by the World Health Organization (WHO), will be the biggest cause of death, overcoming coronary diseases[1]. Demographic and epidemiological transitions have caused an increase in cancer cases, especially in developing or underdeveloped countries[2].

In relation to Brasil, it is expected that there will be an increase in cancer mortality, especially in the

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North and Northeast regions of the country by 2030[3].

There are several risk factors that cause neoplasms, which are a sum of environmental and genetic factors. Thirty-five percent of cancer deaths worldwide could be attributed to the combined effect of nine risk factors, separated into five groups: diet and physical inactivity, addictive substances (use of tobacco and alcohol), sexual and reproductive health (sexually transmitted diseases), environmental risks (air pollution, solid fuels, passive smoking) and venous contamination by hepatitis B and C viruses[4].

The risk of cancer in a given population depends directly on the biological and behavioral characteristics of the individuals that compose it, as well as on the social, environmental, political and economic conditions that surround them. This understanding is essential in defining investments in risk assessment research and in effective prevention actions[5].

Even if we consider that the knowledge of the causal mechanism of different types of cancer is not complete, in practice, from the point of view of public health, the identification of only one component may be sufficient for major advances in prevention, based on the choice of preventive measures. Primary prevention, with an emphasis on factors associated with lifestyle at all ages and with interventions to fight environmental and occupational cancer agents, can bring good results in reducing cancer[5].

Thus, it is essential that the population knows the risk factors for the development of cancer in order to prevent them in primary care, thus avoiding spending on tertiary services. This study aims to assess the perception of risk factors for neoplastic diseases in patients treated at a specialty clinic at FMABC.

METHODS

Descriptive, cross-sectional and observational study, in which the perception of the population in the ABC region about the risk factors for neoplasms was evaluated.

The evaluation was carried out by means of a questionnaire to be answered by the patients who visit the outpatient care of clinical and surgical specialties at the ABC Medical School.

The inclusion criteria were signing the Informed Consent Form (ICF) and being a patient at the FMABC specialty outpatient clinics. The exclusion criterion was being under 18 years old.

In the questionnaire, epidemiological and demographic factors of the patient were addressed, such as age, sex, race, marital status. Patients' habits were evaluated: use of alcohol, smoking and illicit drugs; practice of physical activity; hours of sleep a day.

We also assessed personal and family history of neoplasms. Finally, we analyzed socioeconomic data, such as average family income in minimum wages.

To assess knowledge about risk factors, patients were asked whether or not the factor addressed is a risk factor for the development of cancer. The factors were selected based on the "American Institute for Cancer Research's 2015 Cancer Risk Awareness Survey Report"[6].

In addition, the perception of cancer as a disease was assessed using a questionnaire based on the Health Information National Trends Survey (Hints), which asks about the patients' perception of survival rates; pessimism in the face of the disease; the possibility of reducing the risk of the disease and the individual risk of developing cancer[7-9].

The collected data were tabulated and later submitted to statistical analysis.

Categorical variables were analyzed using frequency and percentages. For continuous variables, the description was made by mean or median, depending on the data distribution.

The Shapiro-Wilk test was performed to determine the parametric distribution or not of the data. For continuous data with normal distribution, we used the Pearson correlation test. For continuous non-parametric distribution data, we used the Spearman correlation test.

For qualitative data, the Fisher or Chi-2 test was used, depending on the sample size. Continuous data with qualitative outcome were assessed by the T test, for normal distribution data, and the Mann-Whitney test for non-normal distribution data.

RESULTS

191 patients were included. Epidemiological data are described in Table 1. Most patients were female (63%), married (54%), white (57%) and had completed high school (35%). The median age was 54 years, ranging from 20 to 90 years. The median income was BRL 2,500.00.

Regarding habits, 35% were smokers or former smokers, 13% drank at least once a week and 56% were sedentary.

Regarding the personal history of cancer, 12% had already had cancer, the most common being skin cancer (43%).

Regarding the questionnaire score, out of a total of 29 possible points, the median of correct answers was 13. A weak positive correlation was observed between income and number of correct answers (rho = 0.177, p = 0.02). No relationship was observed between the number of correct answers and educational level, age, sex, marital status, race or patients with a positive family history for cancer. (Table 1)

Regarding the perception of patients about risk factors for cancer, the most listed factors can be seen in Chart 1. The factor most mentioned by the participants was smoking, with 95%, followed by excessive exposure to sun (94%) and use of pesticides (76%). The least mentioned factor was asbestos (12%). (Chart 1)

Patients' perception of the cancer itself was also analyzed (Table 2). The majority (45%) believe that few patients remain alive after five years of diagnosis. In addition, 30% of patients agree that there is no way to effectively prevent cancer, and 65% believe that there are many recommendations for prevention and it is difficult to choose which ones to follow.

Thirty-seven percent disagree that cancer is caused primarily by lifestyle. Fifty-eight percent link cancer to death, and the majority (25%) believe they have more than a 50% chance of developing cancer in the future.

CHART 1. PATIENTS' PERCEPTION OF CANCER RISK FACTORS (RANKING)

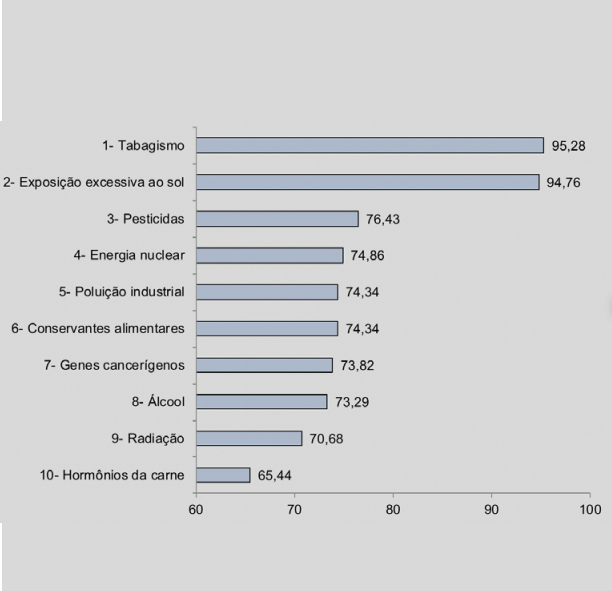

TABLE 1. EPIDEMIOLOGICAL, SOCIOECONOMIC AND HISTORY DATA FOR NEOPLASMS

Variable	N	%
Sex	191	
Male	69	36.12
Female	122	63.87
Marital Status	191	
Married	105	54.97
Single	56	29.31
Divorced	15	7.85
Widow(er)	15	7.85
Race	190	
White	109	57.36
Black	19	10
Brown	57	30
Yellow	5	2.63
Education	191	
Illiterate	5	2.61
Primary Education Uncompleted	44	23.03
Primary Education Completed	24	12.56
Secondary Education Uncompleted	10	5.23
Secondary Education Completed	67	35.07
College Education Uncompleted	7	3.66
College Education Completed	34	17.8
Drinking Habit	191	
Yes	25	13.08
No	166	86.91
Smoking	191	
Yes	23	12.04
No	123	64.39
Former Smoker	45	23.56
Use of Illegal Drugs	191	
Yes	1	0.52
No	190	99.47
Physical Activity	191	
Yes	83	43.45
No	108	56.54
Had cancer	191	
Yes	23	12.04
No	168	87.95
Types of cancer	23	
Skin	10	43.47
Lymphoma	3	13.04
Breast	2	8.69
Prostate	2	8.69
Others	6	26.08
History of cancer in the family	191	
Yes	84	43.97
No	107	56.02
Variable	N	Median and percentiles (25-75)
Age	191	54 (39-63)
Income	170	2,500 (1,500-4,000)
Final score	191	13 (11-15)

TABLE 2. PATIENTS' PERCEPTION OF CANCER (HINTS QUESTIONNAIRE)

Questionnaire questions	N	%
Number of people who develop cancer and stay alive after five years		
Few	87	45.54
Half	43	22.51
Many	42	21.98
Most	7	3.66
Don't know	12	6.28
There are many different recommendations on how to prevent cancer. It is hard to know which of them I should chose		
Agrees	126	65.96
Disagrees	41	21.46
Don't know	24	12.56
There is not much I can do to reduce the chances of developing cancer		
Agrees	58	30.36
Disagrees	118	61.78
Don't know	15	7.85
It seems that everything causes cancer		
Agrees	76	39.79
Disagrees	105	54.97
Don't know	10	5.23
Cancer is a disease that, when detected early, can be cured		
Agrees	180	94.24
Disagrees	8	4.18
Don't know	3	1.57
Someone can say they think they have cancer before it is diagnosed		
Agrees	52	27.22
Disagrees	133	69.15
Don't know	6	3.14
If I do regular exams to check if I have cancer, I can identify cancer when it is easier to treat		
Agrees	182	95.28
Disagrees	4	2.09
Don't know	5	2.61
Cancer is mainly caused by a person's behavior or lifestyle		
Agrees	103	53.92
Disagrees	71	37.17
Don't know	17	8.9
When I think of cancer, I automatically think of death		
Agrees	111	58.11
Disagrees	76	39.79
Don't know	4	2.09
What are the chances of you developing cancer in the future?		
Very low (0-25%)	46	24.08
Low (25-50%)	37	19.37
Medium (50-75%)	48	25.13
High (75-99%)	20	10.47
Very high (100%)	3	1.57
Don't know	37	19.37

DISCUSSION

Oncological diseases represent an important and growing cause of mortality in the world. Knowledge of risk factors for cancer is essential, as it allows the population to understand situations that can prevent new cases of this disease. This study evaluated the perception of risk factors for cancer in a representative sample of the population in the ABC region, in order to explore associations between demographic, socioeconomic data, family history of cancer and perception of cancer risk.

Several factors have already been established in relation to cancer risk, such as alcohol, obesity, lack of physical activity, diets low in vegetables and fruits, and diets rich in red meat. However, the population is increasingly concerned with factors over which they do not have direct control or which have an uncertain relationship with cancer, such as food additives and pesticide residues[6].

The American Institute for Cancer Research (AICR) continuous update project shows that a healthy life can decrease the incidence of cancer in up to – of the cases[6]. The only factors correctly identified by a large part of the population are tobacco and excessive exposure to the sun.

It was possible to observe in our study that there was a positive correlation between income and correct answers in the questionnaire for the perception of risk factors. This finding corroborates data in the literature that justify that individuals of higher socioeconomic level have greater access to information and greater access to some level of study. As a result, individuals with a higher income were more aware of the behavioral risk factors for cancer and also more aware of the behavioral protective factors against cancer[10].

When assessing the perception of cancer as a disease, the Hints questionnaire (Health Information National Trends Survey) was used. This questionnaire was created by the National Cancer Institute of the United States in order to fill the gap on information related to cancer and facilitate communication with the American population. By creating a cyclic population survey, which is repeated every two years, it is expected to assess the progress of health communication in terms of knowledge, attitudes and behaviors of the public studied in relation to cancer (REF).

In our study, a pessimistic attitude towards the disease was observed, since the majority said that few patients remain alive after five years of diagnosis and 58% see the disease as death.

In addition, ways of preventing cancer should be clarified in this population, as 30% of patients agree that they cannot effectively prevent cancer, and 65% believe that there are many recommendations for prevention and it is difficult to choose which to follow. In addition, 37% disagree that cancer is caused mainly by lifestyle, when in fact cancer is caused by genetic factors in only 5% to 10% of cases[11,12].

This is a cross-sectional and observational study, so cause and consequence relationships cannot be established.

CONCLUSIONS

Knowledge about risk factors for cancer in the population in ABC is low, which can contribute to the adoption of risk behaviors for the disease.

RESUMO

OBJETIVO: *Avaliar o conhecimento sobre fatores de risco para câncer em pacientes atendidos nos ambulatórios da Faculdade de Medicina do ABC (FMABC).*

MÉTODOS: *Estudo transversal e observacional conduzido em 2019. Foi utilizado o questionário Cancer Risk Awarness Survey do American Institute for Cancer Research com 29 fatores de risco para câncer, sendo 14 fatores comprovadamente causadores de câncer e 15 sem consenso ou evidência científica de causalidade com o câncer, mas que são frequentemente citados pela população. Variáveis qualitativas foram descritas por frequência e porcentagem, e variáveis quantitativas por média e desvio padrão ou mediana e intervalo a depender da normalidade, avaliada pelo teste de Shapiro-Wilk. Estudo realizado de acordo com a Declaração de Helsinque para pesquisa e aprovado pelo Comitê de Ética em Pesquisa.*

RESULTADOS: *Foram incluídos 191 pacientes. Mediana de idade 54 (20 a 90), 64% do sexo feminino; 35,6% reportaram tabagismo atual ou anterior; 3,1% consumiam mais que cinco doses de álcool por semana; 56% reportaram sedentarismo; 44% tinham pelo menos um caso de câncer em parentes de até segundo grau. A média de acertos na população analisada foi 12,83 ± 3,06. Foi observada correlação positiva fraca entre renda e número de acertos (rho=0,177, p=0,02). Não foram observadas relações entre o número de acertos e nível de escolaridade, idade, sexo, estado civil, cor ou pacientes com história familiar positiva para câncer.*

CONCLUSÃO: *O conhecimento sobre fatores de risco para câncer na população do ABC é baixo, o que pode contribuir para a adoção de comportamentos de risco para a doença.*

PALAVRAS-CHAVE: *Neoplasias. Percepção. Fatores de risco.*

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ORIGINAL ARTICLE

Comparison of radiological scoring systems, clinical scores, neutrophil-lymphocyte ratio and serum C-reactive protein level for severity and mortality in acute pancreatitis

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SUMMARY

Comparison of radiological scoring systems, clinical scores, neutrophil-lymphocyte ratio and serum C-reactive protein level for severity and mortality in acute pancreatitis

BACKGROUND/AIMS: *To compare radiological scoring systems, clinical scores, serum C-reactive protein (CRP) levels and the neutrophil-lymphocyte ratio (NLR) for predicting the severity and mortality of acute pancreatitis (AP).*

MATERIALS AND METHODS: *Demographic, clinical, and radiographic data from 80 patients with AP were retrospectively evaluated. the harmless acute pancreatitis score (HAPS), systemic inflammatory response syndrome (SIRS), bedside index for severity in acute pancreatitis (BISAP), Ranson score, Balthazar score, modified computed tomography severity index (CTSI), extrapancreatic inflammation on computed tomography (EPIC) score and renal rim grade were recorded. The prognostic performance of radiological and clinical scoring systems, NLR at admission, and serum CRP levels at 48 hours were compared for severity and mortality according to the revised Atlanta Criteria. The data were evaluated by calculating the receiver operator characteristic (ROC) curves and area under the ROC (AUROC).*

RESULTS: *Out of 80 patients, 19 (23.8%) had severe AP, and 9 (11.3%) died. The AUROC for the BISAP score was 0.836 (95%CI: 0.735-0.937), with the highest value for severity. With a cut-off of BISAP ≥2, sensitivity and specificity were 68.4% and 78.7%, respectively. The AUROC for NLR was 0.915 (95%CI: 0.790-1), with the highest value for mortality. With a cut-off of NLR >11.91, sensitivity and specificity were 76.5% and 94.1%, respectively. Of all the radiological scoring systems, the EPIC score had the highest AUROC, i.e., 0.773 (95%CI: 0.645-0.900) for severity and 0.851 (95%CI: 0.718-0.983) for mortality, with a cut-off value ≥6.*

CONCLUSION: *The BISAP score and NLR might be preferred as early determinants of severity and mortality in AP. The EPIC score might be suggested from the current radiological scoring systems.*

KEYWORDS: *Pancreatitis, acute necrotizing. Pancreatitis. Mortality. C-reactive protein. Neutrophils. Lymphocyte count.*

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INTRODUCTION

Acute pancreatitis (AP) is usually a self-limiting disease with minimal systemic effects and good outcomes. However, in 10-20% of the cases, the clinical course results in systemic inflammatory response syndrome (SIRS) and multiple organ failure[1]. This severe form of the disease is associated with a mortality of up to 50%. Early recognition of patients with the severe presentation of the disease has been shown to improve prognosis and reduce mortality by establishing close monitoring, early intensive therapy, and proper timing of interventions[2].

Older age, obesity, alcohol and tobacco consumption, hematocrit, serum urea and creatinine levels, a variety of cytokines, chemokines, and other inflammatory response markers have been shown to be associated with severity and mortality of AP. Besides the routine clinical and laboratory data, the presence of systemic inflammatory response syndrome (SIRS), harmless acute pancreatitis score (HAPS), multifactorial clinical and laboratory scoring systems, such as the Ranson score, bedside index of severity in AP (BISAP) score, acute physiology and chronic health evaluation II (APACHE II) score, have been identified to predict severity and prognosis of AP[3-6]. The Balthazar grade, modified computed tomography severity index (MCTSI), extrapancreatic inflammation on computed tomography (EPIC) score, and renal rim sign are among the radiologic scoring systems used in the assessment of AP prognosis. Among all these current scoring systems, APACHE II (score ≥8) is regarded as the gold standard[7].

Among several serum biochemical markers, serum procalcitonin (>1.8 ng/mL) and C-reactive protein (CRP) ≥ 150 mg/L at 48 hours post-admission have been adopted as prognostic factors for the management of AP[8]. Also, serum levels of the inflammatory mediators interleukin (IL) 6, 8, and IL-10 have been found to be accurate for predicting persistent organ dysfunction in AP patients[9,10]. However, these serum markers are expensive, not readily available, and cannot adequately predict the prognosis or severity of AP. Recently, neutrophil-lymphocyte ratio (NLR), platelet to lymphocyte ratio, and peripheral blood CD4+ T cell count have been proposed as widely available markers that provide a rapid evaluation of the extent of the inflammatory process in AP patients[11,12].

Estimation of the magnitude of the inflammatory response to injury in AP patients during the first 48 hours of hospitalization is valuable to guide the appropriate screening of patients, predict complications, and decide whether or not intensive support will be needed. However, comparative data on the clinical and radiological scoring systems for predicting the outcomes in AP are limited and controversial[13]. The purpose of this study was to compare clinical and radiological scoring systems, the serum CRP level, and NLR in predicting the severity and mortality of AP.

METHODS

Patient population

The study protocol was approved by the institute ethics committee. The demographic and clinical data of AP patients between the ages of 18-100 years who were admitted to our hospital and hospitalized from 2015 to 2018 were retrospectively collected from our hospital's written and electronic medical records. A total of 80 patients with AP whose computed tomography (CT) images were obtained 48-72 hours after the onset of symptoms and 3-4 weeks after the initial imaging were included in the study.

The patients who presented symptoms for more than 3 days at admission, with incomplete clinical data, early or late initial CT examination were excluded from the study. The patients who did not undergo CT 3-4 weeks after the initial imaging or who had chronic pancreatitis were not included in the study.

Diagnostic criteria and classification of AP

The criteria for AP diagnosis was the presence of at least two of the following three manifestations: acute onset of characteristic upper abdominal pain (persistent, severe, and usually radiating to the back), elevated levels of pancreatic enzymes (serum amylase and/or lipase > 3 times the upper limit of normal), and findings of ultrasonography (US), CT, or MRI suggesting AP. CT images obtained 3-4 weeks after the initial imaging were assessed to determine the severity of the disease and diagnosis of necrotic pancreatitis.

The etiology of AP was classified as gallstones, alcohol, hyper-triglyceridemia, and others (hypercalcemia, drugs, traumatic, autoimmune, endoscopic procedures). In the remaining cases, the etiology was classified as unknown.

The study group was categorized into mild-moderate and severe AP groups according to the revised Atlanta Criteria[14].

Data collection

HAPS was calculated based on the presence of guarding or rebound tenderness, serum creatinine level (abnormal, ≥ 2 mg/dL), and hematocrit level (abnormal, > 43% in men and > 39.6% in women) on presentation. The BISAP score was calculated using data from the first 24 hours following admission, based on how many of the following characteristics the patient presented: blood urea nitrogen level > 25 mg/dL, impaired mental status, systemic inflammatory response syndrome, age > 60, and pleural effusion on imaging studies. Each determinant was given one point. The Ranson score was calculated using data from the first 48 hours following admission. The presence of features of systemic inflammatory response syndrome (SIRS) was recorded within 24 hours of admission. NLR at admission and serum CRP level (mg/L) at 48 hours were also recorded.

CT images obtained 48-72 hours after the onset of symptoms were assessed for radiologic scoring systems. The Balthazar score was determined and MCTSI was calculated by the sum of the Balthazar and necrosis scores. The grades of the Balthazar score were as follows: Grade A (0 points) for normal CT, grade B (1 point) for focal or diffuse pancreatic enlargement, grade C (2 points) for peripancreatic inflammation or gland abnormalities, grade D (3 points) for single fluid collection, and grade E (4 points) for two or more fluid collections or adjacent gas bubbles. Contrast-enhanced CT images were scored for necrosis as follows: 2 points if regions of necrosis were less than the equivalent in size to the pancreatic head (< 30%), 4 points for necrosis of 30-50% of the gland, and 6 points for necrosis of more than 50% of the gland. Necrosis was scored as 0 if uniform pancreatic enhancement was observed. The EPIC score was determined based on extrapancreatic complications. The components of the EPIC score are presented in Table 1.

TABLE 1. COMPONENTS OF EXTRAPANCREATIC INFLAMMATION ON COMPUTED TOMOGRAPHY SCORE.

Pleural effusion	Points
None	0
Unilateral	1
Bilateral	2
Ascites in any of these locations: perisplenic, perihepatic, interloop, pelvis	
None	0
One location	1
More than one location	2
Retroperitoneal inflammation	
None	0
Unilateral	1
Bilateral	2
Mesenteric inflammation	
Absent	0
Present	1

The renal rim grade was scored as grade 1 when increases in the attenuation of the anterior pararenal and the perirenal spaces were observed. Grade 2 was defined as increases in the attenuation of the pancreatic side of the Gerota's fascia (pararenal space). Grade 3 was characterized as increases in the attenuation of both the pararenal and the perirenal spaces.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (SPSS Inc, Chicago, Illinois). Continuous variables are presented as mean ± SD. The prognostic performance of all radiological and clinical scoring systems, NLR, and serum CRP levels were compared for mortality and severity according to the revised Atlanta Criteria. The data were also evaluated regarding the ability of each scoring system to predict AP mortality and severity by calculating receiver operator characteristic (ROC) curves using the MedCalc software (SolidWorks, Concord, MA). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each individual scoring system were calculated based on the Youden index. The area under the ROC (AUROC) was used to evaluate the discriminative ability of ten parameters (HAPS, BISAP score, Ranson score, NLR, CRP level, Balthazar score, MCTSI, EPIC score, and renal rim grade) for predicting severity and mortality. The association of SIRS with severity and mortality was analyzed using the Chi-square test. A P-value < 0.05 was considered significant.

RESULTS

The present study enrolled 80 AP patients between the ages of 18 and 85 years (mean 55±17). Men constituted 42.5% of the study group. The main cause for AP was cholelithiasis, diagnosed in 57.5% of patients, followed by alcohol (7.6%), hyper-triglyceridemia (8.8%), and others (5.1%). In the remaining cases (21%), the etiology was unknown. Necrotic pancreatitis confirmed by CT scan was diagnosed in 26 (32.5%) patients. Severe AP was diagnosed in 19 (23.8%) patients, and 9

of them (11.3%) died. The patients' mean ages were 52 and 64 years in mild-moderate and severe AP groups, respectively. Nineteen patients (23.8%) developed persistent organ failure. Nine of them (11.3%) had pulmonary insufficiency, 4 (5%) had renal failure, and 6 (7.5%) developed both pulmonary insufficiency and renal failure.

Comparison of scoring systems, NLR, and CRP levels for severity

The ROC curves of the scoring systems yielded an AUC of 0.616 (95% CI, 0.474–0.757) for HAPS, 0.836 (95% CI, 0.735–0.937) for the BISAP score, 0.690 (95% CI, 0.548–0.831) for the Ranson score, 0.726 (95% CI, 0.565–0.886) for NLR, 0.728 (95% CI, 0.578–0.878) for CRP level, 0.651 (95% CI, 0.521–0.782) for the Balthazar score, 0.619 (95% CI, 0.495–0.744) for MCTSI, 0.773 (95% CI, 0.645–0.900) for the EPIC score, and 0.836 (95% CI, 0.735–0.937) for renal rim grade in predicting severe AP (Figure). The best cutoff values calculated for the HAPS, BISAP score, Ranson score, NLR, CRP level, Balthazar score, MCTSI, EPIC score, and renal rim grade were > 1, 1, 1, 6.66, 204.3, 3, 3, 5, and 1, respectively. Using these cutoff values, the sensitivity, specificity, PPV, and NPV of various scoring systems in predicting severe AP were calculated. Performance statistics of variables for predicting severity in AP are shown in Table 2. The presence of SIRS was significantly associated with severe AP (P=0.002).

Comparison of scoring systems, NLR and CRP level for mortality

The receiver-operating characteristic (ROC) curves yielded an AUC of 0.660 (95% CI, 0.489–0.830) for HAPS, 0.847 (95% CI, 0.711–0.982) for the BISAP score, 0.669 (95% CI, 0.479–0.859) for Ranson, 0.915 (95% CI, 0.790–1) for NLR, 0.799 (95% CI, 0.558–0.1) for CRP level, 0.690 (95% CI, 0.548–0.832) for the Balthazar score, 0.615 (95% CI, 0.481–0.749) for MCTSI, 0.851 (95% CI, 0.718–0.983) for the EPIC score, and 0.564 (95% CI, 0.735–0.937) for renal rim grade in predicting severe AP.

The best cutoff values calculated for the HAPS, BISAP score, Ranson score, NLR, CRP level, Balthazar score, MCTSI, EPIC score, and renal rim grade

TABLE 2. PERFORMANCE STATISTICS OF VARIABLES FOR PREDICTING SEVERITY IN ACUTE PANCREATITIS.

Variable	AUC*	Cutoff	Sensitivity*	Specificity*
HAPS	0.616 (0.474-0.757)	≥ 2	57.9 (33.5-79.7)	62.3 (49-74.4)
BISAP score	0.836 (0.735-0.937)	≥ 2	68.4 (43.4-87.4)	78.7 (66.3-88.1)
Ranson score	0.690 (0.548-0.831)	≥ 2	63.2 (38.4-83.7)	68.8 (55.7-80.1)
NLR	0.726 (0.565-0.886)	> 6.66	94.4 (72.7-99.9)	52.5 (39.3-65.4)
CRP	0.728 (0.578-0.878)	> 204.3	75 (47.6-92.7)	63.2 (49.3-75.6)
Balthazar score	0.651 (0.521-0.782)	≥ 4	89.5 (66.9-98.7)	41 (28.6-54.3)
MCTSI	0.619 (0.495-0.744)	≥ 4	94.7 (74-99.9)	41 (28.6-54.3)
EPIC score	0.773 (0.645-0.900)	≥ 6	63.2 (38.4-83.7)	82 (70-91)
Renal rim grade	0.706 (0.574-0.839)	≥ 2	47.4 (24.4-71.1)	85.2 (73.8-93)

*95% confidence interval is shown in parentheses. AUC = area under the curve, HAPS = harmless acute pancreatitis score, BISAP = bedside index of severity in acute pancreatitis, NLR = neutrophil-lymphocyte ratio, CRP = serum C-reactive protein, MCTSI = CT severity index, EPIC = extrapancreatic inflammation on computed tomography

TABLE 3. PERFORMANCE STATISTICS OF VARIABLES FOR PREDICTING MORTALITY IN ACUTE PANCREATITIS.

Variable	AUC	Cutoff	Sensitivity*	Specificity*
HAPS	0.660 (0.489-0.830)	≥ 2	66.7 (29.9-92.5)	60.6 (48.3-72)
BISAP score	0.847 (0.711-0.982)	≥ 2	77.8 (40-97.2)	73.2 (61.4-83.1)
Ranson score	0.669 (0.479-0.859)	≥ 3	44.4 (13.7-78.8)	83.1 (72.3-91)
NLR	0.915 (0.790-1)	> 11.91	61.5 (31.6-86.1)	73.8 (61.5-84)
CRP	0.799 (0.558-1)	> 229	78.6 (49.2-95.3)	70.7 (57.3-81.9)
Balthazar score	0.615 (0.481-0.749)	≥ 4	100 (76.8-100)	41.5 (29.4-54.4)
MCTSI	0.615 (0.481-0.749)	≥ 4	100 (76.8-100)	40 (28-52.9)
EPIC score	0.851 (0.718-0.983)	≥ 6	78.6 (49.2-95.3)	81.5 (70-90.1)
Renal rim grade	0.564 (0.379-0.749)	≥ 2	42.9 (17.7-71.1)	81.5 (70-90.1)

*95% confidence interval is shown in parentheses. AUC = area under the curve, HAPS = harmless acute pancreatitis score, BISAP = bedside index of severity in acute pancreatitis, NLR = neutrophil-lymphocyte ratio, CRP = serum C-reactive protein, MCTSI = CT severity index, EPIC = extrapancreatic inflammation on computed tomography

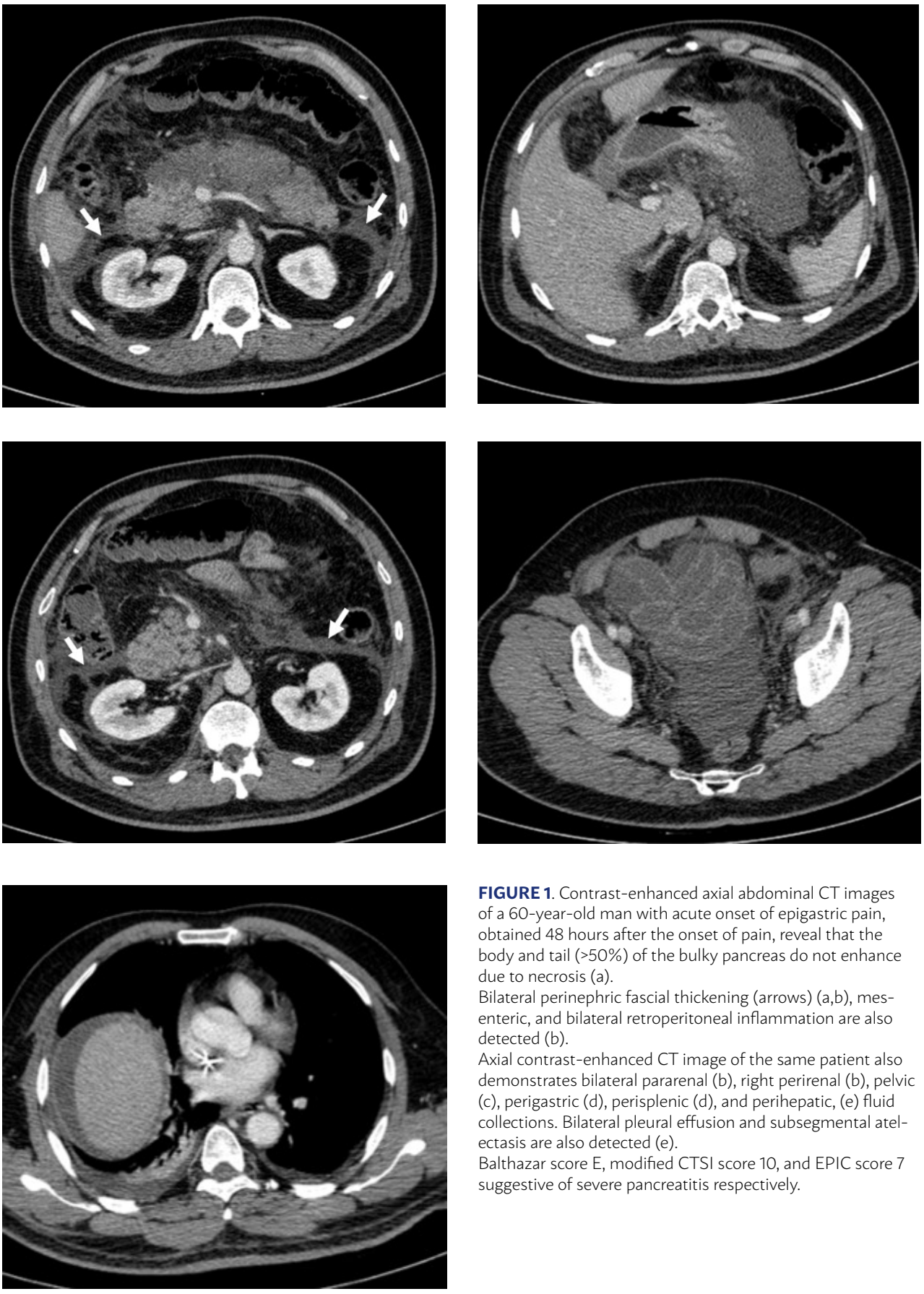

FIGURE 1. Contrast-enhanced axial abdominal CT images of a 60-year-old man with acute onset of epigastric pain, obtained 48 hours after the onset of pain, reveal that the body and tail (>50%) of the bulky pancreas do not enhance due to necrosis (a).
Bilateral perinephric fascial thickening (arrows) (a,b), mesenteric, and bilateral retroperitoneal inflammation are also detected (b).
Axial contrast-enhanced CT image of the same patient also demonstrates bilateral pararenal (b), right perirenal (b), pelvic (c), perigastric (d), perisplenic (d), and perihepatic, (e) fluid collections. Bilateral pleural effusion and subsegmental atelectasis are also detected (e).
Balthazar score E, modified CTSI score 10, and EPIC score 7 suggestive of severe pancreatitis respectively.

were >1, 1, 2, 11.91, 229, 3, 3, 5, and 1, respectively. Using these cutoff values, the sensitivity, specificity, PPV, and NPV of the various scoring systems in predicting severe AP were calculated. the performance statistics of the variables for predicting mortality in AP are shown in Table 3. The presence of SIRS was significantly associated with mortality (P=0.001).

DISCUSSION

In this study, the BISAP score was the most useful among all clinical and radiological scoring systems for severity assessment. A BISAP score ≥ 2, was the most valuable of all variables to predict severe cases of AP with a sensitivity and specificity of 68.4% and 78.7%, respectively. On the other hand, NLR had the highest value to predict mortality. With a cut-off value of > 11.91, the sensitivity and specificity of NLR were 76.5% and 94.1%, respectively. Among the radiological scoring systems, the EPIC score had the highest value to predict both severity and mortality of AP. With a cut-off value of ≥ 6 for the EPIC score, the sensitivity and specificity for severity were 63.2% and 82%, while sensitivity and specificity for mortality were 78.6% and 81.5%.

In a recent review by Shah et al.[7], it was declared that the APACHE II scoring system had the highest accuracy for predicting severe AP when compared with other scoring systems. However, the APACHE II scoring system is exhaustive and cannot be widely adopted for AP patients outside the intensive care setting[15]. Also in a previous study, it was demonstrated that the APACHE II score had just a 67% PPV at 24 h after admission and was even less accurate for identifying patients with specific complications such as peripancreatic fluid collections or major organ failure[16]. In recent years, researchers have been interested in determining the most practical and accurate parameter indicative of the severity and prognosis of AP. Some researchers have found that no statistically significant pairwise differences were observed between the APACHE-II and the other scoring systems, including CRP value at 24 hours, BISAP, Ranson, and Balthazar scores[2,7,17]. Leung et al.[18] found that the Ranson and APACHE II scores had lower sensitivity for complications, mortality, and the length of stay for AP than the Balthazar score. On the other hand, in a study of 105 patients with AP, Sharma et al.[13] found that BISAP (best cutoff value ≥ 3) was more valuable than CT scores (Balthazar score, MCTSI, EPIC, renal rim) in terms of correlation with organ failure. For this reason, they suggested that performing a CT solely for predicting the severity of AP may not provide additional information to what is already provided by BISAP. In our study, even with a lower cutoff value (≥ 2) we found BISAP was the most useful for severity assessment in comparison with other variables (HAPS, Ranson score, NLR, CRP level, Balthazar score, MCTSI, EPIC score, and renal rim grade). Chen et al.[17] also found that the best cutoff value for BISAP was 2 in predicting severity. They compared the BISAP, APACHE II, and Ranson scoring systems in 497 patients and found that the BISAP performed similarly to the Ranson and APACHE II scores in predicting severe AP in terms of AUC, sensitivity, and specificity, suggesting that the BISAP is a reliable scoring system for predicting severity (AUC of 0.762 for BISAP, 0.755 for APACHE II, and 0.801 for Ranson).

Chen et al.[17] also demonstrated that the Balthazar score was superior to other variables (AUC of 0.762 for BISAP, 0.755 for APACHE II, and 0.801 for Ranson) in predicting mortality. On the other hand, Sharma et al.[13] found that the BISAP had the highest value (AUC 0.90) for predicting mortality in AP compared with the SIRS, Balthazar score, MCTSI, Renal rim grade, and EPIC score. They declared that BISAP 3-5 had 100% sensitivity and 75.3% specificity for mortality. In our study, we demonstrated that even a lower BISAP cutoff value (≥2) was valuable for predicting mortality (AUC 0.847). On the other hand, Sharma et al.[13] did include NLR in their study, which was found to be the most valuable for predicting mortality among all variables, including BISAP in our study (AUC 0.915) with a cutoff value ≥ 11.9.

CRP has also been proven to be a reliable and easily accessible marker in AP, providing good prognostic accuracy for severity assessment, prediction of pancreatic necrosis, and in-hospital mortality when measured at 48-72 hours following hospital admission[8,19]. Thus, CRP ≥ 15 mg/dL is adopted as a prognostic factor in AP. However, in our study, another inexpensive and widely available parameter, NLR, had almost the same value as CRP for predicting severe AP and even markedly higher value to predict mortality than CRP value.

Recently, many researchers have investigated the value of NLR in predicting severity and outcomes across a variety of conditions, such as inflammation, cardiovascular disease, neoplastic states, and

preeclampsia[11,20,21]. As an indicator of uncontrolled SIRS and its progression to multi-organ dysfunction syndrome, increased NLR has also been shown to be associated with poor outcomes in severe AP. Although activation and modulation of neutrophils and platelets play a central role in establishing host defenses in settings of systemic inflammation, excessive inflammatory response results in massive cell transmigration to the pancreas, which in turn results in the destruction of the pancreas and organ failure subsequent to release of aggressive defense molecules[22]. Azab et al.[23] were the first to demonstrate that NLR was significantly increased in patients who developed severe AP. Jeon and Park[24] also demonstrated that high baseline NLR was associated with severe AP and organ failure. Han et al.[25] declared that NLR on admission within 48h had the highest AUC for predicting severe AP, with a cut-off value of 6.66, and NLR was also significantly positively correlated with the Ranson score and hospital stays. Zhang et al.[11] suggested that high NLR is associated with persistent organ failure, extended duration of intensive care, and also a higher mortality rate. In line with their study, we found that NLR had the highest value to predict mortality compared with other scoring systems and biomarkers. But in contrast with the literature, the BISAP score was the most useful for severity assessment in our study among all the variables. Compatible with our findings, Gulen et al.[26] found that NLR was not a significant independent prognostic factor for mortality in AP compared with HAPS and red-cell distribution. A superior aspect of our study in comparison with previous literature is that we were able to compare numerous scoring systems and biomarkers.

In line with the literature, the presence of SIRS was found to be significantly associated with AP severity and mortality in this study. Although imaging plays an important role in the diagnosis and management of acute pancreatitis, it does not provide information on systemic inflammatory response and organ failure. In contrast with the literature, the EPIC score accuracy in predicting organ failure was found to be similar to that of BISAP and SIRS scores in a study by Chen et al.[27]; however, it still was not found to be beneficial in differentiating the severity and number of failed organs in the early stage of AP. Since we found that none of the radiologic scoring systems were superior to other clinical, biochemical or hematologic variables, our study also suggests imaging could only be utilized to identify pancreatic or peripancreatic complications and guide therapeutic interventions. Nevertheless, when we made a comparison among radiologic scoring systems including the Balthazar score, MTSI, EPIC, and renal rim grade, an EPIC score ≥ 6 was the most valuable for predicting both severity and mortality. an EPIC score ≥ 6 and renal rim grade ≥ 2 were markedly specific, while a Balthazar score ≥ 4 and MCTSI ≥ 4 were markedly sensitive for both severity and mortality. In line with our study, Sharma et al.[13] also declared that the EPIC score was the most specific (39.2%) for mortality among all four of these radiologic scoring systems.

Our study had some limitations. First of all, it had a retrospective design. Thus, we were not able to evaluate the parameters which are not routinely used for AP patients in our hospital, such as procalcitonin level. For the same reason, the APACHE II score was not included in the variables of our study. Second, the number of patients enrolled in this study was small because patients with missing data in their hospital files and those without CT imaging at the appropriate time were not included. Another limitation of this study was that the variables were not compared in terms of etiology, even though it could influence the systemic response to AP.

Various scoring systems, biochemical, and hematologic markers are shown to have high predictive accuracy for AP severity and mortality AP in recent studies. In this study, the BISAP with a cut-off value of ≥ 2 was found to be more valuable than the HAPS, BISAP score, Ranson score, NLR, CRP level, Balthazar score, MCTSI, EPIC score, and renal rim grade for severity assessment, with sensitivity and specificity of 68.4% and 78.7%, respectively. NLR with a cut-off value of >11.91, had the highest value to predict mortality among all variables, with sensitivity and specificity of 76.5% and 94.1%, respectively. Although radiologic scoring systems do not provide information on systemic inflammatory response and organ failure, the EPIC score had the highest value to predict both severity and mortality of AP in comparison with the others. The EPIC score, with a cut-off of ≥ 6, presented sensitivity and specificity of 63.2% and 82%, respectively, for severity, and 78.6% and 81.5%, respectively for severity. In conclusion, the BISAP score and NLR might be preferred as early determinants of severity and mortality in AP. The EPIC score might be suggested among current radiological scoring systems.

Ethics Committee Approval

This study was approved by the Ethics Committee of the Dokuz Eylül University School of Medicine.

Author Contributions

Concept – N.S.G., G.B., Ö.S.T., O.D.; Design – N.S.G., G.B., Ö.S.T.; Supervision – Ö.S.T., O.D.; Materials – G.B., Ö.S.T., P.E.E.; Data Collection and/or Processing – A.B., P.E.E.; Analysis and/or Interpretation – N.S. G. A.B., P.E.E.; Literature Review - A.B., P.E.E.; Writing - N.S.G., G.B.; Critical Review - Ö.S.T., O.D.

Conflict of Interest

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declare that this study has received no financial support.

RESUMO

Comparação dos sistemas de escores radiológicos, escores clínicos razão neutrófilo/linfócito e níveis séricos de proteína C-reativa para determinação da gravidade e mortalidade em casos de pancreatite aguda

OBJETIVO: *Comparar sistemas de escores radiológicos, escores clínicos, os níveis séricos de proteína C-reativa (PCR) e a razão neutrófilo/linfócitos (RNL) como métodos de previsão de gravidade e mortalidade em casos de pancreatite aguda (PA).*

MATERIAIS E MÉTODOS: *Dados demográficos, clínicos e radiográficos de 80 pacientes com PA foram avaliados retrospectivamente. Os valores de Harmless Acute Pancreatitis Score (HAPS), Síndrome da Resposta Inflamatória Sistêmica (SIRS), Índice de Gravidade na Pancreatite Aguda à Beira do Leito (BISAP), escore de Ranson, escore de Balthazar, Índice Modificado de Gravidade por Tomografia Computadorizada (CTSI), escore de Inflamação Extrapancreática em Tomografia Computadorizada (EPIC) e grau renal foram registrados. O desempenho prognóstico dos sistemas de escores clínicos e radiológicos e RNL no momento da internação e os níveis séricos de PCR após 48 horas foram comparados quanto à gravidade, de acordo com os critérios de Atlanta revisados e mortalidade. Os dados foram avaliados pelo cálculo das curvas ROC e da área sob a curva ROC (AUROC).*

RESULTADOS: *De 80 pacientes, 19 (23,8%) tinham PA grave e 9 (11,3%) morreram. A AUROC para o escore BISAP foi de 0,836 (95%CI: 0.735-0.937), com o valor mais alto de gravidade. Com um valor de corte de BISAP ≥ 2 , a sensibilidade e a especificidade foram de 68,4% e 78,7%, respectivamente. A AUROC para o a RNL foi de 0,915 (95%CI: 0.790-1), com o valor mais alto de mortalidade. Com um valor de corte de RNL > 11,91, a sensibilidade e a especificidade foram de 76,5% e 94,1%, respectivamente. Entre os sistemas de escore radiológico, o EPIC apresentou o maior valor de AUROC, 0,773 (95%CI: 0.645-0.900) para gravidade e 0,851 (95%CI: 0.718-0.983) para mortalidade com um valor de corte ≥6.*

CONCLUSÃO: *O escore BISAP e a RNL podem ser preferíveis como determinantes precoces de gravidade e mortalidade na PA. O escore EPIC pode ser sugerido entre os atuais sistemas de escores radiológicos.*

PALAVRAS-CHAVE: *Pancreatite necrosante aguda. Pancreatite. Mortalidade. Proteína C-reativa. Neutrófilos. Contagem de linfócitos.*

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ORIGINAL ARTICLE

Effects of four types of integrated Chinese and Western medicines for the treatment of COVID-19 in China: a network meta-analysis

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SUMMARY

OBJECTIVE: *Various integrated Chinese and Western medicines might be beneficial for the treatment of Coronavirus disease 2019 (COVID-19). This study aims to evaluate the efficacy of lung computed tomography (CT) of four integrated Chinese and Western medicines in the treatment of COVID-19 using network meta-analysis (NMA).*

METHODS: *Multiple databases were consulted to find randomized controlled trials of four different types of integrated Chinese and Western medicines for the treatment of COVID-19. NMA was conducted on the data using Stata (13.0) software. The odds ratio (OR) was calculated. The studies included in this paper were divided into a control group (Western medicine) and an observation group (one of four integrated Chinese and Western medicines).*

RESULTS: *5 eligible publications were identified. A total of 598 cases were included in the study, and the results showed that the four types of integrated Chinese and Western medicines (symptomatic and supportive care with Qingfei Touxie Fuzheng, Lianhua Qingke, and Xuebijing) were significantly superior ($P < 0.05$) to symptomatic and supportive care alone, except for symptomatic and supportive care with Lianhua Qingwen. The combination of symptomatic and supportive care with Lianhua Qingke had the highest probability of being the most clinically efficacious intervention, with a surface under the cumulative ranking (SUCRA) curve of 85.7.*

CONCLUSIONS: *A combination of symptomatic and supportive care with Lianhua Qingke is the best option among the four integrated Chinese and Western medicines considered for the treatment of COVID-19.*

KEYWORDS: *Coronavirus Infections. Coronavirus. Medicine, Chinese Traditional. Drugs, Chinese Herbal. Meta-Analysis.*

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is an acute respiratory-tract-based clinical syndrome caused by a new coronavirus, which is currently widespread on a global scale. The infectious disease seriously endangers human health and public safety, and this damage continues to increase every day[1]. Clinicians mainly use symptomatic support therapy since there is currently no specific medicine that can be used to cure the disease. Some studies have shown that the combination of Chinese and Western medicine can

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achieve some effect on the treatment of COVID-19[2].

A network meta-analysis (NMA) is a meta-analysis that combines traditional direct/head-to-head comparison and indirect comparison. Traditional meta analysis (TMA) focuses on comparing two groups, while NMA emphasizes comparing multiple interventions under the same conditions based on its high statistical power and precision, so it is also called multiple treatments meta-analysis[3].

The aim of this study was to evaluate the lung computed tomography (CT) efficacy of four types of integrated Chinese and western medicines in the treatment of COVID-19 using NMA and rank them according to their performance. Combinations included symptomatic and supportive care with Qingfei Touxie Fuzheng recipe, Lianhua Qingwen granule, Lianhua Qingke granule, and Xuebijing injection. This study may provide a useful guide for the selection of medication treatments for COVID-19.

METHODS

Search Strategy

The databases used for this study included PubMed, EMBASE, Web of Science, SciFinder and Sino Med, Cochrane Library, BIOSIS Previews, China National Knowledge Index, China Biomedical Medicine, Wan fang, Chinese Science Citation, Chongqing VIP Network, China Science and Technology Journal, and China Academic Journal Network Publishing. The search words used included Chinese medicine, combination therapy, Qingfei Touxie Fuzheng, Lianhua Qingwen, Lianhua Qingke, Xuebijing, efficacy, drug therapy, randomized controlled trials, pneumonia, and coronavirus disease 2019.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) randomized controlled trials; (2) patients with COVID-19 who met the criteria of Coronavirus disease 2019 diagnosis, and the treatment plan (6th edition) of the Chinese National Health Commission[4]; (3) patients aged 18 years and above; (4) provision of an effective number of cases for measurement of outcomes; (5) studies that used symptomatic and supportive care[4] as the control group, while the observation group was one of the integrated Chinese and Western medicines for the treatment of COVID-19.

The exclusion criteria were as follows: (1) studies with incomplete or repeated data; (2) purely descriptive studies with no control group; (3) research types that were summaries of experience, theoretical discussions, case reports, animal-based experiments, and reviews; (4) patients with dementia or severe mental illness; (5) patients with severe liver, kidney, or heart damage, tumor, or autoimmune disease.

Efficacy Evaluation Criteria

The curative effect in this study was based on lung computed tomography (CT) findings. The total effective cases were defined as the number of patients whose pulmonary lesions had significant change or progression compared with the previous CT.

Data Extraction and Quality Evaluation

Two reviewers searched the literature and extracted data independently based on the exclusion and inclusion criteria. The contents of extracted data included the following: (1) basic information of the publication; (2) outcome indicators of CT findings; (3) quality indicators of the publication. Disagreements were resolved through discussion with a third evaluator. The Jadad quality scoring standard was used to evaluate the quality of the publications.

Statistical Analysis

Commands of the network package in Stata (13.0) were used to construct the network, evidence contribution, confidence interval (CI), funnel, and ranking plots. The efficacy of interventions was ranked according to the value of the surface under the cumulative ranking (SUCRA) curve. The selected indicators were count data, while OR was used as the combined effect, and the confidence interval (CI) was set at 95 %. A P-value < 0.05 was defined as statistically significant.

Ethics statement

All analyses were adapted from previously published work. Thus, no ethical approval and patient consent were required.

RESULTS

Characteristics of Included Studies

A total of 5 randomized controlled trials involving 598 patients were ultimately included in this study[5-9]. Figure 1 shows the selection details of the studies included, while their basic information is presented in Table 1.

FIGURE 1.

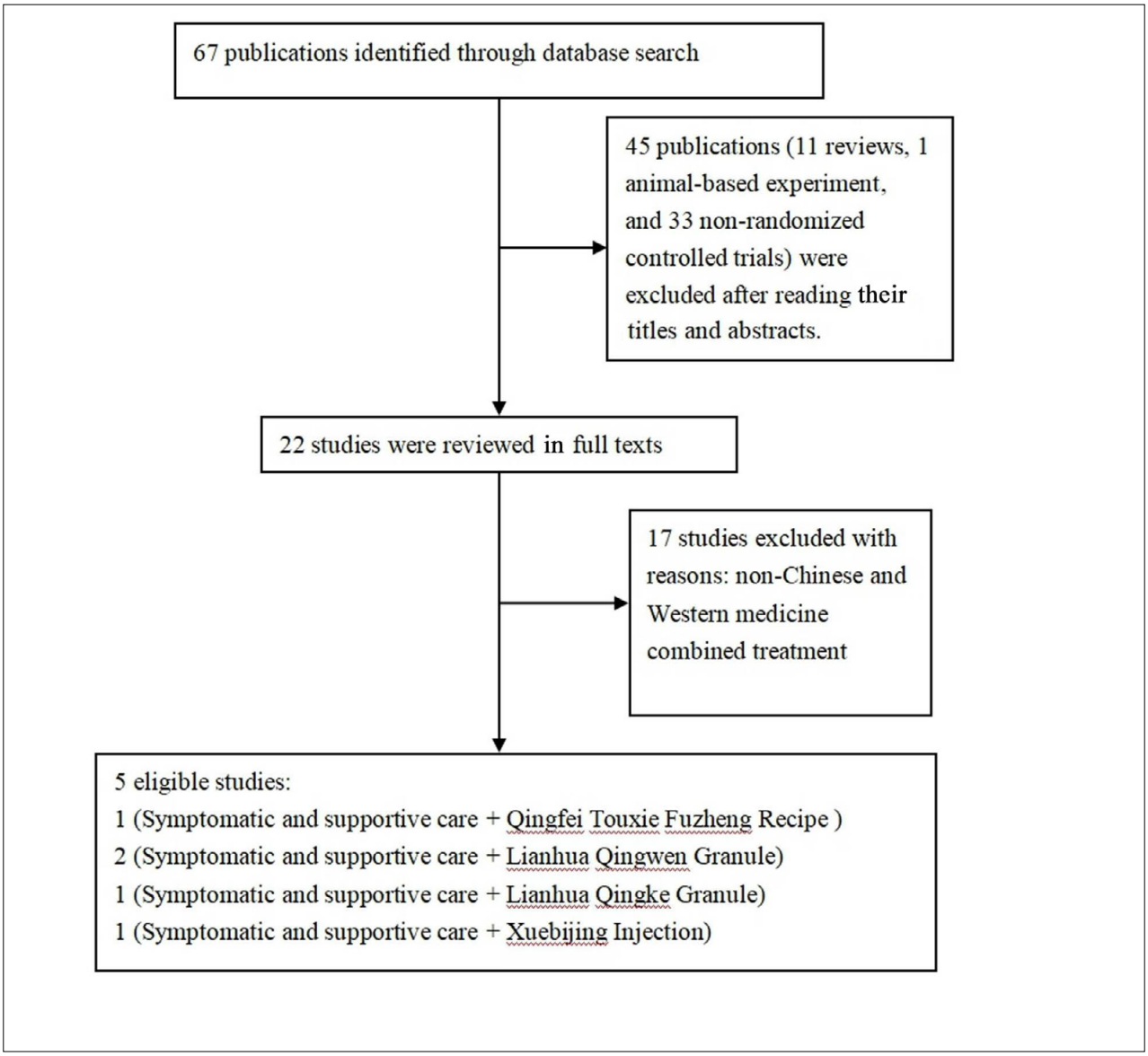


TABLE 1. BASIC INFORMATION OF STUDIES INCLUDED IN THE NETWORK META-ANALYSIS

Author	Year	Comparison	Country	Simple size (Total effective cases/Total cases)					Dose	Duration (Days)	Jadad quality score
				A	B	C	D	E	Combined Chinese medicine		
Ding et al.[5]	2020	B vs A	China	21/49	32/51	-	-	-	150 milliliters each time, 2 times a day	10	3
Cheng et al.[6]	2020	C vs A	China	23/51	-	28/51	-	-	6 grams each time, 3 times a day	7	2
Yu et al.[7]	2020	C vs A	China	93/148	-	102/147	-	-	6 grams each time, 3 times a day	7	3
Sun et al.[8]	2020	D vs A	China	18/25	-	-	31/32	-	1 bag each time, 3 times a day	14	3
Zhang et al.[9]	2020	E vs A	China	15/22	-	-	-	21/22	50 milliliters each time, 2 times a day	7	1

Notes: A, Symptomatic and supportive care; B, Symptomatic and supportive care + Qingfei Touxie Fuzheng Recipe; C, Symptomatic and supportive care + Lianhua Qingwen Granule; D, Symptomatic and supportive care + Lianhua Qingke Granule; E, Symptomatic and supportive care + Xuebijing Injection.

Network Meta-analysis

Network Plot of Four Types of Integrated Chinese and Western Medicines

Of the 5 studies, publications on the combination of symptomatic and supportive care with Lianhua Qingwen were the most frequent. The group of symptomatic and supportive care alone had the highest number of subjects, while symptomatic and supportive care + Xuebijing had the lowest number of subjects (Figure 2).

FIGURE 2.

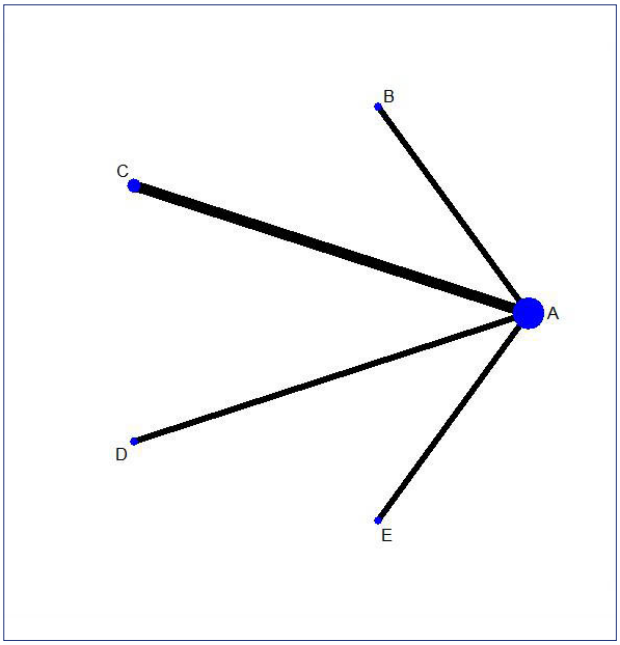
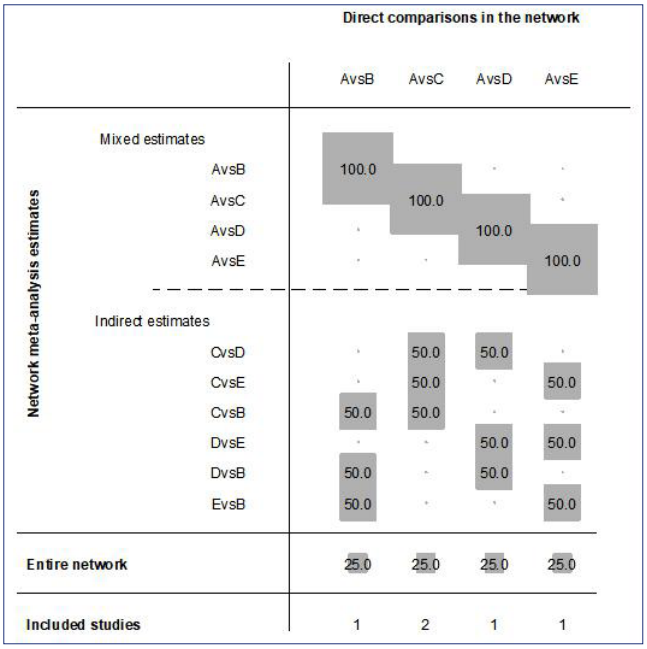


FIGURE 3.



Evidence Contribution Plot

Figure 3 shows that the direct comparison of symptomatic and supportive care alone and the combination of symptomatic and supportive care with Qingfei Touxie Fuzheng had a 100 % effect on the mixed comparison. The direct comparison between symptomatic and supportive care alone and symptomatic and supportive care + Qingfei Touxie Fuzheng had a 50 % effect on the indirect comparison between symptomatic and supportive care + Qingfei Touxie Fuzheng, and symptomatic and supportive care + Lianhua Qingwen. The direct comparison of symptomatic and supportive care alone and symptomatic and supportive care + Qingfei Touxie Fuzheng had a 25.0 % effect on the results of the meta-analysis.

Confidence Interval (CI) Plot

The meta-analysis results showed that the pooled OR and 95% CI of COVID-19 improvement compared with symptomatic and supportive care alone was 2.25 (1.01 to 5.01) for symptomatic and supportive care + Qingfei Touxie Fuzheng, 1.38 (0.91 to 2.08) for symptomatic and supportive care + Lianhua Qingwen, 12.06 (1.37 to 106.04) for symptomatic and supportive care + Lianhua Qingke, and 9.80 (1.09 to 88.23) for symptomatic and supportive care + Xuebijing, which indicates a significant difference in lung CT efficacy, except for symptomatic and supportive care + Lianhua Qingwen. For the comparison between Chinese and western combinations, no significant differences were found. The OR for the network estimates along with 95% CI is presented in Figure 4.

Publication Bias

Regarding publication bias, all the outcomes in the study were almost symmetrical (Figure 5), indicating that the publication bias may not have existed.

Ranking Plot

The distribution of probabilities for each treatment ranked for their efficacy in COVID-19 according to SUCRA values are shown in Figure 6 and Table 2. The order of SUCRA values for different types of integrated Chinese and Western medicines was as follows: symptomatic and supportive care + Lianhua Qingke (85.7), symptomatic and supportive care + Xuebijing (82.1), symptomatic and supportive care + Qingfei Touxie Fuzheng (50.5), and symptomatic and

TABLE 2. SUCRA RANKINGS OF MYCOPLASMA PNEUMONIA IN CHILDREN TREATMENTS

Treatment	SUCRA	Pr Best	Mean rank
A	0.0	0.0	4.9
B	50.5	1.2	3.0
C	28.8	0.1	3.8
D	85.7	54.0	1.6
E	82.1	44.7	1.7

Notes: A, Symptomatic and supportive care; B, Symptomatic and supportive care + Qingfei Touxie Fuzheng Recipe; C, Symptomatic and supportive care + Lianhua Qingwen Granule; D, Symptomatic and supportive care + Lianhua Qingke Granule; E, Symptomatic and supportive care + Xuebijing Injection.

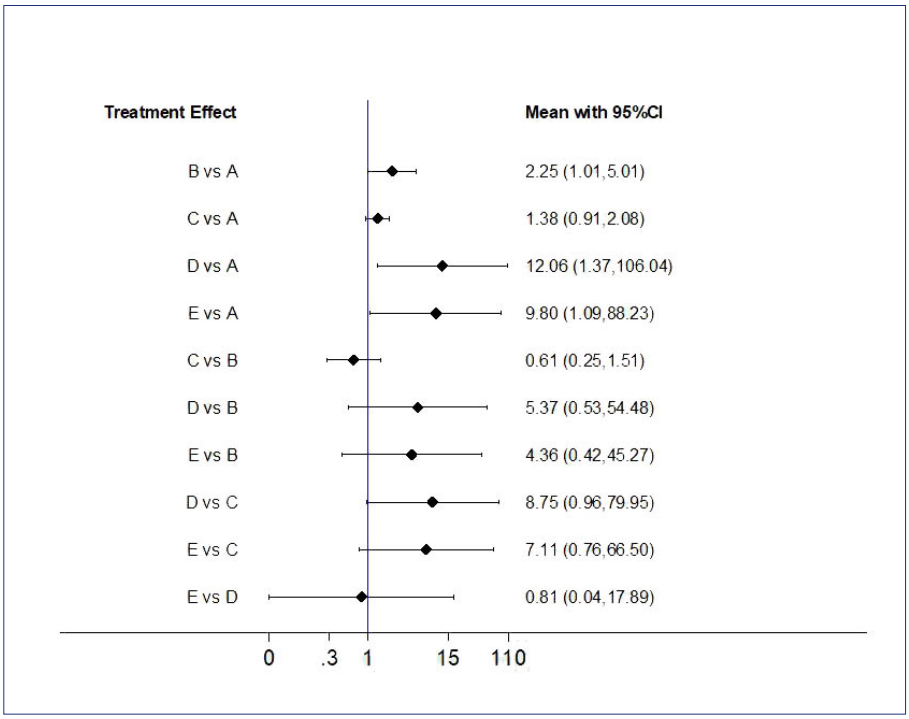


FIGURE 4.

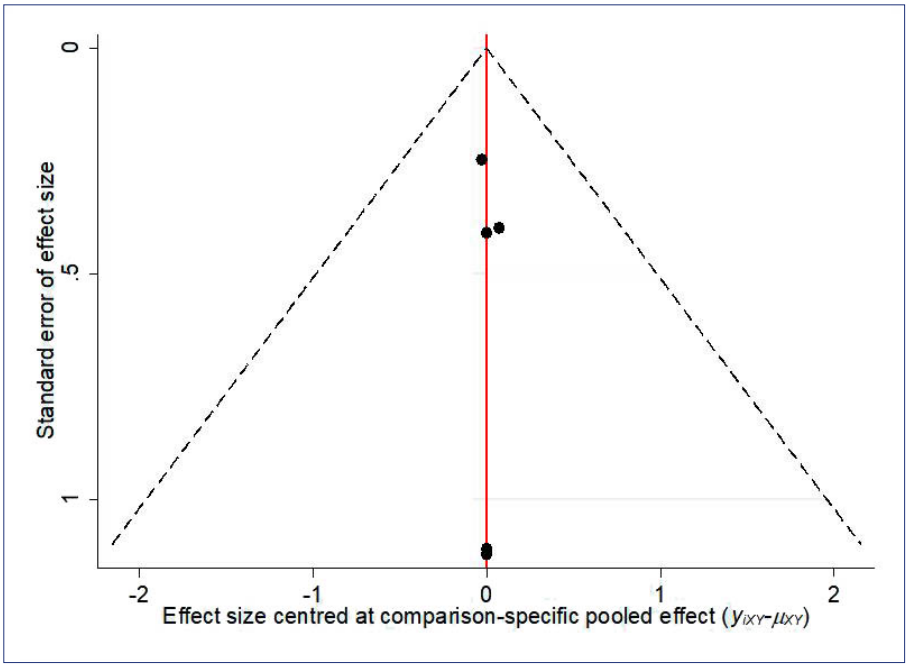


FIGURE 5.

supportive care + Lianhua Qingwen (28.8). Therefore, the combination of symptomatic and supportive care with Lianhua Qingke had the highest probability of being the best intervention option in terms of lung CT efficacy.

DISCUSSION

This study analyzed four types of integrated Chinese and Western medicines and conducted a pairwise comparison. The resulting network plot makes the results more intuitive. The results showed that the combination of symptomatic and supportive care with Qingfei Touxie Fuzheng, Lianhua Qingke, and Xuebijing was more effective when compared to symptomatic and supportive care alone in the treatment of COVID-19. The combination of symptomatic and supportive care with Lianhua Qingke had the highest SUCRA value and the highest probability of being the best treatment option.

As the COVID-19 epidemic continues to spread, the respiratory infectious disease poses a serious threat to human health and is characterized by high infectivity. Fever, dry cough, and fatigue are the main

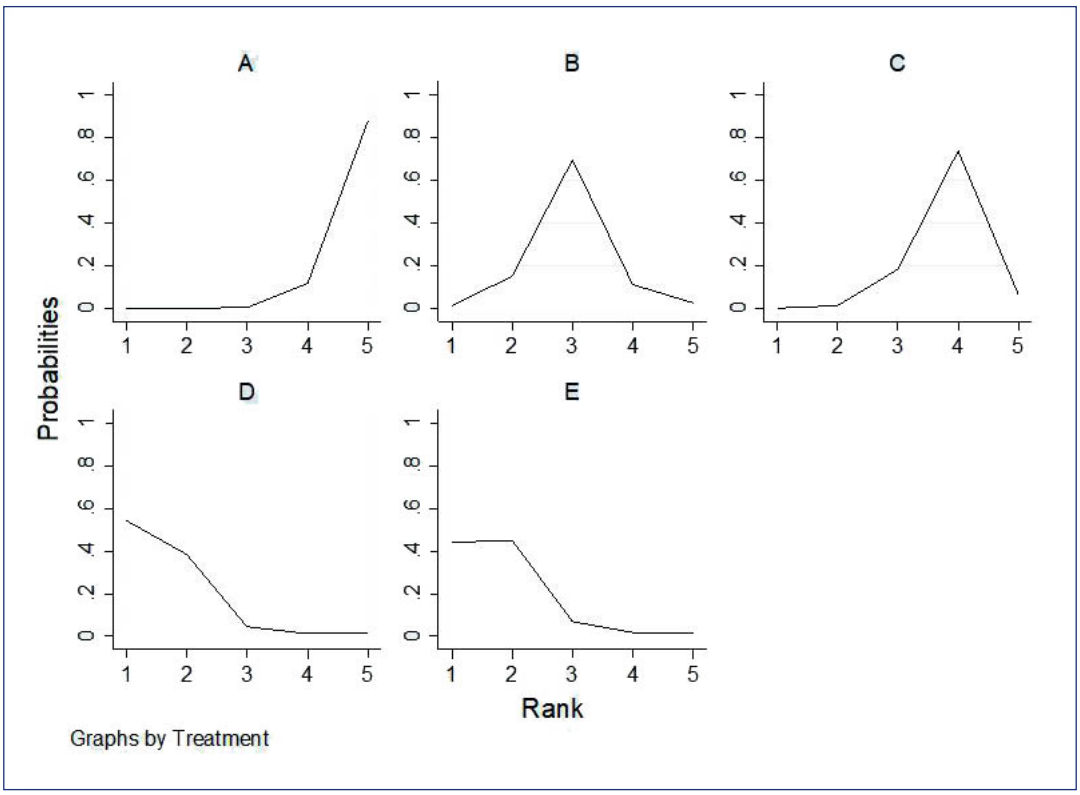


FIGURE 6.

clinical manifestations of COVID-19 in patients[10]. Pathological anatomy shows the formation of mucus and mucus plugs in the bronchial lumen, and the formation of serous, fibrinous exudate and transparent membrane in the alveolar cavity[11]. These viscous exudates obstruct the airways and are difficult to be coughed out, seriously affecting the ventilation function of the lungs, reducing the efficacy of oxygen therapy and mechanical ventilation, and even causing suffocation and death[11]. In the early stage of COVID-19, the lungs show multiple small patchy shadows and interstitial changes, especially in the extrapulmonary zone. Then it develops multiple ground glass shadows and infiltration shadows. In severe cases, lung consolidation may occur, and pleural effusions are rare[11].

For clinical treatment, no specific drugs have been found. At present, Western medicine mainly adopts symptomatic supportive treatments such as anti-virus, oxygen therapy, and nutritional support[4]. Chinese medicine has a long history in epidemic treatment and has accumulated rich clinical experience, such as outstanding contributions to the outbreak of Severe acute respiratory syndrome (SARS)[12].

Respiratory mucus or phlegm belongs to the category of "phlegm" in traditional Chinese medicine[8]. Lianhuaqingke granule is an innovative Chinese medicine developed under the guidance of this Chinese medicine theory during the outbreak of the new coronavirus pneumonia[8]. The combination of symptomatic and supportive care with Lianhua Qingke had the highest probability of being the best intervention option in terms of lung CT efficacy, indicating that Lianhua Qingke has a better improvement effect on the lung deterioration. Lianhua Qingke is composed of ephedra, gypsum, forsythia, mulberry skin, fried bitter almond, honeysuckle, rhubarb, and platycodon grandiflorum, which have the function of clearing away heat and detoxifying, clearing the lungs, and relieving fever, cough, and phlegm. Lianhuaqingke can block the cascade reaction chain with airway inflammation as the core while antiviral and bacteriostatic drugs regulate immune function, relieve airway spasm, protect airway mucosa, reduce airway resistance, and improve respiratory function. In addition, no adverse events related to Lianhua Qingke have been found[8].

This study may be very helpful for the clinical treatment of COVID-19, and its limitations include the following: (1) the number of publications included in this paper is small; (2) lack of uniformity in the duration of treatment; and (3) there is no quantitative analysis of clinical symptoms or immune system cytokines. Future studies involving a large sample size

and high-quality RCT are needed in the treatment of COVID-19.

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None.

Conflict of interest

All authors have no conflicts of interest to declare and have approved the submitted manuscript and are responsible for the reported research. Approval from the Research Ethics Committee is not applicable to this study.

Authors' contributions

Lairun Jin was responsible for the concept and design of the manuscript. Hui Yuan and Yan Xu were responsible for data collection. Lairun Jin and Hui Yuan were responsible for the analysis and interpretation of data. Lairun Jin and Yan Xu were responsible for the writing and revision. All authors approved the final version of the submitted manuscript.

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Special thanks to Miss Wang for bringing me good luck.

RESUMO

OBJETIVO: *Vários medicamentos chineses e ocidentais integrados podem ser benéficos para o tratamento da COVID-19. O objetivo deste estudo é avaliar a eficácia da tomografia computadorizada (TC) de pulmão de quatro medicamentos chineses e ocidentais integrados para o tratamento da COVID-19 usando uma meta-análise em rede (NMA).*

MÉTODOS: *Vários bancos de dados foram consultados para encontrar ensaios clínicos randomizados de quatro tipos diferentes de medicamentos chineses e ocidentais integrados para o tratamento da COVID-19. A NMA foi realizada nos dados usando o software Stata (13.0). O odds ratio (OR) foi calculado. Os estudos incluídos neste artigo foram divididos em um grupo de controle (medicina ocidental) e um grupo de observação (um dos quatro medicamentos chineses e ocidentais integrados).*

RESULTADOS: *5 publicações elegíveis foram identificadas. Um total de 598 casos foram incluídos no estudo, e os resultados mostraram que os quatro tipos de medicamentos chineses e ocidentais integrados (tratamento sintomático e de suporte com Qingfei Touxie Fuzheng, Lianhua Qingke e Xuebijing) foram significativamente superiores (P <0,05) a somente cuidados sintomáticos e de suporte, exceto cuidados sintomáticos e de suporte com Lianhua Qingwen. A combinação de cuidados sintomáticos e de suporte com Lianhua Qingke teve a maior probabilidade de ser a intervenção clinicamente mais eficaz, com uma superfície abaixo da curva de classificação cumulativa (SUCRA) de 85,7.*

CONCLUSÕES: *Uma combinação de tratamento sintomático e de suporte com Lianhua Qingke é a melhor opção entre os quatro medicamentos integrados chineses e ocidentais considerados para o tratamento de COVID-19.*

PALAVRAS-CHAVE: *Infecções por Coronavirus. Coronavirus. Medicina Tradicional Chinesa. Medicamentos de Ervas Chinesas. Metanálise.*

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ORIGINAL ARTICLE

Identification of key genes for type 1 diabetes mellitus by network-based guilt by association

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SUMMARY

OBJECTIVE: *This study aimed to propose a co-expression-network (CEN) based gene functional inference by extending the "Guilt by Association" (GBA) principle to predict candidate gene functions for type 1 diabetes mellitus (T1DM).*

METHODS: *Firstly, transcriptome data of T1DM were retrieved from the genomics data repository for differentially expressed gene (DEGs) analysis, and a weighted differential CEN was generated. The area under the receiver operating characteristics curve (AUC) was chosen to determine the performance metric for each Gene Ontology (GO) term. Differential expression analysis identified 325 DEGs in T1DM, and co-expression analysis generated a differential CEN of edge weight > 0.8.*

RESULTS: *A total of 282 GO annotations with DEGs > 20 remained for functional inference. By calculating the multifunctionality score of genes, gene function inference was performed to identify the optimal gene functions for T1DM based on the optimal ranking gene list. Considering an AUC > 0.7, six optimal gene functions for T1DM were identified, such as regulation of immune system process and receptor activity.*

CONCLUSIONS: *CEN-based gene functional inference by extending the GBA principle predicted 6 optimal gene functions for T1DM. The results may be potential paths for therapeutic or preventive treatments of T1DM.*

KEYWORDS: *Diabetes mellitus, type 1. Protein binding. Genetic association studies. Genetics.*

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a disorder of glucose homeostasis characterized by progressive insulin deficiency, which results in hyperglycemia that develops as a result of autoimmune destruction of the pancreatic β-cell[1]. The morbidity of T1DM has increased worldwide in the last decades, especially in childhood and developed countries[2].

Increasingly, high-throughput genome-wide association studies have resulted in a paradigm shift in the way that researchers view complex diseases. As a powerful approach for molecular research, high-throughput genome-wide analysis has been applied for unprecedented discovery in various diseases. Transcriptome analysis has yielded huge

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amounts of genes influencing the likelihood of developing T1DM[3-5]. Understanding the function of uncharacterized genes is one of the major challenges of biology[6,7]. While most biological functions arise from integrated activities between many genes, making gene function prediction complex[8]. Gene interaction usually involves participation in the same or related cellular functions. Thus, gene interactions can be used to infer gene functional relationships. The function of a protein can be inferred by observing whether it interacts with another protein of known function, which is an example of the "guilt by association" concept[9,10]. Gene networks have been widely used to predict gene function using the neighbor voting algorithm, a basic application of the "guilt by association" principle[9,11].

In this study, we performed a network-based gene function inference to explore informative genes and gene functions involved in the development of T1DM by expanding the "guilt-by-association" method.

METHODS

Our gene function prediction procedure contained two main core steps: network characterization across thousands of gene ontology (GO) annotation sets using a fully vectorized neighbor voting algorithm, and gene multifunctionality assessment to determine the optimal gene functions.

Differential co-expression network from transcriptome data

Here, the transcriptome data of T1DM retrieved from the public functional genomics data repository Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/), under the accessing number of GSE55098[12], were utilized to determine gene differential expression and gene co-expression. The microarray data were obtained from peripheral blood mononuclear cells of 12 patients with newly diagnosed T1DM and 10 normal controls and presented on the GPL570 (Affymetrix U133 Plus 2.0) platform. Detailed sample characteristics and microarray experiments have been shown in a previous study[12]. Gene expression levels were normalized using the robust multiarray average (RMA) procedure and normalized using the median method.

Gene differential expressions between T1DM and normal controls were measured by Linear Models for Microarray Data (Limma) package in R. By assimilating a set of gene-specific t-tests and a Benjamini-Hochberg false-discovery-rate (FDR) based method[13] to adjust the p-values, differentially expressed genes were determined under the threshold values of $p < 0.05$ and $|\log2FoldChange| \geq 2$. Then, a network was generated based on these differentially expressed genes, and the Spearman's correlation coefficient (SCC), a measure of the correlation between two genes, was used to re-weight the gene network. In the differential co-expression network, SCC gives a value of edge connection between -1 and +1 inclusive, and the SCC absolute value was considered as the weight value of the edge; a weight value close to 0 represents a weaker connection between two genes, and a weight value close to 1, a stronger connection between two genes.

In this representation of the differential co-expression network, each row and column indicated a node, and the connection between the two was indicated by the corresponding entry in the adjacency matrix. Moreover, Cytoscape (http://cytoscape.org/) was employed to visualize the differential co-expression network.

Furthermore, topological centrality is effective for identifying essential molecules in well-characterized interaction networks. Here we analyzed the topological centrality of the differential co-expression network. Degree quantifies the local topology of each node, by summing up the number of its adjacent nodes. Genes with high node degrees tend to be associated with many functions. Thus, degree centrality of the differential co-expression network was investigated.

GO annotation

After representing the differential co-expression network as a matrix, we performed a gene attribute analysis to determine the gene set label vectors and assess gene multifunctionality. The GO consortium (http://geneontology.org/) contains 19,003 human GO terms, covering 18,402 genes. To improve the prediction performance, only GO terms with differentially expressed genes > 20 remained in the subsequent analysis.

Network-based gene function prediction

After obtaining both the network and the annotations, we assessed the network through its topology and the annotations through gene multifunctionality calculation.

Neighbor voting for gene function prediction

Neighbor voting algorithm based on the "guilt-by-association" principle was employed to perform the gene function prediction. In the "guilt-by-association" principle, genes with shared functions are preferentially connected. In a network, genes with similar neighbors may share common properties, giving rise to a prediction metric based on the similarity of neighbors. In this study, we applied the neighbor voting algorithm based on the differential co-expression network and GO annotations. Specifically, we hid a subset of gene labels in one GO term and assessed whether the remaining genes in this GO term could predict the identities of the hidden genes using information inferred from the differential co-expression network.

Multifunctionality assessment

Gene multifunctionality refers to genes possessing multiple molecular functions, each of which can be characterized by the set of genes inferred to be interacting in a particular biological context[14]. Moreover, node degree is unambiguously linked to multifunctionality, and multifunctional genes often are expected to exhibit a higher node degree. As with node degree, multifunctionality is also a key factor in explaining the results of gene function prediction. Given a gene i, its multifunctionality was defined as:

$$MF_i = \sum_{k|i \in GO_k} \frac{1}{n_k * n'_k}$$

Where nk is the number of genes within the GO term k, and n'k is the number of genes outside the GO term k. The more highly annotated or multifunctional a gene is, the higher the chances of predicting them as good candidates for having any annotation. A comparison of gene multifunctionality with the neighbor voting performance AUC presented an indication of the degree to which generic predictions dominate results. Thus, as a control of the annotations in the neighbor voting algorithm, we performed a gene multifunctionality assessment using GO annotations and generated a ranked score for each gene.

RESULTS

Objects

We firstly obtained the accessible expression data of T1DM to identify the differentially expressed genes and generate the gene co-expression. We constructed the co-expression adjacency matrix of 325 differentially expressed genes (covering 52,650 interactions), where the entry indicated the connection between two genes (Figure 1A).

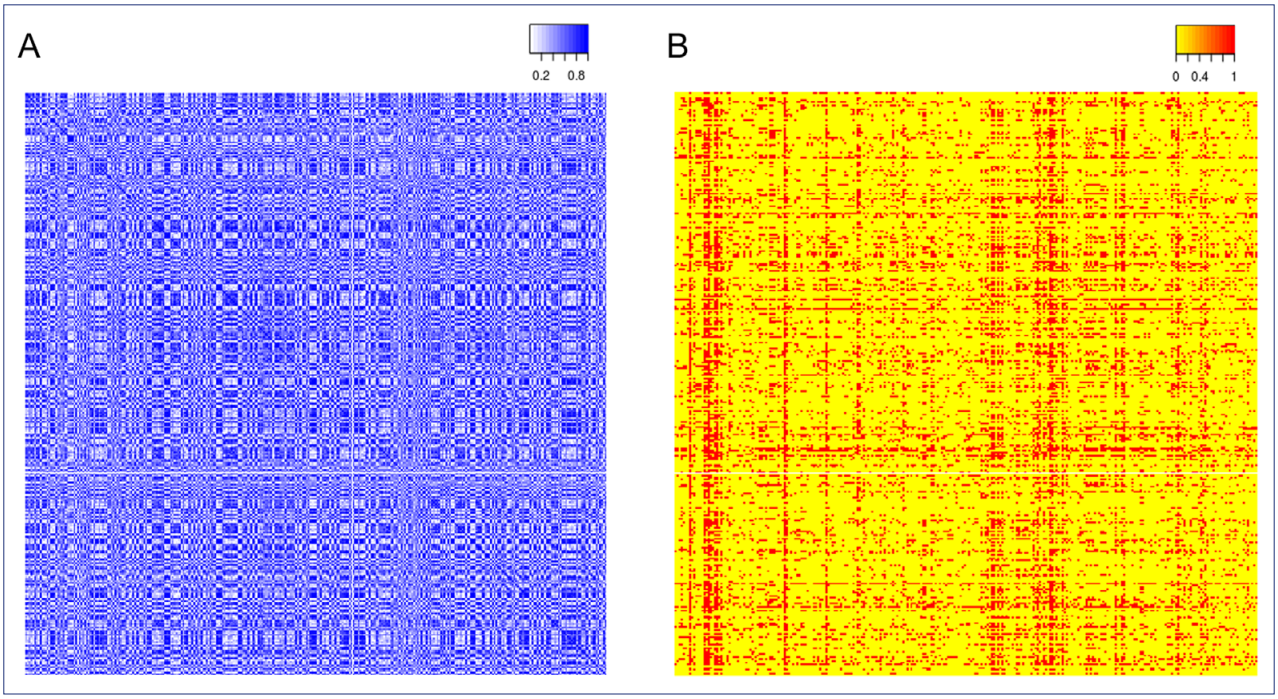


FIGURE1. Two necessary objects for the network-based function inference by extending the "guilt by association" method. A: Differential co-expression network matrix; B: Gene set annotation vectors.

FIGURE 2. A: The distribution of node degree of the co-expression network. B: Differential co-expression network with edge weight > 0.8 and node degree > 1.

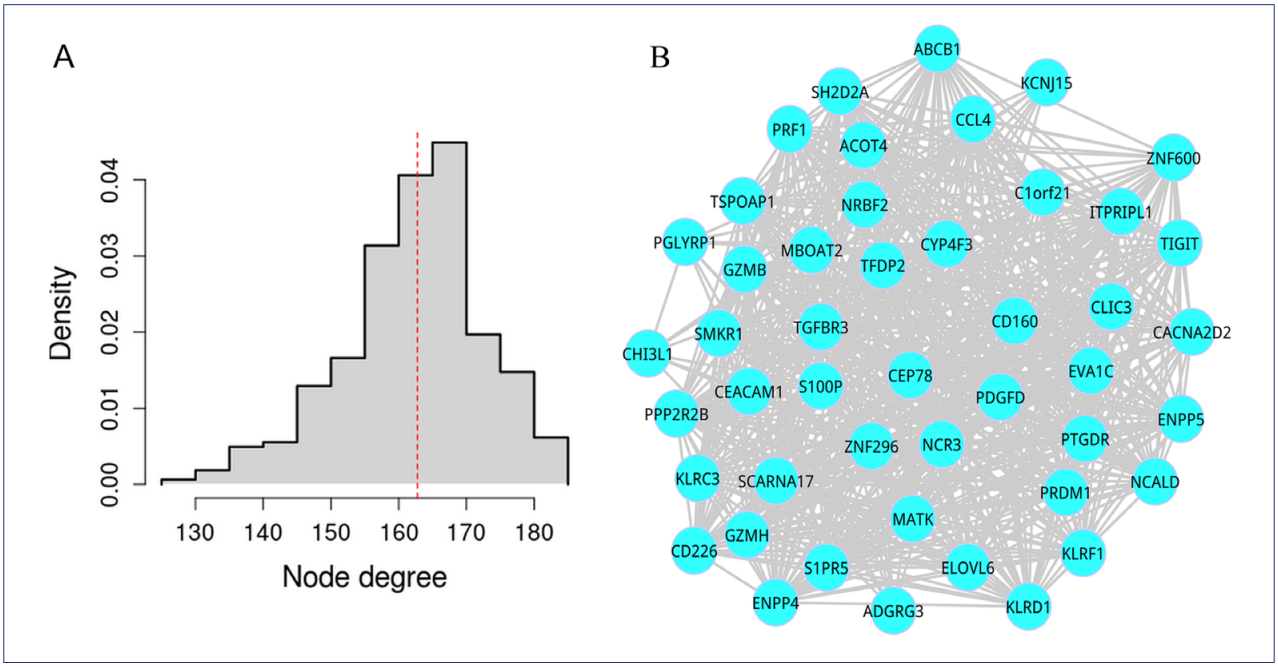
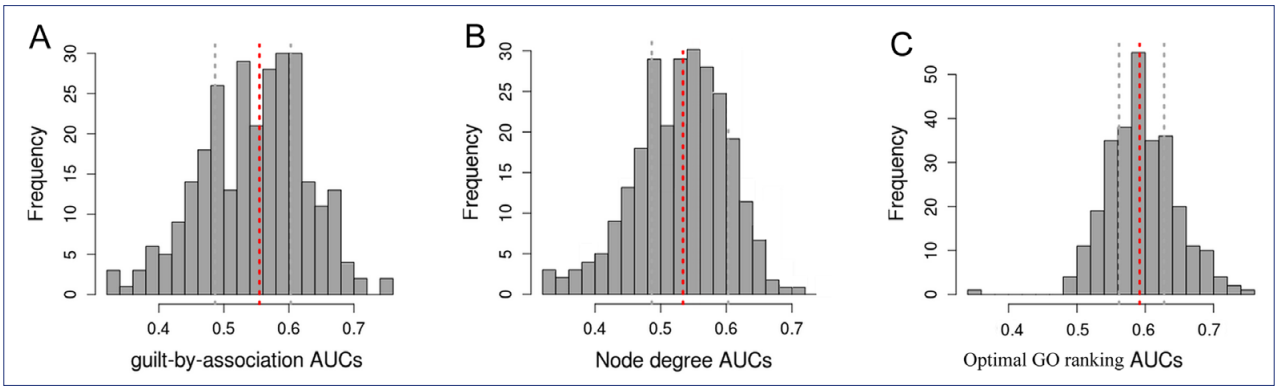


FIGURE 3. The distributions of the area under the receiver operating characteristics curve (AUC) scores. A: Distribution of AUC scores from the neighbor voting algorithm. B: Distribution of AUC scores for node degree ranking. C: Distribution of AUC scores from multifunctionality assessment. red: median, grey: inter-quartile ranges.



Subsequently, these GO terms were represented as a binary vector, where each entry corresponded to a differentially expressed gene, with a 1 indicating that the differentially expressed gene was a member of this GO term, and a 0 if it was not (Figure1B).

Differential co-expression network

After generating a co-expression matrix of 325 differentially expressed genes, gene interactions with weight values < 0.8 were removed, and the remaining gene interactions were used to construct the differential co-expression network, including 45 differentially expressed genes and 675 interactions (Figure 2A). A degree centrality analysis was performed for the co-expression network and illustrated the distribution of node degree in Figure2B.

Gene function inference

Generally, genes with similar neighbors may share common properties. Thus, we performed gene function inference for T1DM based on the differential co-expression network. A node degree analysis is an important assessment of the network. Genes with high node degrees tend to be involved in many GO annotations set. Thus, we analyzed the gene node degree and calculated the node degree AUC (Figures 3A and 3B). A total of 176 terms were identified with AUC > 0.5. Moreover, one GO term defense response to another organism (AUC = 0.718) showed good performance with AUC > 0.7.

Based on the optimal ranking gene list, we generated the distribution of AUCs for 282 GO terms (Figure 3C). Go terms with AUC > 0.7 were defined

as the optimal gene functions for T1DM, including regulation of immune system process (AUC = 0.741), positive regulation of immune system process (AUC = 0.738), system process (AUC = 0.725), signal transducer activity (AUC = 0.717), transmembrane signaling receptor activity (AUC = 0.713), and receptor activity (AUC = 0.713).

DISCUSSION

In this study, we proposed a co-expression network-based gene functional inference by extending the "Guilt by Association" strategy to predict candidate gene functions for T1DM from the GO consortium, which is important for revealing the molecular mechanisms and future applications of therapeutic decisions.

By extending the "Guilt by Association" strategy on the differential co-expression network, we generated several optimal gene functions for T1DM by assessing gene multifunctionalities, such as regulation of the immune system process and receptor activity. Regulation of the immune system process is defined as any process that modulates the frequency, rate, or extent of an immune system process. It is well known that T1DM is a chronic autoimmune disorder with the destruction of pancreatic β cells in genetically predisposed individuals with impaired immune regulation. Previous studies have revealed altered immune regulation in patients with T1DM[15,16]. Dys-regulation of the immune system contributes to the breakdown of immune regulation, leading to T1DM[17]. Eizirik et al.[18] indicated that innate immunity and inflammatory mediators play important roles in the process of T1DM. Moreover, several genetic variants in T1DM have been proven to have functional features of impaired immune regulation[19]. Regulation of the immune system process might enable investigators to restore immune imbalances with therapeutic interventions.

CONCLUSIONS

Using a co-expression network-based gene functional inference based on the "Guilt by Association" principle, our study predicted 6 optimal gene functions related to the regulation of immune system process and receptor activity, which might lead to potential paths for therapeutic or preventive treatments of T1DM and its complications.

Conflict of interest

None declared

Author Contributions

Conceptualization, Hao-Ren Wang; formal analysis, Shan-Shan Li; writing and original draft preparation, Hao-Ren Wang; writing and review and editing, Jia-Mei Tian; supervision, Tong-Huan Wei; funding acquisition, Hao-Ren Wang.

RESUMO

OBJETIVO: *O objetivo deste estudo é realizar uma inferência funcional genética baseada na rede de coexpressão (CEN), expandindo o escopo do princípio de "Culpa por Associação" (GBA - Guilt by Association) para prever as funções genéticas do diabetes mellitus tipo 1 (T1DM).*

MÉTODOS: *Primeiro, os dados transcritos do T1DM foram recuperados do repositório de dados genômicos para a análise dos genes diferenciais (DEGs), e foi gerada uma CEN diferencial ponderada. A área sob a curva ROC (AUC) foi escolhida para determinar a métrica de desempenho para cada termo de Ontologia Genética (GO). A análise da expressão diferencial identificou 325 DEGs no T1DM, e a análise de coexpressão gerou uma CEN diferencial com aresta de peso >0,8.*

RESULTADOS: *Um total de 282 anotações de GO com DEGs >20 foram mantidas para inferência funcional. Ao calcular a pontuação de multifuncionalidade dos genes, a inferência da função genética foi realizada para identificar as funções genéticas ideais para T1DM com base na lista de classificação genética ideal. Considerando um valor de AUC >0,7, foram identificadas seis funções genéticas ideais para a T1DM, tais como a regulação do processo imunológico e da atividade dos receptores.*

CONCLUSÕES: *A inferência funcional genética baseada em CEN, ao expandir o princípio de GBA, previu seis funções genéticas ideais para o T1DM. Os resultados podem ser caminhos potenciais para tratamentos terapêuticos ou preventivos do T1DM.*

PALAVRAS-CHAVE: *Diabetes mellitus tipo 1. Ligação proteica. Estudos de associação genética. Genética.*

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ORIGINAL ARTICLE

TMPO-AS1 is an independent prognostic factor for patients with laryngeal squamous cell carcinoma

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SUMMARY

OBJECTIVE: *Long noncoding RNA (lncRNAs) are frequently abnormally expressed in tumors and involved in the occurrence and progression of human cancer. Recently, a disease-related lncRNA, TMPO antisense RNA 1 (TMPO-AS1), was identified to be dysregulated in several tumors. Hence, we aimed to demonstrate whether TMPO-AS1 could be a promising prognostic marker for patients with laryngeal squamous cell carcinoma (LSCC).*

METHODS: *RT-PCR was performed to test TMPO-AS1 expressions in 187 LSCC specimens compared with matched normal specimens. Chi-squared tests were used to determine the associations between TMPO-AS1 expressions and the clinicopathological characteristics of LSCC patients. Then, the clinical outcome of LSCC patients who had lower or higher TMPO-AS1 expression was analyzed using Kaplan-Meier assays. Finally, a Cox proportional hazards model was carried out to evaluate the prognostic values of TMPO-AS1 and other clinical features.*

RESULTS: *We found that TMPO-AS1 was distinctly upregulated in human LSCC tissues compared with corresponding normal specimens ($p < 0.01$). Higher expressions of TMPO-AS1 were observed to be positively associated with the clinical stage ($p = 0.020$) and lymph node metastasis ($p = 0.027$). A clinical study in 187 patients revealed that patients with TMPO-AS1 low expressions had poorer survival than those with TMPO-AS1 high expressions ($p = 0.0012$). In addition, the result of multivariate assays demonstrated TMPO-AS1 expression is an independent predictor for the overall survival of LSCC patients.*

CONCLUSIONS: *TMPO-AS1 might be considered a novel molecule involved in LSCC progression, which provides a possible prognostic biomarker.*

KEYWORDS: *Larynx. Carcinoma, squamous cell. Laryngeal neoplasms. Biomarkers. Gene expression regulation, neoplastic.*

INTRODUCTION

Laryngeal squamous cell carcinoma (LSCC) is one of the most common head and neck tumors in the world, accounting for > 15 % of head and neck squamous cell carcinoma[s1]. According to a tumor report, the incidence of LSCC in China, particularly in the Guangdong Province, has been rising gradually[2]. Up to date, the clinical application of surgery or radiotherapy has made early-stage LSCC curable. However, the five-year overall

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survival rates of most patients with advanced tumor remain unsatisfactory, despite therapeutic advances[3,4]. The high mortality rate of LSCC may be attributed to distant metastasis. Therefore, the identification of novel diagnostic and prognostic markers is urgently needed.

Long noncoding RNAs (lncRNAs) are a class of non-protein-coding RNAs longer than 210 nucleotides that are typically recognized as mRNAs-like transcripts[5]. Previously, most lncRNAs were originally considered transcriptional "noises," but growing research in epigenetics has confirmed they play crucial regulatory functions in various gene expressions[6,7]. In recent years, the potential function of lncRNAs as promoters or inhibitors in a wide variety of tumor processes, such as growth, apoptosis, and metastasis has been described[8,9]. For instance, lncRNA MIAT, a highly expressed lncRNA in gastric cancer, was shown to be positively associated with advanced TNM stages and distant metastasis and promote tumor-cells metastasis by modulating the miRNA-141/DDX5 axis[10]. LncRNA LOC554202, a well-studied lncRNA whose overexpression was frequently reported in several tumors, was suggested as an oncogenic factor in LSCC progression because its upregulation promoted LSCC cell growth and invasion via sponging miRNA-31[11]. Up to date, a large number of functional lncRNAs had been identified and their functions were also studied *in vitro* and *in vivo*[12]. However, only a few lncRNAs have been functionally characterized in LSCC.

TMPO antisense RNA 1 (TMPO-AS1), located at 12q23.1, was first identified as an abnormally expressed lncRNA in lung adenocarcinoma by Li et al.[13]. The potential of TMPO-AS1 as a possible prognostic biomarker was also preliminarily explored using bioinformatics analysis. Then, functional assays by Qin et al.[14] confirmed TMPO-AS1 acted as a tumor promoter in lung cancer. Recently, TMPO-AS1 was also demonstrated to be overexpressed in prostate cancer and predicted advanced clinical stages as well as poor clinical outcome[15]. However, the expression and effects of TMPO-AS1 in other tumors remain unknown. In this study, we asked whether there were abnormalities of this lncRNA in LSCC.

METHODS

Patients and Specimens

LSCC tissues and adjacent normal tissues from 187 patients with LSCC who had undergone medicinal resection were collected between 2011 and 2014 from the Jining No.1 People's Hospital. All of the specimens were checked by two pathologists. All specimens were obtained at the time of surgery and immediately snap-frozen in liquid for the subsequent experiments. None of these patients underwent local or systemic therapies before the operations. This study was approved by the Medical Ethics Committee of the Jining No.1 People's Hospital and informed consent was obtained from all participants. The demographic and clinicopathological data are listed in Table 1.

Quantitative PCR analysis

For the extraction of total RNA from LSCC specimens and normal samples, Trizol reagent was purchased from Life Technologies (Haidian, Beijing, China) and used based on the company's protocol. The synthesization of cDNA was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Pudong, Shanghai, China). The levels of lncRNA were examined by applying qRT-PCR, which was performed using Power SYBR Green PCR Master Mix (Biosystems, Nanjing, Jiangsu, China). The RT-PCR assays were carried out for 40 cycles with the successive arrangements: 94°C for 10 min, 55°C for 30 s, and 72°C for 20 s. GAPDH was applied as

TABLE 1. RELATIONSHIP BETWEEN LNCRNA TMPO-AS1 EXPRESSION LEVELS AND CLINICOPATHOLOGICAL PARAMETERS OF LSCC PATIENTS.

Clinicopathological features	Number of cases	TMPO-AS1 expression		p
		High	Low	
Age				0.270
<55 years	96	45	51	
≥55 years	91	50	41	
Gender				0.437
Male	125	61	64	
Female	62	34	28	
Tobacco exposure				0.127
Smoker	100	56	44	
Nonsmoker	87	39	48	
Differentiation				0.304
Well	115	55	60	
Moderately/poorly	72	40	32	
Clinical stage				0.020
I/II	125	56	69	
III/IV	62	39	23	
Lymph node metastasis				0.027
Negative	13 9	64	75	
Positive	48	31	17	

TABLE II. UNIVARIATE AND MULTIVARIATE ANALYSIS OF OVERALL SURVIVAL IN LSCC PATIENTS

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p	HR	95% CI	p
Age	1.786	0.662-2.433	0.155	-	-	-
Gender	1.553	0.754-2.412	0.118	-	-	-
Tobacco exposure	1.449	0.824-2.325	0.155	-	-	-
Differentiation	1.728	1.028-2.58	0.114	-	-	-
Clinical stage	2.892	1.375-5.018	0.011	2.683	1.217-4.653	0.021
Lymph node metastasis	3.127	1.472-5.213	0.008	2.884	1.215-4.886	0.013
TMPO-AS1 expression	3.035	1.365-4.735	0.013	2.885	1.217-4.357	0.017

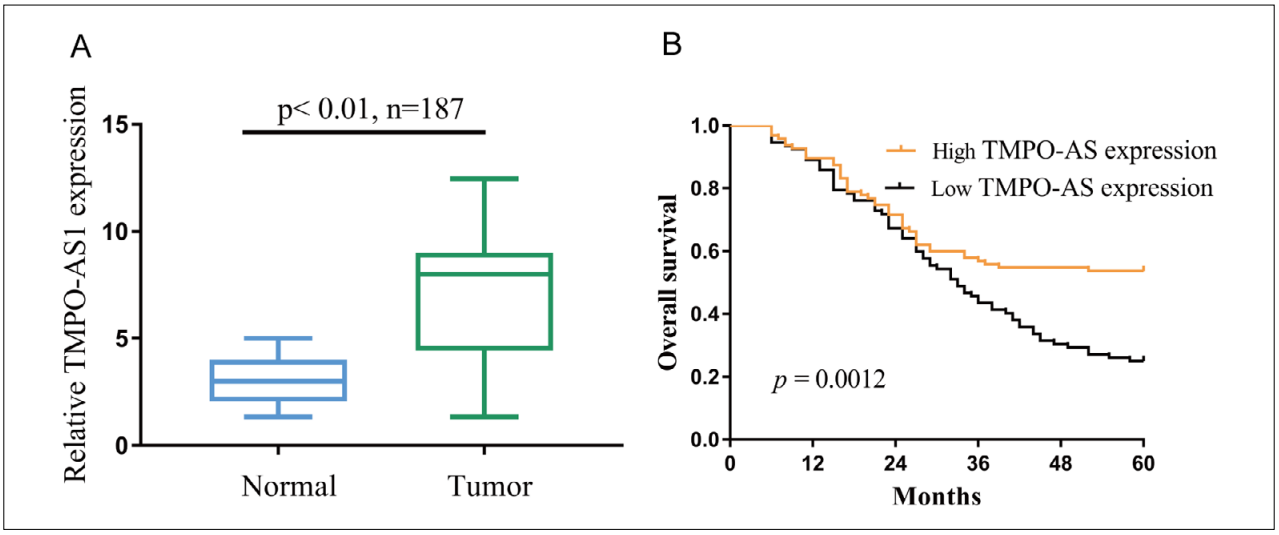

FIGURE 1. The associations between TMPO-AS1 levels and survival time in LSCC patients. (A) The expressions of TMPO-AS1 were determined by RT-PCR in 187 paired LSCC and adjacent normal tissues. (B) The associations between TMPO-AS1 levels and survival time in LSCC patients.

an endogenous control for the normalization of TMPO-AS1 expressions. The data were analyzed and expressed relative to the threshold cycle (CT) values. The primers' sequences for RT-PCR were as follows: TMPO-AS1, 5'- AGCCCACACACTACAGGCAG-3' (forward) and 5'- GCACAAAAGCAGTACGACCTA-3' (reverse); GAPDH, 5'- ACTCATGACCACAGTCCATGCC-3' (forward) and 5'- AGAGGCAGGGATGATGTTCTGA-3' (reverse).

Statistical analysis

The statistical assays were performed by SPSS software package (SPSS Inc., Chicago, IL, USA). Student's t-tests were used for the assays of the differences between the two groups. The correlations between clinicopathological parameters and TMPO-AS1 levels were determined using chi-square tests. Survival curves were plotted by the use of the Kaplan-Meier methods and possible differences in survival time were determined by the log-rank tests. The prognostic relevance of several variables to overall survival was analyzed using multivariate assays. A value of $p<0.05$ was considered statistically significant.

RESULTS

A significant upregulation of TMPO-AS1 was observed in LSCC tissues

To explore whether TMPO-AS1 was abnormally expressed in LSCC, our group used qRT-PCR to detect the TMPO-AS1 levels in 187 LSCC patients. As presented in Figure 1A, TMPO-AS1 was found to be distinctly higher in LSCC specimens compared with matched non-tumor samples ($p < 0.01$). Our data

suggested TMPO-AS1 as a new player that may display an oncogenic function in LSCC cells.

The correlation between TMPO-AS1 and clinical parameters of LSCC

Then, our group investigated the clinical significance of TMPO-AS1 in LSCC patients. The cutoff value, determined using the median expression of TMPO-AS1, was used to divide the 187 LSCC patients into two groups (High: n =95 and Low: n =92). The associations between TMPO-AS1 expressions and the clinical parameters of LSCC were summarized in Table 1; the results revealed that increased expressions of TMPO-AS1 positively correlated with the clinical stage (p = 0.020) and lymph node metastasis (p = 0.027), but not with age, gender, tobacco exposure, or differentiation (p >0.05). These observations revealed increased expressions of TMPO-AS1 predicted distinctly aggressive clinical features, suggesting that this lncRNA may promote LSCC progression.

Association of TMPO-AS1 expressions with patients' survival time

To further verify whether the overexpression of TMPO-AS1 had prognostic value in LSCC patients, our group performed a five-year follow-up analysis and collected clinical information on 5-year survivors from 122 to 187 patients. Then, Kaplan-Meier assays were applied and the results indicated that patients with higher TMPO-AS1 expressions had worse overall survival than those with lower TMPO-AS1 expressions (p= 0.0012, Figure 1B). Subsequently, univariate and multivariate analysis was used for further determining the practicability of TMPO-AS1 as a biomarker. In univariate assays, TMPO-AS1 expression, clinical stage, and lymph node metastasis were confirmed to be associated with the overall survival of LSCC patients (All p > 0.05). Moreover, in multivariate analysis, we confirmed high TMPO-AS1 expression (HR= 2.885, 95% CI: 1.217-4.357, p =0.017), together with clinical stage and lymph node metastasis, as an independent prognostic biomarker for LSCC patients.

DISCUSSIONS

In this study, for the first time, we provided strong evidence that TMPO-AS1 levels were frequently up-regulated in LSCC tissues, which suggested positive associations between the dysregulation of TMPO-AS1 and LSCC progression. Then, we analyzed whether higher levels of TMPO-AS1 were related to several clinical factors, finding that patients with upregulation of TMPO-AS1 exhibited advanced clinical stage and lymph node metastasis. It was known to us that most tumor patients with metastasis have a poor clinical outcome. Thus, we wondered whether TMPO-AS1 may influence the clinical prognosis of LSCC patients. As expected, in a clinical assay with 187 LSCC patients, we observed that in LSCC patients with increased TMPO-AS1 expressions. For further study of the clinical application of TMPO-AS1 as a prognostic biomarker, multivariate analysis was then performed and the results confirmed that TMPO-AS1 served as an independent biomarker for predicting the clinical outcome of LSCC patients.

LSCC is one of the most common malignant neoplasms. In order to improve the clinical prognosis of this tumor, sensitive biomarkers need to be identified that can be used to predict how well the body responds to a therapeutic schedule[16,17]. In recent years, critical molecular events during LSCC progression have been identified due to the development of genomics and proteomics[18]. These advances have resulted in the disclosure of new LSCC biomarkers, such as mRNAs, proteins, and ncRNAs[19-21]. These diverse markers could be detectable in plasma, marrow, and tumor specimens. Importantly, lncRNAs are considered to be ideal biomarkers for diagnosing LSCC and predicting prognosis because they are easy to detect, stable, and positively correlated with frequent tumor metastasis and clinical outcome[22].

To our best knowledge, this is the first time the possible prognostic value of TMPO-AS1 in LSCC patients has been reported. Several limitations of our experiments should be considered. Firstly, there was a relatively small sample size enrolled, which may result in statistical discrepancy. Secondly, our findings suggested TMPO-AS as a tumor promoter in LSCC. However, due to the funds and time available, functional explorations using *in vitro* and *in vivo* assays were not performed in the current study. Thirdly, the potential mechanism involved in the advanced progression of LSCC mediated by TMPO-AS1 overexpression needs to be explored.

CONCLUSIONS

Our findings highlighted the great value of TMPO-AS as a novel marker and possible therapeutic target.

Conflict of interest

The authors declare no conflicts of interest.

Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Lihua Zhang and Yu Zhang contributed equally to the work.

RESUMO

OBJETIVO: *RNAs longos não-codificantes (INcRNAs) são frequentemente expressos anormalmente em tumores e estão envolvidos na ocorrência e progressão do câncer humano. Recentemente, um INcRNA relacionado com a doença, o TMPO antisense RNA 1 (TMPO-AS1), foi identificado como desregulado em vários tumores. Por isso, procuramos demonstrar se o TMPO-AS1 poderia ser um marcador de prognóstico promissor para pacientes com carcinoma de células escamosas da laringe (LSCC).*

MÉTODOS: *RT-PCR foi realizado para medir as expressões do TMPO-AS1 em 187 espécimes de LSCC em comparação com espécimes normais correspondentes. Foram utilizados testes Qui-quadrado para determinar as associações entre as expressões do TMPO-AS1 e as características clínicas dos pacientes com LSCC. Em seguida, o desfecho clínico dos pacientes com LSCC que tinham uma expressão do TMPO-AS1 inferior ou superior foi analisado com ensaios Kaplan-Meier. Por último, o modelo de riscos proporcionais de Cox foi utilizado para avaliar o valor prognóstico do TMPO-AS1 e outras características clínicas.*

RESULTADOS: *Observamos que o TMPO-AS1 estava claramente super-regulado nos tecidos de LSCC humanos em comparação com os espécimes normais correspondentes (p<0,01). Expressões mais elevadas de TMPO-AS1 estavam positivamente associadas ao estágio clínico (p=0,020) e à metástase linfática (p=0,027). Um estudo clínico com 187 pacientes revelou que aqueles com expressões mais baixas de TMPO-AS1 tiveram uma sobrevida pior do que aqueles com expressões elevadas de TMPO-AS1 (p=0,0012). Além disso, o resultado de ensaios multivariados demonstrou que a expressão do TMPO-AS1 é um preditor independente para a sobrevida global de pacientes com LSCC.*

CONCLUSÕES: *TMPO-AS1 pode ser considerado uma molécula nova envolvida na progressão do LSCC, o que proporciona um possível biomarcador de prognóstico.*

PALAVRAS-CHAVE: *Laringe. Carcinoma de células escamosas. Neoplasias laríngeas. Biomarcadores. Regulação neoplásica da expressão gênica.*

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ORIGINAL ARTICLE

Evaluation of Toxoplasma, Rubella, and Cytomegalovirus serological results in women of childbearing age

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SUMMARY

OBJECTIVE: *This study aimed to determine the rates of IgG and IgM antibodies against cytomegalovirus, rubella, and Toxoplasma gondii (all of which may cause congenital infections) in women of childbearing age who were admitted to Bolu Abant İzzet Baysal University Training and Research Hospital.*

METHODS: *Between January 2015 and December 2017, Toxoplasma gondii, rubella, and cytomegalovirus IgM and IgG antibody levels were studied using the ELISA method (Architect i2000SR, Abbott, Germany) in patients aged 15 to 45 who attended the obstetrics and gynecology outpatient clinics. Toxoplasma gondii and cytomegalovirus IgG avidity levels were analyzed retrospectively.*

RESULTS: *A total of 13.470 tests were conducted in the laboratory. Seropositivity percentages of IgM antibodies were found to be 1.3%, 0.5%, and 1.6% for Toxoplasma (n = 3607), rubella (n = 3931), and cytomegalovirus (n = 3795), respectively. The seropositivity percentages of IgG antibodies were 22%, 94.2%, and 98.2% for Toxoplasma (n = 702), rubella (n = 693), and cytomegalovirus (n = 679), respectively. Primary infection (acute, recently acquired) was found in 7 (35%) patients with low Toxoplasma IgG avidity. One (3%) patient with low cytomegalovirus IgG avidity had a primary infection.*

CONCLUSION: *Toxoplasma gondii seronegativity was found to be high in the region. Therefore, screening women of childbearing age may be important for the prevention of congenital infections caused by Toxoplasma gondii.*

KEYWORDS: *Toxoplasma. Rubella. Cytomegalovirus. Immunoglobulin G. Immunoglobulin M.*

INTRODUCTION

Toxoplasma gondii, rubella, and cytomegalovirus (CMV) are microorganisms that can cause intrauterine infections and congenital anomalies in the fetus if they are transmitted during pregnancy[1]. In developing countries, infections that cause congenital anomalies are one of the most predominant causes of perinatal morbidity and mortality[2]. *Toxoplasma gondii* is a parasite that can cause hydrocephalus, intracranial calcifications, and chorioretinitis in fetus[3]. It is known as congenital toxoplasmosis if it is passed from mother to fetus during pregnancy[3]. Rubella is a virus that can cause congenital rubella infection, which can lead to low birth weight, deafness, myopia, cataracts, glaucoma, congenital heart disease, and intellectual disability in the fetus[4]. Finally, CMV is one of the largest viruses of the herpesviridae family, a common

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double-stranded DNA genome[5]. Mothers who have a CMV infection during pregnancy may experience sequelae such as mental retardation, chorioretinitis, and cerebral calcification in their fetuses[6].

The only way to prevent the risks of such infections during pregnancy is by serological screening of women of childbearing age. Informing prospective mothers in the risk group as a result of serological screening will help prevent congenital infections during pregnancy. Serological methods are used in the diagnosis of these infections, which can cause similar clinical manifestations. The IgG and IgM antibodies detected in the TORCH (*Toxoplasma gondii*, rubella, CMV, and HSV) group of microorganisms in human serum can assist in the diagnosis of acute, past, or recurrent infection[7,8]. This study aimed to determine the seroprevalence of *T. gondii*, rubella, and CMV infections through antenatal screening in the Bolu region, Turkey.

METHODS

The study was approved by the Bolu Abant Izzet Baysal University Medical Faculty Human Ethics Committee (2018/278). *Toxoplasma*, rubella, and CMV antibodies were investigated in the serum samples of patients between the ages of 15 to 45 from January 2015 to December 2017. Positive serum samples were examined retrospectively.

Toxoplasma gondii, rubella, and CMV IgM and IgG antibody tests were performed using the ELISA method (Architect i2000SR, Abbott, Germany). Only the first result of each patient was considered; other repetitive results of the same patient were not included in the study. All positive or borderline IgM test results were checked twice. Specific IgM and IgG test results were interpreted as negative, borderline, or positive. *Toxoplasma* and CMV IgG avidity test results were evaluated as low (<30%), borderline (30% to 40%), or high (>40%) on the avidity index according to the manufacturer's instructions. IgG avidity tests are methods used to determine whether the infection is primary (acute, recently acquired) or secondary (previously passed and immunized). In primary infections, the agent-specific IgG avidity is low, but in secondary infections it is high.

RESULTS

A total of 13.470 tests were conducted in the laboratory. Anti-*Toxoplasma* IgM was positive in 50 of the 3607 serum samples (1.3%) in which *Toxoplasma* IgM was examined. Anti-rubella IgM was found to be positive in 22 of 2231 serum samples (0.5%). Anti-CMV IgM was positive in 64 of 3795 serum samples (1.6%) of CMV IgM. Anti-CMV IgG was positive in 669 of 679 serum samples (98.2%) of CMV IgG. (Table 1).

Low avidity was detected in seven patients (35%) according to the avidity test performed on 20 patients with positive *Toxoplasma* IgM. High avidity was detected in eight patients (40%) according to the avidity test performed for *Toxoplasma*. One (3%) of the 33 patients with positive CMV IgM had low avidity. High avidity was detected in 32 patients (97%) according to the avidity test performed for CMV. Seropositivity rates for *Toxoplasma* (n = 702), rubella (n = 693), and CMV (n = 679) based on age group are shown in Table 2.

DISCUSSION

TORCH group infections can affect all age groups, but the transmission of these infections to the fetus during pregnancy is of growing concern, as these infections cause congenital anomalies in the fetus[9]. Conducting these screening tests during pregnancy or early pregnancy contributes to the early diagnosis of congenital anomalies that may occur in the fetus and also determines the regional seroprevalence[10].

The seroprevalence of *Toxoplasma gondii* in the world may vary depending on a variety of factors, including dietary habits, lifestyle, socioeconomic

TABLE 1. THE RATES OF IGM AND IGG FOR RUBELLA, TOXOPLASMA GONDII, AND CYTOMEGALOVIRUS (CMV) INFECTIONS.

Test	Negative (n %)	Borderline (n %)	Pozitive (n %)	Total
Toxoplasma IgM	3544 (98.3)	13 (0.4)	50 (1.3)	3607
Rubella IgM	3893 (99)	16(0.4)	22(0.5)	3931
CMV IgM	3697(97.5)	34(0.9)	64 (1.6)	3795
Toxoplasma IgG	538 (76.6)	9 (1.3)	155 (22.1)	702
Rubella IgG	17(2.4)	23(3.3)	653(94.2)	693
CMV IgG	12 (1.8)	0	667 (98.2)	679

status, and geographical conditions[11]. Some serological studies of *Toxoplasma gondii* in Turkey and other countries are shown in Table 3[12-18]. Indian studies have shown varied results, with seroprevalence ranging from 11% to 55%[19]. In the present study, *Toxoplasma* IgM was 1.3%, and *Toxoplasma* IgG was 22%; these levels were lower than those found by the studies conducted abroad (especially in India and Saudi Arabia). In Turkey, *Toxoplasma* IgM and IgG levels were similar, except for those found by the study in Kilis. The high seropositivity in the Kilis study may have been due to the consumption of undercooked meat and raw vegetables.

Rubella is a common viral infection that is frequently seen in children and young adults. In women of childbearing age, this infection is critical; it causes congenital rubella syndrome. In countries using the rubella vaccine (MMR) in their national vaccination programs, congenital rubella infections are less common[20]. For example, in the routine vaccination program organized by the Ministry of Health in Turkey, rubella vaccination is performed in the first month of childhood and in the first grade of primary school. In studies conducted in different regions of Turkey, rubella seropositivity has been reported as ranging between 86.5% and 96.2%[11,19,21-23]. In the present study, the rate of rubella IgG was 94.2%. According to this ratio, and considering the fact that no vaccination information was obtained from the women of childbearing age, seronegativity has been found for women in Turkey. Therefore, vaccination will be beneficial, as these women are at risk for congenital rubella syndrome.

CMV can be transmitted via vertical and horizontal contact, blood transfusion, and organ transplants. Seroprevalence increases with age and differs according to geographical regions and socioeconomic level[5]. While seropositivity rates in developed countries range from 50% to 60%, rates in developing countries are between 90% and 100%[21]. The results of serological studies of CMV in Turkey and other countries are shown in Table 4[4,6,11,23]. In the present study, the rate

TABLE 2. DISTRIBUTION OF TOXOPLASMOSIS, CYTOMEGALOVIRUS (CMV), AND RUBELLA SEROPOSITIVITY ACCORDING TO AGE GROUPS

Age (years)	Toxo IgG (n = 702)			CMV IgG (n = 679)			Rubella IgG (n = 693)		
	Positive	Negative	Borderline	Positive	Negative	Borderline	Positive	Negative	Borderline
<20	4(%28.6)	10(%71.4)	-	8(%72.7)	3(%27.3)	-	8(%88.9)	-	1(%11.1)
20-30	71(%19.2)	292(%78.9)	7(%1.9)	346(%99.9)	3(%0.1)	-	348(%94.5)	8(%2.2)	12(%3.3)
31-40	75(%24.4)	230(%74.9)	2(%0.7)	290(%97.9)	6(%2.1)	-	280(%92.7)	16(%5.3)	6(%2)
>40	4(%40)	6(%60)	-	23(%100)	-	-	17(%70.8)	3(%12.5)	4
Total	155(22.1)	538(76.6)	9(1.3)	667(98.2)	12(1.8)	-	653(94.2)	17(2.4)	23(3.3)

TABLE 3. COMPARISON OF STUDIES EVALUATING TOXOPLASMA SEROPREVALENCES FROM DIFFERENT REGIONS OF TURKEY AND OTHER COUNTRIES.

Study	Location, setting of the study	Test	Result(%)
Sen et al.[12]	India	*Toxoplasma* IgM	19.4%
Yasodhara et al.[13]	India	*Toxoplasma* IgM	13.1%
Khurana et al.[14]	India	*Toxoplasma* IgG, IgM	15.3% IgG, 3% IgM
Ghazi et al.[15]	Saudi Arabia	*Toxoplasma* IgG	35.6%
Demiroğlu et al.[16]	Kilis/Turkey	*Toxoplasma* IgG, IgM	63.4% IgG,4% IgM
Aşci et al.[17]	Afyon/Turkey	*Toxoplasma* IgG, IgM	23, 6% IgG, 1.9% IgM
Sirin et al.[18]	Izmir/Turkey	*Toxoplasma* IgG, IgM	32.3% IgG, 1.9% IgM

TABLE 4. COMPARISON OF STUDIES EVALUATING CMV SEROPREVALENCES FROM DIFFERENT REGIONS OF TURKEY

Study	Location, setting of the study	Test	Result(%)
Efe et al.[4]	Van/Turkey	CMV IgG, IgM	99.5% IgG, 1.7% IgM
Tamer et al.[23]	Kocaeli/Turkey	CMV IgG, IgM	96.4% IgG, o.7% IgM
Ocak et al.[11]	Hatay/Turkey	CMV IgG, IgM	94.9% IgG, 0.4% IgM
Bakacak et al.[6]	Kahramanmaras/Turkey	CMV IgG, IgM	99.3% IgG, 3.2% IgM

of CMV IgG was 98.2% and IgM was 1.6%. The highest CMV IgG results of studies conducted in Turkey was found by Efe et al[4]. The high seropositivity in this study indicates that the risk of primary infection due to crowded living conditions in our region may be high. Therefore, screening CMV IG and IgM antibodies in women of child bearing age will prevent the risk of CMV congenital infection in the future.

Avidity tests are used to differentiate whether an infection is a primary infection, re-infection, or secondary (pre-established and immunocompromised) infection. In primary infections, the specific IgG avidity (antigen-binding force) is low, while it is high in secondary infections[24]. In this study, the number of positive patients with *Toxoplasma* IgM/G was 50, and the number of positive patients with CMV IgM/G was 64. However, the avidity test counts were 20 for *Toxoplasma* IgG avidity and 33 for CMV IgG avidity. In this study, *Toxoplasma* IgG was found to have a low avidity of 35%, and CMV IgG had a low avidity of 3%. In this study, low avidity detection in CMV IgG and *Toxoplasma* IgG avidity tests shows that there may still be acute CMV infections in our region. Therefore, it is important to evaluate avidity tests in women of childbearing age to differentiate between acute, past, and recurrent infections. Previous avidity studies in this country have been limited. Şimşek et al.[10] found that *Toxoplasma* IgG avidity was 27% (low) and CMV IgG was 27% (low). In the present study, CMV IgG avidity values were lower, and *Toxoplasma* IgG avidity values were similar.

This study faced a few limitations. *Toxoplasma*, rubella, and CMV IgG and IgM test numbers were not studied equally. For example, the avidity IgG test was not studied in all patients with *Toxoplasma* and CMV IgG/M positivity. Another important limitation was that the vaccination history of the patients was unknown. In addition, the patients' antibody levels were tested only at two years. The number of seropositive patients would have been higher if the study had been conducted over a longer period of time.

CONCLUSION

In conclusion, this screening could be an effective approach for women of childbearing age due to the high rate of *Toxoplasma gondii* seronegativity among women in Turkey. Finally, the high seroprevalence of these agents, in our society, calls for preventive strategies such as reproductive hygiene and immunization to circumvent the otherwise inevitable fetal outcomes. This study showed that the seropositivity of rubella and CMV are similar to the results found by other studies conducted in this country.

Author contributions

FA, MB collected the data; MB, MGK reviewed the literature; FA designed the study; FA wrote the manuscript; FA, MB, MGK approved the final version of the manuscript.

RESUMO

OBJETIVO: *O objetivo deste estudo foi determinar as taxas de anticorpos IgG e IgM contra citomegalovírus, rubéola e Toxoplasma gondii (todos os quais podem causar infecções congênitas) em mulheres em idade fértil que foram admitidas no Hospital de Pesquisa e Treinamento da Universidade Bolu Abant İzzet Baysal.*

MÉTODOS: *Entre janeiro de 2015 e dezembro de 2017, os níveis de anticorpos IgG e IgM para Toxoplasma gondii, rubéola e citomegalovírus foram estudados usando o método Elisa (Architect i2000SR, Abbott, Alemanha) em pacientes de 15 a 45 anos que compareceram a ambulatórios de obstetrícia e ginecologia. Os níveis de avidez de IgG para Toxoplasma gondii e citomegalovírus foram analisados retrospectivamente.*

RESULTADOS: *Um total de 13.470 testes foram realizados em laboratório. As porcentagens de soropositividade dos anticorpos IgM foram de 1,3%, 0,5% e 1,6% para Toxoplasma (n=3.607), rubéola (n=3.931) e citomegalovírus (n=3.795), respectivamente. As porcentagens de soropositividade dos anticorpos IgG foram 22%, 94,2% e 98,2% para Toxoplasma (n=702), rubéola (n=693) e citomegalovírus (n=679), respectivamente. Infecção primária (aguda, adquirida recentemente) foi encontrada em sete (35%) pacientes com baixa avidez para Toxoplasma IgG. Um (3%) paciente com baixa avidez para citomegalovírus IgG teve uma infecção primária.*

CONCLUSÃO: *A soronegatividade do Toxoplasma gondii foi alta na região. Portanto, testar mulheres em idade fértil pode ser importante para a prevenção de infecções congênitas causadas pelo Toxoplasma gondii.*

PALAVRAS-CHAVE: *Toxoplasma. Rubéola. Citomegalovírus. Imunoglobulina G. Imunoglobulina M.*

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ORIGINAL ARTICLE

Homeobox B2 is a potential prognostic biomarker of glioblastoma

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SUMMARY

OBJECTIVES: *HOXB2 is a new prognostic indicator for lung cancer. But it is unclear whether HOXB2 holds an effect in glioblastoma (GBM) progression. The purpose of this article was to probe the influences of HOXB2 on GBM pathogenesis.*

METHODS: *HOXB2 expression level and prognostic power in GBM patients were analyzed. Then the mRNA and protein expression levels of HOXB2 in GBM cell lines were tested by qRT-PCR and western blotting. Cell proliferation, invasion, and migration were determined by CCK8 and transwell assay, severally. The protein levels of PI3K/AKT-pathway associated proteins were analyzed by western blotting.*

RESULTS: *The results indicated that HOXB2 was distinctly overexpressed in GBM patients and high expression of HOXB2 was related to a poor prognosis. Moreover, the expression of HOXB2 was higher in all GBM cell lines U251, U-87MG, GOS-3 than that in HEB cells (normal control). Meanwhile, decreased expression of p-PI3K and p-AKT were identified after HOXB2 knockdown.*

CONCLUSIONS: *These data demonstrated that HOXB2 had a vital role in GBM progression and could serve as a promising target for GBM treatment.*

KEYWORDS: *Glioblastoma. Biomarkers. Homeodomain proteins.*

INTRODUCTION

Glioblastoma (GBM) is the most prevalent and aggressive malignant brain cancer[1]. Currently, the standard treatment methods are surgery, chemotherapy, and radiation therapy[2]. A recent study found that surgical resection extent in elderly patients with glioblastoma has an impact on survival[3]. In recent years, researchers have been working to explore the molecular mechanisms of gliomas, discovering telomerase reverse transcriptase and many other molecules[4,5]. The homeobox (HOX) genes, containing a 183-nucleotide sequence, are a regulated gene family that encodes particular nuclear proteins into transcription factors[6]. The HOX genes contribute to the assignment of segmental identity in the development of hindbrain[7]. In humans, there are four clusters, i.e., HOXA, HOXB, HOXC, and HOXD, which are organized by 39 HOX genes[8]. One piece of evidence revealed that the expression of ectopic HOXB2 in pancreatic cancer may be related to a poor prognosis[9]. However, there were few reports on GBM.

In the present review, we verified the role of HOXB2 in GBM from the following aspects: i) Differential gene analysis and prognosis analysis; ii) the effect of HOXB2 on GBM proliferation, invasion, and

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migration; iii) Study on the mechanism of HOXB2 on phenotype.

METHODS

Analyses of HOXB2 expression profile from ONCOMINE

In this paper, the expression data of GBM patients and healthy individuals were obtained from the ONCOMINE (https://www.oncomine.org/resource/login.html) database. According to the median expression value of HOXB2, GBM patients were distributed into high and low expression groups. The statistical significance was determined by the log-rank test.

Culture of GBM cell lines

Human GBM cell lines U251, U-87MG, GOS-3 and normal human astrocytes HEB were obtained from ATCC (MD, USA). The cells were routinely cultured in RPMI-1640 medium supplemented with FBS (10%), penicillin (100 U/ml), and streptomycin (0.1 mg/ml) with 5% CO_2 at 37 °C.

Cell transfection

RNA interference was utilized to silence HOXB2 expression in U251 cells. The scrambled siRNA was used as a negative control. Full-length sequences of HOXB2 were cloned into the pcDNA3.1 vector to over-express the HOXB2 level, termed pcDNA3.1-HOXB2 and the negative control pcDNA3.1 were used to over-express HOXB2 expression in GSO-3 cells. si-HOXB2#1, si-HOXB2#2, si-control, pcDNA3.1-HOXB2, and pcDNA3.1 were all synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China) and transfected into human clone cancer cells according to the Lipofectamine2000 transfection kit (Invitrogen, Shanghai, China). The siRNA sequences for HOXB2 were the following.

si-control: F: 5'-AATTCTCCGAACG GTCACGT-3'
si-HOXB2#1: F: 5'-TACTGAATTAGCGTTTAATC-3'
si-HOXB2#2: F: 5'-CAATCAAGGAGTCGACATTA-3'

qRT-PCR

The extraction of total RNA was carried out with an RNA extraction kit following its instructions. After reverse transcription into cDNA, the expression of HOXB2 was detected by qRT-PCR. Actin was utilized as an internal control. PCR reaction system: 95 °C for 5 min, 40 cycles of 95 °C for 30 sec, 60 °C for 45 sec, and annealing at 72°C for 30 min. There were 3 sets of holes in each group. The HOXB2 expression level was calculated by the $2^{-\Delta\Delta^{Ct}}$ method. This was repeated three times independently. The primer sequence was as follows:

HOXB2: F: 5' CGCGAGATGGAAGGAGAGTC 3'
R: 5' AGGGCCTGTCTAGTCCTCTG 3'
ACTIN: F: 5'CCCGAGCCGTGTTTCCT 3'
R: 5' GTCCCAGTTGGTGACGATGC 3'

CCK8

To measure cell proliferation, the number of viable cells at 0, 24, 48, and 72 h after transfection was assessed by Cell Counting Kit 8 (CCK8; Dojindo Laboratories, Kumamoto, Japan). The OD_{450} value was measured by a microplate reader (Bio-Rad, CA, USA) and the proliferation curve was drawn by GraphPad Prism 7.0.

Transwell migration and invasion experiments

Transwell migration and invasion assays were conducted in 24-well Transwell chambers. The upper surface of transwell was precoated with (invasion assay) or without (migration assay) Matrigel (BD Transduction). The transfectants (1×10^5 cells per well) were transferred into the upper chamber. In the lower chamber of 24-well plates, 500 µl of complete medium was added. Following 22 h of incubation, the migrated or invasive cells on the lower surface of the filters were fixed and stained with 0.1% crystal violet for twenty minutes. After being washed by PBS, the outcomes were photographed under the microscope (Olympus, Tokyo, Japan) and 5 fields of view were randomly selected. The stained cells were counted directly in triplicate.

Western blotting

Cells were directly lysed in RIPA lysis buffer (Thermo Scientific, Waltham, MA, USA). We used 10% SDS-PAGE, and 20 µg protein were separated. Then the proteins were transferred onto a PVDF membrane. After being enclosed with 5% non-fat milk at room temperature for one hour, the membrane was then hatched with the primary antibodies at 4°C overnight and the secondary antibodies at 37°C for two hours. Finally, the membrane was visualized by ECL. QUANTITY ONE software to scan the gray value and Tubulin was employed as an internal control.

Data statistics

The outcomes were presented as mean ± SD. All data analyses were executed utilizing SPSS 22.0 software, and all histograms were drawn employing GraphPad Prism version 7.0. Intergroup differences were assessed by Student's t-tests (between two groups) or analysis of variance (between more than two groups), as appropriate. Post hoc analysis was executed utilizing the Bonferroni test. The outcomes were considered as statistically significant when at $P < 0.01$.

RESULTS

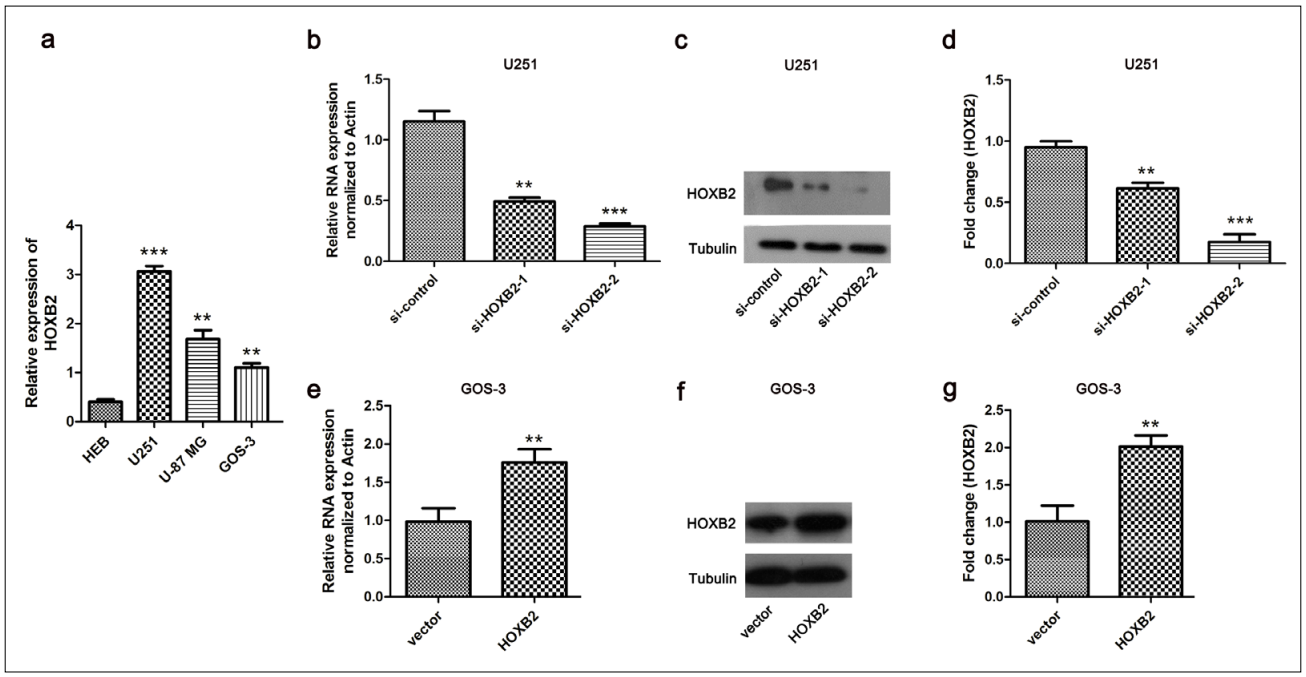
Increased HOXB2 was discovered in GBM tissues and related to worse prognosis in GBM patients

In the paper, HOXB2 expression in GBM and normal tissues was investigated using the ONCOMINE database. The results indicated that HOXB2 was visibly up-regulated in GBM tissues compared with normal tissues (Figure 1a, P = 2.93E-9; Figure 1b, P = 3.31E-9). Besides, based on the TCGA database, the expression of HOXB2 in GBM patients was drawn utilizing GEPIA. According to the median value of HOXB2 expression, patients were distributed into high and low expression groups. GBM patients with HOXB2 high expression were related to worse overall survival compared to those with low expression (P = 0.00076).

Over-expression and ablation of HOXB2 in GBM cell lines

We further studied the expression of HOXB2 in GBM cell lines. HEB is a normal brain cell line that we used to compare with five different GBM cell lines U251, U-87 MG, and GOS-3. A significant over-expression of HOXB2 was found in these three GBM cell lines compared to HEB cells. Furthermore, HOXB2 showed the highest expression in the U251 cell line while exhibited the lowest expression in the GOS-3 cell line compared to other GBM cells using mRNA levels for comparison (Figure 1a). Hence, in the following experiments, detection of the HOXB2 knockout effect was executed in the U251 cell line, and detection of the influences of HOXB2 over-expression was carried out in the GOS-3 cell line. The HOXB2 si RNA was transfected into the U251 cell line, and the non-specific sequence si-control was transfected as a control. After 48 h of transfection, the total RNA and protein were extracted and detected by qPCR and western blotting. The results indicated that si RNA-1 and si RNA-2 could distinctly reduce HOXB2 expression both in mRNA and protein levels in U251 cells, and si RNA-2 displayed the lower expression of HOXB2. We utilized si-HOXB2#2, and the knockout efficiency was over 80% (Figure 1b-d). Furthermore, as illustrated in Figure 1e-g, pcDNA3.1-HOXB2 could remarkably elevate HOXB2 expression both at mRNA and protein levels in GOS-3 cells.

FIGURE 1. OVER-EXPRESSION AND KNOCKDOWN OF HOXB2 IN DIFFERENT GBM CELL LINES.



Knockdown of HOXB2 represses U251 cell proliferation, invasion and migration whilst HOXB2 high-expression accelerates GOS-3 cell proliferation, invasion, and migration

For the purpose of studying the impact of HOXB2 on GBM cell proliferation, CCK-8 assay was executed. As displayed in Figure 2a, the OD_{450} value of U251 cells was lower in the si-HOXB2 group compared to the si-control group, proving that HOXB2 deficiency reduced U251 cell proliferation (P<0.01, P<0.01). The influences of HOXB2 on the invasion and migration of the U251 cells were investigated utilizing transwell assays. The silencing of HOXB2 visibly decreased the number of invasive and migrated crystal violet-stained cells in the transwell assay (Figure 2b-c). Conversely, the over-expression of HOXB2 sharply facilitated GOS-3 cell proliferation after being cultured for 48 h and 72 h, but there was no significant effect at 24 h (P<0.01, P<0.01, Figure 2d). Moreover, HOXB2 high-expression distinctly increased the number of crystal violet-stained GOS-3 cells in the transwell assay (P<0.01, P<0.01, Figure 2e-f).

Down-regulation of HOXB2 suppresses the PI3K/AKT pathway in U251 cells, and over-expression of HOXB2 promotes the PI3K/AKT pathway in GOS-3 cells

The PI3K/AKT signaling pathway is an important signaling pathway in tumors. The western blot results indicated that the p-PI3K and p-AKT expression levels were decreased significantly in U251 cells transfected with si-HOXB2 compared to the si-control group (Figure 3a-b). Nevertheless, the protein levels of p-PI3K and p-AKT were remarkably improved by the over-expression of HOXB2 (P<0.01, P<0.01, Figure 3c-d).

DISCUSSION

In this paper, we discovered that HOXB2 high expression was linked to a poor prognosis of GBM patients. Furthermore, knockdown of HOXB2 significantly suppressed cell proliferation, invasion, and migration, possibly by activating the PI3K/AKT pathway in U251 cells.

Our results showed that si-HOXB2 overtly reduced the mRNA and protein expression levels of HOXB2 in U251 cells. HOXB2, as part of the HOX genes family, affects normal vertebrate organ and limb development[10]. Recently, Hoxb2 was identified as a target gene of PLZF and may be associated with many developmental systems, such as hematopoiesis and the central nervous system (CNS)[11]. There is evidence to suggest that HOXB2 is expressed in the mature

FIGURE 2. HOXB2 DEPLETION RESTRAINED THE PROLIFERATION, MIGRATION, AND INVASION OF U251 CELLS, AND THE OVER-EXPRESSION OF HOXB2 ACCELERATES THE PROLIFERATION, MIGRATION, AND INVASION OF GOS-3 CELLS.

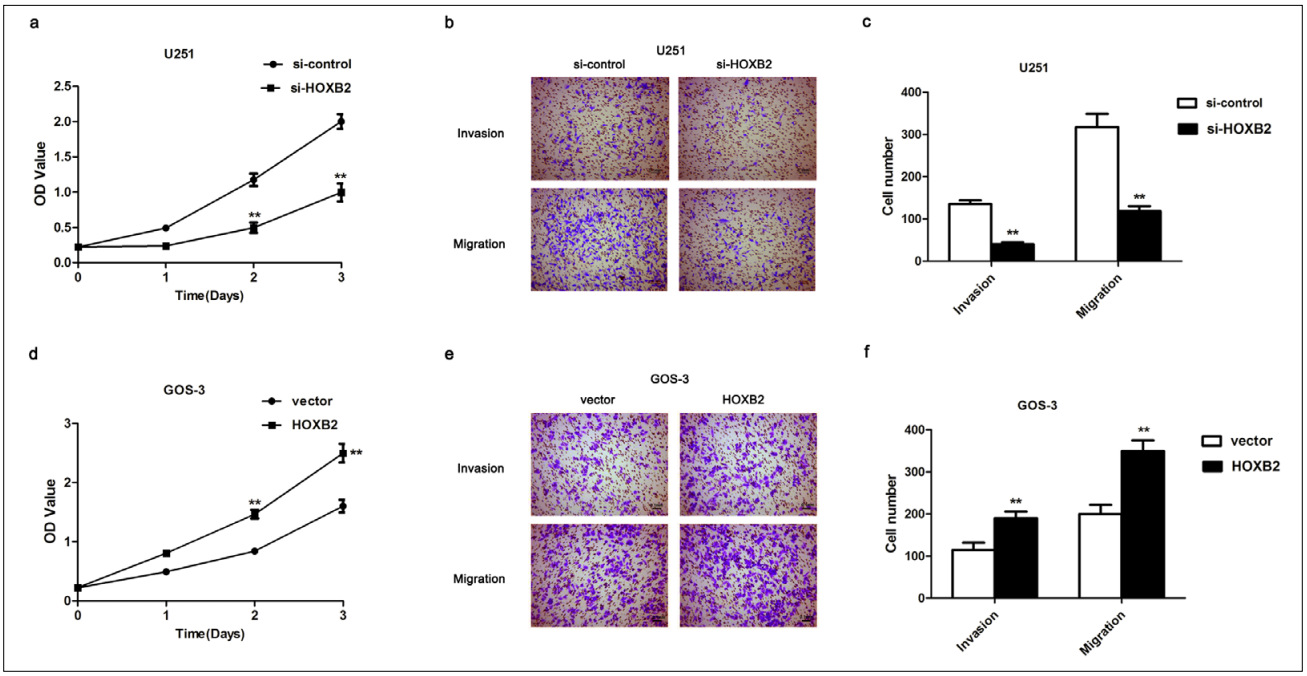
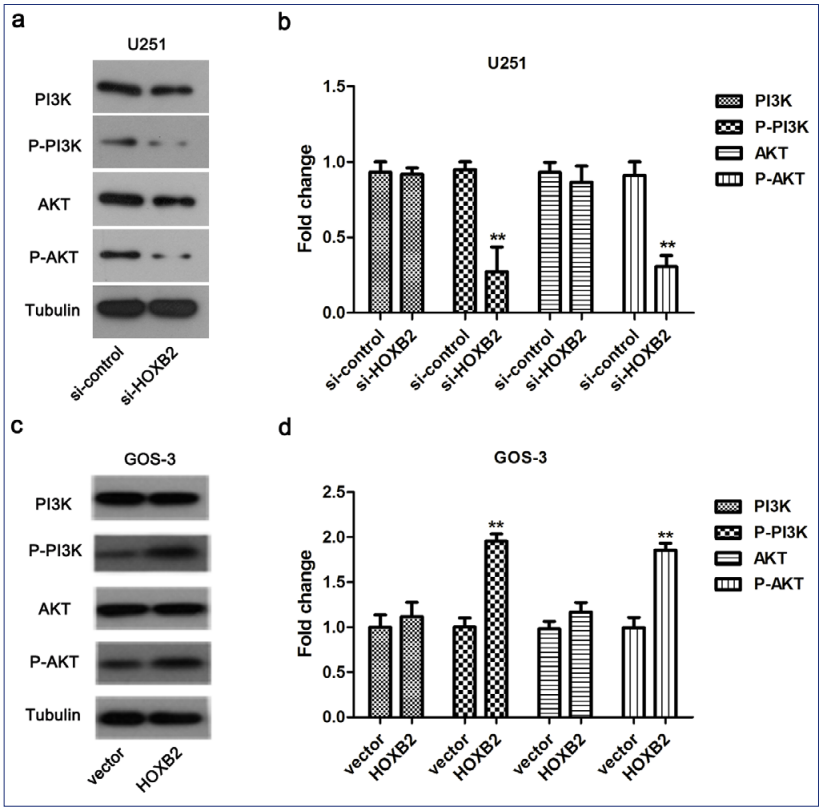


FIGURE 3. IMPACTS OF HOXB2 ON THE PI3K/AKT SIGNALING PATHWAY IN GBM CELLS.



brain and is related to motor neurons in neuronal cell lines[12]. According to a recent report, HOXB2 and HOXB1 had genetic interaction during facial-nerve motor-nuclear development[13]. Boimel et al.[14] hold the view that HOXB2 has a vital role in the regulation of breast cancer growth. It has been indicated that HOXB2 can be used as a novel biomarker for pancreatic cancer[9]. These data have motivated us to study whether HOXB2 has a role in GBM. In our study, we found that the down-regulation of HOXB2 suppressed the proliferation, invasion, and migration of U251 cells, manifesting that HOXB2 had a promoting role in GBM progression.

Studies have reported that the activation of PI3K/AKT participated in the regulation of GBM proliferation[15]. PI3K/AKT, as a significant signaling pathway in reacting to extracellular signals, can mediate many cellular processes including protein transcription, apoptosis, vascular metabolism, production, and cell viability[16,17]. As a major downstream PI3K effector, AKT regulates the activity of many targets, including transcription factors, kinases, among others[18]. AKT participates in the modulation of cell proliferation and anti-apoptosis[19,20].

CONCLUSION

In summary, the present research demonstrated that HOXB2 played a promoting role in GBM progression, partially through mediating the PI3K/AKT pathway. Thus HOXB2 may be a novel promising target for GBM treatment. A limitation of our study is the lack of *in vivo* experiments, which will be reported in future studies.

Conflict of Interest

The authors declare that they have no conflict of interests.

Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

RESUMO

OBJETIVOS: *A HOXB2 é um novo indicador prognóstico para o câncer de pulmão. Mas não está claro se a HOXB2 tem algum efeito na progressão do glioblastoma (GBM). O objetivo deste artigo foi sondar as influências da HOXB2 na patogênese do GBM.*

MÉTODOS: *Foram analisados o nível de expressão e o poder prognóstico da HOXB2 em pacientes com GBM. Em seguida, os níveis de expressão proteica e mRNA da HOXB2 em linhagens de células de GBM foram testados por qRT-PCR e western blotting. A proliferação, a invasão e migração celular foram determinadas por CCK8 e ensaios transwell, várias vezes. Os níveis proteicos das proteínas associadas à via PI3K/AKT foram analisados pelo método western blotting.*

RESULTADOS: *Os resultados indicaram que havia uma clara superrexpressão da HOXB2 em pacientes com GBM e que a alta expressão da HOXB2 estava relacionada a um prognóstico negativo. Além disso, a expressão da HOXB2 foi mais elevada em todas as linhagens de células do GBM U251, U-87MG, GOS-3 do que nas células HEB (controle normal). Entretanto, a diminuição da expressão de P-PI3K e p-AKT foi identificada após a redução da expressão da HOXB2.*

CONCLUSÕES: *Esses dados demonstram que a HOXB2 desempenha um papel vital na progressão do GBM, podendo ser um alvo promissor para o tratamento do GBM.*

PALAVRAS-CHAVE: *Glioblastoma. Biomarcadores. Proteínas de homeodomínio.*

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ORIGINAL ARTICLE

Incidence of aspirin resistance is higher in patients with acute coronary syndrome and atrial fibrillation than without atrial fibrillation

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SUMMARY

In patients with atrial fibrillation, standard anticoagulation with a vitamin K antagonist plus dual antiplatelet therapy with a P2Y12 inhibitor and aspirin is the standard of care after percutaneous coronary intervention (PCI). While this therapy reduces the risk of thrombosis and stroke, it increases the risk of bleeding. It is unclear whether the antiplatelet effect of aspirin and clopidogrel may worsen atrial fibrillation (AF).

OBJECTIVE: *Thus we aimed to analyze platelet aspirin resistance (AR) and clopidogrel resistance (CR) in acute coronary (ACS) patients based on sinus rhythm (SR) and AF.*

METHODS: *In this prospective trial, we included 543 patients (mean age: 62± 12 years; range: 26 - 89 years) who were on aspirin and clopidogrel therapy after the diagnosis of acute coronary syndrome. AR and CR were analyzed by a Multiplate® MP-0120 device by using the method of whole blood aggregometry.*

RESULTS: *AF patients had significantly higher age, mean platelet volume, and High-Sensitivity C-Reactive Protein (p< 0.01 for each parameter). Similarly, Arachidonic-acid induced (ASPI) aggregation was higher in AF patients compared to SR patients (666±218 vs. 187±179, p<0.001). Among the ACS patients, significantly more female patients had AF (p<0.001). The incidence of hypertension in the AF group was higher compared to the SR group (p<0.001). However, adenosine diphosphate levels were not at a significant level in the two groups.*

CONCLUSION: *Our findings indicate that the platelet inhibitory effect of Aspirin was worse for patients with AF, suggesting that the effectiveness of aspirin may be less in the prophylaxis of thromboembolism and more a bleeding risk.*

KEYWORDS: *Aspirin. Clopidogrel. Drug resistance. Acute coronary syndrome. Atrial fibrillation.*

INTRODUCTION

The incidence of atrial fibrillation in patients who undergo percutaneous coronary intervention is approximately 5% to 8%[1,2]. Oral anticoagulation (OAC) is indicated in these patients for the prevention of stroke and systemic embolism[3]. In addition, these patients must be administrated dual antiplatelet therapy with a P2Y12 inhibitor plus aspirin for the prevention of cardiovascular events, including stent

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thrombosis[4]. Until recently, most guidelines recommended both anticoagulation and dual antiplatelet therapy (triple therapy)[3,5]. However, it can be difficult to balance the prevention of thrombosis with the risk of bleeding[6]. In a recent study, ınvestigators omitted the use of aspirin from the standard regimen and used a single P2Y12 inhibitor in combination with an oral anticoagulant. They found that the risk of bleeding was lower with a regimen of reduced-dose rivaroxaban plus a P2Y12 inhibitor than with the standard triple therapy[7]. Another study supported the use of triple therapy for a short duration[8]. RE-DUAL PCI has shown that dual therapy was not inferior to triple therapy concerning the risk of thromboembolic events[9]. In the PIONEER trial, investigators reported that the administration of either low-dose rivaroxaban plus a P2Y12 inhibitor for 12 months or very-low-dose rivaroxaban plus DAPT for 1, 6, or 12 months was associated with a lower rate of clinically significant bleeding than standard therapy with a vitamin K antagonist plus DAPT for 1, 6, or 12 months[10]. In the AUGUSTUS trial, similar results were found too. P2Y12 inhibitor plus apixaban without aspirin resulted in less bleeding and fewer hospitalizations without significant differences in the incidence of ischemic events than regimens that included a vitamin K antagonist, aspirin, or both[11]. OAC plus aspirin or a single P2Y12 inhibitor regimen has seemed to be adequate for the prevention of ischemic events. However, the optimal antithrombotic therapy for these patients remains controversial. There is no clear recommendation on the use of ASA or clopidogrel with anticoagulant therapy after ACS. We aimed to investigate whether there is an association between AF and ASA resistance in patients with ACS.

METHODS

This was a prospective observational sub-study parallel to an investigation of resistance to aspirin and clopidogrel in patients in the Isparta area of Turkey. The protocol was obtained from the patients or their substitutes. All patients were followed-up prospectively and retrospectively analyzed for this study. The overall study population included 628 patients with ACS. The inclusion criteria were the following: age greater than 18 years and the presence of ACS. The exclusion criteria included the following: clinical indications of prolonged use of heparin and fondaparinux, clinical indications for the use of ASA doses of >100 mg/day or clopidogrel at doses of >75 mg/day prior to enrolment in the study, rheumatic mitral disease, use of prasugrel, ticagrelor, clinical indications of use of oral anticoagulants, cardiogenic shock at admittance to the hospital, heart failure categorized as Class III or IV in the NYHA scale at admittance to the hospital, thrombocytopenia (<100 x 10 9 g/L), purpura, anemia with hemoglobin concentration <100 g/L, clinically present active inflammation or thrombosis in-stent revealed in the interview or during hospitalization, and co-existence of diseases with poor prognosis (less than a year of life). According to these criteria, 85 patients were excluded due to thrombocytopenia (n=5), high-dose clopidogrel usage (n=40), oral anticoagulant usage (n= 30), and cardiogenic shock (n= 10). Finally, 543 patients were included in this sub-study. The institutional ethics committee approved the study and all participants provided written informed consent.

Diagnoses were recorded by the participating physicians based on clinical, electrocardiographic, and biochemical (elevated troponin levels) criteria. The type of myocardial infarction (ST-elevation vs non-ST-elevation) and unstable angina were homogeneously defined based on current guidelines[4].

Each patient was questioned about major cardiovascular risk factors including their family history of coronary artery disease, current smoking status, hyperlipidemia, hypertension, diabetes mellitus, and obesity. A family history of coronary artery disease was defined as the manifestation of the disease in first-grade male relatives younger than 55 years or in first-grade female relatives younger than 65 years of age. Hyperlipidemia was defined as fasting total cholesterol level >200 mg/dL or pharmacotherapy with lipid-lowering agents. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg measured before hospitalization or pharmacotherapy with antihypertensive drugs. Diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dL or pharmacotherapy with insulin or oral antidiabetic agents. Obesity was defined as body mass index >30 kg/m^2. Patients who were smoking prior to hospitalization were characterized as smokers.

The patients' clinical data, their previous medication history, and medications started after hospitalization were recorded. The patients were divided into two groups: with AF and without AF. A 12-lead electrocardiogram was recorded upon admission to the hospital. AF was defined as an irregular

rhythm with the absence of discrete P waves in the 12-lead electrocardiogram[12].

Patients were given 300 mg loading of ASA, 600 mg of clopidogrel, and, subsequently, maintenance doses of 75 mg clopidogrel and 100 mg ASA daily.

Blood sampling and analyses

Platelet function analysis was performed in the patients after they received a 600-mg clopidogrel loading dose in addition to pre-treatment with ASA. Blood samples were obtained 24h after percutaneous coronary intervention. Whole blood (5 ml) was collected using heparin as the anticoagulant in a Lithium Heparin bottle (non-gel). The blood was analyzed in a Multiplate Platelet Function Analyzer, which analyzes platelet function in whole blood samples based on impedance aggregometry, (Dynabyte Medical, Munich, Germany) 2006, using 20 µl of the activator. The following tests were performed: (i) ADP test with adenosine diphosphate (ADP) to assess P2Y12-dependent platelet aggregation; (ii) ASPI test with arachidonic acid (AA) to assess cyclooxygenase-dependent platelet aggregation

Arachidonic acid (ASPI) test reagent (20 µl of 15 mM stock solution) contains arachidonic acid. This triggers platelet aggregation via platelet cyclooxygenase, which is blocked by aspirin. Adenosine diphosphate (ADP, 20 µl of 0.2 mM stock solution) which triggers platelet activation via platelet ADP receptors (i.e. P2Y12 receptor that is inhibited by clopidogrel). All tests were performed within 2 h of blood sampling. The results were defined as the area under the curve (AUC) at the end of the 6 min measurement period. An AUC value of 500 min for ASA and 470 min for clopidogrel was considered as the minimum resistance value for patients under dual antiplatelet therapy[13-15].

Statistical analysis

SPSS version 16.0 software package program was used in the statistical analyses of the study. Categorical variables were expressed as frequency (%) and compared with the $\chi 2$ test. A Kolmogorov-Smirnov test was used to test the distribution of numeric variables, and those with normal distribution were expressed as mean ± standard deviation and were compared with the Student's t-test. Data without normal distribution were expressed as median (Inter-quartile range (IQR) of 25%-75% percentiles) and were compared with the Mann-Whitney U test. In all statistical analyses, p values <0.05 were considered as statistically significant.

RESULTS

A total of 543 patients (mean age: 62± 12 years; range: 26 - 89 years) were included in this study. At admission, 32 patients (10 %) had AF. The demographic and clinical characteristics of the patients with and without AF are listed in Table 1. The patients with AF were predominantly female when compared to patients without AF (p<0.001). Diabetes mellitus and hypertension were more common (p=0.06, p<0.001, respectively), but smoking was less commonly seen in patients with AF as compared to those without AF (p = 0.069). The incidence of obesity was similar in both patient populations (p=0.541). Total cholesterol, triglycerides, low-density lipoprotein cholesterol, and HDL levels were similar between the two groups of patients (p= 0.283, p= 0.071, p= 0.282, p=0.343, respectively). ASPI levels were higher in the AF group (p<0.001). Mean platelet volume (MPV) and Hs-CRP levels were higher in AF patients (p<0.001, p=0.001, respectively). The presence of AF was positively correlated with MPV (r=0.16 p<0,001), Hs-CRP (r=0.45, p<0.001). Univariate analysis showed that the presence of AF was a predictor of AR (OR 5.18, 95% CI 1.88-14.2, p < 0.001).

DISCUSSION

The present study showed that AF patients with ACS had increased AR compared to SR patients with ACS. Moreover, ASA was observed to be inadequate for the treatment of AF.

Antiplatelet therapy is the cornerstone of the therapeutic approach in coronary artery disease for the prevention of stent thrombosis and the reduction of cardiovascular events in patients who undergo coronary stenting and suffer acute coronary syndromes. Anticoagulation is needed for stroke prevention in patients with atrial fibrillation. In general, guidelines advise the continuation of anticoagulation and using triple oral antithrombotic therapy (TOAT), for a short period after PCI, the duration of which depends on bleeding risk and stent type. In addition, they recommended using bare-metal stents, targeting an INR range of 2.0–2.5 for patients receiving TOAT, and using radial access during PCI. They recommend OAC and single oral antiplatelet therapy (SAPT) with ASA or a P2Y12 receptor antagonist, for a short period after PCI[3,16]. However, there is no clear opinion on whether SAPT is ASA or a P2Y12 receptor antagonist.

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE PATIENTS WITH AND WITHOUT ATRIAL FIBRILLATION (AF).

	Patients without AF (n=511)	Patients with AF (n= 32)	P value
Age, year	61± 11	71± 7	< 0.001
Female Gender n(%)	103 (20%)	16 (50%)	< 0.001
BMI (kg/m^2)	26± 3	27± 3	0.541
Smoking n(%)	269 (52%)	12 (37%)	0.069
Diabetes Mellitus n(%)	177 (34.6%)	16 (50%)	0.06
Hyperlipidemia n(%)	162 (31.7%)	8 (25%)	0.281
Hypertension n(%)	216 (42%)	26 (81%)	<0.001
Total Cholesterol (mg/dL)	183 ± 39	191± 48	0.283
HDL Cholesterol (mg/dL)	39 ± 11	40 ± 7	0.343
LDL Cholesterol (mg/dL)	110 ± 32	117 ± 43	0.282
Triglyceride Cholesterol (mg/dL)	160 ± 84	199 ± 115	0.071
BUN (mg/dL)	18 ± 8	21± 9	0.023
Creatinine (mg/dL)	1.6 ±7	1.0 ± 0.3	0.094
ASPI	187 ± 179	666 ± 218	<0.001
ADP	263	306	0.261
HGB(g/dL)	13.8 ± 1.7	13.8 ± 1.7	0.848
Platelet count (x10^3/mm^3)	230 ± 68	228± 58	0.848
Mean platelet volume (fL)	8.2 ±0.9	9.2±1.4	<0.001
Hs CRP (mg/dL) (mean)	131	250	<0.001

BMI: Body mass ındex, HDL: High density lıpoprotein, LDL: Low density lıpoprotein, ADP: adenosine diphosphate, ASPI: Arachidonic acid HGB: Hemoglobin, Hs-CRP: High sensitive C reactive protein

In the Re-DUAL PCI study, investigators showed that, among patients with atrial fibrillation who had undergone PCI, dual therapy with OAC and a P2Y12 inhibitor had a lower bleeding risk than with triple therapy with warfarin, a P2Y12 inhibitor, and aspirin. Additionally, dual therapy with dabigatran was non-inferior to triple therapy with warfarin concerning the rate of thromboembolic events[9]. In the WOEST trial, patients were assigned to receive clopidogrel alone (double therapy) or clopidogrel and aspirin (triple therapy). They established that treatment with clopidogrel and oral anticoagulants was associated with a significantly lower risk of bleeding complications than aspirin, clopidogrel, and oral anticoagulation in patients with atrial fibrillation who had undergone PCI. Furthermore, they did not determine an increased risk of thrombotic events by omitting aspirin[7]. In our study, AR was higher in patients with AF. Additionally, AF is an independent risk factor of AR, based on multivariate analysis. Our findings are supported by recent studies. Clopidogrel can be recommended in patients with atrial fibrillation who had undergone PCI with an OAC according to these findings.

Increased platelet turnover resulting from infection, inflammation, diabetes mellitus, or hypertension can result in an increased proportion of non-aspirinated platelets[17]. Additionally, hypertension and diabetes mellitus were risk factors for the development of AF[18]. Moreover, the overall effect of aspirin-induced inhibition of platelet aggregation may be diminished. Our results showed that the incidence of diabetes mellitus and hypertension were higher in patients with AF. Thus, an increase in the proportion of non-aspirinated platelets may contribute to pathogenesis in these patients.

An association between inflammation and AF has been indicated in the literature[19,20]. Activated platelets release inflammatory mediators and induce the expression of these mediators in monocytes/macrophages and granulocytes[21]. Inflammation is an important factor in the initiation and maintenance of AF[22,23]. HsCRP and interleukin-6 levels were reported to be elevated in patients with paroxysmal, persistent, and permanent AF compared to those with sinus rhythm[24]. Moreover, thrombogenesis markers correlated with CRP levels. Erdogan et al.[25] reported that elevated MPV values positively correlated with higher CRP in different types of hypertensive patients. Similarly, in the present study, hsCRP and MPV levels were significantly higher in patients with AF. Increased inflammation in AF may be another factor that could contribute to AR.

CONCLUSION

The present study suggests that in a patient population with ACS, inflammation, prothrombotic state, and ASA resistance were significantly higher in patients with AF compared to those without it. The results of our study support the hypothesis that ASA may be less indicated for prophylaxis of thromboembolism and increases bleeding risk. A P2Y12 inhibitor may be used with OAC instead of ASA for long-term treatment in AF patients who undergo PCI. Nevertheless, this study was not powered to detect differences in the occurrence of thrombotic events, such as stent thrombosis, when aspirin was omitted, and this feature would need to be studied in a larger trial. We think that aspirin does not need to be used in patients receiving oral anticoagulants and undergoing PCI. Further studies are needed to establish the pathophysiological and clinical significance of increased oxidative stress and inflammation and investigate the effect of antioxidant and anti-inflammatory agents in patients with AMI.

Author Contributions

Hasan Aydın Baş: Methodology, Validation; Fatih AKSOY: Conceptualization, Formal analysis, Writing-original draft, Writing-review & editing, Project administration, performing coronary angiography; Ali Bağcı: Formal analysis; Ercan Varol: Final editing; Ahmet Altınbaş: Project administration, performing coronary angiography.

RESUMO

Em pacientes com fibrilação atrial, a anticoagulação padrão com antagonista da vitamina K mais terapia antiplaquetária dupla (DAPT) com inibidor de P2Y12 e aspirina é o padrão de tratamento após intervenção coronária percutânea (ICP). Enquanto essa terapia reduz o risco de trombose e derrame, aumenta o risco de sangramento. Não está claro se o efeito antiplaquetário da aspirina e do clopidogrel pode piorar a fibrilação atrial (FA).

OBJETIVO: *Analisar a resistência à aspirina plaquetária (AR) e ao clopidogrel (CR) em pacientes coronarianos agudos (SCA) com base no ritmo sinusal (SR) e na FA.*

MÉTODOS: *Neste estudo prospectivo, foram incluídos 543 pacientes (idade média: 62±12 anos; intervalo: 26-89 anos) em uso de aspirina e clopidogrel após o diagnóstico de síndrome coronariana aguda. AR e CR foram analisados por um dispositivo Multiplate® MP-0120, utilizando o método de agregometria de sangue total.*

RESULTADOS: *Os pacientes com FA apresentaram valores significativamente maiores para idade, volume médio de plaquetas e proteína C reativa de alta sensibilidade (p<0,01 para cada parâmetro). Da mesma forma, a agregação induzida por ácido araquidônico (Aspi) foi maior nos pacientes com FA em comparação com os pacientes com SR (666±218 vs. 187±179, p<0,001). Entre os pacientes com SCA, significativamente mais pacientes do sexo feminino apresentaram FA (p<0,001). A incidência de hipertensão no grupo FA foi maior em comparação com o grupo SR (p<0,001). No entanto, os níveis de difosfato de adenosina não foram expressivamente significativos nos dois grupos.*

CONCLUSÃO: *Nossos achados indicam que o efeito inibitório plaquetário da aspirina foi pior em pacientes com FA, sugerindo que a eficácia da aspirina pode ser menor na profilaxia do tromboembolismo, com maior risco de sangramento.*

PALAVRAS-CHAVE: *Aspirina. Clopidogrel. Resistência a medicamentos. Síndrome coronariana aguda. Fibrilação atrial.*

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ORIGINAL ARTICLE

Physical and financial participation of teaching hospitals in private care in São Paulo - Brasil

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SUMMARY

OBJECTIVE: *To evaluate the physical and financial participation of private health insurance beneficiaries in the THs located in the State of Sao Paulo, regarding the care of Brazilian Unique Health System patients, in the year 2017.*

METHODS: *The research data were obtained from the System of Evaluation of the Teaching Hospitals (SAHE), of the State Department of Health of São Paulo (SES/SP).*

RESULTS: *It was observed that, on average, the THs analyzed provide 17% of their operational vacancies for the Supplementary Health System, and that the financial return is better in the philanthropic ones.*

CONCLUSIONS: *The health care services provided by THs deserve to be deepened, evaluating the real advantages obtained in the provision of services, given that supplementary health care requires differentiated infrastructure.*

KEYWORDS: *Health management. Public health. Supplementary health. Hospitals, teaching. Financial management.*

INTRODUCTION

The Brazilian Constitution of 1988 establishes the Single Health System (SUS) along with the Supplementary Health System (SS), regulated by the National Supplementary Health Agency (ANS), for assistance to Brazilians, having covered 25% of the 208.5 million population in 2018. The systems rely on a network of health units, with emphasis on teaching hospitals (THs), whose certification began in 2004 with the Interministerial Decree Nº 1,000[1], which established an important process to differentiate hospital groups that, in addition to health care, also carry out research and teaching. The country currently has 204 THs that are certified and under contracts, 52 of which are located in the state of São Paulo (ESP)[2], of different legal formats: public (autonomous and direct administration) and private (philanthropic and charities). Of all teaching hospitals in the ESP, 38 are general hospitals and 14 are specialized. They are all equally distributed in 14 of the 17 macroregions of health, being of different sizes and providing assistance to the SUS and the SS, especially in medium and high complexity cases (MAC). Of these, 32 hospitals are under state management and 20 under municipal management. Among them, 28 THs service not only SUS patients, but SS patients covered by Group Medicine, Medical Cooperative, Health Insurance, and Self-Management,

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in addition to the private health insurance plans of philanthropic entities.

It is a well-known fact that the financing of health care procedures by SUS is outdated regarding operational costs, which stimulates some hospitals to increase service to beneficiaries of private health insurance plans to cover the difference in costs in THs and reach solvency. This type of care is deserving of technical study to confirm its advantages. This phenomenon, known as double-door, is found also in Public and Private THs[3-5].

To measure and compare are essential concepts in running a business, especially in healthcare units, whose activities are complex, complicated, highly disruptive, high risk and cost, with greater weight in THs, where research and educational activities are inherent and whose influence deserve physical and financial quantification and qualification for due governance and institutional sustainability.

By teaching hospitals (THs) we mean: University Hospital of ownership or management of a public or private university, or bound to them by assignment of use or leasing arrangements, duly formalized; Teaching Hospital, of ownership or management by single medical schools, public or private, or bound to them by assignment of use or leasing arrangements, duly formalized; Auxiliary Teaching Hospital, one that is not owned or managed by a university or medical school alone, which develop programs of in-service training, undergraduate or post-graduate studies in health, duly under contract with an institution of higher education, per the classification adopted by the MEC[6].

The State Department of Health of São Paulo (SESSP) also defined its own functional classification, comprising the actual University Hospitals, Specialized Hospitals, and Under-Contract or Bound Hospitals (owned by the universities) to health faculties; these last two can have both teaching hospitals and auxiliary teaching hospitals (Table 1).

Table 1 includes the specialties and the total of THs, in addition to the number of those under SS contracts. The 52 THs represent 25.5% of the THs in the Country. The 28 THs that service private health insurance plans represent 58.8% of the THs of the ESP.

Comparisons between health systems are complex, considering the number of intervening variables, as evidenced by Mossialos et al.[7], such as the different types of funding, mechanisms for providing services, and the legal classifications of the units. In THs, there are differences in the specialties offered and, in a single case, there is no provision of outpatient services for SS patients. There are also differences in infrastructure, all of which are factors that affect the amount spent in the provision of care.

It is noteworthy that among the public THs managed by Social Organizations of Health (OSS), none had beds intended to SS patients by legal obligation. It is also important to mention that there have been legislative attempts to allow hospitals managed by OSS to provide 25% of their beds for beneficiaries of private health insurance plans, which were not consolidated, such as State Law nº 1,131 of 2010, which had its effects suspended[8].

TABLE 1. TEACHING HOSPITALS IN THE STATE OF SÃO PAULO BY LEGAL CLASSIFICATION, SPECIALTY, TOTAL, AND THS WITH SS CONTRACTS

SES (and ME) Classification	Legal Classification	Specializations	Number	SS Contracts
University	Autonomous and Foundation*	Overview	8	6
Specialized			14	7
(Teaching Hospitals and Auxiliary Teaching Hospitals)	Social Organization	Oncology	5	3
	Direct Administration (AD)	Maternity	4	1
	Philanthropic	Cardiology	2	2
	Autonomous (A)	Infectious Diseases	1	0
		Renal transplantation	1	1
		Rehabilitation Hospital	1	0
Bound to/Under Contract with Faculties			30	15
(Teaching Hospitals and Auxiliary Teaching Hospitals)	Social Organization	Overview	5	0
	Pub. L. Foundation/(A)/AD**	Overview	8	1
	Santa Casa	Overview	7	6
	Other philanthropic organizations	Overview	10	8
Total			52	28

*Private Legal Foundation**Public Legal Foundation, direct administration, and autonomous

OBJECTIVE

Analyze the physical and financial participation of care provided to beneficiaries of private health insurance plans in THs of the ESP in comparison to SUS patients in 2018.

METHODS

The data used are from the Teaching Hospitals Evaluation System (SAHE) of SESSP and the Hospital Information System (SIH) of the Information Department of SUS (DATASUS), of the Ministry of Health.

The sample consisted of 23 THs, 82.1% of the 28 THs that met the informational requirements on services provided for the SS per SAHE, in 2019 (with data from 2018). Five of them did not meet the requirements, of which four are under Municipal Management and not under contract with the SESSP, but with the Municipal Health Departments. One of the clauses for SESSP contracts requires the provision of information by the hospitals. Those under municipal management that, without contractual obligation, submit data to the SAHE certainly do so because they value the importance of such information for the management of the system and/or due to the information they receive in return from the SESSP when they need it for the assessment and planning of health actions.

From the SAHE we retrieved the following variables about private health insurance plans: total annual revenue, total number of operational beds and those intended for SS, number of patients discharged, and subsidies from the ESP treasury for public and philanthropic hospitals. From the DATASUS we retrieved the following variables: number of HAA, annual values from SUS regarding hospitalizations of high and medium complexity cases (MAC) and strategic cases (FAEC), and the number of hospital discharges of SUS patients.

We established an indicator capable of comparing the funding of the system, i.e., the ratio between the revenue of private health insurance plans and the income from SUS divided by the number of discharges in the same year, using as the definition of discharge when a patient leaves the inpatient unit upon medical discharge (cured, with improved or unchanged health), by evasion, due to withdrawal from treatment, internal transfer, external transfer, or death[9]. (Revenue or SUS Income/number of discharges)

Donations, scholarships for professionals financed by the SESSP, sale of non-clinical services, parliamentary amendments, and revenue from financial markets were not incorporated. It should be emphasized that all nonprofit hospitals analyzed in this study are CEBAS (Charitable Entity of Social Assistance Certificate) certified, which in itself is an indirect source of revenue since it exempts the unit from paying certain charges or taxes. According to Law No. 12,101 of 2009, a hospital is CEBAS certified in the area of healthcare if it services SUS at a minimum 60% percentage, taking into account hospitalizations and outpatient visits.

The THs included in the study were: *Hospitais das Clínicas da Faculdade de Medicina da Universidade de São Paulo, de Ribeirão Preto, de Botucatu e de Marília, Hospital São Paulo, Hospital de Base de São José do Rio Preto, Hospital Amaral Carvalho, Centro Infantil Boldrini, Instituto Dante Pazzanese de Cardiologia, Instituto do Coração da Faculdade de Medicina da Universidade de São Paulo, Hospital do Rim, Hospital e Maternidade Celso Pierro, Santa Casa de Araraquara, Santa Casa de Ribeirão Preto, Santa Casa de Franca, Santa Casa de Fernandópolis, Santa Casa de Limeira, Santa Casa de Santos, Hospital Universitário São Francisco, Hospital Padre Albino, Hospital São Vicente de Paula, Hospital Santa Lucinda,* and *Casa de Saúde Santa Marcelina.* The hospitals are represented by letters.

The THs were divided, according to the SESSP classification, into three groups: six university hospitals (those belonging the universities of São Paulo), five specialized (represented by those linked to universities of São Paulo, philanthropic and of direct administration by the ESP), and 12 philanthropic (general hospitals such as *Santas Casas* and associations). In the specialized group, there are the Auxiliary Teaching Hospitals, and in the group of hospitals under contract, there are Teaching Hospitals and Auxiliary Teaching Hospitals, according to the MS/ME classification.

To analyze the information, we used descriptive statistics.

RESULTS AND DISCUSSION

The integration between the SUS and SS systems, considering a perspective of information and planning, organization, management, and evaluation of the health system as a whole, is highly desired, but aspects such as the use of public beds for SS clients and patients tend to decrease the supply of services to the population that depends on SUS.

In table 2, we present the University Hospitals, with a total of 4,215 beds, of which 11.0% are dedicated to the SS (12.9% of SS discharges). These hospitals account for 11.4% to 22.9% of high-complexity hospitalizations.

It is possible to see that University Hospitals have little financial gain from servicing SS contracts, and the amounts received per discharge vary between 0.6 to 2.8 times what is refunded by the health insurance plans. University hospitals G and M are best paid by the SUS than by the SS. With the exception of the letter C TH, which provides 32.4% of its beds for the SS, the others do not provide more than 9.6%.

The budgetary sources of these hospitals are the resources provided directly by the Ministry of Health and the ESP treasury.

Table 3 contains information on the five specialized THs that service patients covered by the SS; the percentage of high-complexity hospitalizations varies from 30.7% to 88.1%.

Of the 1,387 beds offered, 15.3% are destined to SS contracts, whose percentage of discharges is 16.4%.

The gain from SS contracts presents greater uniformity when analyzed together, ranging from 1.6 to 3.3 times the value paid by SUS. No TH receives a lower amount from health insurance plans than that paid by SUS. This group contains both public hospitals with direct administration and autonomous philanthropic hospitals, except for the hospital of letter B, which provides only 4.2% of its beds to the SS; in all others, the percentage is higher, up to 29.9%.

The specialized THs perform a greater number of high-complexity procedures, which are, therefore, of high complexity and costs, but offer a smaller number of specialties, thus enabling a more rational administration, although with all the difficulties inherent to hospitals.

Table 4 presents the most heterogeneous group of THs, with 12 hospitals in which high-complexity hospitalizations range from 1.7% to 19.0%.

TABLE 2. SS BEDS; TOTALS; SS/SUS RATIO; SS, SUS, TOTAL, AND DISCHARGES; SS/SUS DISCHARGE RATIO; REVENUE/SUS INCOME COMPARISON RATIO SS/SUS FOR SS AND SUS - UNIVERSITY HOSPITALS IN 2018

University	SS Beds	Total Beds	% SS/SUS Beds	SS Discharges	SUS Discharges	Total Discharges SS + SUS	% Discharge SS/SUS	Revenue/ SS Discharges	SUS Income/ SUS Discharges	$ SS/SUS
C	295	910	32.4	18,166	41,013	59,179	30.7	9,520.65	8,397.00	1.1
The	50	575	8.7	1,223	19,902	21,125	5.8	12,376.12	10,182.86	1.2
C	69	1,223	5.6	5,398	50,709	56,107	9.6	(20,875.71)	30,040.46	(0.7)
H	38	759	5.0	889	38,128	39,017	2.3	55,025.27	19,665.66	2.8
G	10	524	1.9	1,309	25,249	26,558	4.9	(8,223.49)	13,364.51	(0.6)
S	6	224	2.7	163	8,011	8,174	2.0	17,838.46	12,302.11	1.5
Subtotal/ perc/ratio	464	4,215	11.0	27,148	183,012	210,160	12.9	20,643.28	15,658.77	1.3

TABLE 3. SS BEDS; TOTALS; SS/SUS RATIO; SS, SUS, TOTAL, AND DISCHARGES; SS/SUS DISCHARGE RATIO; REVENUE/SUS INCOME COMPARISON RATIO SS/SUS FOR SS AND SUS - SPECIALIZED HOSPITALS IN 2018

Specialized	SS Beds	Total Beds	% SS/SUS Beds	SS Discharges	SUS Discharges	Total Discharges SS + SUS	% Discharge SS/SUS	Revenue/ SS Discharges	SUS Income/ SUS Discharges	$ SS/SUS
K	80	380	21.1	3,682	12,460	16,142	22.8	23,607.91	9,026.86	2.6
AT	23	77	29.9	971	2,575	3,546	27.4	17,700.90	10,526.18	1.7
B	16	379	4.2	158	9,323	9,481	1.7	46,249.90	24,779.27	1.9
A	73	400	18.3	2,557	11,914	14,471	17.7	51,251.80	31,505.11	1.6
AC	20	151	13.2	1,213	7,355	8,568	14.2	40,113.40	12,054.42	3.3
Subtotal/ perc/ratio	212	1,387	15.3	8,581	43,627	52,208	16.4	33,927.02	17,578.37	1.9

TABLE 4. SS BEDS; TOTALS; SS/SUS RATIO; SS, SUS, TOTAL, AND DISCHARGES; SS/SUS DISCHARGE RATIO; REVENUE/SUS INCOME COMPARISON RATIO SS/SUS FOR SS AND SUS - UNDER-CONTRACT OR BOUND HOSPITALS IN 2018

Under-Contract	SS Beds	Total Beds	% SS/SUS Beds	SS Discharges	SUS Discharges	Total Discharges SS + SUS	% Discharge SS/SUS	Revenue/ SS Discharges	SUS Income/ SUS Discharges	$ SS/SUS
BI	30	290	10.3	749	19,001	19,750	3.8	18,671.38	5,767.45	3.2
Y	43	227	18.9	2,921	10,978	13,899	21.0	13,901.00	5,967.44	2.3
BF	32	116	27.6	1,298	5,048	6,346	20.5	6,546.95	4,903.34	1.3
AF	327	664	49.2	11,752	11,116	22,868	51.4	33,367.73	4,826.97	6.9
AI	91	254	35.8	4,332	12,214	16,546	26.2	18,226.73	3,206.23	5.7
BE	42	197	21.3	2,154	8,968	11,122	19.4	12,601.77	5,157.04	2.4
AD	141	726	19.4	7,042	26,410	33,452	21.1	34,332.95	8,486.62	4.0
BC	123	319	38.6	10,253	14,199	24,452	41.9	(8,326.20)	12,295.67	(0.7)
P	76	226	33.6	3,369	9,680	13,049	25.8	11,855.29	6,613.55	1.8
AJ	68	160	42.5	4,464	7,663	12,127	36.8	(4,242.66)	4,456.08	(0.9)
L	67	198	33.8	3,892	8,721	12,613	30.9	(6,955.34)	7,601.46	(0.9)
AG	6	238	2.5	146	16,115	16,261	0.9	16,043.77	3,767.27	4.3
Subtotal/ perc/ratio	1,046	3,615	28.9	52,372	150.113	202,485	25.9	15,422.65	6,087.43	2.5
Total/ percentage/ ratio	1,722	9,141	18.7	88,101	376,752	464,853	19.0	23,331.00	13,108.19	1.8

This is the TH group with the greatest number of beds dedicated to the SS, i.e., 28.9%, with 25.9% of discharges, that is, a quarter of their services are provided for the SS.

The financial gain from the SS, in comparison with that from SUS, is of a magnitude that ranges from 0.7 to 6.9 times, and three of the THs receive from SS contracts values lower than from the SUS.

The TH whose most significant source of funding is the SUS faces difficulties since the amounts paid for the procedures listed in the SUS Table have been outdated for years, with no other financial solution than to increase the number of beds destined for SS patients. These THs do not receive budgetary resources from the Treasury, although some do receive some type of subsidy. Only one of them provides 2.5% of its beds, all the other provide over 10.3%, and the TH referred to by the AF acronym provides up to 49.2% of its beds for the SS.

On average, the three groups provide 18.7% of their beds for SS patients and are responsible for 19.0% of all discharges, although the average gain is 1.8 times that of SS on in comparison to the SUS.

Thus, the analysis of each individual group (university, specialized, and philanthropic hospitals) shows a better financial gain in philanthropic THs that service the SS.

CONCLUSIONS

After comparing the amounts received per discharge, although in two groups the gains from the SS were better, there are not enough indicators to establish the cost/benefit of this practice.

It is necessary to know the local and regional geographical, demographic, socioeconomic, and epidemiological conditions to verify the concrete need for the beds in both populations, i.e., SS and SUS.

The study shows that the decision of allocating a particular percentage of beds for the SS and SUS is not supported in technical and management tools. The decision to invest in increased supply for the SS is not a good solution for all cases; there is no evidence regarding the relationship between the different investments in the differentiation of services.

RESUMO

OBJETIVO: *Avaliar a participação física e financeira do atendimento aos beneficiários de planos privados de saúde nos Hospitais de Ensino (HE) do Estado de São Paulo (ESP), em relação ao atendimento a pacientes do Sistema Único de Saúde, no ano de 2018.*

MÉTODOS: *os dados da pesquisa foram obtidos do Sistema de Avaliação dos Hospitais de Ensino (SAHE), da Secretaria de Estado da Saúde do Estado de São Paulo e do Departamento de Informática do SUS (DATASUS) do Ministério da Saúde.*

RESULTADOS: *observou-se que, em média os HE analisados ofertam 18,7% dos leitos operacionais para a Saúde Suplementar (SS), e que o retorno financeiro é melhor nos filantrópicos.*

CONCLUSÕES: *o atendimento a planos de saúde pelos HE merece ser aprofundado, avaliando-se as reais vantagens obtidas na prestação dos serviços e que o atendimento à SS exige infraestrutura diferenciada, e, principalmente o conhecimento de custos operacionais para estipulação de preços dos procedimentos.*

PALAVRAS-CHAVE: *Gestão em saúde. Saúde pública. Saúde suplementar. Hospitais de ensino. Administração financeira.*

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ORIGINAL ARTICLE

Medical Interns and COVID-19: results of national research

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SUMMARY

OBJECTIVE: *Assess the impact of COVID-19 on medical students' internships in public and private institutions in Brasil, in addition to estimating the quality of the measures taken by their respective Universities in the face of the problem and the availability of personal protective equipment (PPE).*

METHODS: *A descriptive cross-sectional quantitative analysis study carried out with 317 students undergoing medical internship from March 31, 2020, to April 12, 2020. The survey was conducted through an online questionnaire using the SurveyMonkey tool with 20 questions. Interns from the fourth to the sixth year of medical schools in the country were randomly included in the study through a survey sent by Whatsapp application. Statistical analysis was performed using the Chi-Square, considering p <0.05 as significant.*

RESULTS: *Four main topics were identified in the research: student demographic data; how classes and courses are being taught; the use and ease of access to personal protective equipment and the students' fears and perspectives for the future.*

CONCLUSION: *The study clarified that although half of the students still have some degree of content and, in their majority, they are satisfied, there is still a lot of difficulty in obtaining personal protective equipment, which prevents students from returning safely to their internships.*

KEYWORDS: *Coronavirus Infections. Personal protective equipment. Internship and Residency.*

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INTRODUCTION

In December 2019, in the Hubei province, in Wuhan, China, there was an outbreak of SARS, which would later be discovered as a new virus in circulation, Covid-19[1-3]. The virus can cause pneumonia, fever, cough, fatigue, or have an asymptomatic presentation. With an incubation period of 3 to 14 days before the onset of symptoms, it went unnoticed, which could explain its worldwide prevalence[1-3].

The WHO recommends that the personal protective equipment (PPE) indicated for health workers who come into contact with many people, many of whom presenting no symptoms, include gloves, goggles or face shield, cap, surgical mask, special masks (N95 or FFP2) and apron[3]. However, from the beginning of the Covid-19 pandemic in Brasil and worldwide, there have been reports of PPE shortage in hospitals, and in many cases, their absence brings concerns about the safety of medical students in hospital environments. The use of PPE is already scarce in this environment, so many were removed from their internship facilities[4,5].

The Covid-19 pandemic changed routines throughout the world with the need for social distancing, quarantines, remote classes, the use of new tools, the aid of digital technology, and work from home. However, medical interns, who are important in hospital environments, find themselves in an intermediary situation, between the regular program, in which remote classes are possible, and the in-person care of patients, which has been modified and carried out by doctors to optimize the use of PPE, of which there is a shortage[6].

Thus, the goal of this study is to evaluate the situation of medical interns in the current Covid-19 pandemic scenario.

METHODS

This study is a cross-sectional quantitative analysis, of descriptive nature, and was approved by the Medical Ethics Committee under number 3,979,279.

The survey was conducted based on an online questionnaire using the SurveyMonkey tool and comprising 20 questions divided into the following subjects: demography; how the program has been carried out so far; use and ease in acquiring PPE; and students' fears and perspectives.

The survey was sent to medical students from the 4th to the 6th year of the program from all over the country, randomly, via WhatsApp application.

This research was carried out between 31 March 2020 and 12 April 2020. The statistical analysis was performed using the chi-square test, considering $p<0.05$ as statistically significant. The presentation of the measured variables is shown by graphs.

RESULTS

A total of 317 students agreed to participate in the research. Figure 1 shows the states from which the answers come.

Most of the respondents study in private institutions (77.9%). From the total number of respondents, 54.5% are 5th-year students, 44.2% 6th-year, and 1.3% 4th-year.

On the contents of the internship, 50.2% (159) have their content completely halted; 42.6% (135) have their contents partially halted, with theoretical content being offered remotely; and 7.2% (23) provided different answers. There was no difference between students from public and private institutions (p=0.0783).

Most students with halted internships required adaptations, and many universities began to offer remote classes. Figure 2 shows how the contents are being offered.

On PPE, 71.6% (227) reported difficulty of access. Of the 44.5% (141) students who have access to PPE, 70 are provided by the hospital or internship facility, 54 have protective equipment provided by the university, and 17 have acquired it themselves. There was no difference between students from public and private institutions.

Figure 3 shows how the training related to PPE was carried out, demonstrating that students from public

FIGURE 1. STATES WHERE STUDENTS STUDY.

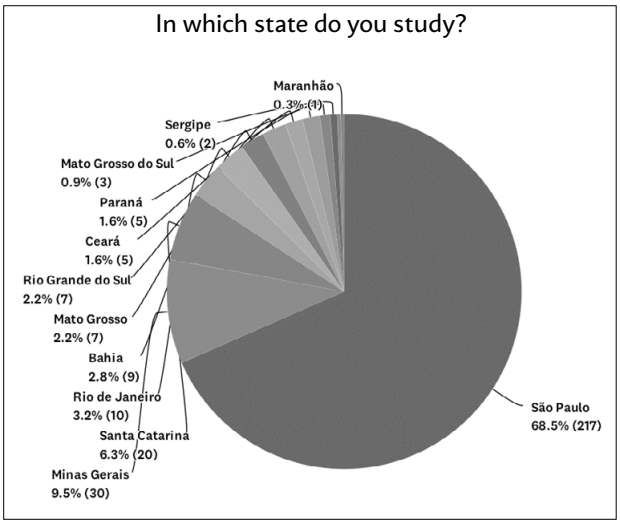


FIGURE 2. DYNAMIC OF ACTIVITIES AND QUALITY OF REMOTE CLASSES DURING THE COVID-19 PANDEMIC, COMPARING SCHOOLS PUBLIC AND PRIVATE INSTITUTIONS (P=1,000)

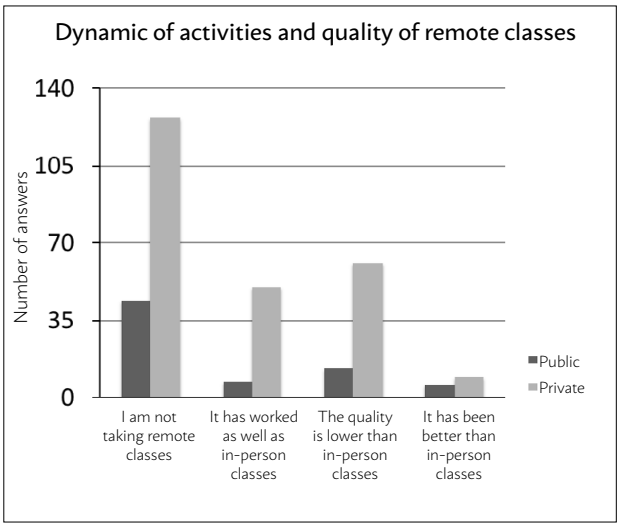
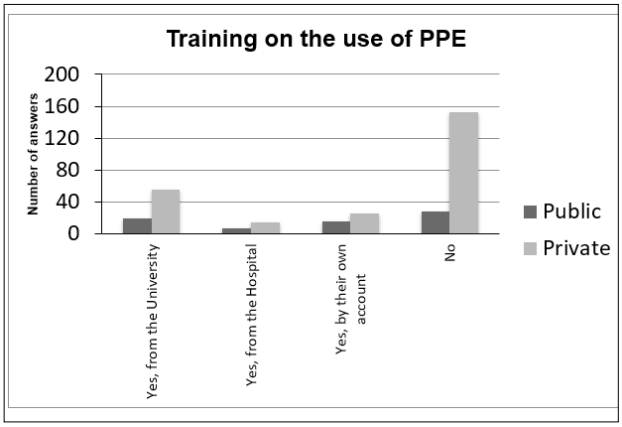


FIGURE 3. TRAINING ON THE USE OF PPE, COMPARING STUDENTS FROM PUBLIC AND PRIVATE INSTITUTIONS (P=0.0015)



institutions received more training. With respect to the theoretical content on Covid-19 and guidance to students, 41.6% (132) received this content, but 58.4% (185) did not receive any content or guidance. When comparing the types of institutions, there was a difference between those who received training (58.6% public *x* 36.8% private, p=0.0015).

Regarding the safety to see patients, 44.2% (140) of the respondents do not feel safe to care for patients in the emergency room, 39.7% (126) consider themselves partially safe, 10.7% (34) consider themselves safe, and 5.4% (17) consider other safety issues regarding the care of patients in the emergency room in the current pandemic. There was no difference between students from public and private institutions.

With respect to the fear of contamination by the virus, 80.8% (256) of the interns are afraid of being contaminated and 19.2% (61) did not have this fear.

If the respondents had the option, 34.7% (110) would prefer to work as volunteers wherever there was a need, 30.6% (97) would stay at home until the pandemic was over, 29.7% (94) would like to have continued with their regular internship, and 5.0% (16) would prefer to perform other activities. There was no difference between students from public and private institutions (p=n.s.)

With regard to their perspectives, 50.0% (158) feel insecure, because they do not know how they will compensate the lost internship time; 21.5% (68) are worried because this situation will delay their graduation; 19.9% (63) are calm, trying to seize the opportunity for medical learning; and 8.5% (27) have other expectations.

When asked about their degree of satisfaction with how the University is handling the situation, 36.0% (114) said they are dissatisfied, 25.2% (80) are satisfied, 20.2% (64) are very dissatisfied, 14.2% (45) are indifferent, and only 4.4% (14) are satisfied with their Universities in this new scenario. There was no difference between students from public and private institutions.

DISCUSSION

The Coronavirus Resource Center, of the Johns Hopkins University, revealed that by 30 April 2020, the current Covid-19 pandemic had already affected more than 3 million people worldwide, of which more than 80 thousand are in Brasil, and had caused the death of over 230,000 people worldwide[7].

This has transformed everyday life throughout the world. One of the first establishments to interrupt activities were schools because they are not considered essential activities. In addition, with the current technological advances, it is possible to offer remote classes in order to maintain the continuity of programs. However, medical students undergoing internships find themselves in an intermediary situation. The internship is the stage in which students begin their in-hospital activities, with some kind of theoretical content as a foundation. And, to exercise their in-hospital activities, PPE is required so they can be adequately protected. In the current pandemic, there is a general lack of protective equipment, leaving medical interns, who do not yet have the autonomy to perform medical practices, as the last ones to have access to PPE. Thus, they may be the class of students who are the most affected by the

current situation, causing them a series of problems and anxiety.

Considering students who are taking remote classes, half of them reported that the classes have equal or superior quality to the traditional in-person classes. Some institutions have trained their teachers, further enhancing these indexes. For example, the Anhembi Morumbi University provided training for 100% of its teachers regarding the new type of media used for the classes and the handling of the remote tool; as a result, 23 of the 35 internship students (65.6%) who are taking remote classes considered them to be of equal or better quality than in-person classes.

An important issue to be considered is that 71.6% of the respondents reported difficulties in getting PPE. Due to this difficulty, universities, hospitals, and students themselves have been making efforts to purchase PPE. This overall difficulty prevents students from safely resuming their internships. Another relevant aspect is the training of students on the disease and the use of PPE. Many reports describe health professionals who were contaminated while putting on or taking off their PPE. Over half of the students reported receiving no theoretical content on Covid-19 (58.4%), nor any training on the use of PPE (57.1%). When comparing students from public and private institutions, we notice that more students from public institutions received specific training than those from private institutions [58.6% *vs.* 36.8%, p=0.0015 and 60% *vs.* 38.1%, p=0.0015, respectively analyzed regarding theoretical content and training on the use of PPE].

Another relevant aspect is that only 10.7% of the respondents considered themselves safe to care for patients in the emergency room during the pandemic, while 80.8% reported being afraid of being contaminated with the virus, which demonstrates widespread insecurity regarding the current situation. This problem is reported by Lai et al., who showed the psychological effects among 1,257 health professionals who worked in Wuhan during the peak of the pandemic and identified high rates of depression (50.4%), anxiety (44.6%), insomnia (34%), and stress (71.5%) among those interviewed[8].

All of these aspects are directed to educational institutions, who must provide swift answers to situations never before experienced or seen. Of all the respondents, 56.2% reported some degree of dissatisfaction with the way their educational institution is handling the current situation. This was expected because, in a moment such as this, virtually everyone had some loss. All sectors of society have varying degrees of dissatisfaction. According to research conducted by the *Instituto Datafolha*[9], for 69% of the population, the pandemic will decrease their income, generating negative prospects for society. It is up to the educational institution to support students at this moment when many of them are isolated from their families, without being able to go to their internships or return home.

We believe that, in universities in general, some points of attention could improve the level of satisfaction of these students, such as streamlining the communication channel between the coordination staff and students and providing psychological support to students. In addition to communication, another key point regards information and knowledge. Thus, there should be investments in technical communication about the disease and the use of PPE and also in general communication that is not specific to this problem. New communication channels should be opened for students, and there should be investments in the individualization of specific problems.

In addition, investments in PPE can be useful for returning to usual activities. Such return can happen in two ways: the situation reaches a point of balance and internships are resumed, or the number of serious cases increases greatly and the role of students as volunteers will have greater relevance. This voluntary work can count as at least part of the students' internship since they will be experiencing a unique moment in medicine that has not happened in the last hundred years; this makes it easier to compensate for lost internship time. These aspects can improve the understanding of students, reduce fears and concerns, and increase the value of educational institutions for students and their families.

This study has some limitations: despite having national coverage it could not equally contemplate all educational institutions. Still, the number of respondents from each of the educational institutions is limited. In addition, the proportion of answers has no statistical calculations to support them.

On the other hand, this is the first study carried out in the country that exposes the situation of medical interns in the Covid-19 pandemic and can serve as a basis for actions planned by the institutions, assisting them in handling medical internships during this period.

CONCLUSION

It can be concluded, based on this study, that remote education is a viable and suitable alternative for part of the medical internship support content and should be used extensively to train all interns on the Covid-19 pandemic and the use of PPE. In addition, the creation of new communication channels during this moment of a pandemic can increase the safety of students and improve their perception of educational institutions and their role in this crisis that involves the entire health system of Brasil and the rest of the world.

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Contribution of the authors

Maria M. C. Carrascosa: Organized and divided the project and its activities. Prepared the questionnaire and helped in its rapid dissemination for the recruitment of subjects for data collection. Wrote the introduction and results. Found and selected references from 1 to 5, which were used in the preparation of the article. Was responsible for the first version of references 1 to 5. Was responsible for the first revision of the article in Portuguese. Translated the title, introduction, results, and images of the article into English, and formatted the work in English and revised it. Formatted and prepared the article according to the requirements of the journal.

Tércio de Campos: Had the idea for the project. Coordinated the project. Wrote the discussion and conclusion. Contributed to improvements in the introduction, methods, and results of the project. Selected references 6 to 9 used in the article. Revised the work. Submitted the work to the Brasil platform and to the Research Ethics Committee.

Jessica E. Sampaio: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Participated in the drafting of the project objectives and title. Standardized the references according to the Vancouver style. Formatted the project in Portuguese.

Rafaella R. F. Souza: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Participated in the drafting of the presentation text of the questionnaire and the questionnaire itself. Participated in the drafting of the project objectives and title. Drafted the abstract for the project.

Vitória L. Ribeiro: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Revised the methodology in Portuguese and translated it into English. Organized the initial bibliography (3, 4, 5).

Maria L. N. Maia: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Participated in the drafting of the introduction and in the translation of the discussion.

Laura C. L. Gama: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Contributed to the drafting of the questionnaire text, helping to draw up the method.

Mariana P. Severino: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Drafted the methodology. Translated the abstract into English.

Nathan K. Semer: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Drafted the abstract and translated it into English.

Otávio Rondon: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Translated the introduction and results.

Juliana B. M. Silva: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Translated the discussion into English.

Mariana Miyazi: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Participated in the drafting of the questionnaire.

Samara R. Domingues: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Drafted the title and objectives.

Nathália E. S. Batalha: Assisted in the rapid dissemination of the questionnaire for the recruitment of subjects for data collection. Translated the methodology.

Délio E. Martins: Conducted a critical review of the project and manuscript, contributing to the discussion.

RESUMO

OBJETIVO: *Avaliar o impacto da Covid-19 durante o internato dos alunos de medicina em escolas públicas e particulares no Brasil, além de estimar a qualidade das medidas tomadas pelas respectivas universidades diante do agravo e da disponibilização de equipamento de proteção individual.*

MÉTODO: *Um estudo de análise quantitativa transversal, com caráter descritivo, foi realizado com 317 alunos cursando o internato médico durante o período de 31 de março de 2020 a 12 de abril de 2020. A pesquisa foi realizada por meio de um questionário on-line com 20 perguntas pela ferramenta SurveyMonkey. Os internos do 4º ao 6º ano das faculdades de medicina do País foram incluídos no estudo de forma randômica ao receberem a pesquisa pelo aplicativo WhatsApp. A análise estatística foi realizada por meio do Qui-quadrado, considerando p<0,05 como significante.*

RESULTADOS: *Quatro temas principais foram identificados na pesquisa: dados demográficos dos alunos; como as aulas e estágios do curso estão sendo ministrados; a utilização e facilidade de aquisição do equipamento de proteção individual e medos e perspectivas futuras dos estudantes.*

CONCLUSÕES: *O estudo mostrou que apesar de metade dos alunos continuarem tendo algum grau de conteúdo e, na maioria, estarem satisfeitos, ainda há muita dificuldade em se obter equipamento de proteção individual, o que impede que os alunos retornem com segurança aos seus campos de estágio, além de causar medo de contaminação e de continuar atendendo os pacientes.*

PALAVRAS-CHAVE: *Infecções por coronavírus. Equipamento de proteção individual. Internato e residência.*

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ORIGINAL ARTICLE

Serum iron and vitamin B 12 deficiency could indicate celiac disease by flexible spectral imaging color enhancement

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SUMMARY

INTRODUCTION: *Celiac disease (CeD) is an autoimmune disease that can be delayed in diagnosis due to the presence of atypical and asymptomatic cases in adulthood. Herein we aimed to study the frequency of CeD and evaluate whether magnified endoscopy and magnified/FICE (flexible spectral imaging color enhancement) techniques contribute to the diagnosis in patients with serum iron and vitamin B12 deficiency.*

METHODS: *We evaluated 50 adult patients (10 males and 40 females) who had serum iron and vitamin B12 deficiency, prospectively. All the patients had undergone upper gastrointestinal system endoscopy by the same endoscopist. The second part of the duodenum was evaluated with white light, magnified, and magnified/FICE endoscopy. Biopsy specimens were evaluated by the same pathologist. The specimens diagnosed as CeD were classified according to the Modified Marsh-Oberhuber criteria.*

RESULTS: *10 of 50 patients (20%) were diagnosed as CeD. The average age was 41±11 years (20-67 years). Thirty percent of CeD diagnosed patients had typical CeD symptoms. Six of 10 patients (60%) who were diagnosed as CeD had typical endoscopic images under white lighted endoscopy. All of these 10 patients (100%) showed villous irregularity, partial villous atrophy, or total villous atrophy consistent with CeD with magnified and magnified/FICE endoscopy.*

CONCLUSION: *The practical use of magnified/FICE endoscopy allows us to differentiate mucosal abnormalities of the duodenum and minimize false-negative results that indicate normal mucosal findings with conventional endoscopy.*

KEYWORDS: *Celiac disease. Vitamin B 12 deficiency. Iron deficiency. Image enhancement/methods. Image processing, computer-assisted.*

INTRODUCTION

Celiac disease (CeD) is an autoimmune disease characterized by nonspecific findings and symptoms in adulthood, which may lead to a delay in diagnosis. The diagnosis of this disease, which is considered to be the tip of the iceberg due to nonspecific signs and symptoms, is important because of comorbid conditions and increased risk of malignancy. Sometimes micronutrient deficiency may be the only finding indicative of the diagnosis, and it is the physician's awareness of CeD that is decisive at this point.

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Recognition of the pathogenesis of increased inflammation of the small bowel mucosa in CeD is the starting point. Symptoms are related to the extent of involvement of mucosal lesions rather than the severity of pathology in the proximal small intestinal mucosa. Although the data are insufficient in this regard, lesional pathology is thought to cover 30-50% of the entire small bowel mucosa[1]. Therefore, it should be kept in mind that patients may have latent (or asymptomatic) disease regardless of proximal mucosal damage.

New endoscopic methods may facilitate the diagnosis of CeD with asymptomatic and silent forms. Magnifying endoscopes and digital chromoendoscopic methods are just two of the approaches that have been increasing in use in recent years. Flexible spectral imaging color enhancement (FICE), which is one of the digital chromoendoscopic methods, can be used to convert the mucosal surface structure into virtual newly arranged image structures by using selected wavelengths to obtain more detailed information about the investigated area.

In this study, we planned to conduct a prospective study on the incidence of CeD and the diagnostic efficacy of magnified endoscopy and FICE in individuals with iron deficiency anemia and vitamin B12 deficiency.

METHODS

Study Group

Patients with vitamin B12 and iron deficiency anemia (IDA) who were referred to the Gastroenterology Clinic of İzmir Tepecik Education and Research Hospital for upper gastrointestinal endoscopy and gave written consent to take part in the study were included in this study. They also tested for CeD serology with tissue transglutaminase IgA.

The study was approved by the local ethical committee of İzmir Tepecik Education and Research Hospital (No: 2015/18).

Endoscopic evaluation

Patients underwent upper gastrointestinal endoscopy after at least 8 hours of fasting and were evaluated with Fujinon EG-490ZW5 high resolution magnified endoscope (Fuji Photo Optical Co., Ltd., Saitama, Japan). A transparent hood was placed on the endoscope tip to ensure good image quality during the procedure and to prevent gastric or duodenal content migration to the area under investigation. After standard videoendoscopic examination by the same endoscopist, standard endoscopic images, magnified and magnified/FICE images of the duodenum were recorded (at least 3 images). Magnification was done by 40-80 times enhancement. Isolated areas in the bulbs and second part of the duodenum were magnified and evaluated, and two biopsies (not four quadrants) were taken for pathological evaluation after imaging.

Evaluation by white light, high resolution magnified endoscope, and FICE images

Endoscopic images were first blindly evaluated under standard white light endoscopy (WLE) and were recorded as with or without any celiac-compatible imaging findings (decrease in the number of circular folds, mosaic/nodular velvety appearance, scalloped duodenal folds, grooves, fissurations, etc.). Subsequently, magnified endoscopy and magnified/FICE images were evaluated and the findings were divided into three groups, i.e., normal, partial villous atrophy, and total atrophy, and recorded.

Pathological examination:

Biopsy specimens were placed in a 10% formol solution and delivered to the pathology department. After macroscopic sampling and standardized close system tissue follow-up, the specimens were embedded into paraffin then sliced into 4-5 micron sections by a semi-automated system. These slices were evaluated by the same pathologist using hematoxylin & eosin staining. For duodenal biopsies in which intraepithelial lymphocytes were increased, immunohistochemically CD3 antibodies were also applied.

Duodenum samples were evaluated according to the Modified Marsh (Marsh-Oberhuber) classification[2], and gastritis classification in gastric biopsies was done according to the revised Sydney System[3] by the same pathologist who was not blinded. Modified Marsh criteria were defined as follows:

Marsh I: infiltrative lesion, normal villous architecture and mucosa, intraepithelial lymphocytes are increased (>40 lymphocytes/enterocytes counted)

Marsh II: hyperplastic lesion, similar to Marsh I, but it also presents crypt hyperplasia

Marsh III: destructive lesion; subdivided into three groups:

Marsh IIIa: partial villous atrophy (mild atrophy)

Marsh IIIb: subtotal villous atrophy (marked atrophy)

Marsh IIIc: total villous atrophy (complete atrophy)

Statistical analysis

Statistical analysis of the study was performed using SPSS 22.0 (IBM Statistical Package for Social Sciences software version 22). Categorical variables were compared with the Chi-square test and continuous variables were compared with the student-T test. P <0.05 was considered a statistically significant difference with a 95% confidence interval.

RESULTS

The mean age of the patients was 41 ± 11 years (20-67 years). Of the 50 patients, 10 were males and 40 were females. CeD was detected in 10 of 50 patients (20%). Three (30%) of 10 males and 7 (17.5%) of 40 females had CeD. Tissue transglutaminase IgA antibody was positive in all 10 celiac patients diagnosed and confirmed by biopsy.

There were no statistically significant differences in hemoglobin, hematocrit, serum iron, ferritin, vitamin B12, and folic acid values between the CeD and non-CeD groups (Table 1). Moderate and severe chronic inflammation was statistically significantly higher in patients with CeD in duodenal pathology (p<0.0001). Similarly, neutrophil and eosinophil ratios in the duodenal epithelium were statistically significantly higher in patients with CeD (p <0.0001), whereas there were no statistically significant differences between the two groups in the proportion of these cells in lamina propria (p> 0.5). The frequency of Brunner's gland hypertrophy in the duodenum was statistically significantly higher in patients with CeD (p = 0.01). Intraepithelial lymphocytes were present in all of the patients with CeD, but only in 8 of 40 non-CeD patients (p< 0.5).

Pathology results and magnified/FICE endoscopic image results were found to be consistent with CeD in all 10 positive patients. CeD was also excluded by pathology results and magnified/FICE images in all individuals with negative antibody. WLE showed a typical endoscopic appearance in six (60%) of the 10 patients diagnosed with CeD (sensitivity 60%, specificity 100%), with mosaic/nodular velvety appearance, scalloped duodenal folds, grooves, and fissurations. In magnified and magnified/FICE imaging, abnormal mucosal findings suggesting CeD were observed in all 10 patients (villous losses in partial or total atrophy, enlargement due to edema in villous, etc.) (sensitivity 100%, specificity 100%). Partial atrophy in the villous was histopathologically compatible with Marsh 2, while total atrophy was consistent with Marsh 3a-3b-3c. The distribution of patients according to the Marsh classification is shown in Table 2.

DISCUSSION

Celiac disease is a chronic disease that affects the small intestine by gluten ingestion in genetically predisposed patients[4]. CeD can commonly present with atypical symptoms, abdominal bloating, constipation, vomiting, weight loss, decreased fertility in women, and malabsorbtion[5-8]. The distribution of CeD varies across the world. The overall frequency of CeD is estimated to be 0.5-1%[9,10]. As wheat and barley are the basis of agriculture in Anatolia, the prevalence of CeD in our country is 1.3%[11].

Micronutrient deficiency is a condition that bothers the person insidiously and often remains behind closed doors. Iron and vitamin B12 deficiency could be ignored in the case of the etiology. CeD could underlie

TABLE 1. LABORATORY VALUES OF PATIENTS WITH AND WITHOUT CED.

	Celiac	Number (n)	Average	Std. Deviation	p
HGB (gr/dL)	none	40	10.37	1.706	>0.05
	present	10	10.47	1.957	>0.05
HTC (%)	none	40	32.54	4.359	>0.05
	present	10	33.19	5.338	>0.05
MCV (fL)	none	40	73.10	8.064	>0.05
	present	10	76.95	13.537	>0.05
RDW (%)	none	40	17.35	2,623	>0.05
	present	10	17.06	5.515	>0.05
Serum Iron (ug/dL)	none	40	30.425	36.7597	>0.05
	present	10	26.600	15.7565	>0.05
Ferritin (ug/dL)	none	40	13.0008	13.98593	>0.05
	present	10	18.8690	26.10889	>0.05
Vitamin B12 (pg/ml)	none	40	155.7375	49.37206	>0.05
	present	10	171.4000	66.73363	>0.05
Folate (µmol/L)	none	40	7.0065	2.42318	>0.05
	present	10	5.7350	2.55595	>0.05

TABLE 2. DISTRIBUTION OF CELIAC PATIENTS ACCORDING TO MARSH CLASSIFICATION.

	Marsh						Total
	0	1	2	3a	3b	3c	
Celiac	0	0	2	4	2	2	10

in about 10% of iron deficiency anemia (IDA)[12,13]. In a Danish study, newly diagnosed CeD patients had 17% vitamin B12 deficiency and 39% IDA[14]. Extraintestinal symptoms and atypical form play an important role in adulthood[15]. In a case report, a patient with chronic anemia was diagnosed with Behçet's disease due to recurrent oral aphthae, probably due to existing micronutrient deficiency, and was subsequently diagnosed with CeD[16]. In one study, the most common accompanying finding at the time of diagnosis in the advanced age group was found to be anemia in 80% of cases and osteopenia/bone disease in 70%[17]. In a study performed by Tesei et al.[18], on 250 treatment-naive celiac patients and a control group of 176 healthy subjects defining the relationship between antibody presence and diagnosis, serology positive silent CeD was detected in 22 patients (8.8%).

Antibodies are of great importance in CeD diagnosis and screening[19-22]. The sensitivity and specificity of antigliadin IgG and IgA antibodies are below 90%, whereas tissue transglutaminase IgA is around 95%[14-16]. Tissue transglutaminase IgA is recommended for CeD screening for patients age two years and older[17]. Using a screening test before endoscopic evaluation is of great importance in terms of cost in individuals without typical symptoms, and it is not inconvenient to say that biopsy is not necessary if there is no pathological appearance under magnified/FICE examination in individuals without antibody positivity.

Endoscopic appearance is also important in the diagnosis of CeD. Circular folds are regular in the normal duodenal mucosa, with a vivid appearance in the mucosa. Similarly, the magnified endoscopy and magnified/FICE shows that the villous are regular, do not tend to atrophy, and the capillary structure within it can be selected. Mosaic/nodular velvety appearance, scalloped duodenal folds, grooves, and fissurations, which are typical endoscopic findings for CeD may lead the endoscopist to the diagnosis of CeD. In the study performed by Uyanıkoğlu et al.[23], CeD was diagnosed based on endoscopic appearance in 7 (0.035%) of 1950 patients who underwent endoscopy for any reason. In our study, 6 of 10 patients diagnosed with CeD had typical endoscopic appearance (Figure 1a). The loss of villous is also evident in the magnified and magnified/FICE images of these patients (Figure 1b, c). However, WLE alone is not sufficient for the diagnosis of CeD. In our study, there were 4 patients (40%) who had celiac disease due to antibody positivity and biopsy but had normal duodenum with WLE. The magnified and magnified/FICE images of these cases were not normal (Figures 2 and 3).

FICE, a digital chromoendoscopic method, has been shown to be a diagnostic endoscopic method with higher sensitivity and specificity than WLE in studies of gastrointestinal lesions such as Barrett's esophagus, early gastric cancer, and colon polyps and their diagnosis[24-28]. In our study, we observed that villous losses (partial, total atrophy, etc.) were clearly observed in the detailed examination of the mucosal structure with magnified and magnified/FICE methods. Specifically, with magnified/FICE, the character of the tissue is seen in more detail. These data are consistent with the study of Cammorata et al.[27], and we see that the magnified and magnified/

FIGURE 1. ENDOSCOPIC EVALUATION WITH WHITE LIGHT, MAGNIFIED ENDOSCOPE, AND MAGNIFIED/FICE IN AN INDIVIDUAL WITH CED. (A) MOSAIC AND NODULAR VELVETY APPEARANCE, SCALLOPED DUODENAL FOLDS UNDER WLE. (B) TOTAL ATROPHY OF THE VILLOUS IS SEEN UNDER MAGNIFYING ENDOSCOPY. (C) UNDER MAGNIFIED/FICE IT IS CLEAR THAT VILLOUS ARE COMPLETELY ATROPHIED. HISTOPATHOLOGICAL EVALUATION WAS CONSISTENT WITH MARSH 3B.

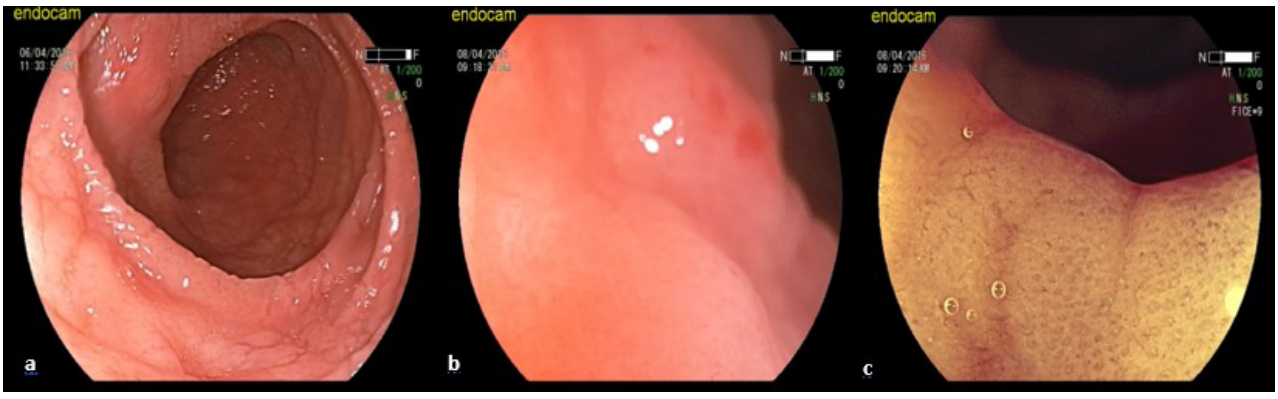


FIGURE 2. (A) IMAGE OF THE DUODENUM WITH WLE SHOWS NORMAL FOLDS AND NO ABNORMAL MUCOSA. (B) IN THE SAME PATIENT'S MAGNIFIED ENDOSCOPY AND (C) MAGNIFIED/FICE IMAGES WE CAN SEE VILLOUS STRUCTURE WAS DISRUPTED AND PARTIAL ATROPHY FINDINGS WERE OBSERVED.

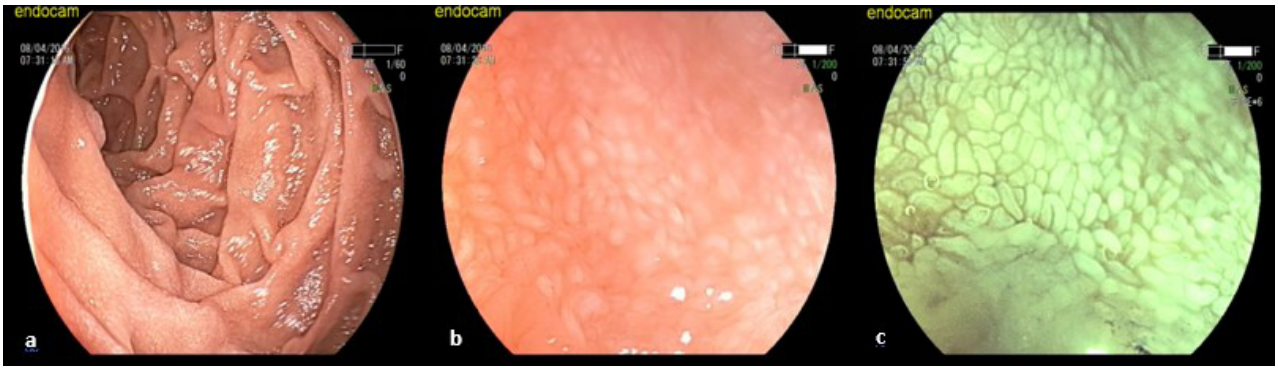
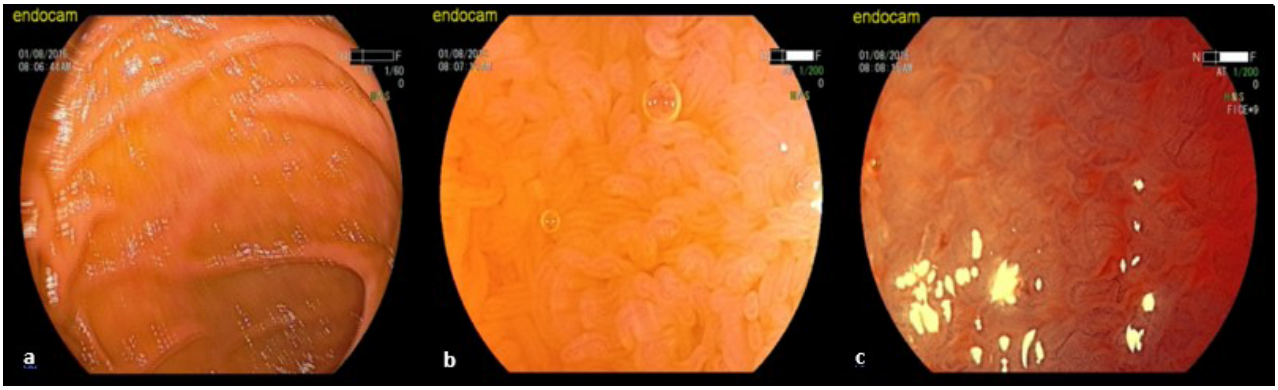


FIGURE 3. (A) IMAGE OF THE DUODENUM WITH WLE SHOWS NORMAL FOLDS AND NO ABNORMAL MUCOSA. (B) MAGNIFIED ENDOSCOPY AND (C) MAGNIFIED/FICE IMAGES OF THE SAME PATIENT SHOW IRRADIATION IN THE VILLOUS AND LOSS OF ORDER. HISTOPATHOLOGICAL EVALUATION WAS CONSISTENT WITH MARSH 2.



FICE method has 100% sensitivity and specificity in the diagnosis of CeD.

As CeD awareness increases, there is a search for more easily accessible and cost-effective diagnostic methods. Easy diagnosis methods are being developed with blood sampling from the fingertip, or urine and stool samples[24]. The sensitivity and specificity of our evaluation with magnified and magnified/FICE were found to be 100% in our group of 50 patients, with 10 of them being diagnosed with CeD. When the narrow-band imaging method and duodenal pathology results of 112 CeD patients performed by Tabibian et al.[29] were compared, the determination of villous atrophy was found to be significantly superior, similar to what was found in our study.

Since our study is prospective, it has some limitations, as follows. The number of patients is limited and we have no findings for magnified and magnified/FICE data in March.

In conclusion, changes in the duodenal mucosa with magnified and magnified/FICE correlate with histopathological findings. In particular, detailed image quality can be achieved with FICE. By using magnified/FICE in addition to conventional WLE, changes in duodenal mucosa are examined in more detail, so false-negative results in individuals with normal duodenal mucosal appearance can be minimized.

Authors' contribution

Concept: SA. Design: SA, OBB. Supervision: HA. Materials: OBB, EC. Data collection and/or processing: SA. Analysis and/or interpretation: SA, OBB. Literature search: SA. Writing: SA, OBB. Critical reviews: SA, OBB, HA.

Conflict of interest

We have no conflict of interest.

RESUMO

INTRODUÇÃO: *A doença celíaca (DC) é uma doença autoimune que pode ter seu diagnóstico atrasado devido à presença de casos atípicos e assintomáticos na idade adulta. Neste trabalho, objetivamos estudar a frequência de DC e avaliar se as técnicas de endoscopia magnificada e magnificada/Fice (flexible spectral imaging color enhancement) contribuem para o diagnóstico em pacientes com deficiência sérica de ferro e vitamina B12.*

MÉTODO: *Foram avaliados prospectivamente 50 pacientes adultos (10 homens e 40 mulheres) com deficiência sérica de ferro e vitamina B12. Todos os pacientes foram submetidos a endoscopia digestiva alta pelo mesmo endoscopista. A segunda parte do duodeno foi avaliada com endoscopia com luz branca, magnificada e magnificada/Fice. As amostras de biópsia foram avaliadas pelo mesmo patologista. Os espécimes diagnosticados como DC foram classificados de acordo com os critérios de Marsh-Oberhuber modificado.*

RESULTADOS: *Dez dos 50 pacientes (% 20) foram diagnosticados como DC. A idade média foi de 41±11 anos (20-67 anos). Trinta por cento dos pacientes diagnosticados com DC apresentaram sintomas típicos de DC. Seis dos dez pacientes (60%) diagnosticados com DC tinham imagens endoscópicas típicas sob endoscopia de luz branca. Todos esses dez pacientes (% 100) apresentaram irregularidade das vilosidades, atrofia das vilosidades parciais ou atrofia das vilosidades totais consistentes com a DC com endoscopia magnificada e magnificada/Fice.*

CONCLUSÃO: *O uso prático da endoscopia magnificada/Fice permite diferenciar anormalidades mucosas do duodeno e minimizar os resultados falso-negativos que apresentam achados mucosais normais com a endoscopia convencional.*

PALAVRAS-CHAVE: *Doença celíaca. Deficiência de vitamina B 12. Deficiência de ferro. Aumento da imagem/métodos. Processamento de imagem assistida por computador.*

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ORIGINAL ARTICLE

Neonatal infection and passive acquisition of serum total IgG and reactive with "Streptococcus" B, anti-LPS of "Klebsiella spp" and "Pseudomonas spp" antibodies in twins

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SUMMARY

OBJECTIVE: *To describe the concentration of total and specific IgG antibodies anti-Streptococcus B, anti-lipopolysaccharide of Klebsiella spp, and anti-lipopolysaccharide of Pseudomonas spp in the umbilical cord of newborn(NB) twins and to analyze the association between neonatal infection and antibody concentration in the umbilical cord blood.*

METHODS: *A prospective cross-sectional study of a cohort of NB twins admitted during the period of 20 months. Patients with malformations and mothers with infection were excluded. Variables analyzed: gestational age(GA); birth weight(BW); antibody concentrations in umbilical cord blood; infection episodes. We used the paired Student t-test, Spearman correlation, and generalized estimation equation.*

RESULTS: *57 pairs of twins were included, 4 excluded, making the sample of 110 newborns. GA=36±1.65weeks and BW=2304.8±460g(mean±SD). Antibody concentrations in twins(mean±SD): total IgG=835.71±190.73mg/dL, anti-StreptococcusB IgG=250.66±295.1 AU/mL, anti-lipopolysaccharide of Pseudomonas spp IgG=280.04±498.66 AU/mL and anti-lipopolysaccharide of Klebsiella spp IgG=504.75±933.93 AU/mL. There was a positive correlation between maternal antibody levels and those observed in newborns(p <0.005). The transplacental transfer of maternal total IgG and anti-LPS Pseudomonas IgG antibodies was significantly lower at NB GA <34 weeks(p <0.05). Five newborns were diagnosed with an infection. Infants with infection had significantly lower total IgG concentration(p <0.05).*

CONCLUSION: *This study showed a positive correlation between maternal and newborn antibodies levels. In infants younger than 34 weeks there is less transfer of total IgG and anti-LPS Pseudomonas IgG. The highest incidence of infection in the newborn group who had significantly lower total IgG serum antibodies reinforces the importance of anti-infectious protection afforded by passive immunity transferred from the mother.*

KEYWORDS: *Infant, newborn. Twins. Maternal-fetal exchange. Immunoglobulin G. Klebsiella. Pseudomonas. Streptococcus.*

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INTRODUCTION

Over the last decades, the incidence of twin pregnancy has increased due to the use of assisted reproduction techniques and the choice of women to have children later in life. Twin pregnancy presents an additional risk of complications for the fetus, newborn, and mother. In comparison with singleton pregnancies, there is a greater risk of congenital malformation, intrauterine growth restriction, cerebral palsy, infection, mortality, and prematurity; the rate of prematurity is approximately 60% in this population[1].

The neonatal immune system has some special features necessary for the period of transition from the intrauterine to the extrauterine environment. Fetuses and neonates have limited antigenic exposure to induce adaptive immunity. The immunocompetence of newborns progresses rapidly in the first three months of life, with the maturation of the cells involved in the adaptive response and acquired antigenic experience[2]. Newborns need to be able to initiate an efficient inflammatory response to ensure protection against infections and, at the same time, allow for their colonization to happen without exacerbated inflammation in response to it. Innate immunity is modulated, with a relative decrease of the *T helper* type 1 (Th1) proinflammatory response and polarization of the *T helper* type 2 (Th2) anti-inflammatory response, which leads to greater susceptibility to infection by intracellular pathogens and lower response to vaccines. Therefore, at the beginning of life, newborns depend on the components of innate response and passive acquisition of antibodies from the mother[3,4].

Epidemiological studies have shown an increased incidence of neonatal sepsis by gram-negative agents such as *Klebsiella* and *Pseudomonas* after the introduction of the recommendation of intrapartum prophylaxis for pregnant women colonized by *Streptococcus* B[5-7].

The objective of this study is to describe the total concentrations of IgG antibodies e specific concentration of anti-*Streptococcus* B, anti-lipopolysaccharides of *Klebsiella* and *Pseudomonas* in the umbilical cord of twins and analyze the possible association between the concentrations of these antibodies and the occurrence of infection.

METHODS

This is a prospective cross-sectional study of a cohort of newborns from twin pregnancies followed-up for 20 months. We excluded newborns with malformation, congenital or maternal infection, and amniorrhexis for more than 12 hours.

The following variables were analyzed: gestational age (GA); birth weight (BW); concentration of antibodies and episodes of infection.

The study was approved by the Research Ethics Committee of the institution. Blood samples from the umbilical cords were collected after a free and informed consent form was signed by the parents. The blood was placed in a tube containing separator gel (BD Vacutainer SST) and sent to the laboratory, where it was centrifuged at 2000 rpm for 10 minutes at 4°C. The sera were divided and stored at -80°C.

The total serum concentrations of IgG antibodies were assessed by nephelometry, using a nephelometer (Behringer, USA) with appropriate standards and controls. The dosages of immunoglobulins and serum dilution of the samples were performed according to the assay protocol a Behringer nephelometer. We used a strain of *Streptococcus sp* from group B type III number H36C, batch 05/85, and lipopolysaccharides of *Klebsiella spp* (L-4268, Sigma, USA), and *Pseudomonas spp* (L-9143, Sigma, USA), both isolated from patients with hospital infections. The IgG antibodies anti-*Streptococcus* B, anti-LPS of *Klebsiella,* and anti-LPS of *Pseudomonas* were analyzed by enzyme-linked immunosorbent assay (ELISA).

To describe the results, we used the relative (percentages) and absolute (n) frequencies of the classes of each qualitative variable. For the quantitative variables, we used the mean, median, minimum value, maximum value, and standard deviation to indicate data variability.

The comparison between birth weight and concentration of antibodies in the umbilical cord blood was done using the paired Student's t-test or the paired Wilcoxon test, and the Mann-Whitney test. The same analyses were carried out between twins with and without infection.

We calculated the Spearman correlations between the levels of maternal and NB antibodies to assess the existence of correlations.

To evaluate neonatal antibodies according to the presence of infection and gestational age, we described the concentrations of antibodies per categories of interest and compared to the values of IgG between the categories, with the use of generalized estimation equations (GEE) with normal marginal distribution and identity function, assuming a symmetrical

component correlation matrix between twins and other antibodies with use of generalized estimation equations with gamma marginal distribution and identity function, assuming a symmetrical component correlation matrix between the twins.

The tests were conducted with a significance level of 5%. The software used for analysis was SPSS and MS Office Excel for Windows.

RESULTS

We studied 59 pairs of twins; four pairs were excluded, three due to insufficient collection of material, and one due to congenital malformation (tetralogy of Fallot). We included 55 pairs of twins, which comprised a sample of 110 newborns.

The most frequent type of delivery was by cesarean section (86%). The maternal age was 29.4 ± 5.9 years (mean ± SD), gestational age was 36 ± 1.65 weeks (mean ± SD) and the birth weight was 2304,8 ± 460g (mean ± SD). The gestational age ranged from 29 to 38 weeks. Most of the newborns presented a gestational age from 34 and 36 6/7 weeks (50.9%), appropriate classification for the gestational age (80.7%), and birth weight between 1500 and 2499g (57%). There was a slight predominance of females (52.7%).

In all serum samples of mothers and their NBs, we detected the total IgG antibodies and specific antibodies anti-GBS, anti-LPS of *Klebsiella*, and anti-LPS of *Pseudomonas*. The antibody concentrations found in 55 mothers and 110 newborns are presented below (mean±SD): Total IgG Mother 830.25±204.47 mg/dL and total IgG NB 835.71±190.73 mg/dL; IgG anti-*Streptococcus* B Mother 438.37±417.24 AU/mL and IgG anti-*Streptococcus* B NB 295.10±250.66 AU/mL; IgG anti-LPS *Pseudomonas* Mother 337.26±694.52 AU/mL and IgG anti-LPS *Pseudomonas* NB 280.04 ± 498.66 AU/mL and IgG anti-LPS *Klebsiella* Mother 715.5±1212,9 AU/mL and IgG anti-LPS *Klebsiella* NB 504.75±933.93 AU/mL.

There was a positive correlation between the levels of maternal antibodies and those in the newborns ($p<0.005$), as shown in Table 1.

The transplacental transfer of maternal antibodies IgG total and IgG anti-LPS *Pseudomonas* was significantly lower in NB with GA < 34 weeks ($p<0.05$). For the serum antibodies of the IgG anti-EGB and anti-LPS of *Klebsiella* class, no difference was observed between the groups. These data are presented in Table 2.

TABLE 1. CORRELATION BETWEEN THE CONCENTRATIONS OF SERUM ANTIBODIES OF THE TOTAL IGG CLASS AND SPECIFIC OF MOTHERS AND NEWBORNS

Variable	NB 1			NB 2		
	Correlation	N	p	Correlation	N	P
IgG Mother (mg/dL)	0.371	55	0.005	0.416	55	0.002
Anti-GBS Mother (Ua/mL)	0.855	55	<0.001	0.925	55	<0.001
Anti-Klebsiella Mother (Ua/mL)	0.895	55	<0.001	0.911	55	<0.001
Anti-Pseudomonas Mother (Ua/mL)	0.954	55	<0.001	0.946	55	<0.001

Results of the Spearman correlation

TABLE 2. CORRELATION BETWEEN THE CONCENTRATIONS OF SERUM ANTIBODIES OF THE TOTAL IGG CLASS AND SPECIFIC OF MOTHERS AND NEWBORNS ACCORDING TO THE GESTATIONAL AGE

Variable	Gestational age (sem)	Average	SD	Median	Minimum	Maximum	N	P
IgG NB (mg/dL)	≥ 34 < 34	851.1 639.4	181.6 207.6	842.5 712.0	273 273	1352 847	100 10	0.013*
Anti-Streptococcus NB (Ua/mL)	≥ 34 < 34	296.0 283.2	256.6 168.1	199.3 214.9	34.6 108.3	1313.8 514.1	100 10	0.918
Anti-Klebsiella NB (Ua/mL)	≥ 34 < 34	527.3 216.9	963.1 309.4	213.5 100.4	13.1 26.7	6205.4 956.9	100 10	0.183
Anti-Pseudomonas NB (Ua/mL)	≥ 34 < 34	295.3 85.1	514.8 27	103.8 87.8	13.3 47.1	3567.8 119.3	100 10	0.032

Results of the Mann-Whitney test; * Results of the Student's t-test

There were five (4.5%) diagnosed cases of infection (late sepsis) with positive blood culture during their hospitalization. The etiologic agents identified in the blood cultures were *Staphylococcus epidermidis*, *Klebsiella sp*, *Acinetobacter baumanni,* and *Staphylococcus aureus*. As for the outcome in the infection group, four NB were discharged from the hospital and one NB evolved to death.

Table 3 shows that the concentration of total IgG antibodies was significantly lower in newborns who presented infections when compared to those without infections (p=0.049). Regarding the specific IgG antibodies, no significant difference was observed between the newborns with and without neonatal sepsis.

DISCUSSION

To our knowledge, this is the first study in Brasil to analyze the transplacental transfer of antibodies in twin newborns and the rate of infection. Stach et al.[8,9] analyzed the transplacental transfer of antibodies in twins according to maternal pathologies, gestational age, chorionicity, changes in the pulsatility of the umbilical artery, placental weight, and restriction of growth. The present study prospectively followed this cohort of newborns and their evolution during hospitalization. We observed that in all serum samples of mothers and their NBs, we detected total IgG antibodies and specific antibodies anti-GBS, anti-LPS of *Klebsiella*, and anti-LPS of *Pseudomonas*. This finding confirms the transplacental transfer of these antibodies, as previously demonstrated by other authors[8,9].

We observed a positive correlation ($r > 0.8$ and $p < 0.05$) between the concentrations of serum total IgG antibodies and the specific antibodies anti-GBS, anti-LPS of *Klebsiella*, and anti-LPS *Pseudomonas* in mothers and NB. These findings are in line with those in the literature; maternal IgG is actively transferred to the fetus via endocytosis. This process is mediated by the neonatal Fc receptor (FcRn) and the pH of the medium[10]. The more acid the pH of the medium, the greater the affinity of IgG with the FcRn. The IgG molecule from the maternal circulation binds to the FcRn receptor in the syncytiotrophoblast and is internalized in a endosome. To ensure a high affinity between the FcRn receptor and the IgG molecule, and protection against the action of lysosomal enzymes, the endosome is acidified. When the gallbladder reaches the fetal circulation, it finds a physiological pH and releases the IgG molecule to the fetus[10-12].

In 10 NB (8.7%) whose gestational age was less than 34 weeks, the average concentration of total serum IgG antibodies (p=0.013) and IgG anti-LPS *Pseudomonas* (p=0.032) was significantly lower. This behavior was expected since the transplacental transfer of antibodies is influenced by the concentration of maternal immunoglobulin, class and subclass of antibody, and gestational age. The transplacental transfer of antibodies begins around 13 weeks and increases until the third quarter[10-12]. Malek et al.[13] found an average increase in the concentration of total serum IgG antibodies from 1.4± 0.7 g/L at 17-22 weeks(10% of the maternal concentration) to 5.6±1.1 g/L at 28-32 weeks (50% of the maternal concentration). This increase in the antibody transfer continues in the third quarter, with a concentration of fetal IgG greater than the maternal by the end of the gestation[10-14]. Brasil et al.[15] studied the transplacental transfer of total IgG and IgG anti-*Streptococcus* B and demonstrated that it is less efficient in premature NB. The positive correlation between the positive concentration and serum concentration of total IgG and IgG anti-*Streptococcus* B and gestational age proves the importance of prematurity as a determinant factor of low serum concentrations of these components in the immune repertoire of NB. Silveira-Lessa et al.[16] studied the passive acquisition

TABLE 3. CONCENTRATIONS OF ANTIBODIES IN THE NB ACCORDING TO THE PRESENCE OF INFECTION AND RESULTS OF THE COMPARATIVE TESTS

Variable	Infection	Average	Median	Minimum	Maximum	SD	N	P
Total IgG RN (mg/dL)	No Yes	849.1 554.8	831 615	273 273	1352 847	178.9 236.8	105 5	0.049*
IgG anti-Streptococcus NB (Ua/mL)	No Yes	298.0 234.8	200.8 190.3	34.6 122	1313.8 438.7	255.1 121.4	105 5	0.654
IgG anti-Klebsiella NB (Ua/mL)	No Yes	519.2 201.3	186.4 170.6	13.1 87.8	6205.4 329.3	953.4 110.6	105 5	0.952
IgG anti- Pseudomonas NB (Ua/mL)	No Yes	290.1 68.1	103.2 64.3	13.3 23.1	3567.8 107	508.3 32.2	105 5	0.447

Results of the EEG with gamma distribution; * Results of the EEG with normal distribution

in term and premature NB of IgG antibodies reactive with lipopolysaccharides of enterobacteria in neonatal infections. The levels of IgG anti-LPS *Klebsiella* and *E.coli* O26, O111, and O6 in premature NB were significantly lower when compared to their mothers. The transfer rates were lower in the group with a gestational age of fewer than 33 weeks (except for *E.coli* O26) and in preterm infants between 33 and 36 6/7 weeks of gestation (except for *Klebsiella* and *E.coli* O111) when compared to term NB. In the present study, regarding the serum antibodies of the IgG anti-EGB and anti-LPS *Klebsiella* class, no statistical difference was observed based on gestational age. This finding is likely due to the smaller number of cases in the group with a gestational age of fewer than 34 weeks.

In the present study, there were five (4.5%) diagnosed cases of infection with positive blood culture during their hospitalization. The concentration of the total IgG antibodies class was significantly lower in NB who presented infections when compared to those without infections (p=0.049). This result was expected, since IgG is the class of immunoglobulins with the highest concentration in the blood, with the role of bacteria opsonization and virus neutralization of[17]. The important role of IgG in immunity can be clinically exemplified by patients who present hypogammaglobulinemia and recurrent infections[18].

Epidemiological studies have documented that maternal antibodies alter the incidence and severity of infections[19]. Lin et al.[20,21] showed that newborns of mothers with concentrations of antibodies anti-*Streptococcus* B serotype Ia greater than 5 µg/mL had 88% less risk of developing sepsis caused by this bacterium in comparison with those of mothers with levels of antibodies anti-*Streptococcus*B serotype Ia less than 0.5 µg/mL. The authors also observed that children of mothers with higher levels of antibodies anti-*Streptococcus* B serotype III above 10 µg/mL had 91% less risk of developing sepsis caused by this bacterium in comparison with the children of mothers with levels of antibodies anti-*Streptococcus* B serotype III below 2 µg/mL. Similar results were found by Baker et al.[22] between the concentration of maternal antibody and relative risk of infection for early sepsis by *Streptococcus* B. The authors demonstrated that the children of mothers with a concentration of anti-*Streptococcus* B Ia, III, V antibodies greater or equal to 1.0 µg/mL had 70% less chance of developing sepsis by this agent. Larsson et al.[23] also found an association between the concentration of antibodies and infection. The authors demonstrated that there is a transplacental transfer of antibodies reactive with proteins and Rib (capsular protein of *Streptococcus* B) and that low concentrations of these antibodies are associated with invasive infection by *Streptococcus* B that express these proteins.

The pair of twins with the lowest gestational age (29 weeks) presented infection by *Acinetobacter baumani* and *Staphylococcus aureus*, respectively, and the second twin died. Although the passive acquisition of antibodies against these agents was not the object of this study, it is likely that the transfer of antibodies to the fetus was greatly reduced during the time of pregnancy. Considering the great vulnerability of preterm infants to infection[24,25], it is essential to know specifically how the transplacental transfer of antibodies occurs in relation to the most frequent bacteria in neonatal sepsis.

Among the limitations of this study, we emphasize the small number of NB with infections confirmed by blood culture. In the face of the results obtained, future studies with larger samples of NB twin and stratification of the groups for analysis according to gestational age are recommended.

CONCLUSION

We demonstrated a linear correlation between the levels of antibodies of mothers and newborns, proving that the total IgG antibodies and specific antibodies anti-*Streptococcus* B, anti-LPS of *Klebsiella*, and anti-LPS of *Pseudomonas* are transferred across the placenta barrier.

In newborns younger than 34 weeks, there was a lower transfer of total IgG and IgG anti-LPS *Pseudomonas*. The association between lower gestational age and decreased levels of anti-LPS of *Pseudomonas* antibodies confirms the greater vulnerability of these neonates to infection by this bacterium.

In preterm NB with infections, the total IgG concentration was significantly lower, which demonstrates the greater vulnerability and risk of infection in this population and the importance of passive immunity transferred through the placenta.

RESUMO

OBJETIVOS: *Descrever o título de anticorpos IgG total e específico anti-Streptococcus B, anti-lipopolissacarídeos(LPS) de Klebsiella e Pseudomonas no cordão umbilical em gêmeos e analisar a possível associação entre os títulos desses anticorpos e a ocorrência de infecção.*

MÉTODOS: *Estudo prospectivo transversal de uma coorte de recém-nascidos (RN) gemelares em 20 meses. Excluídos: malformação, infecção congênita ou materna. Variáveis estudadas: idade gestacional(IG); peso de nascimento(PN); título de anticorpos e episódios de infecção. Foram utilizados testes t-Student pareado, correlação de Spearman e equações de estimação generalizadas.*

RESULTADOS: *Elegíveis 59 pares de gêmeos, excluídos 4 e incluídos 55 pares (n=110RN). A IG foi 36±1,65semanas e o PN foi 2304,8±460g (média±DP). Concentrações de anticorpos dos RN(média±DP): IgG total=835,71±190,73 mg/dL, IgG anti-Streptococcus B=295,1±250,66 UA/mL, IgG anti-LPS Pseudomonas=280,04±498,66 UA/mL e IgG anti-LPS Klebsiella=504,75± 933,93UA/mL. Houve correlação positiva entre níveis de anticorpos maternos e aqueles observados nos RN (p<0,005). A transferência transplacentária de anticorpos maternos IgG total e IgG anti-LPS Pseudomonas foi significativamente menor em RN IG < 34semanas (p<0,05). Foram diagnosticados 5 RN com infecção. Os RN que apresentaram infecção tinham concentração de IgG total significativamente menor (p<0,05).*

CONCLUSÕES: *Na população estudada existe correlação entre os anticorpos maternos e os níveis de anticorpos no RN. Nos gêmeos menores que 34 semanas há menor transferência de IgG total e IgG anti-LPS Pseudomonas. Nos RN com infecção a concentração de IgG total é significativamente menor, o que demonstra a maior vulnerabilidade e risco de infecção dessa população e a importância da imunidade passiva transferida pela placenta.*

PALAVRAS-CHAVE: *Recém-nascido. Gêmeos. Troca materno-fetal. Imunoglobulina G. Klebsiella. Pseudomonas. Streptococcus.*

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ORIGINAL ARTICLE

Prevalence and associated factors in community-dwelling subjects - a population-based study

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SUMMARY

OBJECTIVES: *To assess the prevalence of nocturia and identify factors associated with it in a community-dwelling population.*

METHODS: *A cross-sectional study was conducted in subjects aged 45 years or more and registered with a Family Doctor Program. Information was collected about nocturia, other urinary symptoms, physical examination, co-morbidities, demographics, socio-economic, and lifestyle factors. Multiple logistics regression models were developed to analyze associated factors for nocturia according to gender and the number of nocturnal micturitions(≥1 and ≥2).*

RESULTS: *Out of the 661 individuals included in the study, 62.3% were women. Among the women, the prevalence rates for nocturia ≥1 time and ≥2 times were, respectively, 68.4% and 49%, whereas, among the men, they were 64.3% and 43.8%. Among the women, nocturia ≥1 time was associated with brown skin, a higher BMI, lower schooling, and calcium channel blockers(CCB) use, while nocturia ≥2 times showed association with higher BMI, lower schooling, obstructive sleep apnea (OSA), and the use of CCB. Among the men, nocturia ≥1 time was associated positively with age, alcohol intake, and OSA, and negatively with angiotensin receptor blockers and beta-blockers use. Besides, nocturia ≥2 times was associated with age, not having health insurance, and OSA*

CONCLUSIONS: *Nocturia is a condition highly prevalent in the studied population. For the female subjects, a higher BMI, lower schooling, and the use of CCB were associated with nocturia regardless of the definition used, whereas, among the men, that same association was found with age, not having health insurance, and OSA.*

KEYWORDS: *Aging. Nocturia. Prevalence. Primary Health Care.*

INTRODUCTION

In 2010, a joint ICS and IUGA report on the terminology of female pelvic floor dysfunction defined nocturia as the need to wake up at night one or more times to micturate, and each nocturnal micturition is preceded and followed by sleep.[1]

Based on studies showing that a single episode of nocturia seems not to cause discomfort, this definition was called into question, with the suggestion that nocturia would be clinically significant when there are 2 or more episodes per night.[2]

The incidence of nocturia increases with age, and its prevalence is so high in the elderly that most people often do not consider it a condition worth treating, but rather part of the physiological process of aging.[3] A

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systematic review of the literature showed that 29-59% of men over 70 years of age reported two or more episodes of nocturnal micturition. The prevalence of women in this same age group varied from 28 to 62%.[4]

Even if both patients and physicians may consider nocturia to be more of an annoyance than a serious condition, it has been associated with various health problems, in both men and women, such as obesity, hypertension, diabetes mellitus (DM), heart failure, obstructive sleep apnea (OSA), coronary artery disease, a history of stroke, asthma, overactive bladder syndrome, and the use of medications.[2,5,6]

Regarding its etiology, there are specific factors that cause nocturia. In women, it is associated with obesity, the post-menopause period, and a history of DM, hypertension, hysterectomy, and uterine prolapse, among other conditions.[5,7-9] Among men, it is associated with factors such as age, DM, benign prostate hyperplasia, prostate cancer, prostatitis, and the use of antidepressants.[5,6]

Elderly persons with nocturia are at a higher risk of suffering falls and fractures, which tends to increase their morbidity and mortality.[10] In addition, the condition is also associated with a higher risk of mortality in general, especially in young men and middle-aged men and women, and the survival rate decreases as the number of nocturnal micturitions increases.[11]

Brazilians are undergoing a rapid process of aging, and interest is growing in the study of conditions that affect the health and quality of life of this large population group. A study on the prevalence of lower urinary tract symptoms, including nocturia, conducted in a group of subjects in the north-eastern part of the country, found overall rates of 64.4% and 71.2% in men and women, respectively, when the definition used was the presence of one or more nocturnal micturitions. When the definition was two or more micturitions, the rates decreased by 50%. In both cases, the prevalence increased with the age group, but factors associated with nocturia were not studied.[12]

Given that nocturia is a common condition in the elderly that is associated with a poorer quality of life and the risk of death, and may be overlooked, it is essential that we alert healthcare professionals to the problem, mainly those who provide health care to the elderly.[13]

The objectives of the present study were to assess the prevalence of nocturia and identify demographic, socio-economic, lifestyle, and clinical factors associated with this condition.

METHODS

Study design

This study is part of the Digitalis study, a cross-sectional investigation of the prevalence of chronic diseases and their association with risk factors, using a random, two-stage sample (sector and individuals in the sector) of a group of people aged 45 years or more registered with the Niteroi Family Doctor Program (FDP), in the State of Rio de Janeiro, Brasil.[14] The sample size was calculated to estimate the prevalence and the measures of association, taking the feasibility of the study into consideration. Our calculations predicted that it would be possible to assess 600 subjects, plus 10% for incomplete assessments (losses), in 18 months.

The units (sectors) to be included in the study were randomly selected from the official list of FDP sectors. For each sector, approximately 80 subjects of both genders, between 45 and 99 years of age, were randomly selected from the records of residents kept on file by the program. It was expected that 30 examinations would be conducted per visit. Thus, it was recommended that 50 residents be invited to participate, to take into account non-attendance, and that 30 other names be collected for possible substitutions.

Visits were carried out from August 2011 to November 2012. The participants responded to a questionnaire asking for information related to co-morbidities and demographics, as well as socio-economic and lifestyle factors. Physicians and nurses were in charge of each patient's medical history and physical examination. Besides, urine and fasting blood samples were collected.

The Digitalis questionnaire investigated the presence of various co-morbidities, including urinary disorders. Among other conditions, patients were asked if they had to get up at night to micturate and, if they gave a positive response, how many times that occurred per night. Individuals who reported waking up to micturate at least once at night were deemed to have nocturia.[1] Other urinary symptoms, such as the absence of the perception of the desire to void (the ability to recognize the sensations of bladder filling), the inability to reach the toilet in time (meaning that they had a strong desire to void and urine loss before reaching the toilet), stress urinary incontinence (SUI), defined as involuntary urine loss on sneezing or coughing, the inability to initiate or inhibit voiding, and the inability to completely empty the bladder were also investigated.

Skin color was classified by the participants

themselves as black, brown, or white.

Subjects with blood pressure ≥ 140 mmHg (systolic) or ≥ 90 mmHg (diastolic) were considered hypertensive, as were those who reported using anti-hypertensive drugs. Participants whose fasting glucose was ≥ 126 mg/dL and those who reported oral use of hypoglycemic agents and/or insulin were considered to be diabetic.

The Digitalis questionnaire also included questions about whether alcoholic beverages were consumed and with what frequency. Men who consumed, on average, more than two daily doses of alcohol, or women who consumed more than one dose, were considered to be at-risk drinkers.

Chronic kidney disease (CKD) was defined using the KDIGO criteria, and heart failure according to the guidelines of the Brazilian Cardiology Society for chronic heart failure.[15,16]

The risk of having OSA was assessed using the Brazilian version of the Berlin Questionnaire.[17] That questionnaire contains 19 items, divided into three categories: a history of snoring and apnea, daytime drowsiness, and hypertension or obesity. Subjects who had scores in at least two of the symptom categories were deemed to be at high risk for Obstructive Sleep Apnea Syndrome.[17]

For the classification of depression, the responses from the Patient Health Questionnaire-9 (PHQ-9) were used. Subjects with a score of 3, 4 or 5 (moderate, moderately severe, and severe depression) were considered to be positive.[18]

Body mass index (BMI) was calculated as the ratio of weight in kilograms to height in square meters. Patients with a BMI (kg/m2) ≥ 30 were considered to be obese.

STATISTICAL ANALYSIS

Our analyses were done separately for men and women.

The continuous variables were expressed as mean ± standard deviation (SD) in the event of normal distribution. Category variables were expressed as absolute and relative frequencies. Comparisons between the groups were done using the Student t-test. Frequencies were compared using Pearson's chi-square test, with consistency correction using Fisher's exact test, whenever necessary.

Measures of association were calculated based on two different diagnoses of nocturia: one or more episodes per night, and two or more episodes per night, in the latter, using none or one episode per night as the control group for purposes of comparison.

Multiple regression models were developed to analyze associated factors for nocturia using those variables that presented a p-value ≤ 0.10 in the univariate analysis. We thus calculated the odds ratios (OR) and their confidence intervals at 95% (CI95). All the multivariate analyses were done taking a p-value ≤ 0.05 as statistically significant.

Statistical analysis was done using SPSS software, version 23.0 for Windows (IBM, Chicago, USA).

RESULTS

The study included 661 subjects, of which 412 (62.3%) were women, and 286 (43.3%) were more than 60 years old.

Based on the ICS/IUGA definition, 444 (67.2%) of the patients had nocturia. However, when the symptom of nocturia was considered to be the presence of two or more nocturnal micturitions, its frequency dropped to 47% (311 cases).

Among the women, we found the prevalence of nocturia to be 68.4% (one or more nocturnal micturitions) and 49% (two or more). Among the men, we found frequency rates of 64.3% and 43.8%, based on the definition used.

Among the female subjects, there was an association (p≤0.10) between the occurrence of nocturia (one or more nocturnal micturitions) and the variables of skin color, low level of education, hypertension, DM, BMI (the higher the BMI, the higher the occurrence), symptoms of the inability to reach the toilet in time, SUI, and the use of calcium channel blockers (CCB), beta-blockers, glibenclamide, metformin, and insulin. (Table 1).

When these variables were subjected to multiple logistic regression analyses, we found that the presence of at least one nocturnal micturition showed a positive, independent association with brown skin color, higher BMI, low level of education, and the use of CCB. (Table 2)

Also in the female subjects, when the criterion for nocturia was two or more nocturnal micturitions, we found an association with a low level of education, BMI (the higher the BMI, the higher the rate), hypertension, DM, depression, SUI, a higher risk for OSA and the use of CCB, beta-blockers, ACEI, diuretics, glibenclamide, and metformin (Table 1).

TABLE 1. ASSOCIATION OF DEMOGRAPHIC FACTORS, COMORBIDITIES AND MEDICINE USE WITH NOCTURIA ≥1 OR ≥2 NOCTURNAL MICTURITIONS ACCORDING TO THE GENDER IN COMMUNITY-DWELLING SUBJECTS AGED 45 OR MORE FROM THE NITERÓI FAMILY DOCTOR PROGRAM, RIO DE JANEIRO, BRASIL.

	Women		Men	
Nocturnal micturions	≥1[a]	≥2[b]	≥1[a]	≥2[b]
Age	P=0.25	P=0.23	P=0.02	P<0.001
Mean ± SD	59,66±10,13	59,88±10,08	60,71±10,54	61,91±10,85
BMI	P<0.001	P<0.001	P=0.63	P=0.62
Mean ± SD	29,58±5,75	29,78±5,87	26,61±4,82	26,33±4,66
Skin color	P= 0.095	P=0.84	P=0.18	P=0.005
White	98 (68.1%)	71 (49.3%)	56 (59.6%)	31 (33%)
Brown	112 (74.2%)	75 (50%)	68 (63.6%)	48 (44.9%)
Black	71 (62.3%)	53 (46.9%)	34 (75.6%)	28 (62.2%)
Schooling	0.047	P=0.007	P=0.034	P=0.007
Up to 4 yrs	135 (74.2%)	103 (56.6%)	70 (72.2%)	53 (54.6%)
≥ 5 yrs	149 (65.1%)	99 (43.2%)	89 (58.9%)	56 (37.1%)
Health insurance	0.23	P=0.13	0.087	0.013
No	246 (69.9%)	177 (50.3%)	134 (66.3%)	95 (47%)
Yes	36 (62.1%)	23 (39.7%)	22 (52.4%)	11 (26.2%)
Alcohol (risk dose)	0.73	0.26	P=0.067	P=0.65
No	266(68.7%)	187(48.3%)	132(62%)	92(43.2%)
Yes	18 (72%)	15 (60%)	28 (77.8%)	17 (47.2%)
Hypertension	0.001	0.001	0.99	0.20
No	54(55.7%)	33 (34%)	54(64.3%)	32(38.1%)
Yes	230(73%)	169(53.7%)	106(64.2%)	77(46.7%)
Diabetes	0.002	0.001	0.29	0.20
No	195(65%)	134(44.7)	118(62.4)	78(41.3)
Yes	85(81)	66(62.9)	40(70.2)	29(50.9)
Depression	0.85	0.10	0.13	0.039
No	203(68.6)	138(46.6)	138(63)	92(42)
Yes	80(69.6%)	64(55.7)	21(77.8)	17(63)
ACEI[c]	0.100	0.063	0.032	0.100
No	188(66.4)	130(45.9)	101(59.8)	68(40.2)
Yes	96(74.4)	72(55.8)	59(73.8)	41(51.3)
ARB[d]	0.79	0.37	0.001	0.12
No	248(68.7)	174(48.2)	157(66.5)	106(44.9)
Yes	36(70.6)	28(54.9)	3(23.1)	3(23.1)
CCB[e]	0.002	0.000	0.58	0.68
No	228(65.9)	155(44.8)	144(63.7)	98(43.4)
Yes	56(84.8)	47(71.2)	16(69.6)	11(47.8)
Beta-blockers	0.020	0.004	0.002	0.008
No	224(66.5)	154(45.7)	149(67.7)	103(46.8)
Yes	60(80)	48(64)	11(37.9)	6(20.7)
Diuretics	0.850	0.067	0.440	0.059
No	168(68.6)	111(45.3)	117(62.9)	75(40.3)
Yes	116(69.5)	91(54.5)	43(68.3)	34(54)
Kidney diseases	0.51	0.12	0.12	0.03
KDIGO[f] 1 and 2	242(68.6)	168(47.6)	136(62.7)	89(41)
KDIGO 3,4 and 5	33(73.3)	27(60)	21(77.8)	17(63)
High risk for OSA[g]	0.27	0.044	0.045	0.012
No	231(68.3)	158(46.7)	123(60.6)	80(39.4)
Yes	38 (76)	31(62)	21(80.8)	17(65.4)

	Women		Men	
Nocturnal micturions	≥1[a]	≥2[b]	≥1[a]	≥2[b]
Inability to reach the toilet in time	0.092	0.42	0.31	0.013
No	254(68.3)	179(48.1)	139(62.9)	92(41.6)
Yes	18(85.7)	12(57.1)	10(76.9)	10(76.9)
SUI[h]	0.054	0.063	0.49	0.33
Não	159(65.7)	109(45)	131(64.5)	86(42.4)
Sim	111(75)	81(54.7)	18(58.1)	16(51.6)
Glibenclamide use	0.037	0.065	0.97	0.91
No	259(67.6)	183 (47.8)	153(64.3)	104(43.7)
Yes	25(86.2)	19(65.5)	7(63.6)	5(45.5)
Metformin use	0.002	0.001	0.76	0.57
No	234(66.1)	162(45.8)	144(64.6)	99(44.4)
Yes	50(86.2)	40(69)	16(61.5)	10(38.5)
Insulin use	0.055	0.14	0.93	0.42
No	276(68.3)	196(48.5)	158(64.2)	107(43.5)
Yes	8 (100)	6(75)	2(66.7)	2(66.7)
Prostate disease	_	_	0.39	0.085
No	_	_	142(63.4)	94(42)
Yes	_	_	18(72)	15(60)

Age and BMI – Student-test; others variables: Pearson Chi-square test.
Legend: **a** The tests refer to the comparison between nocturia ≥1 nocturnal micturitions versus no nocturnal micturition for each gender. **b** The tests refer to the comparison between nocturia ≥2 nocturnal micturitions versus < 2 nocturnal micturition for each gender. **c** ACEI - angiotensin-converting enzyme inhibitors; d ARB - angiotensin receptor blockers e CCB - calcium channel blockers; f KDIGO - Kidney Disease: Improving Global Outcomes classification of chronic kidney disease; g OSA – obstructive sleep apnea; h SUI – Stress urinary incontinence

The multiple logistics regression analysis, in turn, showed a positive, independent association with a higher BMI, a low level of education, a higher risk for OSA, and the use of CCB. (Table 3)

Among the male subjects, there was an association (p≤0.10) between the occurrence of nocturia (one or more nocturnal micturitions) and the variables of age (the older the age, the higher the occurrence), a low level of education, not having health insurance, the intake of risky levels of alcohol, a higher risk for OSA, the use of ACEI, the use of angiotensin receptor blockers (ARB) (lower chance) and beta-blockers (lower chance). (Table 1).

When the variables were subjected to multiple logistic regression analysis, we found that the presence of at least one nocturnal micturition showed a positive, independent association with age, intake of risky levels of alcohol and a higher risk for OSA, and a negative, independent association with the use of ARB and beta-blockers. (Table 4)

When two or more nocturnal micturitions was used as the criterion, we found an association with age (the older, the higher the chance), low level of education, not having health insurance, skin color, chronic kidney disease, depression, the use of diuretics, beta-blockers (lower chance), the inability to micturate in proper places, a higher risk for OSA, and a history of prostate disease (Table 1). The multiple logistics regression analysis showed a positive, independent association with higher age, not having health insurance, and a higher risk for OSA. (Table 5)

The variables heart failure, absence of the perception of the desire to void, inability to initiate or inhibit

TABLE 2. ADJUSTED ODDS RATIOS (MULTIPLE LOGISTIC ANALYSIS) FOR FACTORS ASSOCIATED WITH ≥ 1 NOCTURNAL MICTURITIONS IN IN COMMUNITY-DWELLING WOMEN AGED 45 OR MORE FROM THE NITERÓI FAMILY DOCTOR PROGRAM. RIO DE JANEIRO. BRASIL.

Variable	ORa (95% CI)
BMI[a]	1.09 (1.04-1.15) p<0.01
Skin color	
Black	1
White	1.72 (0.97-3.03) p=0.06
Brown	2.23 (1.24-4.01) p <0.01
Schooling	P=0.01
Up to 4 yrs	1.84 (1.14-2.95)
≥ 5 yrs	1
CCB[b] use	P<0.01
Yes	3.20 (1.47-6.97)
No	1

Legend: **a** BMI – body mass index; **b** CCB - calcium channel blockers

TABLE 3. ADJUSTED ODDS RATIOS (MULTIPLE LOGISTIC ANALYSIS) FOR FACTORS ASSOCIATED WITH ≥ 2 NOCTURNAL MICTURITIONS IN IN COMMUNITY-DWELLING WOMEN AGED 45 OR MORE FROM THE NITERÓI FAMILY DOCTOR PROGRAM. RIO DE JANEIRO. BRASIL.

Variable	ORa (95% CI)
BMI [a]	1.06 (1.02-1.10) p < 0.01
High risk for OSA	P<0.05
Yes	2.05 (1.06-3.96)
No	1
Schooling	P<0.01
Up to 4 yrs	1.94 (1.26-2.99)
≥ 5 yrs	1
CCB[b] use	P<0.01
Yes	3.10 (1.67-5.77)
No	1

Legend: **a** BMI – body mass index; **b** CCB - calcium channel blockers

TABLE 4. ADJUSTED ODDS RATIOS (MULTIPLE LOGISTIC ANALYSIS) FOR FACTORS ASSOCIATED WITH ≥ 1 NOCTURNAL MICTURITIONS IN IN COMMUNITY-DWELLING MEN AGED 45 OR MORE FROM THE NITERÓI FAMILY DOCTOR PROGRAM. RIO DE JANEIRO. BRASIL.

Variable	ORa (95% CI)
Age	1.04 (1.01-1.08) p <0.01
Alcohol (risk dose)	P<0.05
Yes	2.84 (1.12-7.20)
No	1
ARB[a] use	P<0.05
Yes	0.16 (0.03-0.75)
No	1
Beta blockers use	P<0.01
Yes	0.18 (0.07-0.47)
No	1
High risk for OSA[b]	P<0.01
Yes	4.61 (1.46-14.59)
No	1

Legend: **a** ARB - angiotensin receptor blockers; **b** OSA – obstructive sleep apnea

TABLE 5. ADJUSTED ODDS RATIOS (MULTIPLE LOGISTIC ANALYSIS) FOR FACTORS ASSOCIATED WITH ≥ 2 NOCTURNAL MICTURITIONS IN IN COMMUNITY-DWELLING MEN AGED 45 OR MORE FROM THE NITERÓI FAMILY DOCTOR PROGRAM. RIO DE JANEIRO. BRASIL.

Variable	ORa (95% CI)
Age	1.05 (1.02-1.08) P<0.01
High risk for OSA	P<0.01
Yes	3.22 (1.33-7.78)
No	1
Health insurance	P<0.01
Yes	3.35 (1.38-8.13)
No	1

Legend: [a] OSA – obstructive sleep apnea

voiding, and inability to completely empty the bladder did not present association with any of the study groups (data not shown).

DISCUSSION

The comparison of the prevalence of nocturia found in the present study and those found in the literature becomes problematic due to the existence of two criteria for defining the condition, the various methods of subject selection (Internet search, medical record data, and interviews with researchers), and different age groups included by the various authors.

The prevalence of nocturia in our study, defined as at least one nocturnal micturition, was similar to that found by Moreira Jr. et al.[12]. However, when two nocturnal micturitions are used, even though there was a decrease in the prevalence rates in both studies, it was less marked in the present study, which may be due to the inclusion of only subjects over age 45 years in the study group. Likewise, when our results are compared with international studies, the prevalence rates are similar in some cases and dissimilar in those where, mainly, the age group of the subjects was lower.[5,6,9,11]

Based on the results obtained, age was an associated factor in men, regardless of the definition of the symptom, and that result is in agreement with the literature.[5,8,19]

That association can be explained by a higher prevalence of co-morbidities and chronic systemic diseases as a man's age increases, especially prostate disorders, for which nocturia is one of the symptoms. Although no association of a history of prostate disorders with nocturia was found in the study group, that may only mean that the subjects were unaware of having prostate disease, but does not rule out the possibility of a subject having it. Furthermore, aging itself may lead to irreversible, age-dependent changes in the lower urinary tract, both functional and structural, that contribute to nocturia.[20]

Based on the results obtained, the higher the BMI, the higher the risk of nocturia in women, which is in agreement with the literature.[5-8,21,22] Women over age 45 years are in the peri- and postmenopausal periods, in which there is a weight gain due to metabolic changes. Added to that is the association of obesity with chronic diseases and, consequently, the use of medications to treat them, and with an increased risk for OSA, all factors associated with nocturia.[23,24]

Subjects with a higher risk of having OSA also have a higher risk of nocturia, both men and women,

although for women, in this case, only when it is defined as two or more nocturnal micturition episodes. This finding is in accordance with various other studies.[23,25,26] Both conditions - OSA and nocturia - are associated with cardiovascular disease, type II diabetes, renal disease, the use of alcohol and smoking, sedentariness, and the use of medications, which may justify the results we obtained.

Some socio-economic variables showed an association with nocturia. An association between low level of education and nocturia was found only in the women, whereas in the men, two or more episodes of nocturnal micturition were associated with not having health insurance. Some studies obtained similar results, while others failed to show these associations.[7,9,19] A low level of education is associated with a low socio-economic level, which reduces the likelihood that a person will have private health insurance, which may be a factor related to less access to health care, lower adherence to the treatments for their co-morbidities, and guidelines for changes in lifestyle, which are associated with nocturia.[27]

Concerning the association between drugs and nocturia, only CCB increased the risk of nocturia in women in the multivariate analysis. This finding agrees with that of the BACH study, and the mechanism for this relationship seems to be due to direct blockage of the re-absorption of sodium in the proximal tube of the kidneys, to an increase in the levels of the atrial natriuretic peptide, or even to peripheral edema in the lower members.[28,29] However, the use of angiotensin blockers appears to be a protective factor for nocturia defined as at least one nocturnal micturition in men. Any condition that causes a pathological increase in angiotensin II may cause primary polydipsia since that substance is a potent central stimulant of thirst. One can thus assume that the beneficial effect of the angiotensin blocker would be less consumption of liquids and thereby a lower production of urine.[30]

Our findings should be interpreted in light of their limitations. Since this was a cross-sectional study, one cannot infer causality. The Digitalis study was designed to be an epidemiological study of chronic disorders, and the results presented here were obtained from a secondary analysis of the data. Other limitations are the sample size, the use of non-standardized questions to identify the presence of nocturia, and the use of a questionnaire for the risk of OSA, without a confirmed diagnosis. However, this study contributes to our knowledge of a condition rarely studied in the Brazilian population, that affects not only the quality of life of the subjects but also their morbidity and risk of death since nocturia is more common in the elderly and related to risks of falls and death. Other strong points are the fact that it was done on the general population, that our analyses were done based on two definitions of nocturia - taking into consideration the influence of socio-economic factors, clinical history and co-morbidities diagnosed by the use of a physical examination and by additional tests.

CONCLUSIONS

Nocturia is a symptom prevalent in the population studied, regardless of the definition chosen (one or more nocturnal micturitions/two or more nocturnal micturitions), and is slightly more predominant in women.

Among the women, the BMI, a low level of education and the use of calcium channel blockers were associated with nocturia regardless of the definition used, whereas among the men, that same association was found with age, the fact of not having health insurance, and an elevated risk of obstructive sleep apnea.

Authors contribuition

All authors contributed equally to the work

RESUMO

OBJETIVOS: *Estimar a prevalência de noctúria e identificar fatores demográficos, socioeconômicos, clínicos e de estilo de vida associados ao sintoma em uma população comunitária.*

MÉTODO: *Estudo transversal em indivíduos com 45 anos ou mais. Foram obtidas informações demográficas, socioeconômicas, sobre noctúria, outros sintomas urinários, exame físico, comorbidades e estilo de vida. As análises foram feitas separadamente de acordo com o gênero e com o número de micções noturnas (≥1 vez e ≥2 vezes).*

RESULTADOS: *Dentre os 661 indivíduos incluídos, 62,3% eram mulheres. Entre elas, a prevalência de noctúria ≥1 vez e ≥2 vezes foi, respectivamente, de 68,4% e 49%, enquanto entre os homens foi de 64,3% e 43,8%. Entre as mulheres, a noctúria ≥1 mostrou associação com cor da pele parda, maior IMC, baixa escolaridade e uso de bloqueadores dos canais de cálcio (BCC), enquanto noctúria ≥2 vezes mostrou associação com maior IMC, baixa escolaridade, apneia obstrutiva do sono (AOS) e uso de BCC. Entre os homens, a noctúria ≥1 vez esteve associada positivamente com idade, ingestão de álcool e AOS, e negativamente com uso*

de bloqueadores dos receptores da angiotensina e de beta-bloqueadores. Além disso, noctúria ≥2 vezes associou-se a idade, não ter plano de saúde e AOS.

CONCLUSÕES: *A noctúria é uma condição altamente prevalente na população estudada. Para as mulheres, IMC elevado, baixa escolaridade e uso de BCC estiveram associados com noctúria independente da definição, enquanto que, para os homens, a mesma associação foi identificada com idade, não ter plano de saúde e AOS.*

PALAVRAS-CHAVE: *Envelhecimento. Noctúria. Prevalência. Atenção primária à saúde.*

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REVIEW ARTICLE

Use of remdesivir for patients with Covid-19: a review article

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SUMMARY

The etiological agent of COVID-19, which causes severe respiratory diseases such as pneumonia and pulmonary insufficiency, has been confirmed as a new coronavirus, now known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). There is currently no authorized medication for the treatment of COVID-19. No vaccines have been authorized. Thus, this study aimed at conducting a review of the use of Remdesivir in patients with COVID-19. The following electronic databases were used MEDLINE, SCIELO, LILACS, and PUBMED. On May 1, Remdesivir received emergency use authorization from the Food and Drug Administration. Remdesivir is currently the most promising molecule in the treatment of COVID-19, taking into account its broad antiviral spectrum (considering the genetic sequences of the virus, it is expected to maintain activity against SARS-CoV-2). There is in vitro and in vivo information available for coronaviruses, as well as an extensive clinical safety database (from a clinical trial of the Ebola virus and in the context of the Monitored Emergency Use of Unregistered and Investigational Interventions - MEURI). Further studies are relevant as available data on the efficacy and safety of Remdesivir against SARS-nCoV-2 are limited.

KEYWORDS: *Betacoronavirus. Coronavirus Infections/therapy. Coronavirus.*

INTRODUCTION

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a newly-emerging human infectious coronavirus, originating in Wuhan, China, that has been spreading rapidly in China and other countries since December 2019[1]. SARS-CoV-2 is a β-coronavirus, which is an enveloped non-segmented positive-sense RNA virus (subgenus sarbecovirus, Orthocoronavirinae subfamily)[2].

The World Health Organization (WHO) named this novel coronavirus disease COVID-19, and there have been confirmed cases in 189 countries or territories outside China, including Japan, the United States

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of America, Italy, Iran, and Brasil[3]. In Brasil, 8,412 deaths have been caused by COVID-19 and there have been 123,809 confirmed cases of the disease recorded nationwide as of May, 6th, 2020[4].

The clinical features of COVID-19 are varied, ranging from an asymptomatic state to acute respiratory distress syndrome and multi-organ dysfunction. The common clinical features include fever (not in all cases), cough, sore throat, headache, fatigue, myalgia, and breathlessness. Conjunctivitis (pink eye) has also been described[5].

There is no evidence from randomized clinical trials (RCTs) that any potential therapy improves outcomes in patients with suspected or confirmed COVID-19. This review summarizes current evidence on the use of Remdesivir for COVID-19 and provides a summary of the current clinical experience and treatment guidelines for this epidemic Novel Coronavirus.

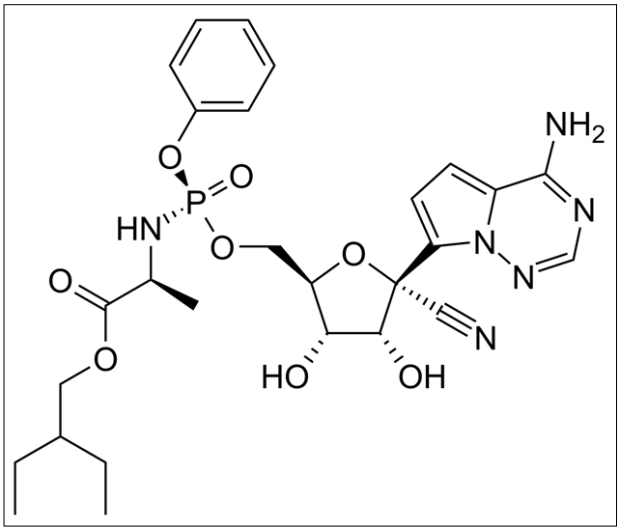
METHODS

A literature search was performed in a Medical Literature Analysis and Retrieval System Online (MEDLINE) via PubMed (1966 to January 2020), available through the following link: https://www.ncbi.nlm.nih.gov/pubmed/; Scientific Electronic Library Online (SCIELO), available at https://www.scielo.org/ and Literatura Latino Americana e do Caribe em Ciências da Saúde (LILACS), available through the following link: https://bvsalud.org/, using the following terms: 2019-nCoV, COVID-19, SARS-CoV-2, Coronavirus and Treatment, to find articles published from January 5 to April 30, 2020. Moreover, we used the findings of literature retrieved by searching authoritative texts and manual searches in WHO reports. We checked the reference lists of all studies identified by the above methods. Studies were excluded if old data was used, if the topics were inappropriate or not pertinent to the purpose of the study.

Use of Antiviral Remdesivir

The Food and Drug Administration (FDA), the agency that regulates medicines in the United States, has approved the use of Antiviral Remdesivir (GS-5734™) (Figure 1) in the treatment of severe cases of COVID-19, the disease caused by the new coronavirus (Sars-CoV-2)[6]. The drug, from drugmaker Gilead, was originally developed to fight Ebola, but with no success[7].

FIGURE 1. CHEMICAL STRUCTURE OF REMDESIVIR.



Remdesivir (also GS-5734) is a monophosphoramidate prodrug of an adenosine analog that has a broad antiviral spectrum including filoviruses, paramyxoviruses, pneumoviruses, and coronaviruses[8]. Remdesivir is a prodrug that is metabolized into its active form GS-441524, an adenine nucleotide analog that interferes with the activity of viral RNA polymerase and promotes evasion of proofreading by viral exoribonuclease, leading to inhibition of viral RNA synthesis. Remdesivir acts early in infection and decreases viral RNA levels in a dose-dependent manner that parallels impairment of viral load *in vitro*[8].

Remdesivir has demonstrated *in vitro* and *in vivo* activity in animal models against the viral pathogens that cause MERS and SARS, which are coronaviruses structurally similar to SARS-CoV-2, the coronavirus that causes COVID-19[9,10].

Concerning its metabolism, it has been shown that, upon intravenous (IV) administration of a 10 mg/kg dose in Rhesus Monkeys, Remdesivir exhibited a short plasma half-life (t1=2 = 0.39 h) with rapid systemic elimination followed by the appearance of transient systemic levels of a key intracellular intermediate alanine metabolite and more persistent levels of GS-441524 (detectable for over 24h in plasma)[11].

Regarding the clinical use of Remdesivir, the results were published by Grein et al.[9], in which patients received a 10-day course of Remdesivir, consisting of 200 mg administered intravenously on day 1, followed by 100 mg daily for the remaining 9 days of the treatment. It was observed, at the end of the follow-up, that of the 61 patients who received at least one dose of Remdesivir, data from 8 could not be analyzed (including 7 patients with no post-treatment data

and 1 with a dosing error). Of the 53 patients whose data were analyzed, 22 were in the United States, 22 in Europe or Canada, and 9 were in Japan. At baseline, 30 patients (57%) were receiving mechanical ventilation and 4 (8%) were receiving extracorporeal membrane oxygenation.

During a median follow-up of 18 days, 36 patients (68%) had an improvement in oxygen-support class, and 17 of 30 patients (57%) receiving mechanical ventilation were extubated. A total of 25 patients (47%) were discharged, and 7 patients (13%) died; mortality was 18% (6 out of 34) among patients receiving invasive ventilation and 5% (1 of 19) among those not receiving invasive ventilation. In this cohort of patients hospitalized for severe Covid-19 who were treated with compassionate-use Remdesivir, clinical improvement was observed in 36 of 53 patients (68%). Measuring the efficacy of this approach will require ongoing, randomized, placebo-controlled trials of Remdesivir therapy.

In another published study, eligible patients were adults (aged ≥18 years) admitted to the hospital with laboratory-confirmed SARS-CoV-2 infection, with an interval from symptom onset to the moment of enrollment of 12 days or less, oxygen saturation of 94% or less on room air, or a ratio of arterial oxygen partial pressure to fractional inspired oxygen of 300 mm Hg or less, and radiologically confirmed pneumonia. Patients were randomly assigned in a 2:1 ratio to intravenous Remdesivir (200 mg on day 1 followed by 100 mg on days 2–10 in single daily infusions) or the same volume of placebo infusions for 10 days. 237 patients were enrolled and randomly assigned to a treatment group (158 to Remdesivir and 79 to placebo); One patient in the placebo group who withdrew after randomization was not included in the ITT population.

Remdesivir use was not associated with any difference in the time for clinical improvement (hazard ratio 1·23 [95% CI 0·87–1·75]). Although not statistically significant, patients receiving Remdesivir had a numerically faster time to clinical improvement than those receiving placebo, among patients with symptom duration of 10 days or less (hazard ratio 1·52 [0·95–2·43]). In this study of adult patients admitted to hospital for severe COVID-19, Remdesivir was not associated with statistically significant clinical benefits. However, the numerical reduction in the time for clinical improvement in those treated earlier requires confirmation in larger studies[10]. At least 23 studies on Remdesivir are currently listed on various trial registers, intending to study 23,500 patients, but fewer than a quarter are double-blind, and some are uncontrolled observational studies.

CONCLUSION

Thusly the studies demonstrated that intravenous doses of Remdesivir were adequately tolerated in patients; this medication was not utilized in patients seriously infected by COVID-19, necessitating control clinical trials. Because of this, more scientific information is needed to reach conclusions about Remdesivir, its benefits, and in what situations it should be used.

Conflicts of Interest

The authors declare that there are no conflicts of interest that may have influenced this work.

Authors' Contributions

TCPA, ARVSC, MLCF, PCPA, and RNSF performed searches in the databases. TJMR, CFSR, FTB, and FWSR selected the articles that would be included in the research. FWSR corrected the writing in English. All authors performed the other parts of the research in an equal way. All authors have reviewed and approved the final text of this article and are responsible for its content.

RESUMO

O agente etiológico da COVID-19, que causa doenças respiratórias graves, como pneumonia e insuficiência pulmonar, foi confirmado como um novo coronavírus, agora conhecido como coronavirus de síndrome respiratória aguda grave 2 (SARS-CoV-2). Não existem atualmente medicamentos autorizados para o tratamento de COVID-19, nem estão também autorizadas quaisquer vacinas. Assim, o estudo teve como objetivo realizar uma revisão sobre a utilização de Remdesivir em pacientes com COVID-19. As seguintes bases de dados eletrônicas foram utilizadas MEDLINE, SCIELO, LILACS e PUBMED. Em primeiro de maio, o Redemsivir recebeu autorização de uso de emergência da Food and Drug Administration. Remdesivir é presentemente a molécula promissora no tratamento da COVID-19 tendo em conta o seu largo espetro antiviral (considerando as sequências genéticas do vírus, é expectável que mantenha atividade contra o SARS-CoV-2). A informação in vitro e in vivo está disponível para os coronavírus, assim como a extensiva base de dados de

segurança clínica (proveniente de ensaio clínico do vírus Ebola e no contexto do Monitored Emergency Use of Unregistered and Investigational Interventions - MEURI). A realização de novos estudos torna-se relevantes uma vez que os dados disponíveis são limitados sobre eficácia e segurança do Remdesivir contra SARS-nCoV-2.

PALAVRAS-CHAVE: *Betacoronavirus. Infecções por Coronavirus/terapia. Coronavírus.*

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REVIEW ARTICLE

Fibrinogen and D-dimer variances and anticoagulation recommendations in Covid-19: current literature review

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SUMMARY

INTRODUCTION: *Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a newly described virus responsible for the outbreak of the coronavirus disease 2019 (Covid-19), named by the World Health Organization (WHO) in February/2020. Patients with Covid-19 have an incidence of acute respiratory distress syndrome (ARDS) of 15.9-29% and sepsis is observed in all deceased patients. Moreover, disseminated intravascular coagulation (DIC) is one of the major underlying causes of death among these patients. In patients with DIC, there is a decrease in fibrinogen and an increase in D-dimer levels. Some studies have shown that fibrinogen and one of its end products, D-dimer, might have a predictive value for mortality in patients with non-Covid sepsis secondary to complications of DIC. Therefore, anticoagulation, considering its mortality benefits in cases of non-Covid sepsis, may also have an important role in the treatment of Covid-19.*

METHODS: *We reviewed the literature of all studies published by April 2020 on patients infected with Covid-19. Our review was limited to D-dimer and fibrinogen changes and anticoagulation recommendations.*

RESULTS: *Anticoagulation therapy can be started following the DIC diagnosis in Covid-19 patients despite the bleeding risks. In addition, the current evidence suggests a routine use of anticoagulation, particularly in patients with higher D-dimer levels (> 3.0 µg/mL).*

CONCLUSION: *Covid-19 is a systemic, hypercoagulable disease requiring more studies concerning treatment. Aanticoagulation is still an issue to be studied, but D-dimer rise and disease severity are the indicative factors to start treatment as soon as possible.*

KEYWORDS: *Coronavirus Infections. Anticoagulants. Disseminated intravascular coagulation. Fibrin fibrinogen degradation products. Blood Coagulation.*

INTRODUCTION

An outbreak of a newly described virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has appeared worldwide with a frequent occurrence of pneumonia and its local or/and systemic complications. The virus has been named SARS-CoV-2 in China, following its first isolation from patients with pneumonia[1]. The disease has been later referred to as coronavirus disease 2019 (Covid-19) by the World Health Organization (WHO) in February/2020[2]. The severity of Covid-19 increases due to the development

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of acute respiratory distress syndrome (ARDS) and sepsis if not limited to pneumonia. Patients with Covid-19 have an incidence of ARDS of 15.9-29%, in addition to ARDS, sepsis is observed in all deceased patients[3-5]. Moreover, disseminated intravascular coagulation (DIC) is one of the major underlying causes of death in these patients. Consumption coagulopathy, which should be obviated in order to decrease mortality, arises in DIC with a decrease in fibrinogen and an increase in D-dimer levels. In fact, fibrinogen and one of its end products, D-dimer, have also been reported to have predictive value regarding the mortality of patients with non-Covid sepsis secondary to complications of DIC[6,7]. Therefore, anticoagulation, considering its mortality benefits in non-Covid sepsis, may also have an important role in the treatment of Covid-19[8].

In the current treatment strategy, patients with Covid-19 receive hydroxychloroquine and/or azithromycin as a first-line therapy[9]. Favipiravir, remdesivir, and lopinavir/ritonavir are the second line available after the failure of first-line therapy, but they can also be used as a first-line agent[10]. Despite limited data concerning drug therapies against Covid-19, biologic agents (tocilizumab, anakinra, etc.), Jak inhibitors, and corticosteroids are also available options if hemophagocytic lymphohistiocytosis occurs due to cytokine storm[11]. Furthermore, there is limited data and no consensus in terms of the management of patients who can take advantage of anticoagulant treatment throughout their disease course. Thus, our review article aims not only to summarize and analyze existing literature reporting on fibrinogen and D-dimer in patients infected with Covid-19 but also to discuss the role of anticoagulation strategies in Covid-19 patients as a complement to standard therapies.

METHODS

A review of the literature has been implemented on the subject of anticoagulation treatment in patients infected with Covid-19. We have included the Pubmed, Embase, and Cochrane databases. They were searched on April 2020 using the following search inputs: 'covid-19, coagulation' (21 outputs), 'covid-19, coagulant' (5 outputs), 'covid-19, thrombus (17 outputs)' and the word 'coronavirus' was used instead of covid-19 in all inputs. If eligible manuscripts featured coagulation abnormalities and potential anticoagulation regimens in the treatment of Covid-19 patients, they were included in our review. Any further articles were obtained after the examination of the references from the relevant articles. Our review is limited to D-dimer and fibrinogen changes and anticoagulation recommendations, thus we did not include articles on the use of anti-platelets or anti-agregants in Covid-19 patients.

Fibrinogen and D-dimer

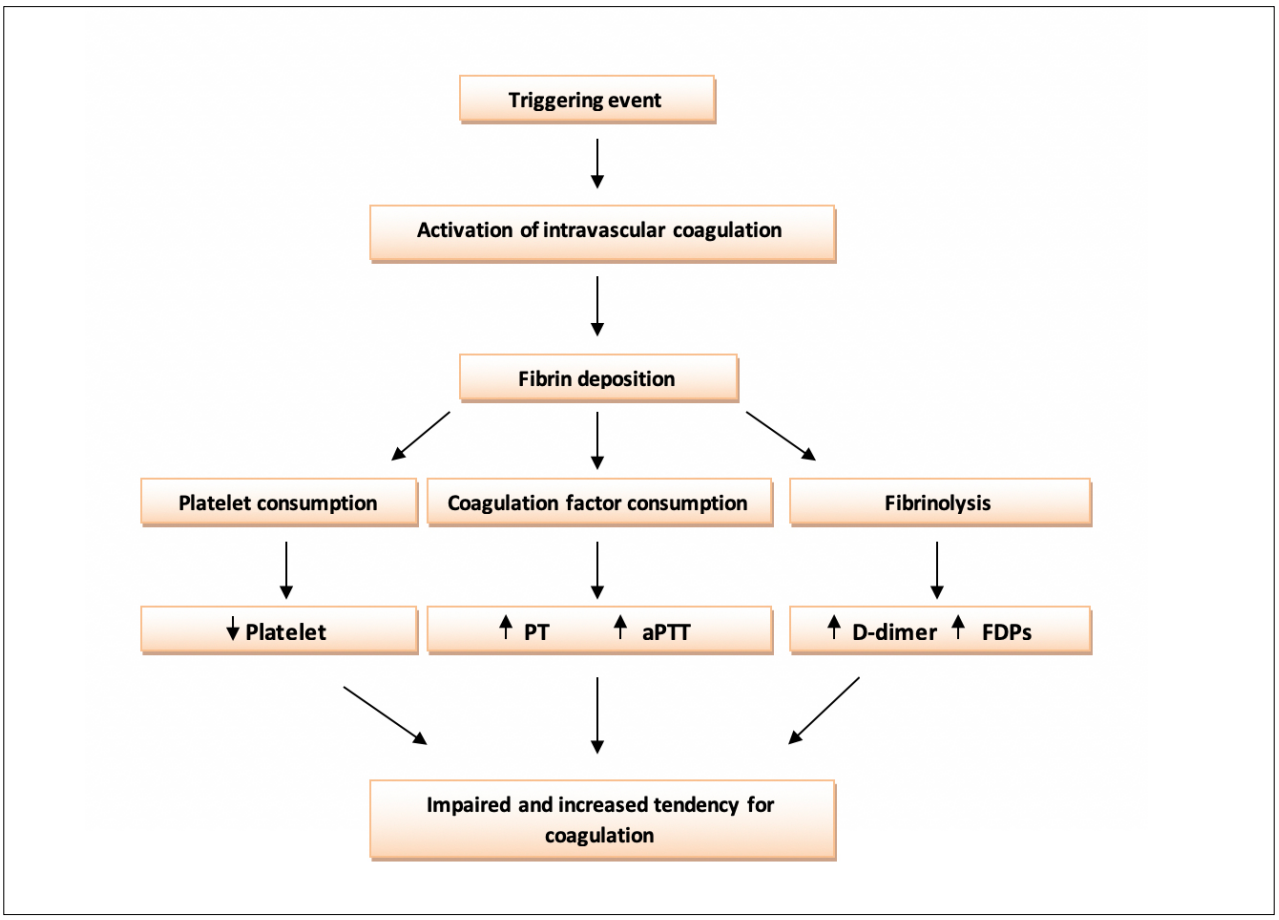
Fibrinogen, which is known as one of the acute phase proteins, is synthesized in high quantity by the liver in response to IL-1 and IL-6 derived stimulation, as well as is involved in fibrin formation as the last step of a triggered coagulation activity[12]. The value of fibrinogen has already been demonstrated it has been chosen as one of the scoring parameters in DIC diagnosis according to The International Society for Thrombosis and Haemostasis (ISTH)[12]. Hence, fibrinogen has intrinsically appeared as the subject of investigation in the Covid-19 pandemic era due to the close relationship between Covid-19 and DIC. The dynamic changes in fibrinogen levels are remarkable and need to be addressed in Covid-19 patients. Table 1 summarizes all the investigations applied in Covid-19 patients concerning fibrinogen and D-dimer. Han et al.[13] have investigated the changes in blood coagulation of patients infected with Covid-19 by comparing them with healthy controls. It is obvious that the level of fibrinogen and its degradation products are not only higher in Covid-19 patients compared to healthy controls (5.02 vs. 2.90 g/L, $p< 0.001$) but also higher in critical Covid-19 patients compared to mild or moderate cases (5.59 vs. 5.10 g/L, $p< 0.01$)[13]. On the other hand, fibrinogen is reported to be non-significant between surviving and non-surviving Covid-19 patients in a different cohort (5.16 vs. 4.51 g/L, $p= 0.149$)[14]. Thus, it is not difficult to interpret this as an admission that fibrinogen is expected to be higher than normal levels in hospitalized patients; however, it might not have a predictive value for mortality in Covid-19 patients. Fibrinogen should be evaluated together with D-dimer levels in order to have more proper prognostic assumptions since its gradual decrease together with higher D-dimer levels have a role in diagnosing DIC status as early as possible in sepsis.

D-dimer is the soluble plasmin-mediated degradation product of fibrin, which is produced after activation of coagulation and fibrinolysis [25] (Figure 1). D-dimer, which is also determined as one of the

TABLE 1. STUDIES PRESENT THE COAGULATION PARAMETERS IN PATIENTS WITH COVID-19 INFECTION

	Study design	Study population	Coagulation parameters	Results	P value	Supplementary information
Zhou et al.[2]	Retrospective	Covid-19 patients survivor (n= 137) vs. Covid-19 patients non-survivor (n=54)	D-dimer, µg/mL	0.6 vs. 5.2	< 0.001	*Value of D-dimer above 1 was associated with 18-fold higher mortality [OR= 18.42, 95%CI: 2.64-128.55, p= 0.0033].
Han et al.[13]	Retrospective	Control (n=40) vs. Covid-19 patients (n=94)	D-dimer, mg/dl Fibrinogen, g/L	0.26 vs. 10.36 2.90 vs. 5.02	< 0.001 < 0.001	*Significantly higher D-dimer and fibrinogen levels were present in patients with Covid-19 diseases.
Tang et al.[14]	Retrospective	Covid-19 surviving patients (n= 162) vs. Covid-19 non-surviving patients (n= 21)	D-dimer, mg/dl Fibrinogen, g/L	0.61 vs. 2.12 4.51 vs. 5.16	<0.001 0.149	*D-dimer level above 3 and fibrinogen level below 1 were present in 85.7% and 28.6%, respectively, in Covid-19 patients who developed DIC.
Cui et al.[15]	Retrospective	Covid-19 non-VTE patients (n=61) vs. Covid-19 VTE patients (n= 20)	D-dimer, µg/mL	0.8 vs. 5.2	< 0.001	*> 1.5 µg/mL as the D-dimer cut-off value to predicting VTE
Liu et al.[16]	Retrospective	Mild Covid-19 patients (n= 26) vs. severe Covid-19 patients (n=4)	D-dimer, mg/dl	0.26 vs. 1.54	<0.001	*Severe Covid-19 patient had higher D-dimer values compared to mild cases.
Qui et al.[17]	Retrospective	Mild Covid-19 pediatric patients (n= 17) vs. moderate Covid-19 pediatric patients (n=19)	D-dimer, µg/mL	0.21 vs. 0.36	0.028	*Moderate Covid-19 pediatric patients had increased D-dimer levels compared to those with mild Covid-19 disease
Chen et al.[18]	Retrospective	Moderate Covid-19 patients (n= 10) vs. severe Covid-19 patients (n= 11)	D-dimer, µg/mL	0.3 vs. 2.6	0.029	*Severe Covid-19 patients had increased D-dimer levels compared to those with moderate Covid-19 disease
Zhang et al.[19]	Retrospective	Non-severe Covid-19 patients (n= 82) vs. severe Covid-19 patients (n=56)	D-dimer, mg/dl	0.2 vs. 0.4	<0.001	*More elevated D-dimer levels were found in severe Covid-19 patients compared to non-severe Covid-19 patients
Zhou et al.[20]	Retrospective	Covid-19 patients without aggravation (n= 12) vs. Covid-19 patients with aggravation (n=5)	D-dimer, mg/dl	0.29 vs. 0.28	0.922	*D-dimer was not a factor associated with disease progression in patients infected with Covid-19
Wu et al.[21]	Retrospective	Covid-19 patients without ARDS (n= 117) vs. Covid-19 patients with ARDS (n= 84)	D-dimer, µg/mL	0.52 vs. 1.16	0.001	*D-dimer was associated with a higher risk of the development of ARDS [OR= 1.03, 95%CI: 1.01-1.04; p< 0.001].
Wu et al.[21]	Retrospective	Covid-19 patients with ARDS (alive) (n= 40) vs. Covid-19 patients with ARDS (died) (n=44)	D-dimer, µg/mL	0.49 vs. 3.95	0.001	*D-dimer was associated with a higher risk of death in Covid-19 patients with ARDS [OR= 1.02, 95%CI: 1.01-1.04; p= 0.002].
Yin et al.[22]	Retrospective	Covid-19 patients with severe pneumonia (n= 449) vs. non-Covid-19 patients with severe pneumonia (n=104)	D-dimer, µg/mL	1.94 vs. 2.52	0.140	*When D-dimer exceeded 3.0 µg/mL (six-fold the upper limit of normal), significantly lower mortality in heparin users than nonusers was found in Covid-19 patients (32.8% vs. 52.4%, p=0.017).
Tang et al.[23]	Retrospective	Covid-19 surviving patients (n= 315) vs. Covid-19 non-surviving patients (n= 134)	D-dimer, mg/dl	1.47 vs. 4.70	<0.001	*D-dimer was positively correlated with 28-day mortality in multivariate analysis [OR= 1.058, 95%CI: 1.028-1.090; p< 0.001]. *The 28-day mortality of heparin users was lower than that of nonusers in patients with D-dimer > 6 fold the upper limit of normal (32.8% vs. 52.4%, p=0.017).
Zhang et al.[24]	Retrospective	Covid-19 surviving patients (n= 89) vs. Covid-19 non-surviving patients (n= 6)	D-dimer, mg/L	The D-dimer level was present as ≤ 1 and >1, respectively	0.001	*For the >1 mg/L group, 81.2% of the patients were severe Covid-19 cases, and 71.9% of the patients reached the composite endpoints including intensive care unit admission or death. *Higher D-dimer level was strongly related to severe Covid-19 pneumonia and composite endpoints, including intensive care unit admission or death (p< 0.001).

Abbreviations: VTE - venous thromboembolism; ARDS - acute respiratory distress . syndrome; DIC - disseminated intravascular coagulation; OR - odds ratio; CI - confidence interval.



diagnostic criteria of DIC, is usually used in order to diagnose or exclude thrombotic events such as deep venous thrombosis or pulmonary embolism[15]. Venous thromboembolism (VTE) is a frequent complication of critical illnesses, particularly in patients treated in intensive care units (ICU). The frequency of VTE has been reported to be 13% in patients of a respiratory intensive care unit[26]. On the other hand, the incidence of VTE has been 25% in patients with severe Covid-19 in the ICU, which also represents a hypercoagulable process in Covid-19 patients[15]. The D-dimer levels are also higher in patients with DVT compared to non-DVT. (5.2 vs. 0.8 µg/mL, p< 0.001) D-dimer within normal limits has been already known to have a higher sensitivity but lower specificity for acute thrombosis. Since we experience higher D-dimer levels in most Covid-19 patients, it is also important to determine a specific level to predict thrombosis. Cui et al.[15] have proposed 3.0 µg/mL as the cut off value to predict VTE with a sensitivity of 76.9%, a specificity of 94.9%, and a negative predictive value of 92.5%.

The serum level of D-dimer has been also reported to be higher if the severity of the disease increases[16-19]. Despite expectancy of higher D-dimer levels in severe Covid-19 patients, it is important to observe statistically significant D-dimer levels in moderate Covid-19 pediatric patients compared to milder ones (0.36 vs. 0.21µg/mL, p< 0.028)[17]. However, in a different cohort with fewer patients, there has been no difference in terms of D-dimer level in Covid-19 patients without or with aggravation (0.29 vs. 0.28 mg/dL, p= 0.922) [20]. Nevertheless, if we consider D-dimer as a prognostic marker, it has been significantly higher in patients with ARDS and independently associated with a higher risk of ARDS development (1.16 vs. 0.52 µg/mL, p< 0.001; OR= 1.03, 95%: 1.01 – 1.04; p< 0.001)[21]. Noteworthy, D-dimer has been proved to be higher in non-surviving patients when compared to survivors in all retrospective investigations[2,14,21-24]. Several of these investigations have emphasized D-dimer exceeding 3.0 µg/mL (six-fold the upper limit of normal) as a cut-off value for diagnosing 85.7% of the

patients with DIC and guiding the anticoagulation treatment in order to decrease mortality secondary to Covid-19[14,22]. As a result, D-dimer has shown a promising value to direct anticoagulation strategies in the treatment of Covid-19.

Anticoagulation

Covid-19 causes clinical complications of multiple organs; however, the main complication occurs in the pulmonary system due to the high expression of SARS-CoV-2 receptor ACE2 and TMPRSS2 in the bronchial cells[27]. The destruction in the lungs has been presented in a histopathological study showing alveolar septal vascular congestion, eosinophil and lymphocyte infiltration, alveolar exudation, and thrombosis in pulmonary circulation[28]. The main underlying mechanism, which is accused of higher coagulation tendency during Covid-19, is the over-activation of the immune system causing complement release syndrome. Elevated cytokines such as IL-6 stand as the key modulator in cellular immune response and are the trigger factor for coagulation disorders[29]. Therefore, anticoagulation treatment should be investigated in Covid-19 since a hypercoagulable state has been demonstrated in both cellular and organ levels.

Early anticoagulation has been suggested in order to reduce thrombosis and microthrombus burden by Li et al.[30], a professor who worked in frontline care in Wuhan. However, his suggestions are based upon his individual experience in Covid-19 patients. In fact, there are controversial articles and some comments about anticoagulation treatment in Covid-19. First of all, complement activation is said to cause a systemic thrombotic tendency, thus complementary inhibition therapy should be the first target therapy to prevent thrombotic microangiopathy, according to Campbell and Kahwash[31]. Moreover, similarities of lung findings have been exhibited between high altitude pulmonary edema (HAPE) and Covid-19 in a recent article, which suggested HAPE as an analogous disease to Covid-19. For this reason, acetazolamide, nifedipine, and phosphodiesterase inhibitors have been proposed to be prescribed in Covid-19 since elevated fibrinogen level has been considered as an epiphenomenon of edema rather than coagulation activation[32]. Conversely, a retrospective study has already tested the efficacy of anticoagulant therapy in Covid-19 and resulted in a reduction in mortality when D-dimer exceeded 3.0µg/mL (32.8% vs. 52.4%, p= 0.017)[23]. The results may be due to the hypercoagulable state of the disease, or the anti-inflammatory effect of anticoagulation, or the combination of these two reasons[33]. An interesting study on tissue plasminogen activator (tPA) use for Covid-19 induced ARDS has shown amelioration in the PaO2/FiO2 ratio, which may be considered a clue for the necessity of anticoagulation prior to ARDS onset[34]. Anticoagulation therapy has also been beneficial in non-Covid 19 induced DIC, thus it should be immediately started following the DIC diagnosis in Covid-19 patients despite the bleeding risks[35]. In addition, the current evidence suggests the routine use of anticoagulation, particularly in patients with higher D-dimer levels (> 3.0 µg/mL). However, in light of existing data, the early administration of anticoagulation should be addressed with further investigations in Covid-19 patients without DIC. As a result, thromboprophylaxis in Covid-19 has been already suggested by position papers in several countries[36,37]. However, routine anticoagulation for Covid-19 patients should be tested by more studies since current evidence directs clinicians to the routine use of anticoagulation merely in severe Covid-19 patients.

CONCLUSION

Covid-19 is a systemic, hypercoagulable disease requiring more studies regarding treatment. Routine thromboprophylaxis should be performed in all patients because of the nature of the disease and immobilization during treatment and isolation are required. Anticoagulation is still an issue to be studied, but increased D-dimer and disease severity are the indicative factors to start the treatment as soon as possible.

Conflict of interest

All authors declare they do not have any conflicts of interest.

Author contribution

Conception: M.İ.H., T.Ç., Design: M.İ.H., T.Ç.; Supervision: A.İ.T. Fundings: A.İ.T ; Materials: M.İ.H., T.Ç.; Data Collection: M.İ.H., T.Ç. Analysis: M.İ.H.; Literature Review: T.Ç., A.İ.T. ; Writer: M.İ.H. Critical Review: A.İ.T.

RESUMO

INTRODUÇÃO: *O coronavírus da síndrome respiratória aguda grave 2 (SARS-CoV-2) é o vírus responsável pelo surto recentemente batizado de doença pelo coronavirus 2019 (Covid-19) pela Organização Mundial de Saúde (OMS) em fevereiro/2020. Os doentes com Covid-19 têm uma incidência de síndrome de dificuldade respiratória aguda (SDRA) de 15,9-29% e sepse é observada em todos os pacientes que vêm a óbito. Além disso, a coagulação intravascular disseminada (DIC) é uma das principais causas subjacentes de morte entre esses pacientes. Em pacientes com DIC, ocorre com uma diminuição do fibrinogênio e um aumento dos níveis de dímero D. Alguns estudos mostraram que o fibrinogênio e um dos seus produtos finais, o dímero D, podem ter um valor preditivo para a mortalidade em pacientes com sepse não relacionada à Covid-19 decorrente de complicações da DIC. Portanto, a anticoagulação, considerando seus benefícios quanto à mortalidade na sepse não relacionada à Covid-19, pode também ter um papel importante no tratamento da Covid-19.*

MÉTODOS: *Realizamos uma revisão de todos os estudos publicados até abril de 2020 sobre pacientes infectados com Covid-19. A nossa revisão limitou-se a alterações no dímero D, nos fibrinogênios e recomendações de anticoagulantes.*

RESULTADOS: *A terapêutica anticoagulante pode ser iniciada após o diagnóstico de DIC em pacientes com Covid-19 apesar dos riscos de hemorragia. Além disso, a evidência atual sugere o uso rotineiro da anticoagulação, principalmente em pacientes com níveis mais elevados de dímero D (> 3, 0 µg/mL).*

CONCLUSÃO: *A Covid-19 é uma doença sistêmica e hipercoagulável que requer mais estudos em relação ao tratamento. A anticoagulação ainda é uma questão a ser estudada, mas o aumento de dímeros D e a gravidade da doença são os fatores indicativos para o início do tratamento o mais rápido possível.*

PALAVRAS-CHAVE: *Infecções por Coronavirus. Anticoagulantes. Coagulação intravascular disseminada. Produtos de Degradação da Fibrina e do Fibrinogênio. Coagulação Sanguínea.*

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REVIEW ARTICLE

Promoting cessation in hospitalized smoking patients: a systematic review

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SUMMARY

OBJECTIVES: *The objective of this review was to evaluate high intensity post-discharge follow-up strategies to promote smoking cessation in hospitalized patients.*

METHODS: *A systematic review was performed, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA – P) protocol. The databases used for research were: PubMed, LILACS/BIREME, Scopus, Web of Science, Cochrane and Scielo. The included articles were randomized clinical trials, published from 1990 to 2018, which evaluated in-hospital and post-discharge intervention, and provided a minimum of 30-day care post discharge. The studies aimed to evaluate tobacco cessation.*

RESULTS: *Fourteen studies were selected for analysis. Across studies, pharmacotherapy was consistently effective for smoking cessation. Communication technologies likewise were consistently effective for cessation and post-discharge access.*

CONCLUSION: *Effective strategies exist. The challenge for future trials is to determine the best approaches for different clinical contexts, to promote cessation.*

KEYWORDS: *Tobacco use cessation. Smoking cessation. Patient discharge. Hospitalization. Systematic review.*

INTRODUCTION

In 2018, Datasus[1] registered more than 11 million hospitalizations in the country, which means that a large contingent of patients spent at least one night in a hospital, including many smokers. This situation configures hospitalization as a valuable opportunity to approach these patients. The post-discharge follow-up of smoking patients is considered a key element for the actions implemented in the hospital environment to be sustained in the home environment. Without the follow-up of smoking patients after hospital discharge, interventions in favor of cessation, initiated during hospitalization, lose effectiveness. However,

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post-discharge follow-up remains a challenge for hospitals that offer evidence-based smoking treatment[2-4].

That said, we consider it necessary to evaluate the strategies studied to assist the smoking patient after hospital discharge, seeking to understand which would be the most effective and promising approaches to promote smoking cessation in this group.

There are still few publications with the purpose of evaluating the strategies for approaching smokers after hospital discharge. Brasil occupies a prominent position for its successful tobacco control program, but few national studies address the challenges of post-discharge monitoring.

A meta-analysis that evaluated the approaches to promote the cessation of hospitalized smokers defined high-intensity interventions as those that, in addition to the approach during hospitalization, remained for 30 days after hospital discharge. Interventions classified as high intensity were more effective in promoting cessation[3]. The aim of this study is to contribute to the literature by reviewing studies that evaluated different forms of high intensity approaches in the post-discharge period of smokers to promote cessation.

METHODS

The studies' eligibility was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (Prisma-P) protocol. The characteristics evaluated were study design, studied population, types of intervention, presence of a control group and analyzed outcomes[5].

Randomized clinical trials were selected in order to study interventions in the post-discharge period in smoking patients, with smoking cessation as the main or secondary outcome. Pilot studies were also included, as presented in an important previous review on approaches during the hospitalization period[2-4].

The studies contemplated interventions in smoking patients initiated during the hospitalization period, or at the time of hospital discharge, with the objective of promoting cessation, extended to post-discharge. Post-discharge follow-up should be maintained for a minimum period of 30 days after the patient leaves the hospital, an intervention considered to be of high intensity by previous meta-analysis[6].

The studied population was composed of hospitalized smokers, defined here as individuals who smoked in the last 30 days[2,3,7].

Studies that evaluated the population exclusively of psychiatric patients, who used tobacco in combination with other drugs, and in patients admitted to rehabilitation clinics were excluded.

The control group received the usual care from the various institutions studied.

Studies that presented smoking cessation outcomes, such as self-reported or biochemically proven tobacco abstinence, were included. The period of abstinence assessed after discharge could vary from short-term, such as seven days after discharge, to long-term, established here as 12 months after discharge. The abstinence to be considered could be punctual, for example, in the last seven days, or continuous, for example, since hospital discharge.

The search strategy adopted was to find articles published in English, Spanish and Portuguese between 1990 and 2018. The choice of the review period was motivated by the development and wide access to new communication resources, such as internet, mobile phones and new communication technologies that started at that time.

The following databases were used for screening: PubMed, Lilacs/Bireme, Scopus, Web of Science, Cochrane and SciELO. To search for gray literature, in an attempt to avoid the non-inclusion of studies due to publication bias, the Open Gray platform was used, in addition to performing a manual search for authors of articles already selected. The following Boolean expressions were used: (TOBACCO USE CESSATION) AND (POST-DISCHARGE) AND (HOSPITALIZATION OR INPATIENT).

For data extraction and review of titles and abstracts, four researchers met in pairs; one of the pairs had the participation of an expert in epidemiology and the other, with a specialist in the treatment of smoking. After the initial search, repeated titles in different databases were excluded. Then, articles that did not meet the proposed acceptability criteria for the review were excluded.

The studies selected in this stage were read in full by the researchers, in order to confirm or discard their eligibility. The decision for inclusion was made by consensus among the four reviewers. The article selection process is described in the flowchart in Figure 1.

The risk of bias was assessed individually in each study according to the Cochrane risk assessment tool (Cochrane Risk of Bias Tool - version 5.1.0), which identifies low, high or uncertain risk of bias,

according to the following possibilities: Selection bias, Performance bias (performance), Detection bias, Friction bias, Reporting bias and other biases that do not belong to the aforementioned domains[8,9].

RESULTS

The initial selection on the search platforms resulted in 338 articles, two of which were added manually. Among them, 28 were repeated and 293 were excluded after analyzing the title and abstracts and resolving differences between researchers, as they did not meet the established criteria. Five studies were discarded after their complete reading, as they did not fit the search objectives (exclusively psychiatric population, non-randomized studies, uncontrolled studies, future study protocols).

14 studies carried out in the following countries were selected for the review: United States, Canada, Brasil and Australia. The data were extracted from February 1996 to June 2018. Individual data for each study were obtained from publications, as well as their protocols and records on clinical trial registration platforms (U.S. National Institutes of Health Clinical Trials Registry). The characteristics of the selected studies are shown in Table 1.

The follow-up time for the studies ranged from 3 to 12 months. It is worth noting the finding that the current decade has the largest number of publications on the subject, with 11 studies between 2011 and 2018.

The interventions performed during the hospitalization period varied in different publications. The bedside approach, whether for smoking history, demographic data collection or counseling, was a strategy common to all studies. In one of the studies[11], only the intervention groups received counseling, while the control group received printed informational material.

The pharmacological treatment of smoking, with nicotine replacement during hospitalization, has been used in several studies, with the purpose of reducing abstinence symptoms[6,7,12,13,14,19-21].

Also regarding the hospitalization period, several studies described the referral to community post-discharge care services (quitlines), and in some studies, the way in which the reference to these services was given, whether assisted or not by the researcher, it was the strategy to be studied[7,17].

The post-discharge strategies in the intervention group and in the control group are described in Table 1, which also shows the main characteristics of the population of the selected studies, the outcomes related to cessation and the results. The interventions took place at a distance, with contact, in most studies, mediated by communication technologies, with emphasis on telephone calls and interactive voice response (IVR), a technology that allows the interaction between computers and human beings through the using your phone's voice or keypad. One of the studies intervened via text messages. Sometimes e-mail was used to send information after discharge, but it was not the main intervention mechanism. It is also worth mentioning the attempt of several studies to stimulate adherence to quitline programs, intermediating the enrollment of patients in the programs, in order to promote cessation. The follow-up time for the studies ranged from 3 to 12 months.

All the studies analyzed used some type of pharmacotherapy for smoking cessation at some point in the study. Therapy with nicotine replacement (NRT), bupropion and varenicline appear as alternatives for pharmacotherapeutic treatment, with NRT being the most widely used. The data about this are detailed in Table 2. In most studies, there was a balance in the use of pharmacotherapy between the intervention group and the control group. Among the three studies in which the intervention group received pharmacotherapy more frequently than the control group, in one of them pharmacotherapy was part of the proposed intervention[6,18,20].

The risk of bias was established, in each study, as low (L), high (H) or undetermined (U), considering the following domains: selection, performance, detection, attrition and reporting bias. Table 1 shows the risk of bias in each study. The performance bias was considered high in all studies, in view of the evident difficulty in promoting blindness when offering or receiving interventions, given the nature of the studies. Regarding the detection bias, most studies did not present data regarding the blindness of the outcome evaluators, being, therefore, considered undetermined in most of them. The attrition bias was considered high when the withdrawal of participants was not justified by the authors. In the reporting domain, although some studies have not published a protocol, the outcomes were reported as proposed in the methodology, and therefore, the likelihood of such bias is considered low.

The intention-to-treat analysis was used, considering losses such as still smokers.

CHART 1. CHARACTERISTICS AND RESULTS OF SELECTED STUDIES

Study/ Year of publication	Country/ Follow-up Period	n and special characteristics	Group(s)/Intervention/Post-Discharge Intervention	Control Group/ Post-Discharge Intervention	Cessation outcome/Results (statistically significant difference)
Dornellas et al. 2000	USA Unicentric Feb. 1996 Jan. 1997	100 Patients hospitalized with acute myocardial infarction	Single group (n = 54) • Bedside counseling during hospitalization (20 minutes) and post-discharge telephone counseling, with calls at 1, 4, 8, 12, 16, 20, 26 weeks post-discharge	(n = 46) • Oriented to access institutional video during hospital stay • No interventions after discharge	Self-reported abstinence for 7 days and confirmed by cohabiting, 6 and 12 months after discharge Results There was a difference between the groups • 6 months: - Intervention group = 67% - Control group = 43% (p <0.05) • 12 months: - Intervention group = 55% - Control Group = 34% (p <0.05)
Hennrikus et al. 2005	USA Multicenter (4 hospitals) Jan. 1997 Jul. 1999	2,095 Smokers hospitalized for multiple causes	Group 1 (n = 703) • Modified standard care and note highlighted in the patient's record, recommending advice by the assistant team Group 2 (n = 696) • Modified standard care, note highlighted in the medical record and additional telephone counseling sessions - ≥ 1 - 617 (90%) - ≥ 4- 318 (63%) - ≥ 7 -88 (13%)	(n = 696) • Modified standard care, 2 manuals and referral to community cessation programs	• Outcome 1: Self-reported abstinence for 7 days, 7 days post-discharge • Outcome 2: Self-reported abstinence for 7 days, 12 months after discharge • Outcome 3: Abstinence for 7 days, 12 months after discharge confirmed by salivary cotinine Results There was a difference in relation to outcome 2 • Outcome 1: - Group 1 = 24%; - Group 2 = 25.2% - Control group = 26% - (p> 0.05) • Outcome 2: - Group 1 = 15.2% - Group 2 = 19.8% - Control group = 15% - (p <0.05). • Outcome 3: - Group 1 = 10% - Group 2 = 9.9% - Control group = 8.8% - (p> 0.05)
Reid et al. 2007	Canada Pilot Unicentric Nov. 2004 May 2015	100 Smokers hospitalized for coronary disease	Single group (n = 50) Standard care during hospitalization, RIV 3, 14 and 30 days post discharge and additional counseling as needed	(n = 50) • Standard care during hospitalization, access to NRT and printed material. No other interventions after discharge	Self-reported abstinence in the last 7 days, 52 weeks after discharge Results There was no difference between groups Intervention group = 46% Control group = 34.7% (p = 0.25)
Regan et al. 2011	USA Unicentric Dec. 2007 Jul. 2008	738 Smokers hospitalized for multiple causes	Single group (n = 368) • IVR (4 times) in the 30 days after discharge and possibility of requesting a callback by the counselor	(n = 379) • An IVR 2 weeks after discharge	Self-report of cessation 2 and 12 weeks after discharge Results There was no difference between the groups studied • Withdrawal 2 weeks after discharge - Intervention group = 39% - Control group = 39% - RR 1.02 - CI = 0.85–1.22 • Abstinence 12 weeks after discharge - Intervention group = 29% - Control group = 26% - RR 1.11 - CI = 0.9–1.41

Study/ Year of publication	Country/ Follow-up Period	n and special characteristics	Group(s)/Intervention/Post-Discharge Intervention	Control Group/ Post-Discharge Intervention	Cessation outcome/Results (statistically significant difference)
Rigotti et al. 2014	USA Unicentric Aug. 2010 Apr. 2012	397 Smokers hospitalized for multiple causes that: • Received counseling during hospitalization • Planned to quit smoking • Accepted the pharmacological treatment	Single group (n = 198) • Medication for 30 days, with replacement twice for up to 90 days, 5 IVR (2, 14, 30, 60 and 90 days after discharge) and fax to the primary care physician informing about the treatment	(n = 199) • Recommendation for free call to quitline, individualized recommendation for medication and note in the medical record alerting the attending physician about the prescription of medication	Biochemically proven abstinence for 7 days, 6 months after discharge (cotinine, or monoxymetry for those using NRT), self-reported abstinence for 7 days and abstinence continues 2.3 and 6 months after discharge Results There was a difference between the groups in the main outcome and in some secondary outcomes • Biochemically proven withdrawal: - Intervention group = 26% - Control group = 15% - (p = 0.009) • Self-reported abstinence last 7 days 6 months after discharge: - Intervention group = 40.9% - Control group = 28.1% - (p = 0.008) • Continuous self-reported abstinence 6 months after discharge: - Intervention group = 27.3% - Control group = 16.1% - (p = 0.007) • Other outcomes without statistically significant difference between groups
Cummins et al. 2016	USA Multicenter Jun. 2011 Nov. 2013	1,270 Smoking patients hospitalized in 5 hospitals	Group 1 (n = 320) • Nicotine patches Group 2 (n = 317) • Nicotine patches and telephone advice Group 3 (n = 317) • Telephone advice	(n = 316) • Standard care: quitline	Self-reported abstinence for 7 and 30 days, 2 and 6 months after discharge, and salivary cotinine-confirmed abstinence in those who reported abstinence for 7 days, 6 months after discharge. Result There was no difference between the intervention groups and the control group or between the different intervention groups in any of the analyzed outcomes Main outcome: • Abstinence for 30 days, 6 months after discharge: - Groups without patches = 18.3% - Groups with patches = 22.8% - (p = 0.051) • Counseling groups = 21.1% • Groups without counseling = 20.0% • (p = 0.65)
Fellows et al. 2016	USA Multicenter Nov. 2011 Nov. 2013	898 Smoking patients hospitalized for multiple causes	Single group (n = 597) • Approach during hospitalization • Proactive reference for post discharge assistance • Pharmacotherapy (offered according to a health plan) and 4 IVR 4, 14, 28 and 49 days after discharge	(n = 301) • Approach during hospitalization • Standard care after discharge: - Information on medications and how to access quitline. - A brief follow-up call to assess cessation	Self-reported abstinence in the last 30 days, 6 months after randomization Result: There was no difference in cessation between the two groups in the primary or secondary outcomes related to cessation • Intervention group = 24% • Control group = 22% • (p = 0.159)
Harrington et al. 2016	USA Unicentric Jul. 2011 May 2013	1,488 Smokers hospitalized for multiple causes	Single group (n = 748) • Approach during hospitalization and time of discharge (Standard Care), visit by a team that guided access, registration and use of website with various information on smoking	(n = 740) • Standard care	Self-reported abstinence for 30 days, 6 months after discharge Results: There was no difference between groups • Intervention group = 25.8% • control group = 24.1% • (p = 0.436)

Study/ Year of publication	Country/ Follow-up Period	n and special characteristics	Group(s)/Intervention/Post-Discharge Intervention	Control Group/ Post-Discharge Intervention	Cessation outcome/Results (statistically significant difference)
Sherman et al. 2016	USA Jul. 2011 Apr. 2014	1,619 Smokers hospitalized for multiple causes, with a high number of participants on the street or in temporary housing (25%) alcohol users (40%), and with mental illness (50%) and use of other drugs (60%)	Single group (n = 805) • Post-discharge counseling calls (proactive), 2 weeks after discharge, other calls 1, 3, 7, 14, 30 and 42 days after the first call	(n = 814) • Referred to the quitline	Self-reported abstinence for 30 days, 2 and 6 months after discharge Results: There was a difference between the groups • Withdrawal 2 months after discharge: - Intervention group = 29.0% - Control group = 20.7% - (RR 1.40 CI = 1.13, 1.73) • Withdrawal 6 months after discharge: - Intervention group = 37.4% - Control group = 32.5% - (RR 1.19 CI = 1.01, 1.40)
Richter et al. 2016#	USA Multicenter Jul. 2011 Out. 2014	1,054 Smokers hospitalized for multiple causes	Single group (n = 527) • Assessment of withdrawal symptoms, adjustment of the NRT dosage (standard care). Explanations about the project. Quitline registration mediated by the researcher	(n = 527) • Standard Care, assistance with cessation (quit plan + providing medication prescription after discharge) and forwarding via fax to quitline	• Abstinence in the last 7 days, self-reported, 6 months after discharge • Biochemically confirmed abstinence (cotinine, carbon monoxide, proxy) 6 months after discharge Results There was no difference between the groups studied • Self-reported abstinence for 7 days, 6 months after discharge: - Intervention group = 25.4% - Control group = 25.3% - (p = 0.88) • Abstinence confirmed for 7 days 6 months after discharge (carbon monoxide and cotinine): - Intervention group = 23.7% - Control group = 21.6% - Oradj = 1.02 - 95% CI = 0.77, 1.35 - (p = 0.88) - RR = 1.02 - 95% CI = 0.82; 1.24
Rigotti et al. 2016	USA Multicenter Dec. 2012 Jul. 2014	1,359 Smokers hospitalized for multiple causes who: • Received counseling at hospitalization • Planned to quit smoking • Accepted the pharmacological treatment	Single group (n = 681) • Medication for 30 days, with replacement twice for up to 90 days, 5 IVR (2, 12, 28, 58 and 88 days after discharge) and possibility to access a counselor if necessary	(n = 678) • Recommendation for free call to quitline (1-800-QUIT-NOW), individualized recommendation for medication • Note in the medical record alerting the attending physician about the medication prescription	Biochemically proven abstinence for 7 days, 6 months after discharge (cotinine or monoxymetry for those using NRT) Self-report abstinence assessment at 1, 3 and 6 months Results There was a difference between groups in outcome 2 • Biochemically proven abstinence: - Intervention group = 17% - Control group = 16% - (p = 0.58) • Self-reported abstinence for 7 days, 1 month after discharge: - Intervention group = 43% - Control group = 32% - (p <0.0001) • Self-reported abstinence for 7 days, 3 months after discharge: - Intervention group = 37% - Control group = 30% - (p = 0.008) • Self-reported abstinence 6 months after discharge: - Intervention group = 31% - Control group = 27% - (p = 0.09)

Study/ Year of publication	Country/ Follow-up Period	n and special characteristics	Group(s)/Intervention/Post-Discharge Intervention	Control Group/ Post-Discharge Intervention	Cessation outcome/Results (statistically significant difference)
Thomas et al. 2016	Australia Multicenter Apr. 2012 Jun. 2014	600 Smokers hospitalized for multiple causes	Single group (n = 300) • Behavioral approach (2 sessions in the hospital and 3rd session 4 weeks after discharge). Motivational interview for those who did not want to quit smoking. Pharmacotherapy during hospitalization and 1 week after discharge. Pharmacotherapy for another 28 days according to availability (PBS). Impressive information material, quitline reference, case summary and action plan for assistant physician and community pharmacist and follow-up by telephone at 1, 6 and 12 months after discharge	(n = 300) • Standard care for each hospital: - Brief intervention during hospitalization - Available pharmacotherapy: NRT, bupropion and varenicline maintained for a period of 28 days after discharge according to PBS and follow up by phone at 1, 6 and 12 months after discharge	Abstinence for 30 days, six months after discharge, confirmed with monoxymetry Abstinence for 6 months, 12 months after discharge, also by monoxymetry Self-reported abstinence for 30 days at 1, 6 and 12 months Results There was no difference between groups • Abstinence for 30 days, six months after discharge, confirmed with monoxymetry: - Intervention group = 11.6% - Control group = 12.6% - (OR) = 0.91 - 95% (CI) = 0.55–1.50 • Abstinence for 30 days, 12 months after discharge, confirmed with monoxymetry - Intervention group = 11.6% - Control group = 11.2% - OR = 1.04 - 95% CI = 0.63–1.73 • Self-reported abstinence for 30 days in 1, 6 and 12 months - 1 month: Intervention group = 28.8% Control group = 23.4% OR 1.56 (1.05–2.33) - 6 months: Intervention group = 28.8% Control group = 23.4% OR 1.47 (0.91–2.39) - 12 months: Intervention group = 13.0% Control group = 12.2% OR 1.21 (0.72–2.03)
Busch et al. 2017	USA Pilot Unicentric Out. 2013 Apr. 2015	59 Smoking patients hospitalized for acute coronary syndrome	Single group (n = 28) • Five counseling sessions in weeks 1, 3, 6, 9 and 12 post discharge. Up to 4 additional contacts according to need	(n = 31) • Received informative material at weeks 1, 3, 6, 9 and 12 after discharge	Abstinence in the last 7 days, confirmed by monoxymetry, assessed at the 12th and 24th weeks after discharge. Result There was no difference between the intervention groups and the control group • 12th week: • Intervention group = 48.0% • Control group = 44.8% • 24th week: • Intervention group = 45.8% • Control group = 42.3%
Cruvinel 2016	Brasil Pilot Unicentric Jun. 2015 Mar. 2016	66 patients Hospitalized smokers	Single group (n = 44) • Brief approach, informative material printed during hospitalization, in addition to NRT for 4 weeks (standard care) • And 1 phone call and text messages after discharge	(n = 22) • Standard care and 1 follow-up call 30 days after discharge	Self-reported abstinence in the last 7 days, 30 days after discharge and 90 days after discharge Abstinence confirmed by monoxymetry 90 days after discharge Result There was no difference between the groups in the 1st and 3rd outcomes and there was a difference in the 2nd outcome • 30 days after discharge: - Intervention group = 25.0% abstinence - Control group = 9.1% - (p = 0.13) • 90 days after discharge: - Intervention group = 31.8% - Control group = 9.1% - (p = 0.04) • Abstinence confirmed by monoxymetry: - Intervention group = 20.5% - Control group = 4.5% - (p = 0.09)

NRT: Nicotine Replacement Therapy; IVR: Interactive Voice Response. PBS: Pharmaceutical Benefits Scheme. #The intervention still occurs at the time of admission, but with the aim of interfering after discharge.

TABLE 2. USE OF PHARMACOTHERAPY IN THE STUDIES

Study	Type	Initial Dose	Time	Difference between control and intervention groups
Dornelas et al.[10]	NRT	Not informed	Not informed	No. Approximately 24% of participants in both groups. No statistic data
Hennrikus et al.[11]	NRT Bupropion	Not informed	Not informed	No. No additional data (e-mail sent to the author)
Reid et al.[12]	NRT Bupropion	Not informed	Not informed	No. NRT: Intervention Group: 14% Control Group: 14.3% (p = 0.85) Bupropion: Intervention Group: 8% Control Group: 4.1% (p = 0.60)
Regan et al.[13]	NRT Bupropion Varenicline Other non-specified mediation	Not informed	Not informed	No Intervention group: 69% Control group: 52% (p < 0.05)
Rigotti et al.[14]	NRT, Bupropion varenicline (Isolated or combined)	Not informed	Intervention group: up to 90 days Control group: non-specified	Yes. Intervention group: it was provided. Intervention group: 79 % Control group: 59% RR, 1.34 [95% CI, 1.17-1.54]; (p < 0.001)
Cummins et al.[15]	NRT	14 to 21 mg according to number of cigarettes smoked	08 weeks	Yes. Medication was part of the strategy in two of the intervention groups.
Fellows et al.[16]	NRT Bupropion Varenicline	Not informed	Not informed	No. Intervention group: 47.0% - Control group: 38.0% (p = 0.013)
Harrington et.[17]	NRT Bupropion	Not informed	Not informed	No. Intervention group: 25.9% Control group: 26.0% (p = 1.00)
Sherman et al.[18]	NRT	Not informed	Not informed	No. Intervention group: 44% Control group: 44%
Richter et al.[7]	NRT - During hospitalization and prescribed after discharge		Not informed	No Intervention group: 23% Control group: 26% (p = 0.23)
Rigotti et al.[19]	NRT Bupropion Varenicline, (Isolated or combined)	Not informed	Up to 90 days. -Control group: Non-specified	No. Intervention group: 83.7% - Control group: 60.6% RR 1.38 [95% CI, 1.29-1.48]; (p < 0.001)
Thomas et al.[20]	NRT Bupropion Varenicline After discharge, these same medications were offered for 28 days in a co-participation regime.	Not informed	During hospitalization and for at least 28 days after discharge	Yes Intervention group: 43.1% Control group: 28.8% (p < 0.001)
Busch et al.[6]	NRT – Patches	14 to 21 mg according to the number of cigarettes smoked	08 weeks	Intervention group: 67.9% Control Group: 58.1% (p value not informed)
Cruvinel[21]	NRT	14 to 21 mg according to the number of cigarettes smoked	04 weeks	No Intervention group: 27.3% Control Group: 36.4% (p = 0.44)

NRT: Nicotine Replacement Therapy.

DISCUSSION

A meta-analysis published in 2012[3] categorized interventions to promote the cessation of hospitalized smokers into four groups, according to their intensity. The group considered to be the most intense, and which showed results in the cessation outcomes, was the group with interventions that continued for up to 30 days after discharge. In this review, we analyzed the studies that offered high-intensity approaches to assess the strategies they used.

Behavioral interventions, associated with pharmacological interventions, make up the set of measures to promote the cessation of hospitalized smokers[2,22,23]. The search for those that would be the most effective strategies and the best way to offer them has been the subject of studies, especially in the last decade. The six studies that demonstrated statistically significant differences in termination outcomes had in common the use of some distance communication strategy, such as

phone calls, text messages and interactive voice calls, associated with pharmacotherapy[10,11,14,18,19,21].

The emergence of new communication technologies and the population's growing access to these resources drove the development of strategies and the use of these new tools since the 1990s, supported by the increase in access to telephone sets[2,24].

Pharmacological treatment with first-line drugs (NRT, bupropion and varenicline) is an important strategy for cessation[25]. Nicotine replacement therapy was the drug strategy highlighted in this review, being common to all studies. Pharmacotherapy had an equivalent prevalence, between intervention and control groups, in most studies, with the exception of three of them[15,19,20], in which the intervention group received more medication than the control group.

Quitline programs, in which trained counselors provide support for cessation, appear as a consolidated strategy for monitoring smokers after discharge[2,22,26]. The program is part of the standard care adopted by the control group for most studies. The quality of these programs may be one of the explanations for the absence of a statistically significant difference in the responses between the intervention and control groups in these studies. Control groups received interventions already established in the literature as effective, therefore, the standard of effectiveness of experimental treatments was not strong enough for differences to be highlighted. Based on the importance achieved by quitline, studies also seek to find ways to improve adherence to these programs. One of the mechanisms presented was the intermediation, by a researcher, in the participant's access and registration to the quitline, in order to, with this, favor the cessation in post-discharge[7,17].

The studies that showed the efficacy of alternative strategies did not support such a difference when biochemical confirmation criteria were used, demonstrating the fragility of the information provided by self-report[11,19,21]. The exception was a study[14] that used interactive voice messages and guaranteed medication for a period of up to 90 days after hospital discharge.

The follow-up of hospitalized patients still poses great challenges. Understanding the cause of early relapses has been the subject of analysis, demonstrating that relapse is related to factors such as continue to smoke during hospitalization, low self-efficacy, depression, greater dependence on nicotine and not setting a date to quit smoking[27]. The high number of losses in the post-discharge follow-up is another obstacle in conducting treatment, even with the help of modern communication technologies. The difficulty of follow-up and, therefore, of offering the intervention is pointed out in one of the studies as a justification for why established strategies, such as pharmacotherapy and telephone counseling, have failed to demonstrate the expected result in clinical trials[15].

Some authors consider that the negative result of the intervention does not necessarily invalidate it, and it may be necessary to have a better understanding of in which contexts they would be the most effective options[7].

This study has limitations related to the barriers of working with control groups that are not exempt from intervention, due, in fact, to the ethical implications imposed on this issue.

CONCLUSION

The idea that cessation should be promoted at every opportunity to approach smokers reinforces the need to build and apply intervention protocols for hospitalized smokers. The time of hospitalization is an especially opportune occasion for the treatment of smoking.

Pharmacotherapy has been confirmed as an important element in promoting cessation in hospitalized smoking patients. The important role of communication technologies in the monitoring of the patient after discharge was also highlighted.

In Brasil, the population's growing access to cell phones makes the use of communication technologies very promising for the monitoring of smoking patients. It is still a great challenge for future studies to improve technologies to adapt to the social and economic realities of the Brasilian context.

Authors' contribution

Lígia Menezes do Amaral: Conception and planning of the work, interpretation of the evidence, data collection, writing, revision of the preliminary and final versions; Ângela C. D. Albino Destro de Macêdo: Data collection and writing; Isabella Oliveira Lanzieri: Data collection and writing; Rafaela de Oliveira Andrade: Data collection and writing; Kimber P. Richter: Writing, revision of the preliminary and final versions and approval of the final version; Isabel C. Gonçalves Leite: Design and planning of the work, interpretation of the evidence, data collection, writing, review of the preliminary and final versions and approval of the final version.

RESUMO

OBJETIVO: *O objetivo deste estudo foi avaliar as estratégias no acompanhamento pós-alta para a promoção da cessação no paciente tabagista hospitalizado.*

MÉTODOS: *Foi realizada uma revisão sistemática tomando-se por referência o protocolo Preferred Reporting Itens for Systematic Rewiews and Meta-Analyses (Prisma–P). Foram utilizadas as seguintes bases de dados: PubMed, Lilacs/Bireme, Scopus, Web of Science, Cochrane e SciELO. Os artigos incluídos foram ensaios clínicos randomizados, publicados entre 1990 e 2018, que promoveram intervenções durante e após a alta hospitalar, intervenções essas que se mantiveram pelo período mínimo de 30 dias após a alta. Os estudos deveriam ter como desfecho a avaliação da cessação do tabagismo.*

RESULTADOS: *Quatorze estudos foram selecionados para a análise. A revisão dos artigos destacou a farmacoterapia como elemento importante para a promoção da cessação, bem como o uso das novas tecnologias de comunicação no acesso pós-alta.*

CONCLUSÃO: *Ainda se impõe como um desafio o aprimoramento das estratégias de follow-up após a alta hospitalar para se adequarem aos contextos locais e alcançarem melhores taxas de cessação.*

PALAVRAS-CHAVE: *Abandono do uso de tabaco. Abandono do hábito de fumar. Alta do paciente. Hospitalização. Revisão sistemática.*

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