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Clinicopathological response assessment to neoadjuvant chemotherapy in locally advanced breast cancer- A rural population-based case series

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Abstract

Introduction: Breast cancer is the commonest malignancy among women worldwide. Despite a multidisciplinary approach, locally advanced breast cancer remains a clinical challenge as most of the patients have a high rate of locoregional spread and develop distant metastases. Neoadjuvant chemotherapy not only paves the way for a more conservative surgical option but also decreases the incidence of positive nodes. This study was undertaken, to assess the effectiveness of neo-adjuvant chemotherapy and its impact on clinical and pathological response in locally advanced breast cancer. It also, compare patient characteristics, histological type, and hormonal receptor status with response to neo-adjuvant chemotherapy.

Materials and Methods: This is a prospective observational study over a one-year period on 30 locally advanced breast cancer patients from rural background who received neoadjuvant chemotherapy. All patients received a standard neoadjuvant treatment regimen and were evaluated clinically, radiologically, and pathologically pre- and post-chemotherapy. The clinical response was assessed by RECIST criteria, the pathological response was graded according to Chevalier classification, and the overall impact was assessed by AJCC response criteria.

Results: Most of the patients (46.7%) were in the age group of 35-48 years. The premenopausal and postmenopausal groups were 63% and 37%, respectively. In the present study, tumours expressing oestrogen, progesterone, and HER 2 were 73%, 66%, and 27%, respectively. Patients showing clinically complete responses post-neoadjuvant chemotherapy were 4, partial responses were 21, stable disease was 3, and progressive disease was 2. A pathological partial response was achieved in 93% of patients.

Conclusion: Neoadjuvant chemotherapy in locally advanced breast cancer not only downstages the disease but increases the scope of operability and thus makes it possible to resect the disease with a tumour-free margin in most cases.

Keywords: Locally advanced breast cancer (LABC), Neoadjuvant chemotherapy (NACT)

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Introduction

Breast cancer is among the most common cancers in women worldwide. In Globocan India 2020, breast cancer accounted for 13.5% of all new cancer cases in both sexes and 10.6% of all deaths, with a cumulative new case risk of 2.81 (1). The National Comprehensive Cancer Network describes locally advanced breast cancer (LABC) as American Joint Committee on Cancer (AJCC) (7th edition) stage III breast cancer in the absence of distant metastasis as tumours more than 5 cm in size with regional lymphadenopathy (N1–3) or of any size with direct extension to the chest wall (T4a) or skin (T4b) regardless of regional lymphadenopathy or presence of regional lymphadenopathy (clinically fixed or matted axillary lymph nodes, or any of ipsilateral infraclavicular, supraclavicular, or internal mammary lymphadenopathy) regardless of tumour stage. In the AJCC 7th edition, ipsilateral supraclavicular lymphadenopathy was reclassified as regional lymphadenopathy (N3), which was considered in the AJCC 6th edition as distant metastasis (2).

Despite widespread awareness of the benefits of screening and early detection, 10% to 20% of breast cancer patients are diagnosed with locally advanced disease in industrialized nations, compared to up to 50% of incidence cases in developing nations (3). The present study is based on a rural-based population who were diagnosed as LABC on initial presentation and is amenable to less attrition in our health care setup.

Studies show that with only treatment with radical mastectomy or roentgen, the five-year survival rate was 30% (4,5). Neoadjuvant chemotherapy became a part of standard treatment for LABC after the National Surgical Adjuvant Breast and Bowel Project B-18 trial, which found that it not only reduced tumour size but decreased the incidence of positive nodes and also showed benefit in assessing tumour response to it in vivo (6).

Histopathological response to primary chemotherapy was found to be the single most important prognostic factor for both disease-free and overall survival rates. Moreover, the extent of the remaining tumour determines the rate of local recurrence and dictates the necessity for additional loco-regional therapy (7).

Several studies have shown that oestrogen receptor-negative patients achieved a higher pathological complete response than positive patients (8). High tumour grade and tumour size were clinical determinants of pathological complete response (9).

The present study was undertaken to assess the effectiveness of neo-adjuvant chemotherapy and its impact on clinical and pathological response in locally advanced breast cancer and to compare patient characteristics, histological type, and hormonal receptor status with response to neo-adjuvant chemotherapy (NACT).

Materials and Methods

After getting the ethical approval of the project, consent and agreement were obtained from all the patients. In this way, the study was conducted prospectively on 30 rural background-based patients attending the surgery outpatient department of Fakhruddin Ali Ahmed Medical Hospital, Barpeta, Assam, India, from September 2021 to August 2022.

All patients above 18 years of age diagnosed with locally advanced breast cancer who were willing to undergo follow-up were included in the study. Operable Locally advanced breast cancer (T3N1M0), history of prior radiotherapy to the breast, the presence of distant metastases, and patients in whom neoadjuvant chemotherapy is contraindicated were excluded from the study.

Patients presenting with breast lumps were evaluated clinically, radiologically, and pathologically by tru-cut biopsy to confirm the primary tumour as locally advanced breast cancer. The patient baseline and tumour characteristics, including age, menstrual status, tumour size, nodal status, tumour grade, hormonal receptor HER2 status, and histological type, were noted, and metastatic workup was done .

Patients were treated with a standard neoadjuvant chemotherapy regimen of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC). In triple negative cases, dose-dense AC followed by taxane drugs (Paclitaxel or docetaxel) was given. In HER2-positive cases, AC followed by taxane with trastuzumab and or pertuzumab was given.

After the completion of 3-4 cycles or additional neoadjuvant chemotherapy as needed, the tumour was assessed clinically, and radiologically for tumour size, nodal status, and clinical stage, after which all the patients underwent modified radical mastectomy with en bloc axillary dissection, and a specimen was sent for gross and histopathological examination. Clinical response was assessed by RECIST criteria (response evaluation criteria in solid tumours) (Table 1.)

Table 1. RECIST criteria for clinical response evaluation.

Complete Response (CR)	Clinical disappearance of primary tumour
Partial Response (PR)	>30% decrease in longest diameter
Stable Disease (SD)	<30% decrease or <20% increase
Progressive Disease (PD)	>20% increase in longest diameter

Histopathological response is graded according to Chevalier classification (9).

Table 2. Chevalier classification for grading histopathological response.

No cancer in breast and axillary nodes.	**Pathological complete response (pCR)**
Only in situ carcinoma remains, nodes negative	Pathological partial response (pPR)
Invasive carcinoma with stromal fibrosis.	
No or few modifications in stromal fibrosis.	Pathological no response (pNR)

The overall impact will be assessed by AJCC response criteria (8th edition) (10).

Table 3. AJCC response criteria for overall impact evaluation.

Complete response (CR)	Absence of invasive carcinoma in breast and nodes.
Partial response (PR)	Decrease of either or both T & N stage.
No response (NR)	No apparent change in either T or N stage

Results

The mean age of LABC patients ranged from 35 to 48 years (Figure 1). 57% of patients had right-sided breast disease. 37 % of patients were postmenopausal women, and 63% were premenopausal (Figure 2). A positive family history was found only in one patient, with her mother being affected by the disease.

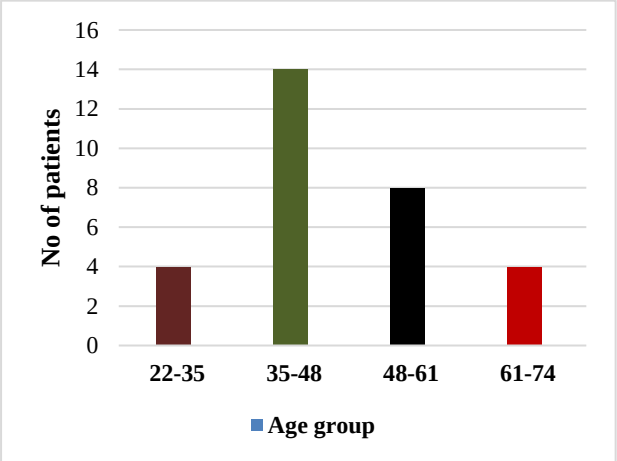

Figure 1. Graph showing age distribution of LABC patients.

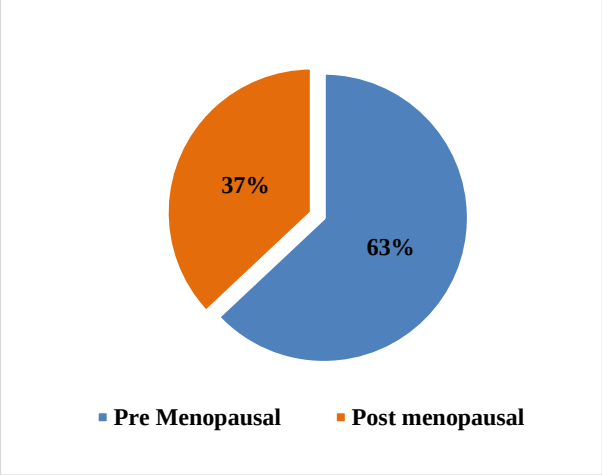

Figure 2. Pie diagram showing menstrual status of LABC patients.

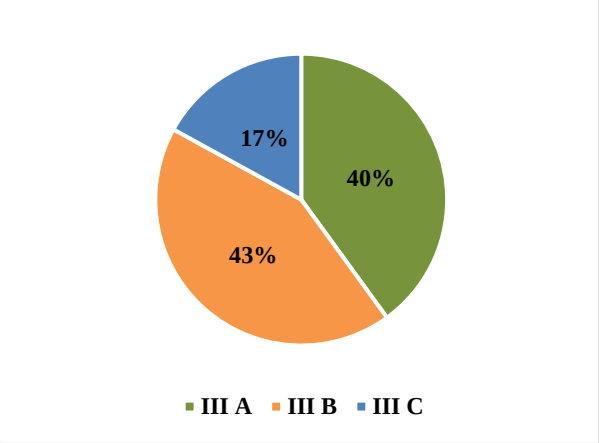

Figure 3. Pie diagram showing tumour stage.

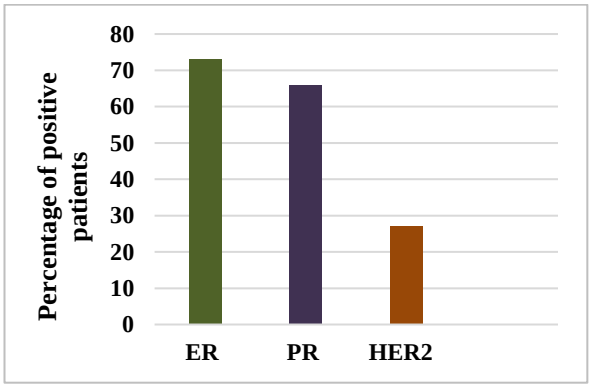

Figure 4. Graph showing tumour ER, PR, HER2 receptor status.

Out of 30 LABC patients, 40% belonged to stage IIIA, 43% were stage IIIB, and 17% had stage IIIC disease (Figure 3). The percentages of oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)-positive tumours were 73%, 66%, and 27% respectively (Figure 4). Most of the patients had invasive ductal carcinoma.

Table 4. Pre-NACT T-Stage vs Post-NACT T-Stage.

		Post chemotherapy tumour stage				
		T0	T1	T2	T3	T4
Pre chemotherapy tumour(T) stage	T2	0	4	0	0	0
	T3	1	1	5	2	0
	T4	3	0	11	1	2

In our study, most patients belonged to T4b 17 before receiving neoadjuvant chemotherapy, but post-therapy, the tumour size reduced, and the majority belonged to T2 17 patients (Table 4). Pre chemotherapy maximum patients belong to N2 18, but post-therapy maximum patients belong to N1 17 patients (Figure 5).

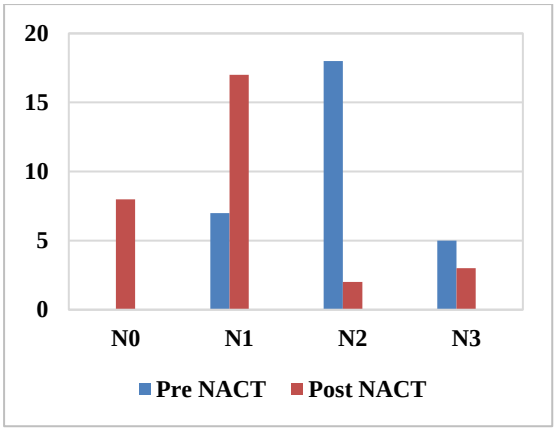

Figure 5. Graph showing pre NACT vs Post NACT N stage.

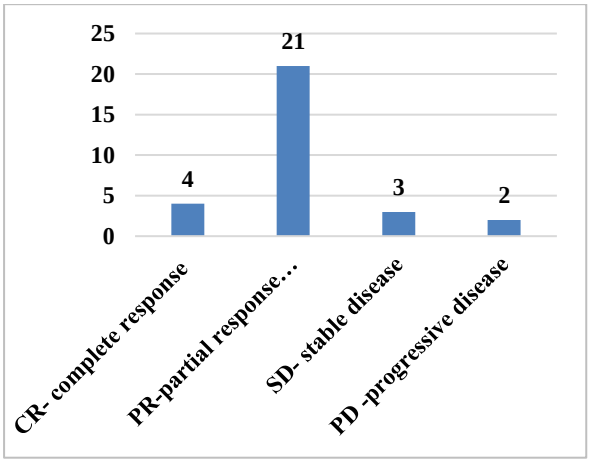

Figure 6. Graph showing clinical response to NACT.

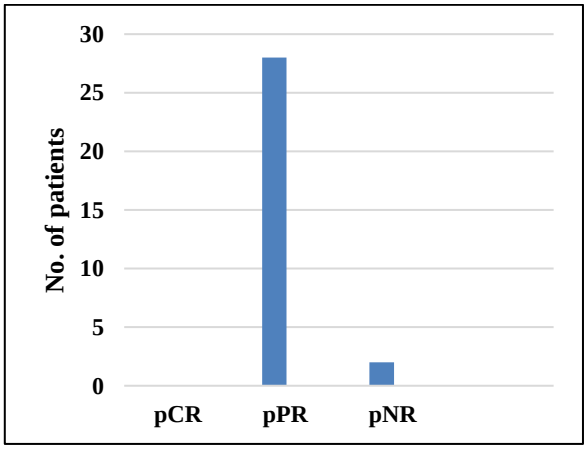

Figure 7. Graph showing pathological response to NACT.

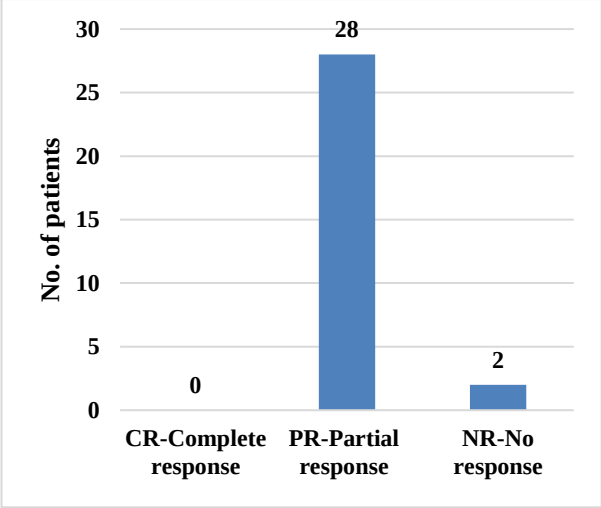

Figure 8. Graph showing overall response to NACT**.**

Among 30 LABC patients, according to RECIST criteria (Table1), clinical complete response post NACT was 13%, partial response 70%, stable disease 10%, and progressive disease 7% (Figure 6). 28 patients showed a pathological partial response according to Chevalier classification (Table 2) (Figure 7), which also corresponds to overall response (Table 3) (Figure 8). The comparison of patient and tumour characteristics with clinical, pathological, and overall response showed the maximum partial clinical response in premenopausal women of less than 50 years having invasive ductal carcinoma and oestrogen progesterone receptor positive, which also corresponds to the partial pathological and overall response (Table 5)**.**

Table 5. Comparison of patient and tumour characteristics with the clinical, pathological and overall response**.**

Characteristics		RECIST				Chevililier classification			Overall response		
		CR	PR	SD	PD	pCR	pPR	pNR	CR	PR	NR
Age	<50 years	2	15	3	2	0	20	2	0	20	2
	51 & above	2	6	0	0	0	8	0	0	8	0
Menopausal	Pre	1	14	3	1	0	18	1	0	18	1
	Post	3	7	0	1	0	10	1	0	10	1
ER status	Positive	3	17	0	2	0	20	2	0	20	2
	Negative	1	4	3	0	0	8	0	0	8	0
PR status	Positive	1	17	0	2	0	18	2	0	18	2
	Negative	3	4	3	0	0	10	0	0	10	0
Histology	Invasive ductal	4	20	3	2	0	27	2	0	27	2
	Invasive lobular	0	1	0	0	0	1	0	0	1	0

Discussion

In the present study, we found that most of the patients 63% were premenopausal and 37% were postmenopausal which was similar to a study by Mishra et al. (12) with 70% cases of premenopausal and 30% cases of postmenopausal.

In our study, post chemotherapy 4 (13.3%) patients had complete clinical remission (cT0). Amongst the 17 (56.6%) patients with pre-chemotherapy T4 disease, only 2 (6.6%) patients remained in T4 stage after chemotherapy, which was comparable to Kunnuru et al. (11) with 32 (53.3%) patients with pre-NACT T4 disease to only eight (13.3%) patients in T4 stage after NACT .

Post chemotherapy, 26.6% of patients had a complete nodal response. In addition to this, N2 stage disease was present in 18 (60%) patients (pre-chemotherapy), which reduced to four (13.3%) patients after chemotherapy.

The usage of NACT in LABC is very effective. In our study, the overall response rate was 93.3% (complete and partial). In patients who achieved a complete clinical response, residual tumours might persist histologically. In our study, 4 patients (13.3%) had a complete clinical response, but all had residual disease histologically. While comparing with another study, the complete clinical response in our study (13.3%) was comparable with Alvarado et al. (12) and Garbhi Olfa et al, (13) which showed a complete clinical response of 12% and 14%, respectively.

In our study group, the relative overall response rates by total number of patients for each factor were as follows: 90.9% in ER-positive tumours, 90% in PR-

positive tumours, and 25% in HER2-positive tumours after three cycles of chemotherapy. A good pathological response of 16.6% was found in triple-negative patients, followed by 90% of 20 luminal A patients.

In the current study, 80% of patients had negative margins post-modified radical mastectomy, probably due to tumour shrinkage following NACT. Very few comparison studies exist stating the same.

Despite being a prospective study due to the short follow-up period of one year, the study faced the challenge of assessing NACT's impact on disease free and overall survival. Due to the small sample size and lack of comparative grouping, generalizing of results became difficult.

Conclusions

In locally advanced breast cancer, NACT not only downstages the disease but also increases the scope of operability for tumours responding to it, and thus making it possible to resect the disease with a tumour free margin in most cases. Including NACT in a protocol-based multimodal approach helps yield better results in locally advanced breast cancer patients.

Author contribution

All authors have equal contribution.

Conflict of interest

The authors have no conflict of interest to declare.

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Investigation of the effect of watery and alcoholic extract of *Arnebia euchroma* on the growth of *Candida* species isolated from patients with COVID-19 associated oral candidiasis using microdilution method

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Abstract

Introduction: Conventional antifungals used to treat fungal infections are no longer as effective, leading to increased mortality. On the other hand, there is an emergence of multidrug-resistant (MDR) fungal strains and for this reason, finding new treatments or substances that have an antifungal effect is noticeable. Therefore, this study aimed to determine the antifungal effects of extracts of *Arnebia euchroma* on the growth of *Candida* species isolated from patients with COVID-19-associated oral candidiasis.

Materials and Methods: Conventional antifungals used to treat fungal infections are no longer as effective, leading to increased mortality. On the other hand, there is an emergence of multidrug-resistant (MDR) fungal strains and for this reason, finding new treatments or substances that have an antifungal effect is noticeable. Therefore, this study aimed to determine the antifungal effects of extracts of *Arnebia euchroma* on the growth of Candida species isolated from patients with COVID-19-associated oral candidiasis.

Results: The results of the present study showed that all the investigated isolates were sensitive to watery and alcoholic extracts of *Arnebia euchroma*. The MIC and MFC of *Arnebia euchroma* watery extract for *Candida albicans* were 512 µg/mL and for *Candida glabrata* were 1024 µg/m, as well as the MIC and MFC of this extract for Candida tropicalis, *Candida parapsilosis*, and *Candida krusei* were 2048 µg/mL. Whereas the MIC and MFC of the *Arnebia euchroma* alcoholic extract for Candida albicans were 0.015625 µg/mL and for *Candida glabrata* were 256 µg/mL, also the MIC and MFC of this extract for *Candida tropicalis* and *Candida parapsilosis* were 512 µg/mL and for *Candida krusei* were 1024 µg/mL.

Conclusion: All the studied *Candida* isolates were sensitive to both types of *Arnebia euchroma* root extract, and the alcoholic extract, compared with the watery extract, inhibited the growth of the tested *Candida* isolates at a lower concentration

Keywords: COVID-19, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*

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Introduction

The immune dysregulation triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been hypothesized as a causal pathway for the increasingly reported oral manifestations associated with coronavirus diseases (COVID-19), especially the ones of fungal origin. Oral candidiasis is a common opportunistic fungal infection of the oral cavity caused by an overgrowth of *Candida* species (1,2). In healthy individuals, *Candida* exists harmlessly in mucus membranes such as the ears, eyes, gastrointestinal tract, mouth, nose, reproductive organs, sinuses, skin, stool, and vagina, but in some patients, it can overgrow and cause symptoms (3). Oral candidiasis causes creamy white lesions, usually on the tongue or inner cheeks. Sometimes it may spread to the roof of the mouth, the gums or tonsils, or the back of the throat (1).

The most common cause of COVID-19-associated oral candidiasis includes *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* (4,5). *Candida albicans* are recovered from 60% of dentate patients' mouths over the age of 60 years.

On the other hand, antifungal resistance represents a major clinical challenge to clinicians responsible for treating oral candidiasis due to the limited arsenal of available antifungal agents. In addition, current drugs may be limited by drug–drug interactions and serious adverse effects/toxicities that prevent their prolonged use or dosage escalation (6). Changes in *Candida* spp. distribution may impact treatment recommendations due to differences in susceptibility to antifungal agents among different spp. Antifungal agents available for the treatment of oral candidiasis are restricted to polyenes, azoles, and the most recent echinocandin class. The emergence of multidrug-resistant strains that are insensitive to several classes of antifungals is a major concern worldwide (6,7). For these reasons, finding new treatments or substances that have an antifungal effect is noticeable.

One of the most common herbal drugs that are used in traditional medication is Abukhals (*Arnebia euchroma*) from the family of Boraginaceae. This plant is herbaceous, with sharp silver pubes and the flower is cluster shaped with stretched and alternate leaves (8-12). One of the most common habitats of this plant is Iran, especially Rasht. The root of this plant was used in reducing the swellings and had anticancer activity. It caused mild constipation and was used in nourishing the liver, kidneys, and spleen. New studies have shown that its extracts contain shikonin which is used in the treatment of burns and dermatitis, proliferation of skin's stem cells, improving arthritis, and inhabitation of inflammation by its antibacterial and antifungal effects (8-12).

In various studies conducted around the world, the antiviral and antibacterial properties of *Arnebia euchroma* have been proven (13,14), but so far there is no comprehensive study evaluating the antifungal effect of this plant in the treatment of oral candidiasis. The economic value of *Arnebia euchroma* as an herbal medicine and its use in cosmetics, food, and personal care products, and the lack of knowledge about antifungal susceptibility profiles of fungal elements causing oral candidiasis against *Arnebia euchroma* among Iranian patients prompted us to conduct a comprehensive study to fill this gap. It is important to have antifungal agents that will treat fungal infections without leading to increased resistance, though the use of azoles and echinocandin antifungal drugs against *Candida* species has seen this happen. As changes are seen in the resistance of fungi to antifungal drugs, in the present study we aimed to assess the antifungal effects of extracts of *Arnebia euchroma* on the growth of fungal agents isolated from COVID-19-associated oral candidiasis in Iran as a new antifungal agent.

Materials and Methods

Collection of plant materials

The roots of *Arnebia euchroma* were collected from the local areas of Rasht, north of Iran. It was authenticated from the proper source and a voucher specimen No: 01 was deposited in the Department of Pharmacognosy, Guilan University of Medical Sciences, Rasht, Iran.

Preparation of Plant Extracts

Collected roots were dried on mats in the shade and at room temperature, spread into thin layers that were not mixed over the 10-day drying period. The extraction process was conducted using 96% ethanol (for

alcoholic extracts) and distilled water (for watery extracts). For preparing alcoholic extract a powdered leaf (100 g) was added to 500 mL of ethanol and for preparing watery extract 100 g of the powder was added to 500 mL of distilled water. The extraction was carried out for 72 hours at room temperature with mild shaking. The extracts were filtered and concentrated at 37° C for 48 hours (15,16).

Fungal species

The antifungal activity was carried out against *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, and *C. parapsilosis* clinical isolates. All the mentioned isolates were previously collected from clinical specimens of patients with COVID-19-associated oral candidiasis hospitalized in Razi Hospital in Rasht City, Guilan, Iran (ethical code: IR.TUMS.SPH.REC.1400.030) and were recognized previously to the species level through sequencing of the internal transcribed spacer (ITS1-5.8s-ITS2) gene. Also, the standard strains of *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 48465), *Candida krusei* (ATCC 2159), *Candida tropicalis* (ATCC 750), and *Candida parapsilosis* (ATCC 22019), which were obtained in lyophilized from the microbial collection of Iranian Biological Resource Center (IBRC, No. 80, West Hoveizeh St, North Sohrevardi Ave, Tehran, Iran) were included in the study.

Antifungal Activity Assessment

In vitro, antifungal susceptibility testing was performed against isolated strains according to the protocols described by the Clinical and Laboratory Standards Institute (CLSI) guidelines, document M27-A3 for yeasts (17). Briefly, by employing 24 hours cultures of yeast isolates on sabouraud dextrose agar (SDA; Difco) homogeneous yeast conidial suspensions were spectrophotometrically measured at the 530 nm wavelength and a percent transmission within the range of 75- 77%. The final inoculum suspension was adjusted to 10^5 conidia/mL in RPMI 1640 medium (GIBCO, UK) buffered at pH 7.0 with 0.165 M morpholino propane sulfonic acid (MOPS, Sigma-Aldrich, St. Louis, MO, USA). For the determination of antimicrobial activities against all of the studied microorganisms, the concentration of each plant extract was diluted two-fold from 4096 µg/mL to 0.00390625 µg/mL. After adding 100 µl of the inoculum suspension the microdilution plates were incubated at 35°C for 48 h; the plates were read visually according to the recommendations proposed by the CLSI M27-A3 document. The microdilution plates were inoculated with 100 µl of the diluted conidial inoculum suspension and incubated at 35 °C for 48 h. The plates were read visually according to the recommendations proposed by the CLSI M27-A3 document. Reference strains of *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) were used for quality control purposes. MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without the development of turbidity.

Ethics Statement

The study was approved by the Research Ethics Committee of Guilan University of Medical Sciences (the number of ethics committee protocol: IR.GUMS.REC.1401.526).

Statistical analysis

MIC values were calculated for clinical and standard samples and the strains were compared. For statistical analysis, a Chi-square test of homogeneity was performed at a significance level of 5 % (18).

Results

In the present study, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of watery and alcoholic extracts of *Arnebia euchroma* on the growth of *Candida* species isolated from patients with COVID-19 associated oral candidiasis (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*) were evaluated using broth microdilution method.

Table 1 presents the MIC of the watery and alcoholic extracts of *Arnebia euchroma* against tested *Candida*. The results demonstrate that all the mentioned *Candida* species against the watery and alcoholic extracts of *Arnebia euchroma* showed sensitivity.

The MIC and MFC of *Arnebia euchroma* watery extract for *Candida albicans* were 512 µg/mL and for *Candida glabrata* were 1024 µg/mL, as well as the MIC and MFC of this extract for *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* were 2048

µg/mL. Whereas the MIC and MFC of *Arnebia euchroma* alcoholic extract for *Candida albicans* were 0.015625 µg/mL and for *Candida glabrata* were 256 µg/mL, also the MIC and MFC of this extract for *Candida tropicalis* and *Candida parapsilosis* were 512 µg/mL and for *Candida krusei* were 1024 µg/mL (Table 1, Figure 1).

Table 1. The minimum inhibitory concentration (MIC) of watery and alcoholic extracts of Arnebia euchroma on the growth of Candida species isolated from patients with COVID-19-associated oral candidiasis by microdilution method.

Type of extract	Yeast	The Concentration of watery and alcoholic extracts of *Arnebia euchroma* (µg/mL) in 96-well microplates											
		4096	2048	1024	512	256	128	64	32	16	8	Positive control	Negative control
Watery	*C. albicans*	-	-	-	-	+	+	+	+	+	+	+	-
Watery	*C.glabrata*	-	-	-	+	+	+	+	+	+	+	+	-
Watery	*C. krusei*	-	-	+	+	+	+	+	+	+	+	+	-
Watery	*C. parapsilosis*	-	-	+	+	+	+	+	+	+	+	+	-
Watery	*C. tropicalis*	-	-	+	+	+	+	+	+	+	+	+	-
96% ethanol	*C. albicans*	-	-	-	-	-	-	-	-	-	-	+	-
96% ethanol	*C. glabrata*	-	-	-	-	-	+	+	+	+	+	+	-
96% ethanol	*C. krusei*	-	-	-	-	+	+	+	+	+	+	+	-
96% ethanol	*C. parapsilosis*	-	-	-	-	+	+	+	+	+	+	+	-
96% ethanol	*C. tropicalis*	-	-	-	+	+	+	+	+	+	+	+	-

Type of extract	Yeast	The Concentration of watery and alcoholic extracts of *Arnebia euchroma* (µg/mL) in 96-well microplates											
		4	2	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.0078125	0. 00390625	Positive control	Negative control
96% ethanol	*C. albicans*	-	-	-	-	-	-	-	-	+	+	+	-

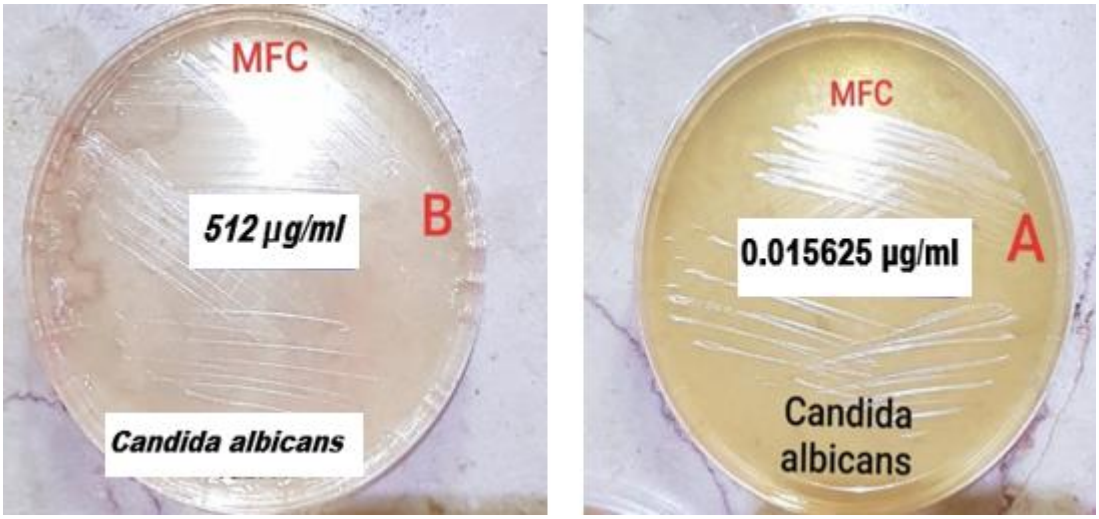

Figure 1. The MFC of the watery (A) and alcoholic (B) extracts of *Arnebia euchroma* against *Candida albicans*.

Discussion

The incidence of fungal infections with high morbidity and mortality has increased globally due to the limited antifungal arsenal and the high toxicity of some drugs. Only five antifungal drug classes are available, including polyenes, azoles, and allylamines that target ergosterol in the cell membrane, pyrimidine analogs that target DNA synthesis, and the new echinocandin class that targets β-glucan in the fungal cell wall. The treatment of oral candidiasis with the use of medicinal plants is of great interest due to fewer side effects, a variety of effective compounds in plants, and lower economic costs. Also, due to the increasing resistance of bacteria and fungi to antimicrobial compounds, the attention of researchers to medicinal plants and natural antimicrobial compounds to treat infections has increased. In various studies conducted around the world, the antiviral and antibacterial properties of *Arnebia euchroma* have been proven (13,14), but so far there is no comprehensive study evaluating the antifungal effect of this plant in the treatment of oral candidiasis according to the type of *Candida* species. For this reason, the purpose of the present study was to investigate the antifungal effects of *Arnebia euchroma* on different *Candida* species (*C. albicans, C. tropicalis, C. parapsilosis, C. krusei,* and *C. glabrata*) isolated from patients with COVID-19 associated oral candidiasis. Determining the possibility of using the watery and alcoholic extract of this plant as an antifungal product in the treatment of oral candidiasis was another purpose of this study.

The findings of the present study demonstrate that all the tested *Candida* species against the watery and alcoholic extract of *Arnebia euchroma* showed sensitivity and the alcoholic extract, compared with the watery extract, inhibited the growth of the tested *Candida* isolates at a lower concentration. Therefore, the alcoholic extract of *Arnebia euchroma* showed more antifungal effects on the tested *Candida* species isolated from the patients with COVID-19-associated oral candidiasis than the watery extract of this plant. In a study conducted by Madarshahi et al., (2022) in Mashhad, northeastern Iran, extracts from *Arnebia euchroma* root have been prepared and its antifungal and anti-aflatoxigenic activities against *Aspergillus flavus* have been investigated. The experiment confirmed the antifungal activity of *Arnebia euchroma* and provided evidence for the potential use of these natural compounds against fungi (8). Also, in a randomized controlled triple-blind trial conducted by Mohammadi et al., (2022) in Ahvaz, southwest of Iran, the effectiveness of *Arnebia euchroma* with vaginal cream clotrimazole 1% United States Pharmacopeia (USP) for the treatment of vulvovaginal candidiasis were compared. The Chi-square showed that there was a significant difference between the culture results in both groups ($p = 0.001$). and confirmed that a vaginal cream containing *Arnebia euchroma* could reduce the complaints of vulvovaginal candidiasis (9). In another

study conducted by Doulah et al., (2014) in Ahvaz, southwest Iran, the antifungal activity of methanolic extract of *Arnebia* species was screened using the disc-diffusion (DD) method and the minimal inhibitory concentration (MIC) using the macro dilution broth technique against *Aspergillus niger, Aspergillus flavus*, and *Candida glabrata*. The tested plant showed mild antimicrobial activity against all tested strains. The results obtained indicate that tested plants may become important in the obtainment of noticeable sources of compounds with health-protective potential, antioxidant, and antimicrobial activity (11). Besides, in a study conducted by Sasaki et al., (2000), in Japan, the antifungal activity of *Arnebia euchroma* was investigated *in vitro* against *Candida albicans*. The results showed that the extract inhibited the fungal growth at MIC 15.6 micrograms/ml (RPMI24 h) or 3.9 micrograms/ml (YNB24 h) (12). The difference in the findings of the present study with other studies could be since the composition of plant extracts and then their antimicrobial effects are different under the influence of endogenous and exogenous factors (environmental light, plant growth location, soil pH, plant genetics, temperature, and humidity). In other words, considering that the geographical location is effective on the amount and even the type of plant metabolites, the plant extracts in different geographical locations can have different antifungal activities.

Conclusions

In general, the findings of the present study showed that the alcoholic extract of *Arnebia euchroma* has a greater antifungal effect than its watery extract on the growth of Candida species isolated from COVID-19-associated candidiasis and can be a suitable alternative for antifungal drugs for the treatment of this infection. Further, it is necessary to conduct more studies in *in vitro* conditions to introduce this extract as a natural and new antifungal agent.

Author contribution

FF investigation, Data curation. **DR** conceptualization, methodology, project administration, and funding acquisition. **ZR** conceptualization, methodology, project administration, writing - original draft, resources, visualization, data curation. writing - review & editing. **KD** methodology, investigation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors have no conflict of interest to declare.

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Long-term outcome for achalasia in patients who underwent laparoscopic Heller myotomy with Dor fundoplication

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Abstract

Introduction: Achalasia is a rare esophageal motility disorder that can require surgical intervention in some cases. This retrospective cross-sectional study aims to evaluate the clinical symptoms of patients with advanced achalasia who underwent laparoscopic Heller myotomy (LHM) and Dor fundoplication.

Materials and Methods: The study included 86 patients (38 men, 48 women) diagnosed with achalasia between 2010 and 2020, of which 20 patients with advanced achalasia underwent LHM and Dor fundoplication. The median follow-up time was 48 months.

Results: The study found that LHM and Dor fundoplication surgery improved dysphagia in 12 patients, with four patients showing improvement in solid food dysphagia and two patients showing improvement in semi-solid dysphagia. Nocturnal cough and slow emptying sensation also improved in 16 cases. Additionally, barium stasis decreased significantly in 14 patients. However, two patients who underwent esophagectomy had hospital mortality.

Conclusion: This study highlights the effectiveness of LHM and Dor fundoplication in reducing dysphagia, nocturnal coughing, regurgitation, and other obstructive symptoms in patients with advanced achalasia. However, the study also underscores the potential risks associated with esophagectomy, suggesting that surgical treatment for achalasia should be carefully considered on a case-by-case basis.

Keywords: Achalasia, Dysphagia, Heller myotomy, Fundoplication, Gastroesophageal reflux

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Introduction

Achalasia is an uncommon but quintessential esophageal motility disorder that occurs equally in men and women (1). Achalasia characterized by reduced relaxation of the lower esophageal sphincter (LES) and absence of esophageal peristalsis resulted in impaired bolus transit, demonstrated with symptoms including dysphagia, retrosternal pain, regurgitation, and weight loss (2).

The disease's pathogenesis is unclear and often misdiagnosed (3). Still, it is suggested to happen because of a virus-related inflammatory neurodegenerative process triggered by an autoimmune and chronic inflammatory process, especially in patients with genetic susceptibility (4). However, at the time of diagnosis, the number of decreased neurons led to significant dysfunction and symptoms. Therefore, the first step of diagnosis is performing endoscopy or radiology, but the gold standard diagnostic method for achalasia is high-resolution manometry (HRM) (5).

According to Chicago classification, achalasia is classified into three subtypes, type I (classic achalasia) refers to the one without any significant pressurization in esophageal, type II is achalasia with compression, which there is no peristalsis and contractile activity, and pan-esophageal pressurization >30 mmHg, and type III is spastic achalasia with rapidly propagated pressurization attributable to an abnormal lumen obliterating contraction (6).

As achalasia progresses, dilation of the esophagus worsens and can resemble a sigmoidal shape. In the end stage of achalasia, patients present dilation of the esophagus with a sigmoid shape (7). Unfortunately, there is no promising treatment for achalasia due to its unknown pathogenesis, and standard treatment options include pharmacological therapy (nitrates and calcium channel blockers), pneumatic dilation, endoscopic myotomy (2,3), Botulinum toxin (Botox) (8), surgical myotomy, and esophagectomy (2,3).

Surgical treatment of achalasia has evolved dramatically over the past 13 years. Since the first report of laparoscopic Heller myotomy by Cuschieri and thoracoscopic Heller myotomy by Pellegrini, minimally invasive surgery has become the gold standard for treating achalasia (9). More recently, the laparoscopic management of esophageal achalasia has achieved widespread acceptance and is now the first line of therapy for patients with achalasia. The satisfactory short-term results of this procedure are well documented in several large series.

Esophagectomy is more aggressive and associated with more significant morbidity/mortality than laparoscopic Heller myotomy (LHM) and Dor fundoplication (10). In this regard, we study the post-surgical side effects and clinical symptoms of patients in two groups who underwent LHD and Dor fundoplication in patients with achalasia.

Materials and Methods

This retrospective cross-sectional study was conducted on 86 patients with achalasia in Razi Hospital, Rasht, Iran, from October 2010 to September 2020. The achalasia was confirmed by clinical findings (endoscopy, radiology, and HRM results). In addition, all demographical data and clinical characteristics of patients were recorded from the patient's archive in the hospital.

The surgery approach was LHM (8 cm over the esophagus and 3 cm over the stomach) and Dor fundoplication (Figure 1). All remnant food was aspirated to prevent pulmonary aspiration after induction of general anesthesia with a tracheal tub. Before the surgery, 16 of the patients had undergone previous dilatations or Botox injections. Longitudinal and circular muscle of the esophagus was cut on the last 8 cm of the esophagus and extended three cm on the gastric wall musculature. Dor fundoplication was performed in all patients. In our study, the perforation and complete myotomy were checked after completion of cardiomyotomy with an ambo-bag, and via a tube in the esophagus air inflate. Postoperative assessments include clinical, radiologic, manometric, and endoscopic evaluation was performed.

A flap of the stomach for coverage was fixed to prevent diverticula formation in the motorized site. Pre and post-operative assessment included symptoms, esophageal emptying observation with barium esophagogram, HMR, and endoscopic evaluation in all patients. The barium esophagogram was obtained under fluoroscopic control.

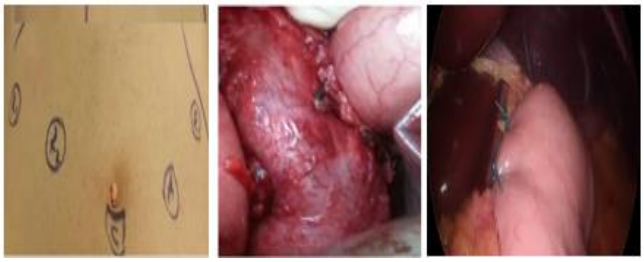

Figure 1. Laparoscopic Heller myotomy in patients with achalasia.

The surgical technique for laparoscopic Heller myotomy was after the pharyngoesophageal ligament that divided the fat pad excised and exposing the anterior gastroesophageal junction; the myotomy was performed by incising the distal 4 to 6 cm of esophageal musculature. Then, the myotomy was extended 2 to 3cm onto the gastric cardia using cautery scissors with an intraesophageally tube; when the EJ junction closed, the esophagus was inflated, mucosal perforations were detected, and the myotomy added a Dor anterior hemifundoplication. Routinely, a contrast swallow was performed on the second day of postoperative in all patients to rule out an occult leakage. For patients with no leak, a clear liquid diet was started on the second postoperative day, and all patients were discharged four days postoperatively. The results were reported in number and percentage.

Results

Among a total number of 86 patients (38 males, 48 females) with a median age of 46 years old, patients had advanced achalasia including lumen dilatation of esophagus between 6 to 12 cm, moderate to severe intra luminal stasis of barium, severe tortoise, recurrent pulmonary aspiration, and recurrent pulmonary infection; and underwent laparotomy for achalasia. These patients failed in pneumatic dilatation and Botox treatment. Dysphagia presented in all patients, and 20 patients experienced an average weight loss of 10 kg before surgery. Pre-surgical clinical characteristics of patients are demonstrated in Table 1.

Table 1. Pre-surgical clinical characteristics of patients with achalasia.

Variables Patients number: 20		Number
Dysphagia	Dysphagia to solid	8
	Dysphagia to liquid	12
Patients with weight lose		14
Regurgitation		20
Nocturnal cough		16
Sigmoid esophagus		8
Size of dilatation		6-10 cm
Pervious intervention	Botox injection	4
	Dilatation	12
Heller myotomy		18
Esophagectomy		2
Duration of hospitalization		8 (8-10) days

Two patients expired, one during operation and another one in five days after surgery due to pneumonia and reparatory failure. Two patients required reoperation for bleeding and gastric herniation. Six patients experienced minor postoperative morbidity, including atelectasis (3n), atrial tachyarrhythmia (4n), and wound infection (3n). The median follow-up days were 30 months (10–48 months).

According to our results, stasis was reported in all patients before the operation. The LES gradient decreased from 32 to 12 mmHg. Endoscopy and biopsy findings demonstrated grade I esophagitis in four patients. Radiological findings represented that barium stasis decreased from 92% to 22%. The post-surgery diameter of the esophagus lumen was 8 cm (8–12 cm), which fell to 6 cm (6-10 cm). Body weight increased after the myotomy [preoperative: 58 kg (38–83 kg), postoperative: 66 kg (48–86 kg)]. No diverticular formation was observed in the motorized zone. Short and long-term functions and symptom improvement in patients with achalasia are illustrated in Table 2.

Table 2. Short and long-term functions and symptom improvement in patients with achalasia.

Short and long-term functions and symptom		Number
Dysphagia improvement (16 n)	<10 month	6
	>48 month	10
Heartburn present	<10 month	8
	>48 month	6
Regurgitation	<10 month	14
	>48 month	4

Slow emptying improvement	<10 month	8
	>48 month	10
Esophagitis post operation	<10 month	4
	>48 month	2
Nocturnal cough improvement	<10 month	12
	>48 month	6

Discussion

Achalasia is a rare esophageal motility disorder for which there is no known etiology, making treatment options challenging. The main goal of treatment is to reduce LES pressure, improve dysphagia and regurgitation, enhance esophageal emptying, and prevent the development of megaesophagus. Surgical management of advanced achalasia is challenging, and esophagectomy is associated with a high incidence of postoperative respiratory complications such as pneumonia. Our results aillustrated that LHM is an effective treatment with a higher patient survival rate and fewer complications.

Previous studies have reported that higher LES resting pressure is associated with better relief of dysphagia after myotomy. The LHM–Dor procedure provides satisfactory long-term results with low morbidity (11,12). Esophagectomy was associated with a high incidence of postoperative respiratory complications, including pneumonia, while LHM is more effective with a higher patient survival rate (13,14). Arain et al. reported that higher LES resting pressure is associated with better relief of dysphagia after myotomy (15). A study demonstrated that extending myotomy three cm over the stomach reduces the postoperative pressure on the LES with no significant difference in reflux when added an anti-reflux procedure (16). Also, Liu et al. reported that esophageal myotomy for achalasia could reduce the resting pressures of the esophageal body and LES and improve esophageal transit and dysphagia (17).

Dor fundoplication added to myotomy reduces the risk of pathologic gastroesophageal reflux, and our study showed that it could be performed in all patients with a low incidence of reflux. Studies have reported favorable responses in more patients even after a long term of follow-up. LHM and Dor fundoplication balance emptying and reflux and could be the selected surgical treatment for patients with achalasia (12,18). In an investigation on a series of 73 patients treated with LHM, favorable responses were reported in more than half of the patients, even after over six years of follow-up (19). Siow et al. demonstrated in their study that LHM and anterior Dor fundoplication are both safe and effective as a definitive treatment for treating achalasia cardia with high patient satisfaction with minimum complications (20). A study by Finley et al. reported that 24 patients who underwent LHM without fundoplication had more significant improvement in esophageal clearance time (21).

Rice et al. represented that the addition of Dor fundoplication decreases the capability of LHM without impairing emptying and reduces reflux. LHM and Dor fundoplication balance emptying and reflux, which could be the selected surgical treatment for patients with achalasia (22). Kummerow et al. illustrated no statistical difference between patient-reported dysphagia or reflux scores in those who underwent an LHM with and without Dor fundoplication (23). In this present study, reflux was reported in nine patients with Do fundoplication. Also, end-stage achalasia treated by LHM with Dor fundoplication showed reduced LES gradient, decreased obstructive symptoms, and improved esophageal emptying.

Performing LHM is an effective treatment with good dysphagia relief and a low incidence of esophageal mucosal perforation (24). Abovementioned studies reported that LHM is an effective treatment with good dysphagia relief and a low incidence of esophageal mucosal perforation. While manometry is sometimes essential for good surgical outcomes, long-term follow-up on dysphagia relief and patient satisfaction is necessary to ensure the effectiveness of therapy. Overall, our study showed that LHM and Dor fundoplication are safe and effective treatments for advanced achalasia, providing significant improvement in obstructive symptoms, decreased LES gradient, and improved esophageal emptying.

Limitations

The limitation of this study was the limited access to the history of patients' underlying disease and incomplete data on individuals' diets and lifestyles.

Conclusions

LHM provided satisfactory symptom improvement in patients with advanced achalasia with promising outcomes. Also, further investigations are required to demonstrate the most effective methods in patients with severe achalasia.

Author contribution

MTA and **MA** wrote the main manuscript text and designed the study. **MSES** and **ASh.** cooperated in data collecting and analysis. All authors reviewed the manuscript.

Conflict of interest

The authors reported no potential conflict of interest.

Ethics approval

Relevant ethical guidelines and regulations were performed for all experiments. This study was done according to the Declaration of Helsinki ethical standards and consent and agreement was obtained from all the patients and was confirmed and approved in the surgery department.

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Prevalence and factors related to post-traumatic stress disorder (PTSD) in patients with COVID-19 and their families admitted to 22 Bahman Hospital in Neyshabur

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Abstract

Diseases such as COVID-19 can be associated with the development of mental disorders such as PTSD in patients or their families, which can last for years. Therefore, this study investigated the prevalence and factors associated with post-traumatic stress disorder (PTSD) in patients with COVID-19 and their families admitted to 22 Bahman Hospital in Neyshabur in 2020. In this descriptive cross-sectional study, 96 patients and 96 family members in Neyshabur were included using available and voluntary sampling. PTSD in individuals was assessed by completing the DSM-5(PCL-5) checklist and interviewing. Demographic information including gender, age, level of education and marital status was also collected. Information related to COVID-19 disease including the patient's pulmonary involvement, duration of hospitalization and ward was recorded. In both groups, the majority of participants were men (56%), married people (90%), and people with a diploma (49%). The mean score of PTSD in the patient group and the patient family group was 35.5 and 33.5, respectively. All subjects in the patient group and the patient group had PTST disorder (PTSD score>18). In the group of patients with COVID-19, the severity of post-traumatic stress disorder was severe in 14.6%, moderate in 21.9%, and mild in the rest. Also, the incidence of PTSD among patients' families was 16.7% severe, 31.2% moderate and the rest mild. Regression analysis showed that the variables of hospitalization and duration of hospitalization could predict stress disorder in patients at 53.9% and 24.2%. Given the widespread coronavirus in communities as well as the prevalence of PTSD in patients and their families, control measures should be considered to improve the mental health of these individuals.

Keywords: Coronavirus, COVID-19, PTSD, Trauma

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Introduction

Post-traumatic stress disorder (PTSD) is the most common and important mental disorder that occurs in a situation where a person experiences a lot of stress with fear of death of themselves or others (1). Certain types of events such as natural disasters such as floods and earthquakes, the spread of infectious diseases as well as physical or sexual abuse are significantly associated with the spread of PTSD (2-5). The prevalence of PTSD is affected by the severity, duration and proximity of the accident (3). Previous studies have shown that PTSD is the most common psychological problem after an epidemic due to traumatic conditions (4). Outbreaks appear to be exacerbated during pregnancy and in patients with dementia (6). Even family members of patients with COVID-19 are experiencing increased stress. Poor knowledge of the structure, behavior, and mechanisms of virus transmission, uncertainty overtime to control the disease, quarantine of patients and families, and death of family members have led to widespread fear and anxiety and loss of confidence in individuals. On the other hand, patients with coronation and their families experience difficult and stressful conditions due to hearing bad news from those around them and the media, the heavy burden of treatment costs and other related factors. The World Health Organization (WHO) estimates that 30 to 50 percent of the population in areas affected by the SARS-CoV-2 coronavirus epidemic suffer from various psychological problems, especially PTSD. At the same time, it has been reported that people with PTSD are more at risk of suicidal ideation, suicide attempt and suicide-related death (5). This is so important that even physicians are advised to include PTSD as part of a common history when taking histories of patients with coronavirus (7, 8). Patients with COVID-19, especially in the severe form of the disease, are usually admitted to infectious wards in isolation. These patients may experience loneliness, anger, anxiety, depression, insomnia, and post-stress symptoms due to perceived social isolation, dangerous conditions, uncertainty about the future, physical discomfort, drug side effects, and fear of transmitting the virus to others. Experience the accident. These effects can negatively affect the social, occupational and quality of life of these people in the short and long term (9). Patients connected to ventilators are restless and confused about what is happening. Therefore, it is predictable that many people who are discharged from intensive care unit survivors after treatment will experience depression, anxiety, post-traumatic stress disorder, and other mental health problems. A UK study reported that more than 50 percent of patients admitted to the intensive care unit showed severe signs of anxiety, depression and post-traumatic stress disorder after discharge (10). In this regard, the prevalence of symptoms and diagnostic criteria for PTSD during the coronavirus epidemic in Italy has been reported up to 49.7% (11). The prevalence of PTSD at the time of the previous epidemic of coronavirus strains was reported to be about 32% (12). Naturally, in such a situation, the mental condition of the family and those around the patients is also unfavorable. In a study in Japan of more than 16,000 participants, including family and friends of patients with COVID 19, the results showed that most of them were fearful and anxious. In this study, PTSD scores in women under 60 were more than was reported from other individuals and age groups (13). The families of patients with COVID-19 have also been identified as a trauma group so that the monitoring of their mental state and, if necessary, counseling measures should be considered for them (14). Otherwise, in the near future we are likely to see an increase in the incidence of PTSD in the whole community (15). PTSD can be very common even long after the initial exposure to trauma. If proper and timely action is not taken for counseling and psychotherapy, this disorder will challenge people for many years to come. Therefore, in the current situation where patients with COVID-19 and their families are under a lot of stress, it seems necessary to study the prevalence of this disorder among patients and their family members to plan counseling and treatment measures. So far, several studies have been conducted on the prevalence of PTSD among COVID-19 survivors worldwide, but studies to examine the psychological consequences of COVID-19 disease among patients and their companions in developing areas such as Iran are limited **(16).** Given that socio-cultural differences, as well as demographic characteristics, affect the prevalence and severity of PTSD in individuals, conducting such a study is a priority. This study aimed to investigate the prevalence and factors associated with post-traumatic stress disorder (PTSD) in patients

with COVID 19 and their families admitted to 22 Bahman Hospital in Neyshabur in 2020.

Materials and Methods

Study design and selection of participants

The present study was a descriptive cross-sectional study conducted in 2020. The study consisted of 19 patients with COVID 19 admitted to 22 Bahman Hospital in Neyshabur and their family members. Sampling was non-random and available and voluntary. In this way, the purpose of the study and the method of work (completing the questionnaire and checklist) was explained to patients and their families. If these individuals met the inclusion criteria and also expressed their consent to participate, a written consent form would be obtained from them. Finally, 96 patients and 96 family members were included in the study. This study aimed to determine the prevalence of PTSD in patients with COVID-19 and their families in Neyshabur and to determine the factors associated with this disorder. Inclusion criteria for patients included: hospitalization based on COVID-19 diagnosis, and ability to answer questions (stability of disease condition). Exclusion criteria for patients included: outpatients with a maximum hospital stay of one day and a history of psychiatric medication use at least one month before admission to hospitalization. Criteria for inclusion of patients' family members in the study were; Be a first-degree relative of the patient who wants to participate in the research and is willing to cooperate at the time of the patient's discharge. Exclusion criteria for a family member should also include relatives of the patient whose patient does not meet the inclusion criteria. Ethical criteria in the research, including ensuring the confidentiality of information, sufficient and necessary explanation of the working method and guidance in referring to counseling centers depending on the severity of the disorder were observed. This research is based on a research design with ethics code IR.NUMS.REC.1399.035.

Data collection

Based on the coordination with the hospital, an arrangement was made to inform the research team one day before the possible discharge of COVID 19 patient, so that one of the research colleagues would be present at the patient's discharge and complete the checklist to diagnose the disorder along with the interview. Data were collected by completing a demographic information questionnaire as well as a DSM-5 (PCL-5) checklist and interview. When completing the checklist and interviewing, the research colleague met with them and their families in full compliance with hygiene principles and at the time of discharge. Demographic information included gender, age, level of education, and marital status. Also, information related to COVID-19 disease including the patient's pulmonary involvement, duration of hospitalization and ward were recorded.

DSM-5 (PCL-5) checklist

The post-traumatic stress disorder checklist was designed based on the DSM-5 (PCL-5). This checklist was prepared by Withers, Leitz, Kane, Palmeier, Marx, and Ashnor (1993), based on the criteria of the Fifth Edition Diagnostic and Statistical Manual of Mental Disorders, for the US National Center for Post-Traumatic Stress Disorder as a diagnostic aid. This checklist contains 17 five-choice items. Of these 17 items, 5 were related to the signs and symptoms of re-experiencing a traumatic event, 7 were related to the signs and symptoms of emotional numbness and avoidance, and 5 were related to the symptoms and symptoms. The scoring method is in the form of Likert from one to five and the total score of the articles (85-17) is considered as the individual score (17). A score of 35 is considered as the cut-off point in most studies (18-20). The validity and reliability of this tool have been reviewed and confirmed in previous studies (17-22). In the present study, Cronbach's alpha coefficient for the whole scale was 0.90. Depending on the scores obtained from the stress checklist after injury and according to the range of scores, individuals were divided into three groups. Thus, individuals with scores ranging from 18-28, 29-56 and 57-85 were divided into mild, moderate and severe PTSD groups, respectively. A score of 17 meant that there was no evidence of this disorder.

Statistical analysis

The number and relative frequency of participants were calculated and reported based on various parameters. Spearman correlation analysis was also used to determine the correlation between individuals' PTSD

scores and demographic variables and COVID-19 disease. Linear regression analysis was used to evaluate the effect of demographic variables and COVID-19 disease on the severity of PTSD. Data were analyzed by SPSS v.16 software at a significance level of 0.05.

Results

Data analysis was performed on 192 patients (96 patients and 96 families of these patients). Demographic characteristics, length of hospital stay, ward, and percentage of lung involvement in patients and families of patients with COVID 19 are listed in Table 1. In both groups, the majority of participants were men (56%), married people (90%), and people with a diploma (49%). The percentage of lung involvement in most of the subjects (78%) was less than 50% who were hospitalized for less than 10 days (58%) in non-specialized wards (80%).

Table 1. Demographic characteristics and COVID-19 disease in study participants.

Variable		Patient group (n=96)		Patient family's group (n=96)	
		Average	Standard deviation	Average	Standard deviation
Age		57.44	15.05	46.14	16.25
		Number	Percentage	Number	Percentage
Gender	Female	40	41.7	31	32.3
	Male	56	58.3	65	67.7
Marital status	Single	6	6.2	6	6.2
	Marriage	90	93.8	90	93.8
	Total	96	100	96	100
Level of Education	Illiterate	3	3.1	3	3.1
	High school	27	28.1	28	29.2
	Diploma	47	49	43	44.8
	Associate degree	3	3.1	5	5.2
	Bachelor	10	10.4	10	10.4
	Master's degree and higher	6	6.2	7	7.3
	Total	96	100	96	100
Percentage of lung involvement	Under 50%	78	81.2	78	81.2
	Above 50%	18	18.8	18	18.8
	Total	96	100	96	100
Duration of hospitalization	Less than 10 days	58	60.4	58	60.4
	More than 10 days	38	39.6	38	39.6
	Total	96	100	96	100
Inpatient department	Non-special	80	83.3	80	83.3
	Special	16	16.7	16	16.7
	Total	96	100	96	100

The amount and severity of post-traumatic stress disorder (PTSD) in patients and families of patients with COVID 19 were assessed using a questionnaire and the results are listed in Table 2. The mean score of PTSD in the patient group and the patient family group was 35.5 and 33.5, respectively. This indicates that, on average, the severity of PTSD in patients was slightly higher than in patients' families. All subjects in the patient group and the patient group had PTSD, meaning that in this study, there was no individual without this disorder among the participants. In the group of patients with COVID-19, the severity of post-traumatic stress disorder was severe in 14.6%, moderate in 21.9% and mild in the rest. The percentage was severe, 31.2% was moderate and the rest was mild.

Table 2. Number and percentage of participants based on the extent and severity of PTSD.

Variable		Patient group (n=96)		Patient family's group (n=96)	
		Average	Standard deviation	Average	Standard deviation
Average PTSD score (17-185)		35.53	18.51	33.500	17.49
		Number	Percentage	Number	Percentage
PTSD score	Mild (18-28)	61	63.5	50	52.1
	Medium (29-56)	21	21.9	30	31.2
	Intense (57-85)	14	14.6	16	16.7
	Total	96	100	96	100

The correlation between PTSD scores in patients and their families with demographic characteristics and disease parameters were examined by the Spearman correlation test and the results are presented in Table 3. According to the results of Table 3, a significant positive correlation was observed between the variables of involvement percentage, type of hospitalization and duration of hospitalization with the severity of post-traumatic stress disorder in both groups.

The longer the hospital stay and the percentage of lung involvement, the higher the stress disorder score was reported. Also, those admitted to the intensive care unit reported higher stress scores. Compared to the patient group, a higher correlation was found between the severity of PTSD and the percentage of pulmonary involvement and inpatient ward among patients' families. If the duration of hospitalization was more correlated with the severity of PTSD among patients. Also, no significant correlation was found between stress disorder and demographic variables such as gender, age, level of education and marital status.

Table 3. Correlation of PTSD scores with demographic variables and parameters of COVID-19 disease in patient groups and patients' families.

Variable		Gender	Age	Level of Education	Marital status	Percentage of lung involvement	Inpatient department	Duration of hospitalization
PTSD patients	Spearman (p)	0.056	0.703	0.245	0.113	0.000	0.000	0.000
	Correlation coefficient (r)	-0.124	0.039	-0.120	0.253	0.461	0.637	0.800
PTSD patients' families	Spearman (p)	0.384	0.802	0.754	0.161	0.000	0.000	0.000
	Correlation coefficient (r)	-0.090	-0.026	-0.033	0.063	0.575	0.7412	0.581

The results of regression analysis to evaluate the effect of demographic variables and parameters of COVID-19 disease on the severity of PTSD are presented in Table 4. In both groups, none of the demographic variables, including age, gender, education, and marital status, affected the severity of PTSD. The results of the regression test showed that the variables of hospitalization, length of hospitalization and percentage of lung involvement of patients can predict stress disorder in the families of patients with 43.1%, 57.5% and 1.10%, respectively. These results mean that family members of patients who have been hospitalized in the intensive care unit for more than 10 days, and their lung involvement rate is more than 50%; Higher levels of stress disorder have also been reported. Also, the results of data analysis by regression test in the patient group showed that only the variables of hospitalization and duration of hospitalization (and not the percentage of lung involvement) can predict stress disorder, at 53.9% and 24.2%. These results indicate that patients who have been hospitalized in the intensive care unit for more than 10 days have also reported a higher rate of stress disorder (Table 4).

Table 4. The effect of demographic variables and parameters of COVID-19 disease on the severity of PTSD in patient groups and patients' families.

		B	Standard deviation	Beta	t	P-value
PTSD patients	Constant	-1.012	0.448		-2.258	0.026
	Gender	0.130	0.098	0.083	1.320	0.190
	Age	-0.003	0.003	-0.062	-0.902	0.370
	Level of Education	-0.010	0.038	-0.017	-0.269	0.789
	Marital status	0.171	0.204	0.056	0.839	0.404
	Percentage of lung involvement	0.327	0.162	0.174	2.014	0.047
	Inpatient department	1.063	0.195	0.539	5.437	0.000
	Duration of hospitalization	0.365	0.115	0.242	3.187	0.002
		F=27.480 P=0.000 ADJ.R^2= 0.664				
PTSD Patient Families	Constant	-.808	0.333		-2.424	0.017
	Gender	-0.031	0.067	-0.020	-0.464	0.644
	Age	-0.002	0.003	-0.035	-0.628	0.531
	Level of Education	-0.016	0.031	-0.025	-0.517	0.607
	Marital status	0.199	0.160	0.064	1.247	0.216
	Percentage of lung involvement	0.022	0.118	0.011	0.186	0.853
	Inpatient department	0.867	0.138	0.431	6.299	0.000
	Duration of hospitalization	0.882	0.081	0.575	10.929	0.000
		F=66.371 P=0.000 ADJ.R^2= 0.828				

Discussion

In this study, the severity of PTSD in patients with COVID-19 and their families in 2020 was evaluated. The effect of some demographic variables as well as parameters related to COVID-19 on the severity of PTSD was also analyzed. According to the results, post-traumatic stress disorder had a significant correlation with the percentage of lung involvement, type of ward and length of hospital stay. In patients, the variables of hospitalization and duration of

hospitalization had a significant effect on the severity of PTSD. Also, three variables of lung involvement percentage, type of hospitalization ward and length of hospitalization in the families of patients with COVID were able to significantly predict stress disorder. The present study showed that 14.6% of patients and 16.7% of their families had a severe type of this disorder and the rest of the participants suffered from moderate and mild types. A previous meta-analysis study found that during previous coronavirus epidemics, the prevalence of PTSD was about 32% (12). In the case of SARS-CoV-2, a study in Italy found that the prevalence of PTSD symptoms among COVID-19 survivors was about 30% (23). However, the results of another study in Italy showed that PTSD was diagnosed in 10.4% of the subjects. The results of this study are more consistent with the present study. In a case-control study, PTSD scores in the case group were significantly higher than in the control group (16, 23). In general, the difference in the prevalence of PTSD among COVID-19 patients can be due to various reasons, including cultural-religious differences, as well as the distance between the test and the completion of COVID-19 disease. Because it has been shown that with increasing duration after discharge, patients' PTSD scores increase by 20% (23). In the present study, contrary to the studies mentioned, the DSM-5 (PCL-5) checklist was completed on the day of discharge. In general, previous studies show that approximately 5 to 10 percent of men and 10 to 12 percent of women will experience the disorder in their lifetime. The lifetime prevalence of this disorder in the general population has been reported to be about 8%. About 5-15% of other people may also have subclinical forms of the disorder (3). The present study showed that PTSD is higher in patients admitted to the intensive care unit and their families who feel the risk of death closer. Previous studies have also shown that post-traumatic stress disorder is more common, especially in people who feel that death is imminent or imminent for any reason. For example, in a study of 16,000 participants in Japan, people who had COVID-19 in one of their family members showed more psychological distress than others (13). Following the outbreak of SARS in 2003, both health care workers and quarantined individuals showed signs of post-traumatic stress disorder. PTSD was also reported to be the most common long-term psychiatric disorder among them. The incidence of this disorder in two years after the outbreak of SARS was 47.8% (4). Risk factors for increasing the severity of PTSD symptoms include female gender, living in a city with the disease, poor sleep quality, and experience with a dangerous physical illness, but there is a significant relationship between PTSD and age and education. In another study, in groups under 60 years of age, respondents who had a patient with COVID-19 had a higher score of psychological stress, regardless of gender, and this difference was statistically significant (13). Another study reported that female gender, poor economic status, and fear could predict the severity of PTSD in patients with coronary heart disease (24). In the present study, patients admitted to intensive care units and their companions showed a higher severity of PTSD, which could be due to fear of death. In general, some differences in the findings between the current study and some previous studies may be due to cultural-religious differences. Another reason may depend on the timing of the study. While the present study was performed approximately 2 years after coronary heart disease, some other studies were performed only a few months after the onset of the disease (11). This suggests that stress levels and mental health problems can probably be reduced over time and familiarity with the risks and how to deal with the disease. Previous studies have also reported that the symptoms of PTSD are moderately high in nurses and physicians and all people working in hospital wards and are higher in women working in these wards (9). Although the disorder is more common in women under normal circumstances (other than coronary heart disease), its prevalence in women can be attributed to the more stressful roles they have to play at home during quarantine, as well as the quarantine effect at home. Staying attributed (25). The prevalence of PTSD, depression and sleep disorders increases during quarantine. Fear of getting sick is the most important factor of psychological distress and living in a completely limited environment is very effective on the duration of sleep and mental health (26). In another study, PTSD and depression and anxiety were more common in medical staff who were directly associated with patients (25). Although exposure rates, work experience, occupational support and social support, quarantine, age, gender and marital status have been reported to be associated with PTSD (27). In the present study age, gender and status Marriage had

nothing to do with PTSD. The reason for this difference could be the difference in the statistical population, because, in the study, participants included the treatment staff of the statistical community and in the present study, the participants were patients admitted to their families.

The results of the present study proved that PTSD is a common disorder among patients and families involved with COVID-19. This disorder can severely affect the quality of life of the person and those around them so that the person is involved with its complications for years after the end of COVID-19. At present, it seems that in addition to efforts at various levels to prevent the spread of coronary heart disease and other worrying conditions, special attention should be paid to mental health issues. Programs offered for screening for psychiatric disorders, including anxiety and depression, especially PTSD among patients and their families, and case management by employing psychiatrists, psychologists, and other related medical groups, especially in quarantine cases due to the severity of vulnerabilities and it seems necessary to create peace and trust in the people (8). This study has strengths and limitations that should be noted. This study is one of the few studies that has been performed on the effect of COVID-19 on the incidence of PTSD in patients and their relatives in the world. Also in this study, the modification effect of several parameters such as gender, education, marital status, length of hospital stay, percentage of pulmonary involvement and hospitalization on PTSD severity were measured. However, the present study has some limitations as well. First, due to the physical and psychological effects of COVID-19, patients' energy and motivation to respond were low. Second, patients' families sometimes had time constraints to respond due to the patient's haste to discharge. Also, the physical distance was observed when communicating with patients and their families, which can affect effective communication with them.

Conclusions

In this study, the rate of PTSD in patients with COVID-19 and their families was assessed by a valid questionnaire. The effect of individual factors and parameters related to COVID-19 on the severity of PTSD was also investigated. The results showed that all subjects in the patient group and the patient family group showed mild to severe degrees of PTSD. On average, the severity of PTSD in patients was slightly higher than in the patients' families. The effect of none of the parameters of gender, age, education and marital status on the incidence of PTSD was significant. However, in the group of patients, the variables of hospitalization and duration of hospitalization had a significant effect on the severity of PTSD. The results of this study indicate that COVID-19 disease and exposure to the resulting fear and anxiety cause PTSD in both patients and their families. Given the widespread of coronavirus in communities and the prevalence of PTSD in patients and their families, control measures should be considered to improve mental health for these individuals. These measures may include screening for psychiatric disorders such as anxiety and depression, especially PTSD among patients and their families, and managing PTSD cases by employing psychiatrists, psychologists, and other medical groups.

Author contribution

MGh managed the manuscript, study design, controlling the project and fulfilled the data processing and compiled some sections of the article. **MB**, **ZJ**, **FKh** and **HO** were involved in some sections of the manuscript like collected data, data processing and performed statistical analyses. **MGh** wrote the whole manuscript. All authors revised the article comprehensively and confirmed the final edited version of the paper.

Conflict of interest

The authors reported no potential conflict of interest.

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Evaluation of serum level of uric acid among patients with exacerbation of asthma and patients with controlled asthma

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Abstract

Introduction: Evidence of increased serum uric acid (UA) levels during asthma exacerbations is still unclear. High levels of UA may lead to increased inflammation; in this regard, we aimed to investigate the level of UA and associated factors in patients with exacerbation of asthma attacks and those with controlled asthma.

Materials and Methods: In this study, demographical and clinical data from 300 patients (150 outpatients and 150 hospitalized patients) with asthma who were referred to Razi Hospital, Rasht, Iran, from August 2018 to March 2019 were collected. Also, the UA and spirometry parameters (FEV1, FEV1/FVC) were assessed for patients. All data were analyzed using SPSS version 21 considering a significant level<0.05.

Results: Among 300 patients with asthma, 158 were male, and 142 were female. A significant association was reported between gender, body mass index (BMI), history of smoking, opium consumption, alcohol consumption, number of asthma attacks, family history of asthma, and history of atopy among hospitalized and outpatients (P<0.05). In addition, there was a significant difference between the level of UA in the two groups at the beginning of treatment (P<0.05). Also, a significant difference between the level of UA in hospitalized patients at the beginning and the end of treatment was observed (P<0.05). In addition, a significant difference between the oxygen saturation level among two groups of outpatients and hospitalized patients at the beginning of treatment was seen (P<0.001).

Conclusion: According to our results, the level of UA might be used as a relative predictive factor in the severity of asthma attacks.

Keywords: Asthma, Chronic obstructive pulmonary disease, Outpatients, Hospitalized patients, Uric acid

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Introduction

Asthma is a chronic inflammatory disease of the airways characterized by increased responsiveness of the tracheobronchial passages to various stimuli. Asthma occurs due to temporary blockage of airflow due to chronic inflammation of the airways, identified by periodic and reversible attacks of wheezing, shallow breathing, shortness of breath, and cough. From the etiological point of view, asthma is a heterogeneous disease that genetics, environment, and allergens contribute to the onset and continuation of it (1,2). The prevalence of asthma is increasing in many parts of the world, and it is estimated that 4-5% of the population of the USA has asthma. Bronchial asthma occurs at any age, with the most frequent onset at the early years of life (3).

Asthma is diagnosed according to some symptoms, physical examination, chest X-ray, and lung function diagnostic tests measuring FEV1, PEF, and most importantly, FEV1/FVC through spirometry (3,4). A disproportionate response of T-helper cells usually causes airway restriction in asthma type 2 (Th2) to allergens. Cytokines produced by Th2 are mainly responsible for regulating many features of asthma and lead to airway inflammation, excessive mucus secretion, and structural changes in the airway path (5,6).

Uric acid (UA) is a product of the purine metabolism pathway first recognized as a danger signal released from dying cells (7). It was reported that the level of UA was increased in the airways of asthmatic patients exposed to allergens. The administration of UA crystals with protein antigen leads to increased Th2 immunogenicity and clinical features of asthma through dendritic cell activation, splenic tyrosine kinase, and inositol triphosphate (IP3) kinase signaling (8).

These studies indicate that UA is an essential initiator and enhancer of Th2 immunogenetic in asthma, reflecting airway inflammation. In addition, strategies that target the inhibition of UA synthesis with allopurinol or the suppression of the uricase enzyme lead to a reduction in the production of Th2 progenitor cytokines, pulmonary inflammation, repair, and fibrosis (9,10). Hypoxia can explain the possible potential mechanism of the impact of UA on asthma during the exacerbation of asthma, which is induced by UA, oxidative stress, and inflammation-inducing lung tissue damage that leads to increased levels of UA. Also, high levels of UA may lead to increased inflammation that ultimately impairs lung function (11,12).

Previous studies have indicated that serum levels of UA increased in hypoxic conditions such as chronic heart failure, primary pulmonary hypertension, and chronic obstructive pulmonary disease (COPD) compared to hyperoxia/normoxia conditions (13–15). Moreover, the exact mechanism also occurs during the exacerbation of asthma and bronchospasm caused by it. However, evidence of increased serum levels of UA during asthma exacerbations is unclear; only a few studies have been conducted on this issue. In this regard, we aimed to compare the serum level of UA among patients with controlled asthma and patients with exacerbation of asthma attacks at the beginning of hospitalization and at the time of discharge from the hospital in Rasht, Iran.

Materials and Methods

Study design

This study collected demographical data and clinical characteristics of 300 patients (150 outpatients and 150 hospitalized patients) with asthma who were referred to Razi hospital, Rasht, Iran, from August 2018 to March 2019. Patients with incomplete data and a history of malignancies were excluded from the study. All data were recorded from the patients' archives. Asthma exacerbation was diagnosed based on GINA guidelines, which include a set of specific clinical findings, including relevant medical history, progressive increase in shortness of breath, cough, wheezing, chest tightness, pulse rate, respiratory rate, oxygen saturation, and peak flow measurement (16). The patients with lung diseases, in addition to asthma, suspected or confirmed malignancy, multiple disorders or infection, acute gastrointestinal bleeding, cardiovascular diseases, kidney failure, and consumption of foods containing large amounts of purine, were excluded from the study. This study was approved by the ethical committee at the Guilan

University of Medical Science [IR.GUMS.REC.1397.357].

Variables

Collected data included age, gender, body mass index (BMI) as low weight (BMI<18.5 kg/m^2), average weight (BMI=18.5–24.99 kg/m^2), overweight (BMI=25–29.9 kg/m^2), and obese (BMI≥30 kg/m^2), a family history of asthma, keeping pets, history of exposure to allergens, history of smoking, alcohol consumption, opium consumption, underlying diseases, asthma medication, obstructive symptoms, sinusitis, mental illness, history of reflux, atopy, level of serum UA, and spirometry indexes such as FEV1 and FEV/FVC. In addition, the level of UV was measured by kit (Bionik, Iran) with the BT3500 (Biotecnica Instruments. SpA -Italy) auto-analyzer.

Statistical analysis

All data were analyzed using SPSS software version 21. The quantitative data were reported as mean ± standard deviation (SD), and qualitative data were also described as numbers and percentages. The normality was measured using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The comparison of serum levels of UA in patients with exacerbation of asthma attacks and those with controlled asthma was measured using the independent t-test (Mann-Whitney if were non-parametric). The correlation between the serum level of UA and variables was measured using Pearson's correlation test (Spearman's if were non-parametric). The comparison of the level of UA in patients with exacerbation of asthma attack at the beginning of hospitalization and at the time of discharge was performed using paired t-test (Wilcoxon's if were non-parametric). Logistic regression was used to investigate the relationship between the level of UA and asthma considering the effects of other intervening variables. The statistical significance level of the data was defined as P<0.05.

Results

Demographical data and clinical characteristics of patients were demonstrated in Table 1. Of the 300 studied patients, 158 were male, and 142 were female, with a mean age of 60.19±16.48 years. The mean age of hospitalized patients was lower than outpatients, 56.96±17.15 years (24-90) and 60.19±15.16 years (27-86), respectively, but no statistically significant difference was reported (P=0.610) between the mean age of two groups. The mean BMI in hospitalized and outpatient groups was 78.78±18.98 kg/m^2 and 73.96±19.22 kg/m^2, respectively, representing a significant difference between the two groups (P=0.010). The average time of asthma diagnosis in outpatients and hospitalized groups was 88.19±117.71 months and 106.71±101.7 months, respectively (P=0.140).

Evaluating the presence or absence of daily exposure to occupational allergens among patients revealed that most hospitalized patients were exposed to allergens compared to the outpatients (P=0.001). Also, keeping pets was more frequent among hospitalized patients than outpatients (P<0.05). Most hospitalized patients had a history of opium consumption, and the frequency of smoking was significantly higher in hospitalized patients than in outpatients (P<0.001). There was a significant difference between the two groups based on alcohol consumption and a history of mental illness (P<0.05).

The average times of asthma attacks in hospitalized and outpatients were 2.02±1.27 months (0-8), and the mean age of 66.68±21.73 years old (1-50) during their lifetime (P<0.001). However, the frequency of hospital administration due to asthma attacks was significantly higher in the hospitalized group compared to the outpatients (57.12±87.78 vs. 2.09± 1.21, P=0.010). In addition, the prevalence of a family history of asthma and consuming asthma medication was higher among hospitalized patients (P<0.05). Also, pulmonary construction symptoms were highly reported in both groups, while the prevalence of atopy was significantly higher among hospitalized patients (P<0.001).

The mean serum level of UA was 4.82±1.19% mg/dL in the outpatient group; and in the group of hospitalized patients, at the beginning of the treatment, the mean serum level of UA was 7.31±1.85 mg/dL and at the end of treatment was 5.69±1.11 mg/dL (P<0.001), Figure 1. There was a significant difference between the serum level of UA in the two groups (P<0.001). The mean oxygen saturation level in the outpatient group was 91.14±1.81%, and in hospitalized patients at the

beginning and the end of treatment, were 78.13±2.95% and 90.65±1.91%, respectively (P<0.001).

Evaluation of the level of FEV1 illustrated that in the group of outpatients, it was 65.59 ± 3.3%. In the group of hospitalized patients, at the beginning of the treatment, the mean serum level of UA was 69.95±44.2 mg/dL, and at the end of treatment was 94.76±62.2 mg/dL (P<0.001). Also, the level of FVC in the group of outpatients was 65.05±2.99, and in hospitalized patients at the beginning and the end of treatment, were 44.2±13.9 and 57.2±46.5, respectively (P<0.001).

Table 1. Demographical data and clinical characteristics of outpatients and hospitalized patients with asthma.

Variables		Hospitalized patients n (%)	Outpatients n (%)	P value
Gender	Male	79 (52.7)	79 (52.7)	0.500
	Female	71 (47.3)	71 (47.3)	
Expose to allergen	Yes	124 (82.7)	34 (22.7)	0.001
	No	26 (17.3)	116 (77.3)	
Keeping pets	Yes	90 (60.0)	58 (38.6)	0.010
	No	60 (40.0)	92 (61.4)	
History of smoking	Yes	82 (54.6)	44 (29.3)	<0.001
	No	68 (45.4)	106 (60.7)	
History of underlying disease	Yes	97 (64.6)	85 (56.6)	0.180
	No	53 (35.4)	65 (33.4)	
History of opium consumption	Yes	68 (45.4)	26 (17.3)	<0.001
	No	82 (54.6)	124 (82.7)	
History of alcohol consumption	Yes	50 (30.0)	34 (22.7)	0.050
	No	150 (70.0)	116 (77.3)	
History of mental illness	Yes	16 (10.7)	30 (20.0)	0.030
	No	134 (89.3)	120 (80.0)	
History of sinusitis	Yes	73 (48.7)	21 (14.0)	<0.001
	No	77 (51.3)	129 (86.0)	
History of reflux	Yes	40 (26.7)	45 (30.0)	0.620
	No	110 (73.3)	105 (70.0)	
Family history of asthma	Yes	64 (42.7)	44 (29.3)	0.020
	No	86 (57.3)	106 (60.7)	
History of asthma medication	Yes	117 (78.0)	95 (63.3)	0.009
	No	33 (22.0)	55 (36.7)	
Presence of obstructive symptoms	Yes	115(76.6)	132 (88.0)	0.001
	No	35 (23.4)	18 (12.0)	
History of atopy	Yes	99 (66.0)	38 (25.4)	<0.001
	No	51 (34.0)	112 (74.6)	

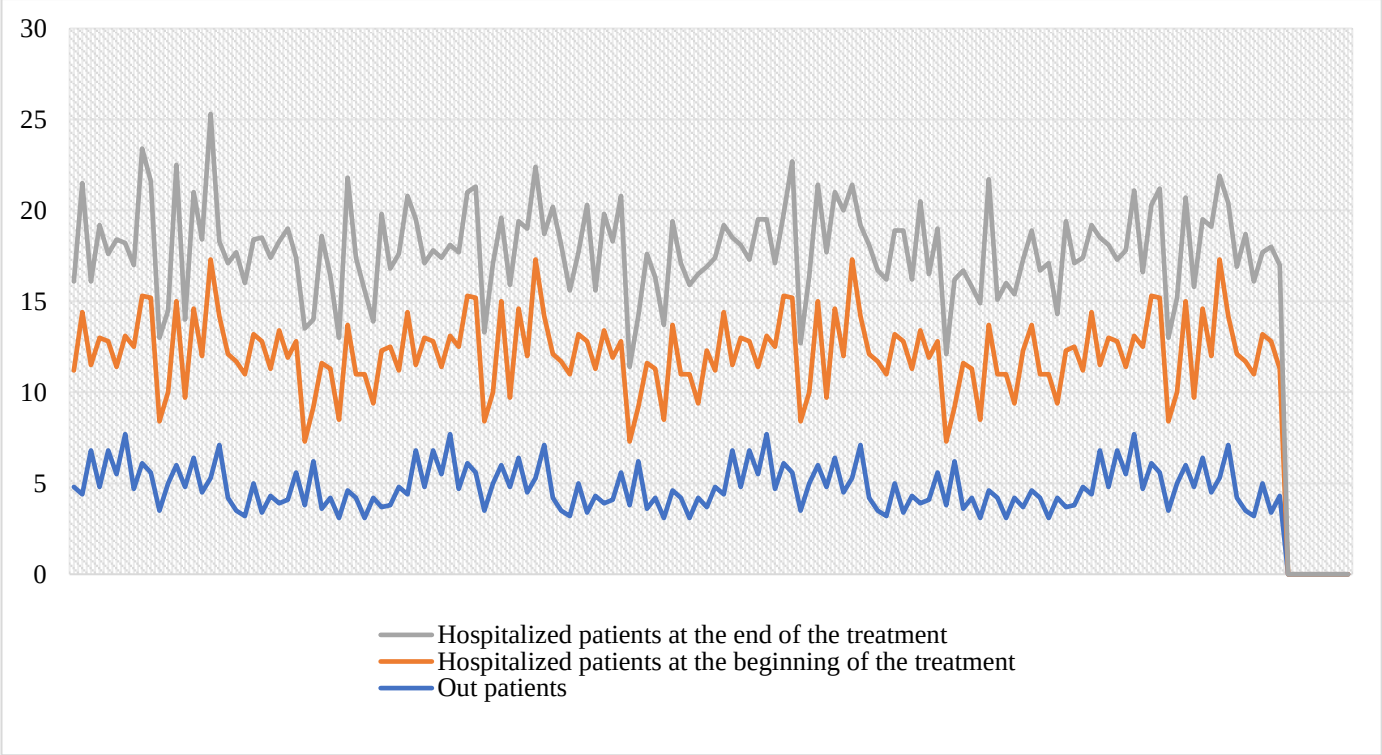

Figure 1. The level of uric acid in the outpatient group, and in the group of hospitalized patients at the beginning and end of the treatment.

No statistically significant association was reported between the serum level of UA and oxygen saturation among hospitalized patients with exacerbation of asthma attack at the time of administration and at the time of discharging (r=0.4, P=0.620 vs. r=0.06, P=0.410), respectively. While a significant association was reported between the level of UA and duration of hospitalization (r=0.92, P=0.008). Assessing the correlation of serum level of UA with spirometry indices (FEV1, FEV1/FVC) among hospitalized patients at the times of administration and discharge represented no statistically significant differences (r=-0.119, P=0.14 vs. r=0.05, P=0.540) for FEV1 and (r=-0.12, P=0.12 vs. r=-0.04, P=0.620) for FVC. In the multivariate logistic regression analysis of significant factors using the Backward method between two groups, the initial level of UA was significantly different (P<0.05). The results of the multivariable logistic regression demonstrated that among variables, only the consumption of medication was significantly associated with the level of UA in hospitalized patients (P<0.05) (Table 2).

Table 2. The results of multivariate logistic regression in the investigation of factors related to the initial UA level; and secondary UA levels in hospitalized patients.

Variables	Regression coefficient	Standard error	Standardized regression coefficient	Test statistics	P-value
Outpatients/Hospitalized patients	2.44	0.18	0.61	13.45	<0.001
Use of medication	-0.491	0.219	-0.182	-2.23	0.027

Discussion

The present study evaluated the serum level of UA among patients with exacerbation of asthma attacks and controlled asthma. Most of the patients in the current study were middle-aged males (52.6%). Due to our findings, the level of UA in hospitalized patients was significantly higher than in outpatients. Also, the level of UA at the beginning of hospitalization was

significantly higher than at the time of discharge, similar to other studies (17–19). In addition, our results illustrated that the level of UA was significantly different in the two groups regarding age, gender, BMI, a history of smoking and opium, alcohol consumption, and asthma medication ($P<0.05$).

This increase may be related to the asthma attack due to the inflammatory state caused by cell necrosis and apoptosis, followed by an increase in purine metabolites, including DNA and RNA. This increase in metabolites elevated the function of the xanthine oxidase (XO) enzyme, which results in higher levels of UA (20). Another mechanism that has been described as the increased UA following an asthma attack is the increase in the degeneration of adenosine three phosphates (ATP) and the increase in the production of UA by the XO enzyme following hypoxia that plays a diverse role in both acute and chronic lung inflammation (21,22). In addition, several studies have mentioned the relationship between the higher level of UA and the increase in the level of inflammatory cytokines such as C reactive protein (CRP), tumor necrosing factor (TNF), and interleukin-1 (IL-1), which are higher in the acute phase compared to the chronic phase (11,18,23).

The current study found no significant association between hospitalization frequency and UA level in the two groups ($P>0.05$). This finding suggests that UA levels may not be a reliable marker for predicting the likelihood of hospitalization in patients with asthma exacerbation or controlled asthma. While previous studies have suggested a potential link between UA and the incidence of asthma (24), our results indicate that other factors might play a more dominant role in determining hospitalization rates. This negative correlation implies higher UA levels may indicate poorer respiratory function and lower oxygen saturation levels. Moreover, there was a negative association between the level of UA and FEV1 and FVC. These findings align with previous research suggesting that UA could contribute to airway inflammation and obstruction, impairing lung function. While their result of the association between hospitalization frequency and UA level did not consistent with our study (18). However, it is essential to note that our study only establishes an association and does not establish a causal relationship.

A study by Lin et al. found a significant relationship between levels of UA and functional lung disorders (17). Other studies reported that the level of UA has an inverse association with spirometry indicators, primarily due to tissue hypoxia during the exacerbation of asthma that causes UA production, and secondly, because the increase in UA levels causes lung tissue inflammation and decreases lung function (25,26).

Recent studies illustrated the impact of the antioxidants such as vitamins E and C in reducing the level of UA, which can indicate that the level of UA may be changed due to cardiovascular diseases, diet patterns, lifestyle, kidney function, and familial purine metabolism problems (27,28). Moreover, the age of the patients can affect the level of UA, which explains the variety of results in different studies (27). Also, some studies have pointed out that vitamin D, E, and C deficiency affects the exacerbation of asthma attacks (29,30).

Limitations

One limitation of this study is referring to the recall bias of the patients to report the duration time of asthma. Also, the impact of consuming supplements was not investigated in the current.

Conclusions

According to our results, the level of UA at the beginning of hospitalization was significantly higher than outpatients and at the time of discharge, which might be used as a predictive factor for asthma severity assessment.

Author contribution

AT, **SAAF**, and **AJ** conceptualization, the original draft writing, investigation, and formal analysis; **AT** and **AJ** conceptualization, supervision, and project administration; **SAAF** and **AJ c**onceptualization, and project administration; **AT**, and **MGH** investigation; **AJ** and **MGH** writing including reviewing and editing and investigation.

Conflict of interest

The authors reported no potential conflict of interest.

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The role of astrocytes in Alzheimer's disease, A systematic review

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Abstract

Introduction: Alzheimer's disease (AD), the most common neurodegenerative disease in the world, appears in two forms, early and late. Pathologically, an amyloid beta peptide is the hallmark of this disease which is followed by synaptic dysfunction, brain atrophy, and accumulation of neuronal tangles. The purpose of this study is to review the researchers on astrocytes' role in the progress of AD.

Materials and Methods: A comprehensive search was conducted in databases articles focusing on key terms "Inflammatory reactions", "Alzheimer's disease", "Inflammatory factors" and "Astrocytes" and Boolean operators. Articles before 2001 were removed.

Results: Finally, after analyzing the selected articles, 20 articles were extracted and included in this review.

Conclusion: Astrocytes are a group of glial cells in the central nervous system .The inflammatory activity of astrocytes plays a role in the development and progression of Alzheimer's disease. They strengthen the function of synapses by secreting neurotrophic factors. They also clear amyloid beta peptides from nerve tissue. Amyloid beta peptides bind to specific receptors on these cells and change the activity of these cells from anti-inflammatory to inflammatory type. It seems that astrocytes play a pivotal role in the development and progression of AD, particularly at the late stage of the disease. Finding a rational strategy to suppress inflammatory A1 phenotype might be a promising tool to slow down the progress of AD.

Keywords: Alzheimer's disease, Astrocytes, Inflammatory factors, Amyloid beta

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Introduction

Alzheimer's disease (AD) is the most important and common neurodegenerative disease in the world. Global statistics state that in 2017, about 44 million people were affected by this disease. In the United States, AD is the only disease without a cure among the 10 leading causes of human death. In 2017, the costs paid in America for these patients were 259 billion dollars. It is predicted that by 2050, these costs can increase to an impressive figure of 1.1 trillion dollars (1, 2). This disease exists in two forms: early or familial and late sporadic (3). The late type affects individuals over 65 years old, and the early type includes a small number of affected people and occurs under 65 years of age (4). Currently, the amyloid beta hypothesis stands as the most accepted hypothesis which states that amyloid beta (Aβ) peptides are the early finding in the brain of affected people. Therefore, excessive accumulation of amyloid peptides in the form of amyloid plaques in the brain tissue disturbed neural connections and initiates neuro inflammation however, in normal brain Aβ is destroyed by various factors such as neprilysin, endothelin-converting enzyme, insulin-degrading enzyme, angiotensin-converting enzyme, plasmin and cathepsin D (5-8).

Other important symptoms of this disease include functional disorders of synapses, brain atrophy, and the creation of neuronal filament coils inside nerve cells, which consist of tau-hyperphosphorylated protein (1, 2).

Despite all the efforts made in the field of understanding this disease and the factors responsible for initiating AD, a suitable and guaranteed treatment has not yet been provided. Therefore finding a new strategy to control the disease and prevent its progression has great importance (9, 10).

Materials and Methods

A complete and comprehensive search was conducted in the literature available in PubMed, Scopus, and Google Scholar databases, and articles were searched using the key terms "inflammatory reactions", "Alzheimer's disease", "inflammatory factors" and "astrocytes".

Key terms were selected using MeSH and Boolean operators such as "AND", "OR" and "NOT" were used to connect these terms. From October 2021 to December 2022, two researchers searched independently.

Results

In this study, articles on Alzheimer's, inflammatory cytokines, memory, and astrocytes were selected. In the following, the articles that were presented about inflammatory diseases, brain, and depression, and also the articles before 2001 were removed. Also, to avoid excluding other valuable studies, a search was conducted to extract other related studies Abstract. Finally, 20 studies were extracted and included in this review.

Discussion

Astrocytes are a group of glial cells present in the central nervous system (CNS) (11). These cells play important and different roles in the CNS. Perhaps their most important role is to initiate immune and inflammatory responses to prevent possible damage to nerve tissue. Astrocytes are the main regulators of magnesium concentration in the brain (11). Along with pre-synaptic and post-synaptic neurons, they are the main components of synapses and play a role in regulating synaptic plasticity by secreting gliotransmitter (12, 13).

Astrocytic dysfunction results in the failure of Aβ clearance.

The balance between the production and clearance of Aβ plays a detrimental role in AD, and an inefficient Aβ clearance may be more susceptible to AD (14). An increasing number of studies have evidenced that astrocytes act as a cellular player in Aβ clearance and degradation from the brain parenchyma into the perivascular space, across BBB (Figure 1), or by enzymatic degradation (15).

The BBB would be a diffusion barrier that impedes the influx into the brain parenchyma of certain molecules based on polarity and size. The principal cellular constituents of the BBB include capillary endothelial cells, perivascular pericytes, and astrocyte end-feet (Figure 1A). Maintaining the normal physiological

function of astrocytes will have a critical role in the transport of Aβ across BBB into the circulation which is mainly mediated by receptor for advanced glycation end products (RAGE) and lipoprotein receptor-related protein 1 (LRP1) in endothelial cells (16). Since RAGE acts as an important transporter via regulating the influx of circulating Aβ into the brain while the efflux of brain-derived Aβ into the circulation via BBB is implemented by LRP1 (14) (Figure 1B). In addition to the direct factor that astrocytic dysfunction leads to the failure transport of Aβ across BBB, astrocytic dysfunction may indirectly result in other avenues which are associated with the failure of Aβ clearance from the brain, such as abnormal interstitial fluid drainage and the failure of microglial phagocytosis (17). Astrocytic dysfunction probably induces the occurrence of neuroinflammation and oxidative stress, and then both neuroinflammation and oxidative stress contribute to abnormal interstitial fluid drainage and the failure of microglial phagocytosis, and the failure of Aβ clearance, finally (18).

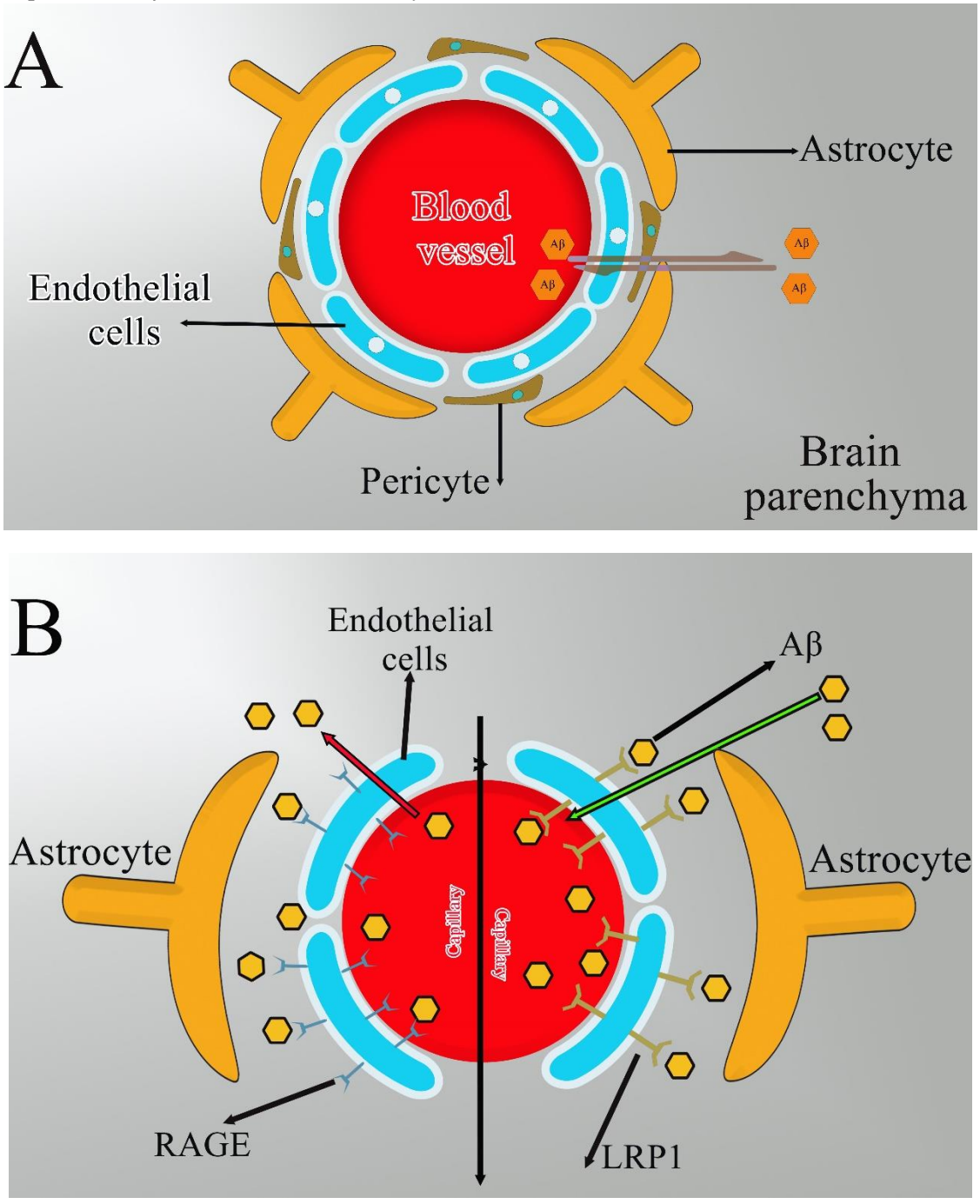

Figure 1. The proposed mechanism where astrocytes are associated with Aβ clearance).

Major Roles of Astrocytes in Alzheimer´s Disease

Alzheimer's disease (AD) is characterized by amyloid beta accumulation (Aβ or senile plaques), formation of hyperphosphorylated tau neurofibrillary tangles, neuroinflammation, synaptic demise, neuronal death, and brain dysfunction leading to severe cognitive impairment. The amyloid hypothesis originally postulated a linearity of progression according to Aβ accumulation, which subsequently led to the formation of tangles and other pathological hallmarks (19). The role of glial cells, and astrocytes in particular, in the neuropathology of many neurodegenerative diseases, is universally acknowledged (20).

The risk of AD is associated with genes mainly expressed by glial cells, either astrocytes, microglia, and/or oligodendrocytes (21).

Apolipoprotein E (APOE), a major genetic risk factor in Late-Onset AD (LOAD), is mainly expressed in astrocytes in the healthy brain (22) and contributes to the accumulation of Aβ in the brain (23).

Other genes associated with AD such as Clusterin (CLU) and Fermitin family member 2 (FERMT2) are similarly predominantly expressed by astrocytes. Reactive astrogliosis is prominent in AD being an early event in human patients and in animal models, possibly even preceding the formation of Aβ Aβ

plaques (24).

These data suggest a crucial role of astrocytes in the pathogenesis of AD. Morphological studies in post-mortem AD patient brains demonstrated close interaction between astrocytes and Aβ depositions (25).

It is however unclear how this close interaction translates into the disease progression. Astrocytes, when associated with senile plaques, become reactive with morphological hypertrophy manifested by thicker processes and increased expression of the intermediate filament proteins glial fibrillary acidic protein (GFAP), vimentin, nestin, and synemin (26).

Reactive astrocytes are found in both human AD patient brains [75] and AD mice models (27)

Pathological signals inducing astrogliosis in AD can be associated with damaged cells; Aβ by itself is a strong instigator of astrocyte reactivity. At the molecular level, Aβ induction of astrogliosis remodeling is mediated by Ca^{2+} release from the endoplasmic reticulum; inhibition of the latter suppresses astrocytic reactivity (28).

In AD, astrocytes undergo relatively mild isomorphic gliosis and astrocytic domains do not overlap, potentially indicating a defensive nature of the astrocytic response. Indeed, inhibition of astrogliosis exacerbates Aβ accumulation and histopathology in AD mice (29). Reactive astrocytes in the vicinity of plaques display aberrant calcium dynamics (30).

In particular, human AD brains are characterized by severe disruption or even complete disappearance of interlaminar astrocytes (31). Atrophic astrocytes are characterized by reduced volume and thinner processes. In the 3xTg-AD mice model, atrophic astrocytes appear as early as 1 month of age in the entorhinal cortex (EC), and the atrophy is sustained after 12 months of age when Aβ plaques begin to appear (32).

Human astrocytes derived from induced pluripotent stem cells (iPSC) from patients with both familial and sporadic forms of AD also show atrophic phenotypes in vitro compared to control cells (33).

While atrophy might lead to loss of astrocyte homeostatic functions and give rise to synaptic dysfunction, increased excitability, and/or damage of the BBB, (Figure 2) very little functional data are available. Finally, the neurodegenerative process may directly damage astrocytes resulting

in clasmatodendrosis, characterized by fragmentation and disappearance of distal fine processes, along with swelling and vacuolation of the cell body (34) (Figure 2).

Astrocytes could be, in principle, involved in Aβ production as they upregulate β-secretase 1 and the amyloid precursor protein (APP) in the diseased brain (35).

However no quantitative data points to astrocytes as the major source of Aβ. Astrocytes are more likely to participate in Aβ clearance and elimination by different mechanisms. Astrocytes express aquaporin 4 (AQP4) water channels in their vascular end-feet and play an

essential role in the glymphatic system implicated in the clearance of Aβ (36) (Figure 2).

They also produce amyloid beta-degrading proteases that cleave the peptide into smaller fragments. The metalloendopeptidases neprilysin (NEP), insulin-degrading enzyme (IDE), and endothelin-converting enzymes 1 and 2 (ECE1 and ECE2) are all expressed in astrocytes and contribute to the degradation of monomeric Aβ species(37).

Astrocytes also express matrix metalloproteinases MMP-2 and MMP-9 which degrade both fibrillar and monomeric Aβ (37) (Figure 2).

Clearance of Aβ can be mediated by extracellular proteins APOE, ApoJ/Clusterin, β1-antichymotrypsin (ACT), and β-2-macroglobulin (β-2-M), all produced by astrocytes (Figure 2); these proteins promote the transport of β-2-macroglobulin Aβ across the BBB to the circulation either alone or in association with LRP1 and VLDLR receptors (37).

Recent studies report that iPSC-derived human astrocytes and mouse astrocytes expressing APOE4 are less efficient in clearing Aβ than those expressing APOE3 (38). Expression of APOE4 also leads to the degeneration of pericytes thus facilitating the breakdown of the BBB further contributing to cognitive impairment in APOE4 carriers (39). In AD, reactive astrocytes interact with neurons, microglia, and oligodendrocytes by releasing feed-forward signals and contributing to the vicious cycle that leads to neurodegeneration. Of note, β-2-macroglobulin β-2-macroglobulin Aβ can activate the NF- κ B pathway in astrocytes, which leads to the release of the complement protein C3 (Figure 2). The C3 binding to the microglial receptor C3aR alters β-2-macroglobulin -amyloid beta phagocytosis while the C3 binding to the neuronal receptor C3aR disrupts dendritic morphology and network function, both effects contributing to AD pathogenesis (40). Both NF- κB and C3 cascades are activated in the human AD brain and AD mouse models (41). About 60% of the astrocytes in the prefrontal cortex of AD patients are C3-expressing astrocytes (41) and could contribute to neuronal damage; although further analyses are needed for confirmation.

In AD, reactive astrocytes participate in shifting the excitation-inhibition balance through secretions of GABA. In a healthy brain, astrocytes do not contribute much to GABA production, however, in AD GABA starts to be synthesized by astrocytes through the putrescine-MAO-B pathway (42). In this way, reactive astrocytes start to secrete GABA thus increasing inhibition, likely to be a defensive response against neuronal hyperexcitability that seems to be a universal result of AD progression (43).

An increase in MAO-B expression in astrocytes, which accompanies AD, also results in a hyperproduction of hydrogen peroxide that may instigate neuronal damage and death (44) metabolic deficits (45) and mitochondrial dysfunction also contribute to AD progression (46). Extensive transcriptomics and proteomics studies revealed deficient mitochondrial bioenergetics in AD brains (47). Exposure of mouse astrocytes to Aβ up-regulates superoxide dismutase thus increasing oxidative stress (48); while the continuous infusion of Aβ into mice brains results in a substantial increase in the production of hydrogen peroxide (49) overproducing astrocytes has been recently detected in the brains of AD model mice (44). The toxic effect of Aβ on astrocytes is manifested by mitochondrial depolarisation with subsequent loss of Ca^{2+} homeostasis (50). At the same time, astrocytes can exert neuroprotection at different stages of AD. Both astrogliosis and microgliosis in response to Aβ increase glial secretion of transforming growth factor (TGF-β) (Figure 2). TGF-β protects neurons from Aβ toxicity and enhances Aβ clearance by microglia (52). Moreover, astrocytes surrounding Aβ plaques demonstrate phagocytic activity and can phagocytose neuritic dystrophies in both mouse models and AD patients' brains, further suggesting the beneficial roles of astrocytes in AD (51). These data show that astrocytes actively contribute to the pathogenesis of AD. At the same time, many questions remain to be addressed. What astroglial states/phenotypes are found at different stages of AD? How do astrocyte states/phenotypes differ between brain regions, which are known to have different vulnerabilities to AD? How do astrocytes crosstalk with other brain cells? Are they able to promote neurodegeneration? How do AD risk genes modulate astroglial responses in AD? New methodologies such as RNA sequencing and spatial transcriptomics in combination with the use of human iPSC-derived

models and CRISPR-based studies are providing a deeper understanding of how astrocytes evolve during the course of AD.

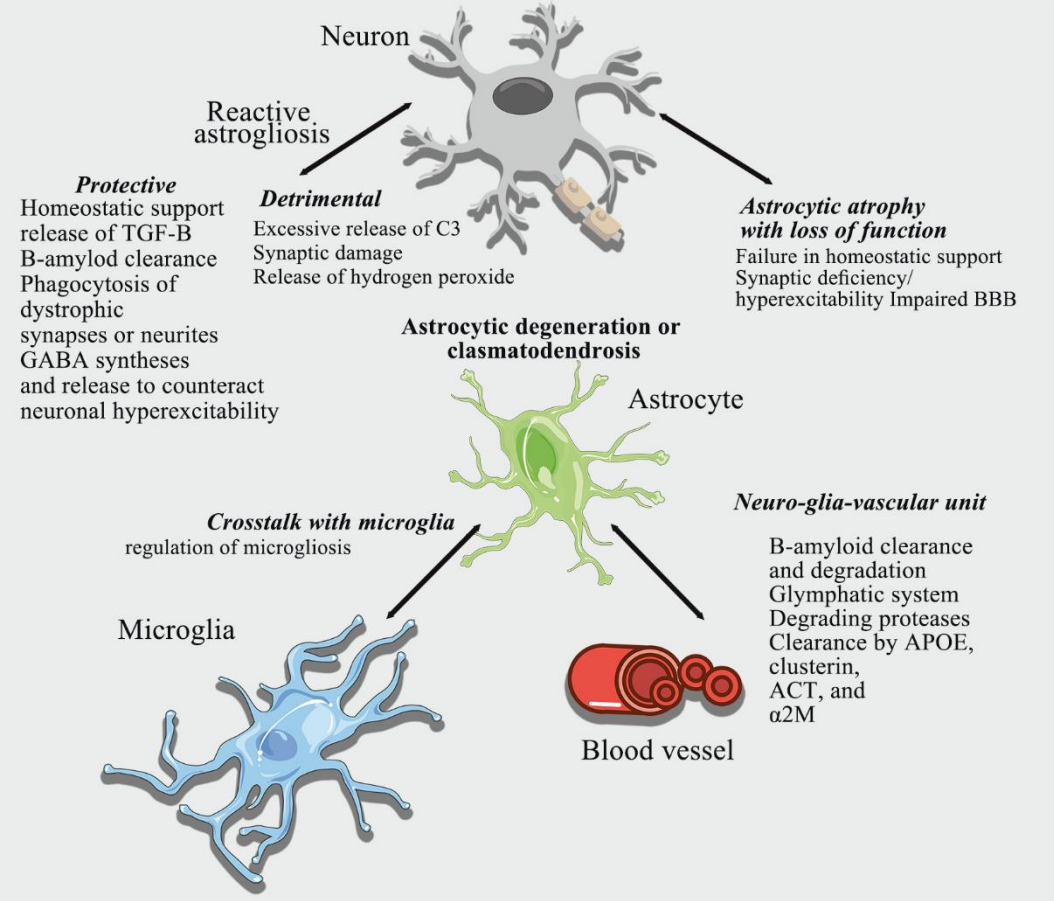

Figure 2. Contribution of astrocytes to Alzheimer´s disease.

By secreting neurotrophic factors such as tumor beta growth factor (TGF-β), brain-derived neurotrophic factor (BDNF), and neuron growth factor (NGF), astrocytes contribute to the growth of dendritic appendages and strengthen the function of the synapse (52) .They are also able to convert glucose into lactic acid and then, neurons use this lactic acid for pyruvate synthesis and metabolic functions (53). Astrocytes, possessing the enzyme glutamine synthetase, receive glutamate, which is the most important neurostimulator mediator in the CNS, and form part of the glutamine-glutamate cycle (54). Astrocyte mitochondria are concentrated near sites of homeostatic transport (50). These mitochondria provide energy for the Na^+/K^+ ATPase pump, which in turn causes the accumulation of neurotransmitters such as glutamate and regulates cytosolic Ca^{2+} concentration (55). A deficiency in ATP supply may affect glutamate clearance and increase excitotoxicity. Mitochondrial dynamics and function are also impaired in human astrocytes with apolipoprotein E1 (APOE) allele (56).

In addition, there are some indications that astrocytic mitochondria can be transferred to neurons and contribute to neuronal bioenergetics. In particular, these processes seem to support neuroprotection after stroke (57). Studies show that astrocytic neuron transfer exerts neuroprotection in the context of Parkinson's disease (58).

Whether this process contributes to AD remains an exciting and unanswered question. Astrocytes seem to express lipoprotein E, neprilysin, insulin-degrading enzyme, endothelin-converting enzyme, angiotensin-converting enzyme, and matrix metalloproteinases, and clear Aβ peptides from nerve tissue (59). Recently the neuroprotective role of astrocytes also was reported (23, 24). They inhibited astrocytes in the AD model and reported that not only was cognition deficit exacerbated

but also neuroinflammation was apparent in their brain indicating the progress of AD in the absence of astrocytes (60).

However, it should be emphasized that astrocytes are a double edge sword playing both inflammatory (A1 type) and anti-inflammatory roles (A2 type). Considering diverse phenotypes of neurodegenerative A1 and neuroprotective A2 astrocytes, and the multidimensional functions of reactive astrocytes (41, 61), understanding the complete role of reactive astrocytes remains at the beginning of its path.

In a series of experiments, two groups of mice with certain characteristics were mated together. The first group was mice that had a gain-of-function mutation in the Aβ precursor protein (APP) gene and the other group was mice that lacked the NLPR3 inflammasome (a mediator molecule in the pathway inflammation related to receptors in astrocytes). Newborn babies showed better spatial memory compared to parents with mutations in APP, lower caspase 1 activity and more clearance of Aβ, and this itself can be proof of the role of astrocytes in the worsening of AD (52, 62).

Investigations show that Aβ peptides are connected to these cells through receptors located on the surface of astrocytes, and then the activity of these cells is changed to ward destruction and damage (52, 63-65).

One of the most important receptors and signaling involved here is the advanced glycation end products (RAGE/NF-κB) pathway, which is activated through the binding of Aβ to the RAGE receptor (56, 65). RAGE has two isoforms: the s-RAGE isoform, which is its soluble type, and the m-RAGE isoform, which is attached to the membrane and can have harmful effects in certain conditions, including bonding with Aβ (66).

The activation of this path causes the activation of a chain of molecular interactions in astrocytes and then in the entire nervous tissue. The nuclear factor kappa light chain enhancer of activated B cells (NF-κB) is a gene transcription complex that is normally inactively located in the cytoplasm. This complex generally consists of two parts. A regulatory part (in this case, called IκB) and an acting part (67). The binding of Aβ to RAGE, through the classical or canonical pathway, activates a kinase that phosphorylates the regulatory part of the NF-κB complex (IKK for short). This kinase, in turn, phosphorylates IκB and separates it from the complex and migrates into the cell nucleus, and promotes the transcription process of cytokine genes with the help of certain factors. Among these factors is bromodomain-containing protein 4 (BRD4). This protein is one of the three members of the benign essential tremors (BET) family. The members of this family share a sequence of about 110 amino acids called bromodomain (12, 67-69). In total, all these events cause the expression of specific inflammatory proteins and cytokines, and adhesion molecules in white blood cells. And in this way, astrocytes change from a neurotrophic state to a neurotoxic state (67).

In the field of various human diseases, numerous animal studies have been planned. Today, many specific animal models are used in medical research, including models of stroke (70), heart failure (71, 72), and kidney failure (73). In the field of mechanism, prevention, and treatment of Alzheimer's disease, many animal studies have been used, for example, the study conducted by Nikkar et al (60) simultaneous administration of bromodomain and histone deacetylase I inhibitors alleviates cognition deficit in Alzheimer’s model of rats .

Among the most important inflammatory cytokines that are secreted, all types of interleukins (ILs) such as IL-1β, IL-6, IL-10, IL-17, IL-18, tumor necrosis factor (TNF-α), interferons (IFNs) especially IFN-γ and chemokines such as Monocyte chemoattractant protein (MCP) and macrophage inflammatory protein (MIP) noted (74, 75).

The release of these cytokines causes neutrophils and macrophages to be called, neurons to be damaged, dendritic spines to be destroyed, and synapse dysfunction, resulting in cognitive defects. The binding of these cytokines to their receptors in neurons causes the activation of mediators such as protein kinase C (PKC), caspase 1, caspase 3, p38 and pathways such as phosphoinositide 3-kinases, caspase 3 activity alone is sufficient to trigger the events leading to neuronal apoptosis. Caspase 3 can also cause abnormal processing of tau protein so that this protein is broken at the place of aspartate 421 root and a product is created that accumulates faster than the natural form of tau in the neuron and shortens the life of the neuron (76, 77).

In addition, these cytokines can affect the 5'-UTR region of the APP gene, causing its overexpression and eventually increasing Aβ (78).

They can also cause the activation of beta and gamma-secretase enzymes in the path of APP amyloidogenic processing and regularly increase the production and secretion of Aβ (79). In response to amyloid beta, calcineurin protein is activated in astrocytes and this causes the activation of a transcription factor called a nuclear factor of activated T-cells (NFAT) in this way, the production and secretion of cytokines will increase (52). By binding to their receptors on the surface of astrocytes, Aβ, and IL-1 can induce the production of sphingomyelinase enzyme in astrocytes. The substrate of this enzyme is sphingomyelin found in cell membranes, and by breaking it down, it produces ceramide, which is a secondary messenger and induces messages related to the death of neurons and even astrocytes themselves (80, 81). IL-1β increases the phosphorylation of tau protein and decreases a pre-synaptic marker called synaptophysin through the p38-MAPK pathway in primary culture media containing neurons and astrocytes (82).

IL-18 can affect N-methyl-D-aspartate (NMDA) receptors and thereby interfere with the long-term potentiation (LTP) process (81). NMDA receptors affect tau protein structure and function in different ways (81). For example, signals generated by these receptors can activate calpains. Calpains stimulate tau phosphorylation by affecting other kinases such as glycogen synthase kinase, cyclin-dependent kinase 5 (CDK5), extracellular signal-regulated kinases (ERK1), and ERK2. Calpain activity also cleaves p35 to p25 and.p35 normally forms a CDK5/p35 complex with cyclin-dependent kinase 5 (CDK5) and this complex phosphorylates tau protein to its normal level. but p25 aggravates this process and tau hyperphosphorylation (81, 83, 84). The research of Farman and his colleagues showed that in APP/PS1 mice, by using the Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT) peptide, which is an interfering factor in the Calcineurin/NFAT[2] pathway, it is possible to reduce the activity of astrocytes as well as the level of Aβ, and the function of synapses and indicators (62). Improve learning and memory (59).

Garwood and his colleagues concluded experiments that using the antibiotic minocycline can prevent the activity of astrocytes and prevent the activation of caspase 3 in neurons and the production of h-tau. Additionally, they were able to demonstrate that adding Aβ to culture media containing both neurons and astrocytes induced neuronal death more rapidly than media containing only neurons. In this way, they clarified the role of astrocytes and inflammation in Alzheimer's pathogenesis (63). In 2004, Bergamaschini and colleagues showed that the use of enoxaparin (a type of low molecular weight heparin) in Alzheimer's mice reduced the number of active astrocytes surrounding amyloid plaques and slowed the progression of the disease (85). Henka and his colleagues showed that the use of pioglitazone and ibuprofen reduces inflammation in glial cells and also reduces the amount of Aβ1-42 in APPV717I transgenic mice (86).

Medeiros and his colleagues showed that the long-term use of IL-1 receptor-blocking antibodies in 3xTg Alzheimer's mice improves cognitive deficits, reduces the damage caused by tau protein, and reduces certain types of Aβ filamentous and oligomeric peptides (87).

In 2017, Yi and his colleagues showed that Boldin, which is extracted from the boldo tree, is effective in improving the condition of Alzheimer's mice by inhibiting the activity of connexins in glial cells, including astrocytes (88).

In 2015, Zhang and colleagues showed that the use of paeoniflorin as an anti-inflammatory in Alzheimer's APP/PS1 mice reduced the activity of glycogen synthase kinase and it also prevents the chain of inflammatory processes in the NF-κB pathway and excessive activation of astrocytes (68). Fragoulis and his colleagues showed that the use of methysticin, an activator of the Nuclear factor E2-related factor 2 (Nrf2) pathway (which is an anti-inflammatory transcription factor), in the form of oral gavage during 6 months with a weekly dose of APP/Psen1 Alzheimer's mice, it reduces astrogliosis, inflammatory cytokines secretion and reduces long-term memory disorders (89).

In 2018, Wilkanik and his colleagues showed that intraperitoneal injection of roscovitine in Alzheimer's

mice prevented CDK5 activity and the process of inflammatory responses (90). Astrocytes, when associated with senile plaques, react with morphological hypertrophy manifested by thickening processes and increased expression of the intermediate filament proteins glial fibrillary acidic protein (GFAP), vimentin, and nestin (87).

These data show that astrocytes are actively involved in the pathogenesis of AD. At the same time, many questions remain to be addressed. What are the astroglial states/phenotypes in different stages of AD? How do astrocytic states/phenotypes differ between brain regions with different vulnerabilities to AD? How do astrocytes communicate with other brain cells? Are they able to detect neurodegenerative disorders? How do AD risk genes modulate astroglial responses in AD?

It is hoped that new methods such as RNA sequencing and spatiotemporal transcription, in combination with human induced pluripotent stem cells (iPSC)-derived models and clustered regularly interspaced short palindromic repeats (CRISPR) based studies, will provide a deeper understanding of how astrocytes evolve during AD (Table 1).

Table 1. Summary of the research conducted on the role of astrocytes and inflammatory mediators in the development of Alzheimer's disease).

The name of the scholar	Year	Reference	Type of Study	Results
Furman et al	2012	(62)	Using the VIVIT peptide Calcineurin/NFAT pathway interfering factor) in APP/PS1 mice.	The activity of astrocytes and the level of amyloid beta decreased, and the function of synapses and learning and memory indicators improved.
Garwood et al	2011	(63)	Conducting tests using the antibiotic minocycline.	It prevented the activity of astrocytes and prevented the activation of caspase 3 in neurons and the production of hyperphosphorylated tau.
Garwood et al	2011	(63)	Comparison of the addition of amyloid beta to vessel media containing neurons and astrocytes with media containing only neurons.	Amyloid beta-induced neuronal death more quickly and revealed the role of astrocytes and inflammation in Alzheimer's pathogenesis.
Bergamaschini et al	2004	(85)	Application of Enoxaparin (a type of low molecular weight heparin) in Alzheimer's rats.	Reducing the number of active astrocytes surrounding amyloid plaques and reducing the speed of disease progression
Heneka et al	2005	(86)	Pioglitazone (PPARγ agonist) and ibuprofen were used.	Reduction of inflammation in glial cells and reduction of Aβ1-42 in APPV717I transgenic mice.
Garwood et al	2011	(63)	Use of minocycline antibiotic in h-tau mice	Reducing the activity of astrocytes and preventing the activation of caspase 3 in neurons and the production of h-tau protein (hyperphosphorylated tau)
Medeiros et al	2011	(87)	Long-term use of IL-1 receptor blocking antibody in 3xTg Alzheimer's mice.	Improvement of cognitive deficits, reduction of damage caused by tau protein, and relative reduction of certain types of amyloid beta filamentous and oligomeric peptides.
Furman et al	2012	(62)	Using the peptide VIVIT, an interfering agent in the Calcineurin/NFAT pathway in APP/PS1 mice.	It reduced the activity of astrocytes as well as the level of amyloid beta and improved the function of synapses and memory.

Zhang et al	2015	(81)	Using Paeoniflorin as an anti-inflammatory in APP/PS1 Alzheimer's mice	Preventing the activity of glycogen synthase kinase enzyme as well as the chain of inflammatory processes in the path of NF-κB and excessive activation of astrocytes.
Yi et al	2017	(88)	Preventing the activity of connexins of glial cells and including astrocytes with the help of Boldine, which was obtained from the Boldo tree.	In improving the disease condition in Alzheimer's mice.
Fragoulis et al	2017	(89)	Using methysticin by oral gavage for 6 months with a dose of once a week in APP/Psen1 Alzheimer's mice.	It reduced astrogliosis, reduced the release of inflammatory cytokines, and reduced long-term memory disorders.
Wilkaniec et al	2018	(90)	Intraperitoneal injection of Roscovitine in Alzheimer's rats.	It prevents the activity of CDK5 (cyclin-dependent kinase 5) and the process of inflammatory responses
Nikkar et al	2022	(60)	Chronic co-inhibition of astrocytes metabolism (with fluorocitrate) and also BRD4 (with JQ1) on cognition deficit at early stages of AD in rats.	Inhibition of astrocytes metabolism by fluorocitrate impaired spatial memory and reduced CREB/PSD95/synaptophysin levels in the hippocampus

Conclusions

Astrocytes have multiple functions in the brain and are essential for protecting neurons and maintaining homeostasis .However, under different pathological conditions including AD, they are associated with loss of function associated with neuroinflammation and neurodegeneration. A thorough characterization of these cellular states, together with morphological and functional analyses, will enhance the understanding of how astrocytes evolve in pathology .Soon, using selective inhibitors for A1 or A2 types of astrocytes, we may be able to correlate different astroglial states with specific stages of Alzheimer's disease and clarify the exact role of these cells in various stages of AD.

Author contribution

All the authors met the standard writing criteria based on the recommendations of the International Committee of Medical Journal Editors and all contributed equally to the writing of the work.

Conflict of interest

The authors hereby declare that there is no conflict of interest regarding the present research.

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Ultrasound-guided (USG) transversus abdominis plane (TAP) block with bupivacaine and dexmedetomidine on the control in postoperative analgesia of inguinal hernia surgery: A randomized clinical trial

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Abstract

Introduction: Transversus abdominis plane block (TAPB) is now a well-established technique in postoperative analgesia for lower abdominal surgeries. We aimed to investigate the effects of adding dexmedetomidine to bupivacaine used in USG (TAP block on postoperative pain and complications in patients undergoing inguinal hernia repair.

Materials and Methods: About 66 eligible patients enrolled in the survey. They were randomly assigned to one of the two groups of 20 CC of bupivacaine 0.5% + 1 CC Normal saline or 20 CC of bupivacaine 0.5% + 100µg dexmedetomidine. The amount of pethidine consumption, postoperative VAS score, and complications were measured. Patients were evaluated at the recovery ward (T0) and 2, 4, 6, 12, and 24 hours after surgery. Regarding the VAS score and if the patient's pain complained from a VAS≥3, pethidine 0.5 mg/kg was administered. The total dose, the average dose of pethidine used, and the first time of pethidine administration after the nerve block was recorded.

Results: Two groups had no significant difference regarding baseline characteristics. A significant difference was found at T4 about VAS (P=0.005). The amount of pethidine consumption was lower in the DEX group but not statistically significant except for T4 (P=0.006). The two groups showed no difference regarding side effects such as PONV.

Conclusion: Injection of dexmedetomidine in combination with bupivacaine for TAPB is an effective and safe drug for controlling pain after hernia surgery.

Keywords: Transversus abdominis plane block; Bupivacaine; Dexmedetomidine; Inguinal hernia

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Introduction

Hernia repair surgery is one of the most common surgeries in the world pain is one of the most common complications after hernia repair surgery. Chronic groin pain has been reported to be about 6-10% in long-term follow-up (1,2). There are several causes of postoperative pain. Include pain from incisions and deeper structures and emotional pain, such as pain when coughing and moving. However, the central pain is caused by an incision in the abdominal wall. Therefore, transversus abdominis muscle block for postoperative pain in lower abdominal surgery, including inguinal hernia, can be very effective, mainly when used as part of a multi-modal analgesic regimen (3). Some methods have been used to reduce postoperative pain. Drugs have been the gold standard for controlling severe pain for many years, but the side effects of these drugs have always posed challenges for physicians. These side effects include gastrointestinal motility disorders, urinary retention, constipation, respiratory depression, pruritus, abuse, postoperative vomiting nausea. Some other methods for postoperative pain control include using Nonsteroidal anti-inflammatory drugs (NSAIDs) and/or acetaminophen, gabapentin or pregabalin, I.V. ketamine, epidural with local anesthetic (with or without opioid), or intrathecal opioid, infusion of opioids with I.V. patient-controlled analgesia (PCA) (4).

Abdominis transversus muscle block has been used as an effective and safe method in providing balance analgesia after abdominal surgery. Rafi first did this block in 2001 (5). The abdominal transversus muscle block creates a sensory block through local anesthetic infiltration between the internal oblique and transverse abdominal muscles. In this block, three layers of muscle, external oblique, internal oblique, and transverse abdominal muscle, are anesthetized. Moreover, the T7–T12 intercostal muscles of the ilioinguinal nerve, the iliohypogastric nerve, the L1–L3 nerves, and the lateral branches of the cutaneous are blocked (6).

The blocking reduces the use of drugs after surgery, the time of the first request for pain relief, and the side effects of drugs. Among the available local anesthetic drugs, long-acting types such as bupivacaine are an excellent choice. Bupivacaine is an amide local anesthetic used in many studies to block (7). To perform this block, using an ultrasound guide is more accurate and practical than blind methods (8,9). It also has fewer side effects because the location of the needle and local anesthetic propagation is entirely recognizable. However, performing this block without an ultrasound guide can rupture the intraperitoneal membrane (10).

On the other hand, in situations where only local anesthetics are used, one of the problems with the block is the limitation of analgesia time. Other drugs as a supplement to the block can increase the quality and time of the block effect and reduce their side effects by decreasing the dose. Studies have shown the addition of various drugs, including dexmedetomidine, morphine, sufentanil, clonidine, adrenaline, and magnesium sulfate (11). Dexmedetomidine has been proposed as an adjunct to local anesthetics. Many studies show that adding dexmedetomidine to local anesthetics increases the duration of action and analgesia time after surgery (12,13). It is a selective alpha two receptor agonist that acts by inhibiting these receptors in the central nervous system to inhibit the release of norepinephrine in a dose-dependent manner (14). It also reduces neuronal activity through inhibitory effects on sodium and potassium channels and exerts its analgesic role by inhibiting the transmission of neural messages in C-fibers. The combination of bupivacaine and dexmedetomidine has been used successfully in some other blocks (15,16).

Due to the importance of the issue and its application in improving the quality of life of patients, providing desirable medical services, and reducing hospitalization days and economic costs, we decided to conduct the present study with the aim of the effectiveness of TAPB with USG in reducing pain after inguinal hernia surgery in Razi Hospital, Rasht, Iran.

Materials and Methods

Study design and variables

This study was a controlled, randomized clinical trial with a double-blind, parallel design on patients who were candidates for elective inguinal hernia surgery. It was performed with an age range of 18 to 65 years and with ASA class I, II. After approving the draft study

and receiving the ethical code (IR.GUMS.REC.1397.449**)** and IRCT code (IRCT20121216011766N5), obtaining informed consent from eligible patients was included. In addition, all methods were performed following the Declaration of Helsinki, and all individuals consented to participate in this study. Patients who met the inclusion criteria were randomly assigned by one of the nurses of the relevant ward who was unaware of the study to two groups of 33 people with Intervention (I) and control (C) marks in intervention and control groups. Random sequences were generated using the Random Generator program. Based on the randomized block method, 15 blocks of size 4 with a ratio of 1 to 1 (as two groups I and C) and one block of six were generated for 66 patients. After generating the list, each person was assigned a unique code, and during the study, the person was identified with this code. All patients underwent general anesthesia, and the patient's vital signs, including heart rate, respiration rate, and blood pressure, were monitored and recorded. Immediately after the operation and before transferring the patient to the recovery unit, by placing the 15 MHz linear probe of the ultrasound device (Midray) in a transverse position, just above the iliac crest and in the maxillary line on the same side of the surgery, after finding the sheet between the internal oblique muscle and the abdominal transversus muscle using ultrasound, in the control group: 20cc of 0.5% bupivacaine with 1cc of normal saline, and intervention group: 20cc of 0.5% bupivacaine with 1cc (100 mcg) of dexmedetomidine were injected (brand name Precedex containing 200 mcg/2ml, manufacturer HOSPIRA reference manufacturer of USA).

In the post-anesthesia care unit (PACU), at intervals of 0, 2, 4, 6, 12, and 24 hours after surgery, pain intensity was measured in two groups by VAS by asking the patient to show their pain intensity on a 10 cm ruler. A score of zero was given for analgesia, and a score of 10 for maximum pain. During the 24 hours, the patients were hospitalized in the ward, 0.5 mg/kg of pethidine was prescribed if they needed analgesia (VAS>3). The total dose, the average dose of pethidine used, and the first time of pethidine administration after the nerve block was recorded. Complications such as nausea, dizziness, and vomiting in the two groups were evaluated. Inclusion criteria are age 18 to 65 years, body mass index 18-35 kg/m^2, drug insensitivity, ASA Class I, II, no infection at the injection site, no alcohol and drug addiction, and type of surgery (Inguinal hernia repair without tension). Also, surgery lasting more than two hours, the need for other operations during inguinal hernia surgery, the requirement to receive drugs in recovery, excessive bleeding, recurrence of hernias, and the need for spinal anesthesia excluded patients from the study.

Statistical Analysis

The required sample size was calculated using G*Power © software version 3.1.0. About 26 patients in each group were estimated by assuming the test power of 80%, and the first type error was 0.05 to determine the effect size of 0.8 (17). Finally, 33 patients in each group were determined by considering the 20% drop coefficient. The SPSS software version 18 was used for all statistical analyses. Fisher and Chi-square tests were used to compare the ratios in the two groups. Also, to compare the quantitative means in the two groups, the Mann-Whitney U test, and to compare the analgesia time, Kaplan-Meier and TaroneWare tests were used. Generalized Linear Models (GLM) and Generalized Estimating Equation (GEE) were used to investigate the effect of drugs on VAS scores during the study period. A value of P< 0.05 was considered statistically significant

Results

Demographic characteristics of patients undergoing inguinal hernia surgery in two groups affected by bupivacaine injection and combined with dexmedetomidine via TAP block with ultrasound guide were examined by chi-square test, which the results are shown in Table 1. It was also found that in the C group, there was a statistically significant difference between the values of VAS in the periods studied (P<0.001). Also, there is a statistically significant difference between the two groups between VAS values in the studied periods (P=0.047). As shown in Table 2, intergroup statistical estimation illustrated that there was a statistically significant difference between the amounts of pethidine intake in bupivacaine+dexmedetomidine (F=44.86, P<0.001) and bupivacaine groups (F=28.6, P<0.001). A significant difference was also found in the statistical

estimation between groups (F=3.89, P<0.001) according to the period. The comparison between the mean of pethidine intake among two groups with (45.3 ± 29.47) and without (60.0 ± 37.79) dexmedetomidine was not statistically significant (P=0.094).

Table 1. Demographic characteristics of patients undergoing inguinal hernia surgery in two groups through TAP block with ultrasound guide.

Variable		Bupivacaine+Dexmedetomidine n (%)	Bupivacaine n (%)	Total n (%)	P-Value
Age (year)	> 40	7 (21.2)	9 (27.3)	16 (24.2)	0.015
	41-60	26 (78.8)	21 (63.6)	47 (71.2)	
	>60	0 (0.0)	3 (9.1)	3 (4.5)	
Mean ±SD	Age (year)	8.3 ± 8.3	48.24 ± 10.51	47.81 ± 9.41	0.717
ASA class	I	27 (81.8)	23 (69.7)	50 (75.8)	0.251
	II	6 (18.2)	10 (30.3)	16 (24.2)	
Mean ±SD	Weight (kg)	69.93 ± 5	67.9 ± 5.02	68.92 ± 5.08	0.105

Table 2. Evaluation of changes in pethidine intake (mg) and comparison of mean pethidine in patients undergoing inguinal hernia surgery in two groups via TAP block.

Time	Bupivacaine + Dexmedetomidine	Bupivacaine	P-Value
Recovery	22.27 ± 16.1	23.78 ± 14.94	0.693
2 hours after surgery	21.06 ± 16.28	21.21 ± 16.39	0.97
4 hours after surgery	1.96 ± 7.89	11.06 ± 15.94	0.005
6 hours after surgery	0 ± 0	2.87 ± 9.27	0.079
12 hours after surgery	0 ± 0	1.06 ± 6.09	0.321
24 hours after surgery	0 ± 0	0 ± 0	-

Moreover, the comparison of mean pethidine in patients in these two groups was shown that no significant differences were found between the amounts of pethidine intake (P=0.094) (Figure 1).

According to the information shown in Figure 2, using a t-test, it was found that only 4 hours after surgery, there was a statistically significant difference between the amounts of pethidine in the two groups (P=0.005). According to Mann-Whitney U test results in Table 3, a significant difference was not observed between the values of analgesia duration (hours) in patients in two groups 24 hours after surgery (P=0.567).

Table 3. Comparison of analgesia duration in patients undergoing inguinal hernia surgery in two groups through TAP block in 24 hours after surgery.

Group	Number	Mean± SD	P-Value
Bupivacaine + Dexmedetomidine	33	6 ± 10.35	0.567
Bupivacaine	33	4.54 ± 9.33	

Based on Chi-square test results, table 4, a statistically significant relationship was not observed between the incidence of side effects (nausea or vomiting, or dizziness) in the two groups 24 hours after surgery (P=0.459).

Table 4. Evaluation of side effects (nausea or vomiting) in patients undergoing inguinal hernia surgery in two groups.

Side effects	Bupivacaine +Dexmedetomidine n (%)	Bupivacaine n (%)	Total n (%)	P-Value
+	16 (48.5)	19 (57.6)	35 (53.0)	0.459
-	17 (51.5)	14 (42.4)	31 (47.0)	
Total	33 (100)	33 (100)	66 (100)	

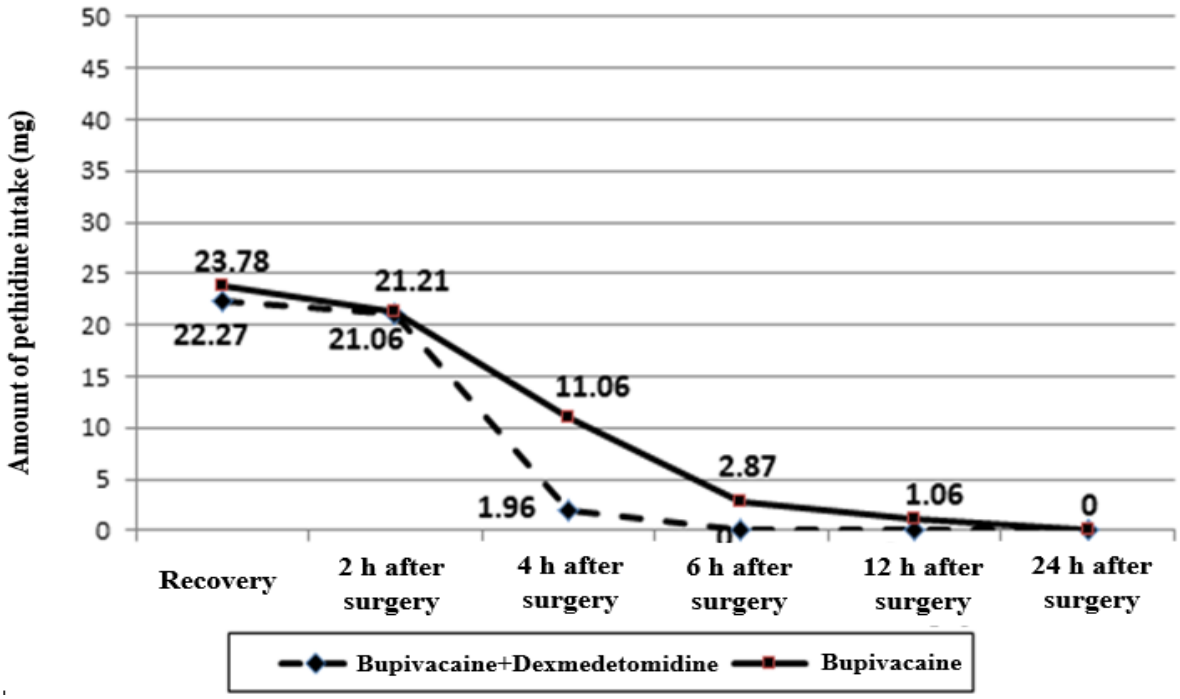

Figure 1. Evaluation of changes in pethidine intake in patients undergoing inguinal hernia surgery in two groups under the influence of bupivacaine injection alone and in combination with dexmedetomidine via TAP block.

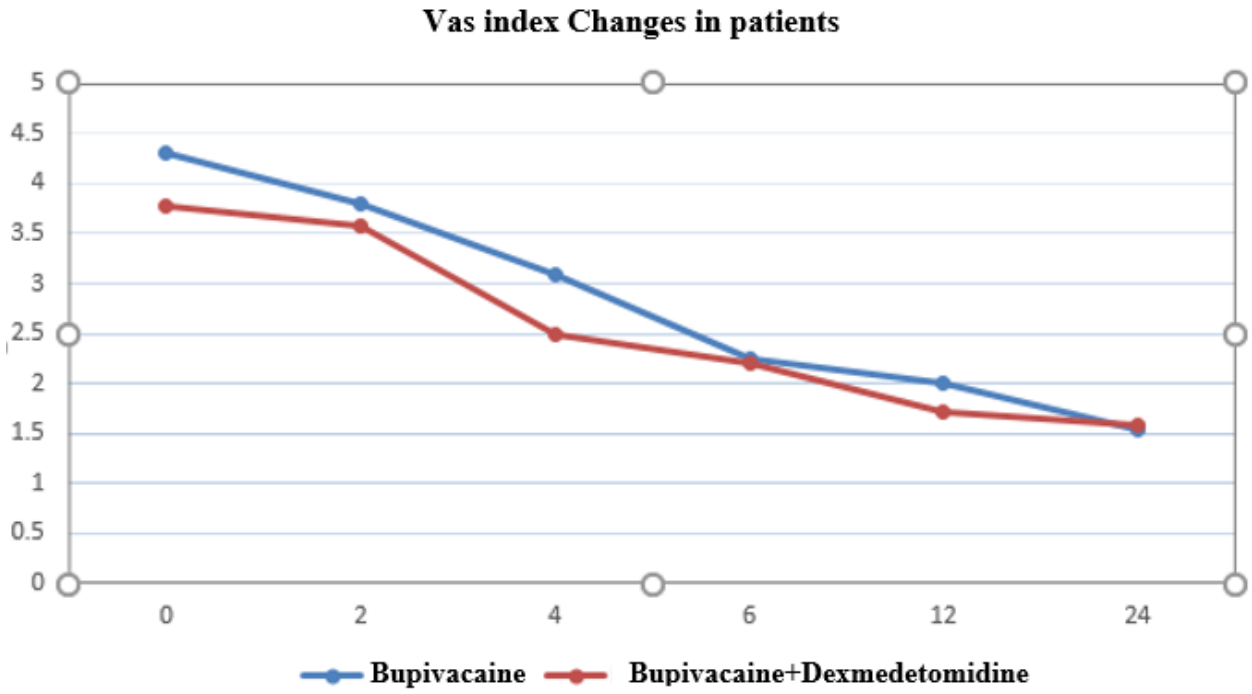

Figure 2. Evaluation of VAS index changes in patients undergoing inguinal hernia surgery in two groups via TAP.

Discussion

Acute postoperative pain strongly predicted persistent pain following both open anterior and endo-laparoscopic hernia repair (18). Physiological responses to surgical pain and trauma include respiratory effects, cardiovascular, Gastrointestinal, urinary system, neuroendocrine, and metabolic, which lead to side effects. Control of physiological processes associated with acute postoperative pain can lead to satisfaction and increase the quality of life in patients (19). Kokoulu et al. described the block as an effective and cost-effective method. Their study reported significantly lower levels of anesthetic drugs in the group that underwent this block than in the group undergoing standard general anesthesia and laparoscopic inguinal hernia surgery (20).

Various methods have been proposed to control acute pain after surgery, including administering nonsteroidal anti-inflammatory drugs, peripheral nerve block, and nerve root block (21). Increasing awareness of drug-related side effects, including respiratory depression, obstruction, and sedation, has led to a shift in drug use to control postoperative pain (22). TAP block has been used as an effective and safe method in providing balance analgesia after abdominal surgery by blocking the abdominal wall nerve. In the USG method, local anesthetics are injected near the nerve and help increase the injection's accuracy (23). Various studies have shown that TAP block is an effective technique for controlling pain and reducing morphine use after surgery, including retropubic prostatectomy, colorectal surgery, a cesarean delivery, abdominal hysterectomy, laparoscopic appendectomy, and abdominal hernia surgery (23–26).

This study evaluated the effectiveness of TAP block with USG in reducing pain after inguinal hernia surgery. It showed that the pain intensity in the two groups significantly differed in the first four hours after surgery. No significant difference in hemodynamic changes (H.R., MAP) was observed in any of the cases during surgery. During the first four hours after surgery, a significant difference was observed between the two groups regarding pethidine consumption. However, in the following hours, between 4 and 12 hours, the amount of pethidine in the two groups was not significantly different. In addition, there was no significant difference between the two groups regarding postoperative side effects.

According to other studies, analgesia time with bupivacaine block is about 4-6 hours. Therefore, the results of this study can be justified that in the first 4 hours, no significant difference was observed between the two groups in terms of VAS score. However, after this time and with the disappearance of bupivacaine effects, the effectiveness of dexmedetomidine increased block efficiency. Furthermore, Aksu et al. reported the satisfaction of patients who have undergone abdominal surgery (26). Therefore, the results of this study are consistent with the present study and suggest that dexmedetomidine is a drug whose addition to bupivacaine in TAP block reduces pain and drug use after surgery. It should be noted that the dose of drugs used in the two studies is the same.

In the study of Feyz et al., the pain of patients in resting and moving positions in the ilioinguinal/iliohypogastric group was less than the TAP block group, which was statistically significant. Besides, satisfaction with analgesia was significantly higher in the ilioinguinal/iliohypogastric group than in the TAP block group. Therefore, it was suggested that the iliohypogastric ultrasound-guided block is more suitable than the TAP block; and to control pain after inguinal hernia surgery, the ultrasound-guided ilioinguinal /iliohypogastric block is more appropriate than the TAP block (27). In the current study, superiority was not observed for the TAP block, which can be justified due to the difference in the method of the present study with this study, which used bupivacaine alone. Due to the high prevalence of this operation and its increasing importance in controlling acute pain after surgery is a topic that requires extensive research.

Limitations

Since the characteristics of people and their interpretation of pain intensity, their expectation of pain, and their level of tolerance are different and can be effective in expressing pain by the patient; it is challenging to assess patients' pain. Furthermore, due to the limited follow-up times, the evaluation of patients was limited in terms of possible complications and the procedure's effectiveness.

Conclusions

According to the results of this study, injection of dexmedetomidine in combination with bupivacaine for TAPB can be used as an effective and safe drug for controlling pain after hernia surgery.

Author contribution

HKH and **HH** researched literature and conceived the study. **CEA** and **ZP** were involved in protocol development, gaining ethical approval, patient recruitment and data analysis. **MF**, **MRH** and **ASN** wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript

Conflict of interest

The authors reported no potential conflict of interest.

Ethics approval

This study was approved by the ethical committee at the Guilan University of Medical Sciences [I.R.GUMS.REC.1397.449].

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