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World Health Organization. WHO Criteria for Diagnosis of Osteoporosis. Last Accessed Date: 15.06.2017. Available from: http://www.4bonehealth.org/education/world-health-organization-criteria-diagnosis-osteoporosis/

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Message from the Editor-in-Chief

Message from the Editor-in-Chief,

Dear colleagues,

As we all know, 2023 is the 100th anniversary of the Republic of Türkiye. In this meaningful year, as GMJ, the official journal of Gülhane Faculty of Medicine, University of Health Sciences Türkiye, we are proud to continue our way by presenting studies that will shed light on science and the future.

We are publishing our 4th issue this December, as we do every year. In this issue, current and interesting information in different medical fields is shared with 5 original articles and 2 case reports. We believe that this content, which blends very interesting and current topics from different clinical departments, will be useful for a multidisciplinary perspective.

As we end 2023, we would like to take this opportunity to thank the readers, for their continued interest throughout the year, the authors who submitted their articles to the journal, to reviewers who provided objective guidance in the evaluation of the articles, and all our editors who ensured that GMJ continued its regular publication life, for their contributions.

As we enter 2024, I hope you all have a good year.

M. Ali Gülçelik, M.D., Prof.
Editor-in-Chief

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Can pre-treatment inflammatory biomarker levels predict the response of tocilizumab therapy in COVID-19 patients?

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Keywords: COVID-19, tocilizumab, prognostic nutritional index, C-reactive protein/albumin ratio, systemic immune-inflammatory index

ABSTRACT

Aims: The results of randomized clinical trials evaluating the effects of tocilizumab in patients with patients are inconsistent. We aimed to investigate the reliability of pre-treatment levels of prognostic nutritional index (PNI), C-reactive protein (CRP)/albumin ratio (CAR), systemic immune-inflammatory index (SII), interleukin-6 (IL-6), and lactate dehydrogenase (LDH) as treatment response biomarkers in hospitalized coronavirus disease-2019 (COVID-19) patients who were administered tocilizumab.

Methods: Adult patients hospitalized for COVID-19 confirmed by severe acute respiratory syndrome-coronavirus-2 polymerase chain reaction and administered tocilizumab because of rapid clinical worsening despite receiving standard care were retrospectively included. Patients who received steroids, anakinra, or IVIG before tocilizumab and had missing data were excluded. Treatment effectiveness was evaluated with the improvement in the clinical status on an eight-category ordinal scale at 28 days of tocilizumab administration.

Results: One hundred and thirty-three COVID-19 patients with a mean age of 62.64±13.66 years and consisting of 93 (69.9%) males were included. At 28 days of tocilizumab administration, 99 (74.4%) patients improved. Improved patients after tocilizumab treatment had significantly lower IL-6, LDH, SII, CAR, and higher PNI. To predict the effectiveness of tocilizumab, IL-6 had the highest area under the curve (AUC) value (AUC=0.782), followed by LDH (AUC=0.761), PNI (AUC=0.696), SII (AUC=0.671), CAR (AUC=0.682), and CRP (AUC=0.643). The cut-off level was 143.12 pg/mL for IL-6 (sensitivity=84.85%, specificity=64.71%), 460U/L for LDH (sensitivity=71.72%, specificity=73.75%), 31.35 for PNI (sensitivity=79.80%, specificity=55.88%), 3895.92 for SII (sensitivity=90.91%, specificity=47.06%), and 61.15 for CAR (sensitivity=67.68%, specificity=61.76%).

Conclusions: In COVID-19 patients with clinically worsening disease, the administration of tocilizumab in the early stage of the hyperinflammatory state may improve the prognosis. Pre-treatment inflammatory biomarker levels may predict tocilizumab response.

Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection may cause various clinical manifestations ranging from asymptomatic to multisystem life-threatening manifestations (1). Although most patients experience only mild to moderate symptoms, a subgroup of patients may progress to severe and critical disease, including Acute respiratory distress syndrome (ARDS), multi-organ failure, and death. Although the pathogenesis of coronavirus disease-2019 (COVID-19) is considered to result from a complex interplay of multiple pathophysiological mechanisms, such as direct viral effect, renin–angiotensin–aldosterone system imbalance, dysregulated immune response, and coagulopathy, an overproduction of pro-inflammatory cytokines, described as cytokine storm, is considered as a major reason for disease progression and death (2,3).

The pleiotropic cytokine interleukin-6 (IL-6) plays a pivotal role in the pathogenesis of COVID-19, and circulating levels of IL-6 are closely associated with clinical outcomes (4,5). Therefore, tocilizumab, an inhibitor of IL-6 receptors, was considered an attractive therapeutic option. However, the results of randomized clinical trials that evaluated the effects of tocilizumab in patients with COVID-19 are inconsistent because of the differences in trial design, initial disease severity used for the inclusion or exclusion, timing of tocilizumab administration, use of co-interventions, and outcome measurements (6).

The prognostic nutritional index (PNI), C-reactive protein (CRP)/albumin ratio (CAR), and systemic immune-inflammatory index (SII), which are novel parameters for measuring the degree of inflammation, have been reported as valuable biomarkers for discriminating disease severity and predicting mortality in COVID-19 (7-15).

As tocilizumab treatment may be beneficial in some SARS-CoV-2-infected patient populations, the ideal patient group and optimal timing for tocilizumab administration are still unknown. Thus, determining the patient populations likely to benefit from tocilizumab is important. In this study, we evaluated the reliability of pre-treatment levels of PNI, CAR, SII, IL-6, and lactate dehydrogenase (LDH) as treatment response biomarkers in hospitalized COVID-19 patients who were administered tocilizumab.

Methods

Study design and participants

This retrospective study was conducted at the University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Ankara, Türkiye. The study protocol was first registered in the data of the Türkiye Republic Ministry of Health Scientific Research Committee and then approved by the Committee on the Human Research Ethics of the University of Health Sciences Türkiye, Gülhane Faculty of Medicine (date: 26.05.2021, number: 2021/35). The study was conducted in accordance with the Declaration of Helsinki. Between May 1 and September 1, 2020, consecutive SARS-CoV-2-infected patients who consulted the rheumatology department because of rapid clinical worsening were evaluated for inclusion. Electronic medical records of the patients were used to collect data.

The inclusion criteria were as follows: (1) age ≥18 years, (2) positive nasopharyngeal swab reverse-transcriptase-PCR for SARS-CoV-2 RNA, and (3) chest computed tomography (CT) findings suggestive of COVID-19. The exclusion criteria were as follows: (1) patients who received steroids, anakinra, or IVIG before tocilizumab administration and (2) patients with missing baseline data.

A total of 133 patients who received tocilizumab were included in the study. Demographic, clinical, laboratory, and treatment data were obtained from the patient's medical records. The clinical status before tocilizumab administration was assessed according to the eight-category ordinal scale (16).

Category 1: Non-hospitalized patients with no activity restrictions.

Category 2: Hospitalized patients with limited activities and/or home oxygen requirements.

Category 3: Hospitalized patients not requiring supplemental oxygen and no longer requiring ongoing medical care (used if hospitalization was extended for infection control or other nonmedical reasons).

Category 4: Hospitalized patients who do not require supplemental oxygen but need ongoing medical care related to COVID-19 or other medical conditions.

Category 5: Hospitalized patients because of the need for supplemental oxygen.

Category 6: Hospitalized patients because of the need for noninvasive ventilation or use of high-flow oxygen devices.

Category 7: Patients receiving invasive mechanical ventilation or extracorporeal membrane oxygenation.

Category 8: Death.

Treatment protocol

The treatment protocol of the Turkish Ministry of Health for COVID-19 was recorded in the patient files. The protocol of the period included hydroxychloroquine (±azitromycin) for 5-10 days and venous thromboembolism prophylaxis in the absence of a major contraindication. When clinical evidence of progressive COVID-19 was observed despite this standard care (defined as oxygen saturation <92% on room air or receiving oxygen therapy, and CRP ≥75 mg/L), available sets of recommendations included the use of single-dose tocilizumab 8 mg/kg (maximum dose: 800 mg) after exclusion of bacterial and fungal co-infection. A second dose was recommended when no improvement was observed after the first dose within 24 hours.

Outcome

The end-points were improvement in the clinical status on an eight-category ordinal scale at 28 days of tocilizumab administration. The patients were divided into two groups: those who improved after tocilizumab therapy and those who did not. Serum alanine transaminase, aspartate transaminase, LDH, urea, creatinine, CRP, albumin, ferritin, fibrinogen, IL-6 levels, and complete blood count were available for all patients. Laboratory results in the last 24 hours before tocilizumab administration were used to calculate PNI, SII, and CAR. PNI was calculated as serum albumin levels (g/L) + 5 total lymphocyte count (10^9/L) (17). SII was determined using the following formula: platelet count neutrophil count/lymphocyte count (18). CAR was obtained by dividing the CRP levels by albumin level (19).

Statistical Analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA) 16.0 program for Windows. Histograms, probability plots, and the Kolmogorov-Smirnov test were used to check the normal distribution. Normally distributed variables were expressed as mean±standard deviation, skewed variables as median (interquartile range) (25^{th} and 75^{th} percentiles), and categorical variables as number and percentage. The performance of IL-6, CAR, PNI, SII, and LDH in predicting the response to tocilizumab was analyzed using receiver operating characteristic curve analysis. The Youden index was used to determine the best cut-off values for these biomarkers. When a significant cut-off value was observed, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated. A test with an area under the curve (AUC) of ≥0.85 was considered accurate. P<0.05 was considered statistically significant.

Results

The study included 132 patients who received tocilizumab treatment because of clinical progression and cytokine storm despite standard care. The clinical characteristics and laboratory findings of the study groups are shown in Table 1. Overall, the patients were predominantly male (69.9%) with a mean age of 62.64±13.66 (range, 22-90 years) years. Eighty-eight (66.2%) patients had at least one comorbid disease, and the most common comorbidity was hypertension (35.3%), followed by diabetes mellitus (31.6%). The frequency of concomitant antibiotic therapy and steroids was similar between the groups.

Patients who improved after tocilizumab therapy (n=99, 74.4%, were significantly younger and had significantly lower IL-6, LDH, SII, CAR, and higher PNI than patients who did not improve (Table 1).

Table 1. Clinical characteristics of the study sample

	Patients who improved (n=99)	Patients who died (n=34)	p-value
Age, mean±SD, years	60.57±13.81	68.68±11.35	**<0.001**
Male sex, n (%)	74 (73.7)	19 (55.9)	0.380
Time from symptom onset to tocilizumab treatment, days, median (IQR)	9.0 (7.0-11.0)	9.5 (6.0-12.3)	0.824
Clinical status, n (%)			**<0.001**
Category 4	6 (6.1)	0	
Category 5	77 (77.8)	6 (17.6)	
Category 6	15 (15.2)	13 (38.2)	
Category 7	1 (1)	15 (44.1)	
Comorbidities, n (%)			
Hypertension	28 (28.3)	19 (55.9)	**0.006**
Diabetes mellitus	27 (27.3)	15 (44.1)	0.087
Intermediate or advanced chronic kidney disease	6 (6.1)	7 (20.6)	**0.021**
Coronary artery disease	19 (19.2)	8 (23.5)	0.587
Congestive heart failure	4 (4)	5 (14.7)	**0.047**
Hyperlipidemia	2 (2)	1 (2.9)	0.755
Treatment, n (%)			
Antibiotic treatment	79 (79.8)	30 (88.2)	0.270
Steroid after tocilizumab administration	12 (12.1)	6 (17.6)	0.416
Laboratory parameters at tocilizumab administration			
White blood cell count (x10^3/μL), median (IQR)	6.70 (5.00-8.30)	9.10 (6.20-11.83)	**<0.001**
Lymphocyte count (x10^3/μL), median (IQR)	0.80 (0.60-1.10)	0.55 (0.40-0.93)	**0.016**
Neutrophil count (x10^3/μL), median (IQR)	5.10 (3.70-6.70)	8.30 (5.03-10.60)	**<0.001**
Platelet count, (x10^3/μL), median (IQR)	230.00 (188.00-280.00)	218.00 (156.25-306.25)	0.400
LDH (U/L), median (IQR)	392.00 (317.00-480.00)	586.50 (414.750-772.00)	**<0.001**
IL-6 (pg/mL), median (IQR)	80.44 (54.97-124.10)	168.26 (103.40-284.47)	**<0.001**
CRP (mg/L), median (IQR)	146.20 (102.40-210.46)	198.15 (139.91-264.00)	**0.013**
Fibrinogen (mg/dL), median (IQR)	636.00 (514.00-769.00)	688.00 (513.00-867.00)	0.407

Table 1. Continued

	Patients who improved (n=99)	Patients who died (n=34)	p-value
D-dimer (mg/L), median (IQR)	0.78 (0.48-1.24)	1.98 (1.18-3.91)	**<0.001**
Albumin (g/dL), median (IQR)	3.07 (2.81-3.35)	2.79 (2.56-3.01)	**<0.001**
CAR, median (IQR)	48.41 (34.67-71.39)	67.90 (45.69-96.54)	**0.002**
PNI, median (IQR)	34.90 (31.90-38.70)	30.95 (26.95-36.50)	**0.001**
SII, median (IQR)	1548.00 (814.77-2692.57)	3167.13 (1111.83-7162.13)	**0.003**

SD: Standard deviation, n: Number, IQR: Interquartile range, LDH: Lactate dehydrogenase, IL-6: Interleukin-6, CRP: C-reactive protein, CAR: C-reactive protein/albumin ratio, PNI: Prognostic nutritional index, SII: Systemic immune-inflammatory index

To predict the response to tocilizumab treatment, IL-6 had the highest AUC value [AUC=0.782, 95% confidence interval (CI): 0.694-0.870], followed by LDH (AUC=0.761, 95% CI: 0.661-0.861), PNI (AUC=0.696, 95% CI: 0.584-807), SII (AUC=0.671, 95% CI: 0.551-0.790), CAR (AUC=0.682, 95% CI: 0.578-0.786), and CRP (AUC=0.643, 95% CI: 0.535-0.751) (Figure 1). A cut-off level of 143.12 pg/mL for IL-6 had 84.85% sensitivity and 64.71% specificity, 460 U/L for LDH had 71.72% sensitivity and 73.75% specificity, 31.35 for PNI had 79.80% sensitivity and 55.88% specificity, 3895.92 for SII had 90.91% sensitivity and 47.06% specificity, and 61.15 for CAR had 67.68% sensitivity and 61.76% specificity. The performance of these markers in predicting the effectiveness of tocilizumab therapy is shown in Table 2.

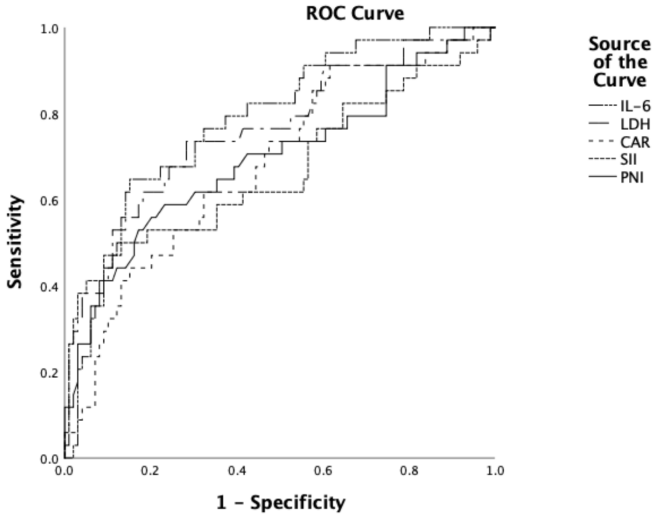

Figure 1. ROC curves of IL-6, LDH, CAR, SII and PNI

IL-6: Interleukin-6, LDH: Lactate dehydrogenase, CAR: C-reactive protein/albumin ratio, SII: Systemic immune-inflammatory, PNI: Prognostic nutritional index, ROC: Receiver operating characteristic

Discussion

To the best of our knowledge, this is the first study to assess the predictive value of PNI, CAR, and SII in determining the response to tocilizumab treatment in patients with COVID-19. Among 133 patients with COVID-19, approximately three-quarters of patients improved after tocilizumab therapy and those had significantly lower IL-6, LDH, SII, CAR, and significantly higher PNI compared with those who failed to receive tocilizumab therapy. To predict the effectiveness of tocilizumab in COVID-19 patients, serum IL-6 level had the highest AUC value, followed by LDH, PNI, CAR, SII, and CRP.

In severe COVID-19, cytokine storm syndrome, an overproduction of proinflammatory cytokines and overactivation of immune cells, can lead to life-threatening medical syndromes including disseminated intravascular coagulation, ARDS, multiorgan failure, and even death if treatment is inadequate (20). Therefore, the timing of diagnosis and treatment are very important strategies for COVID-19 management. Among the cytokines, IL-6 has received particular attention for its relation to COVID-19. The association between IL-6 levels and disease severity has been reported in several studies. Therefore, IL-6 blockade was postulated as an effective therapeutic strategy to reduce inflammation in the cytokine storm associated with COVID-19, and attention was focused on tocilizumab, a recombinant monoclonal antibody against the IL-6 receptor.

Table 2. Predictive performance of inflammatory biomarkers in predicting effectiveness after tocilizumab therapy

	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)	Accuracy (%)	DOR
IL-6 <143.12 pg/mL	84.85	64.71	2.40	0.23	87.50	59.46	79.70	10.27
LDH <460 U/L	71.72	73.75	2.71	0.38	88.75	57.18	72.18	7.04
PNI >31.35	79.80	55.88	1.88	0.36	84.04	48.72	73.68	5.00
SII <3895.92	90.91	47.06	1.72	0.19	83.33	64.00	79.70	8.89
CAR <61.15	67.68	61.76	1.77	0.52	83.75	39.62	66.17	3.38

IL-6: Interleukin-6, LDH: Lactate dehydrogenase, PNI: Prognostic nutritional index, SII: Systemic immune-inflammatory index, CAR: C-reactive protein/albumin ratio, PLR: Positive likelihood ratio, NLR: Negative likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, DOR: Diagnostic odds ratio

Although early observational studies have indicated improved outcomes in COVID-19 patients who received tocilizumab, subsequent randomized clinical trials assessing tocilizumab efficacy reported conflicting results (21-24). However, these trials differed considerably in patient number, study design, disease severity contents of the applied standard of care therapy, and concomitant steroid dosage. Consequently, the benefit of tocilizumab treatment in some patient groups cannot be denied. In this context, we aimed to perform the current study because it was timely and important to define the patients who will benefit from tocilizumab or not, and determine the optimal timing of tocilizumab therapy to prevent drug-related side effects and increase in costs due to unnecessary drug use.

Regarding the reliability of IL-6 as a treatment response biomarker in patients with COVID-19, Flisiak et al. (25) reported that tocilizumab administration reduced mortality and accelerated clinical recovery in patients with pre-treatment IL-6 levels higher than 100 pg/mL who required supplemental oxygen. On the other hand, Li et al. (26) reported that pre-treatment IL-6 levels equal to or higher than 100 pg/mL were associated with poor prognoses after tocilizumab therapy. Similar to the study by Li et al. (26), in our study, serum IL-6 levels were significantly lower in patients who improved after tocilizumab treatment. In addition, we concluded that among patients who had pre-treatment IL-6 levels lower than 143.12 pg/mL, the probability of clinical improvement after tocilizumab therapy increased from 74.44% to 87.23%. If a patient has a pre-treatment IL-6 level higher than 143.12 pg/mL, the probability of mortality increases from 25.56% to 59% (i.e., pretest odds=0.34; posttest odds=1.45; posttest probability=0.59).

PNI has been suggested as a biomarker for assessing the nutritional and immunological status of patients with chronic disease (27). It is calculated using commonly used parameters, including serum lymphocyte count and albumin level (17). Reduced serum lymphocyte count and hypoalbuminemia are predictors of severe disease and poor outcomes in COVID-19 patients (28,29). In addition, lower PNI may serve as a predictor of prognosis in COVID-19 patients (30). Among patients who had pre-treatment PNI levels higher than 31.35, the probability of clinical improvement after tocilizumab therapy was 84.27% (i.e., pretest odds=2.85; posttest odds=5.36; posttest probability=0.84). For the first time in the available literature, we now provide cutoffs of PNI level for predicting response to tocilizumab treatment.

SII can be easily calculated from hemogram parameters (18). Fois et al. (14) reported that SII was an independent predictor of hospital mortality in COVID-19 patients. In their study, patients with higher SII values had lower PaO_2/FiO_2 ratios and higher chest CT severity scores, but there were no differences regarding the comorbidities. They suggested that the SII level would be specifically affected by lung injury occurring in COVID-19 patients, rather than an overall deterioration in their clinical status due to comorbidities (14). Similarly, Muhammad et al. (13) revealed that SII had high sensitivity and specificity in predicting the clinical course of COVID-19 patients. In line with those recently published studies, in our study, patients who did not improve after tocilizumab therapy had significantly higher SII levels than those who improved. If a patient had a pre-treatment SII level <3895.92, the probability of clinical improvement was raised to 83.06% (i.e., pretest odds=2.85; posttest odds=4.90; posttest probability=0.83). For the first time in the available literature, we now provide cutoffs of SII level for predicting response to tocilizumab treatment.

CAR, which reflects both the inflammatory state and nutritional status, can be obtained with easily accessible parameters, including CRP and albumin (19). It has been reported that the CAR level could be used as a prognostic indicator of disease severity and has predictive value for in-hospital mortality in COVID-19 patients (15,31). In our study, patients who improved after tocilizumab therapy had significantly lower CAR than patients who did not improve. Also, a pre-treatment CAR level <61.15 raised the probability of treatment being effective to 83.46% (i.e., pretest odds=2.85; posttest odds=5.05; posttest probability=0.83). For the first time in the available literature, we now provide cutoffs of CAR levels for predicting response to tocilizumab treatment.

LDH is an enzyme in nearly all cells throughout the body. Elevated LDH levels were associated with an increased risk of severe disease and mortality in COVID-19 patients (32,33). Decreased oxygenation, multiple organ injury, and a hypercoagulable state can contribute to the elevation of LDH in COVID-19 patients (32). In our study, COVID-19 patients who improved after tocilizumab administration had lower LDH levels than those who did not. The probability of clinical improvement was increased to 88.54% in patients with pretreatment LDH levels lower than 460 U/L (i.e., pretest odds=2.85; posttest odds=7.72; posttest probability=0.88). Similar to our study, Li et al. (26) reported that patients who died after tocilizumab therapy had significantly higher LDH levels at baseline than those with clinically improved levels. In addition, they showed that, while there was no significant improvement in the post-treatment LDH value of patients who died, LDH levels were significantly decreased in the improved group. However, the performance of LDH in predicting treatment response was not evaluated in that study. For the first time in the available literature, we now provide cutoffs of LDH levels for predicting response to tocilizumab treatment.

Study Limitations

The retrospective design of this study and the lack of a control group are the major limitations. In addition, the data were obtained from a single center through the hospital's

electronic database; therefore, patient selection bias could not be completely eliminated. Because the utility of pre-treatment biomarker levels in predicting tocilizumab response was evaluated, post-treatment changes in these biomarker levels were not evaluated.

Because there is an ongoing debate about the role of tocilizumab in treating patients with COVID-19, the drug may have a substantial benefit in certain populations. It is well known that most patients experience only mild to moderate symptoms during COVID-19, and these patients do not require anti-cytokine therapy. However, mortality occurs in a subset of patients who progress to severe and critically ill and may require anti-cytokine therapy, but the efforts for suppression of inflammation do not necessarily reduce mortality in all cases, especially when the inflammation cascade is excessive and therapy is delayed. Moreover, early use of anti-cytokine therapy may lead to both increased costs and risks, including iatrogenic immunosuppression. From this viewpoint, one can claim that there can be a "window of opportunity" for treating COVID-19. To specify this time interval and the patients who will benefit from anti-cytokine therapy, several biomarkers, including those in our results, can be a reference for future research.

Conclusion

In conclusion, the current study showed that hospitalized COVID-19 patients with lower IL-6, LDH, SII, CAR, and higher PNI levels improved after tocilizumab therapy. Although questions about whether the optimal timing of tocilizumab therapy for COVID-19 therapy can be guided by biomarkers remain unanswered, we proposed some parameters that can be easily obtained or calculated. Further prospective studies in different ethnicities are required to confirm our findings.

Ethics

Ethics Committee Approval: The study protocol was first registered in the data of the Turkish Republic Ministry of Health Scientific Research Committee and then approved by the Committee on the Human Research Ethics of the University of Health Sciences Türkiye, Gülhane Faculty of Medicine (date: 26.05.2021, number: 2021/35).

Informed Consent: This was a retrospective cohort study.

Authorship Contributions

Surgical and Medical Practices: M.N.K., Concept: D.T., Design: D.T., M.Ç., F.B., Data Collection or Processing: M.N.K., F.B., Analysis or Interpretation: D.T., E.T., S.Y., Literature Search: D.T., Writing: D.T., M.Ç., S.Ç.

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Transitioning to oral feeding from other routes in the palliative care unit

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ABSTRACT

Aims: The evaluation of swallowing in the palliative care (PC) unit (PCU) is an important indicator of care. In this study, we investigated how an on-admission swallowing test in the PCU guides the course of the feeding route.

Methods: This single-center, retrospective study included PC patients who underwent bedside swallowing evaluation. The main exclusion criteria were gastrointestinal failure requiring permanent parenteral nutrition, no swallowing test upon admission, and a length of stay shorter than four days. The primary endpoint was the proportion of patients with dysphagia who transitioned to oral feeding.

Results: The study included 63 patients [age, median interquartile range: 80.0 (14) years (37 to 94 years); males: 54.0%]. Thirty-six (57.1%) of 63 admissions had dysphagia, whereas 27 (42.9%) patients had no dysphagia. The route of feeding was modified in 50.8% of the samples during their PCU stay. The proportion of patients who returned to oral feeding was 30.2% (n=19), whereas 6.3% (n=4) and 12.7% (n=8) of the sample underwent nasoenteral tube and percutaneous endoscopic gastrostomy (PEG) placement, respectively. Among the 32 patients who were on tube feeding on admission, 12 (37.5%) returned to oral feeding during their PCU stay. Seven (58.4%) of these subjects who regained oral feeding were on nasoenteral tube feeding and 5 (41.6%) were on PEG feeding on admission.

Conclusions: This study showed a high rate of dysphagia on admission to the PCU. On the other hand, safe and adequate oral feeding could be re-initiated in almost one-third of patients with dysphagia on admission.

Introduction

While traditionally associated with cancer and end-of-life care (hospice), palliative care (PC) unit (PCU) placement is currently considered during a broad range of diseases, alongside curative and restorative treatments (1). Malnutrition leads to deterioration in wound healing, suppression of the immune system, decrease in skeletal muscle mass, atrophy of the intestinal mucosa, development of diffuse edema, and regression in cognitive functions (2). It is common in the PC setting and is associated with a longer length of stay (LOS) (3). Therefore, PC patients (PCP) should be screened for malnutrition, receive nutritional support when necessary, and be monitored at regular intervals (4).

Oral feeding should be a priority in nutritional support; however, if oral intake remains insufficient to meet the required daily energy need, enteral or parenteral support is initiated depending on the condition of the gastrointestinal tract (4). In contrast to oral feeding, which is physiological, controlled

studies have shown no benefit of tube feeding on wound healing and survival (5,6). Swallowing has a dual role, both as part of enjoying food and as a critically important activity for maintaining adequate nutrition and hydration (7). Moreover, sip feeding is effective and cost-saving in combating malnutrition, particularly in acutely ill patients, older adults, and multimorbid patients (8). Observational studies have shown that transitioning to oral eating may be possible in 20-25% of tube-fed patients (9-11). Younger age, lower serum creatinine levels, higher serum albumin levels, and tube placement indication (e.g., head and neck cancers) were related to transitioning to oral eating after tube removal in adult patients (11). However, the available data were generally gathered from diverse clinical settings and, unfortunately, common conditions in a PC were not involved. Moreover, the effect of medically assisted nutrition on the quality and length of life of PCPs is still not evident (12,13).

On the other hand, not admissions to the PCU may undergo a standard swallowing assessment in routine care because most patients' eating status is determined in the clinic or facility where the patient had been followed up before admission to the PCU. On the other hand, in some patients, another feeding plan (e.g., from oral eating to tube feeding or from tube feeding or parenteral nutrition to oral feeding) may be implemented following a comprehensive evaluation on admission. In the current study, we hypothesized that following assessment of swallowing, regaining oral eating could be possible in the PC setting among individuals who are already on tube feeding. Therefore, this study aimed to determine the ratio of transitioning to oral eating in a diverse population of PCU residents.

Methods

In this single-center, retrospective study, adult PCPs who underwent bedside dysphagia evaluation between April 2017 and January 2019 were retrospectively analyzed. The main inclusion criterion was bedside swallowing assessment and dysphagia testing upon admission to the PCU. The exclusion criteria were gastrointestinal failure requiring permanent parenteral nutrition, no swallowing test upon admission, and LOS shorter than four days. Demographic data, diagnoses, length of hospital stay, discharge status, absence of oral intake, artificial patency, and nasogastric or percutaneous endoscopic gastrostomy (PEG) catheter status were examined by screening the hospital registry. The Acute Physiology and Chronic Health Evaluation-II score was calculated for each patient (14). The University of Health Sciences Türkiye Institutional Review Board approved the study protocol (date: 26.02.2019, decision no: 19/33).

Sample size

In the available literature, there was no previous report on the probability of continuing with or returning to oral feeding among PCU patients with dysphagia. Therefore, we calculated the required sample size based on clinical observations. Based on the assumption that approximately 50% in the dysphagia group and 90% in the no dysphagia group would continue with oral eating at discharge, we needed 20 patients in each group to detect a statistically significant between-group difference using the z-test with alpha error probability=0.05 (two-sided) and 1-beta error probability=0.80. Because of the heterogeneity of underlying causes and clinical picture among the PCU admissions, we decided to register all dysphagia records above the minimum sample size required. After the analyses were completed, the numbers in the two groups yielded >95% power.

Swallowing assessment

In the swallowing evaluation, the dysphagia evaluation of patients who were not cooperative and oriented was considered directly positive. Bedside swallowing evaluation was performed in patients considered suitable for the swallowing test. The volume viscosity absorption test is a screening method used to evaluate swallowing safety at different food consistencies and to determine safe bolus volume and viscosity. The patient is seated with the back supported, the head in a neutral position, and the feet on the floor. Three boluses in different volumes (5-10-20 mL) were prepared for the test in liquid, nectar, and pudding consistency. Ingestion efficacy and safety were evaluated. To evaluate swallowing efficiency, lip closure, presence of oral and pharyngeal residues, and repeated swallowing were examined. To test the safety of swallowing, coughing, post-swallowing voice change, and/or a 3% decrease in oxygen saturation are checked (15).

The assessor carefully delivers the bolus to the patient using a syringe. The test begins with the consistency of nectar in increasing volumes. If there is no significant sign of aspiration with nectar consistency, the liquid viscosity is tested. In case of aspiration signs with nectar consistency, the liquid step is skipped, and pudding consistency is tested. In patients with liquid aspiration, nectar consistency is maintained (15).

Fiberoptic endoscopic evaluation of swallowing

In our institution, an otolaryngologist and swallowing physiotherapist performs fiberoptic endoscopic evaluation of swallowing (FEES) (16) routinely in patients with dysphagia during bedside swallowing evaluation. It is performed by entering through the nasal passage from the nasopharynx to the hypopharynx with a flexible fiberoptic nasopharyngolaryngoscope of 3.6 mm diameter and 26 cm length. Its tip can be rotated 90 degrees up and 130 degrees down. The pharyngeal phase of swallowing, hypopharynx, and larynx are directly visualized. It can be applied in ambulatory settings in the upright sitting position and by raising the head of the bed to 45 degrees in bedbound patients. The velopharyngeal port can be adequately visualized in the nasopharynx, and the

patient is asked to swallow during administration to observe velopharyngeal closure. The tip of the scope is then bent down and passed into the oropharynx. The amount of saliva in the hypopharynx is noted as a general indicator of pharyngeal constrictor adequacy. The general appearance of the pharynx and position of the epiglottis are noted. Finally, a point is moved to the posterior of the epiglottis where the laryngeal structures can be clearly observed with the scope. The patient is asked to swallow, hold his breath, cough, and phonate to allow the vocal folds to adduct. Rotation of the larynx or asymmetry of the vocal folds is noted. The patient is then given food and liquid, usually 5 and 10 mL, colored in contrast blue (methylene blue) to swallow. Depending on the purpose of the examination and the patient's condition, different amounts and consistencies of food may also be given. During and after swallowing with FEES material residues, the possibility of aspiration and penetration, coordination of breathing and swallowing, and piriform sinuses can be visualized (17).

Swallowing rehabilitation

The aim of swallowing rehabilitation is to regain control with motor neuron activation or stimulation of alternative motor pathways, and as a result, to prevent aspiration by performing safe and effective swallowing (18). It includes both compensatory and rehabilitative approaches. Compensatory strategies prevent aspiration and ensure safe swallowing by changing the flow direction and speed of the food without changing the physiology. In our routine practice, the rehabilitation program consists of compensatory approaches and direct and indirect therapy. The therapy is directed using data obtained from the bedside evaluation and FEES. Indirect therapy comprising oral motor training methods, sensory stimulations, posture, and maneuvers is applied to non-oral fed patients. When the patient becomes suitable for oral feeding, direct therapy methods and dietary modifications are initiated. The program is applied in 20 sessions, for an average of 45-60 minutes, and 5 days/week.

Statistical Analysis

Statistical Package for Social Sciences (version 20.0, IBM Corp., Armonk, NY, USA) software was used for statistical analysis. The distribution of the data was examined using the Kolmogorov-Smirnov test. Continuous variables are displayed as median [interquartile range (IQR)], and the ratio variables are displayed as count (%). The Mann-Whitney U test was used for between-group comparison of continuous variables. The chi-square test was used to compare ratio variables. $P<0.05$ was considered statistically significant.

Results

We screened 75 admissions to the PCU (Figure 1). Of them, 10 were excluded because of short LOS and 2 were excluded because of permanent intestinal failure. The final sample included 63 patients [age, median (IQR): 80.0 (14) years (37 to 94 years); males: 54.0%). The median (IQR) length of hospital stay was 20 (12) days. Table 1 shows the characteristics of the total sample and comparisons of patients with and without dysphagia. Hypertension, stroke, and dementia were the most common comorbidities. The median (IQR) number of comorbidities and medications was 4.0 (2) and 8.0 (5), respectively. Almost all patients were at risk of malnutrition and had mobilization disabilities. The frequency of pressure ulcers was 52.4%. While 85.7% of the patients were discharged home, 4.8% were transferred to the intensive care unit, 1.6% were transferred to other clinics, and 7.9% died in the PCU.

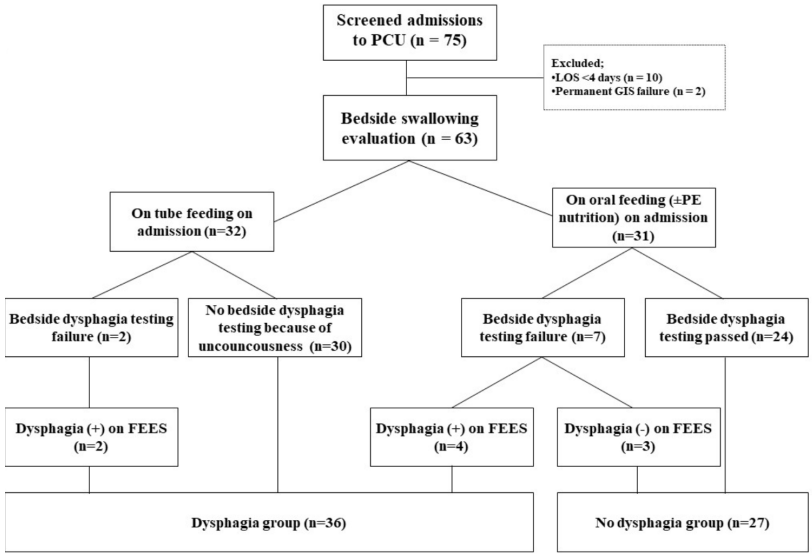

Figure 1. Flow chart on the study

PCU: Palliative care unit, GIS: Gastrointestinal system, PE: Parenteral, FEES: Fiberoptic endoscopic evaluation of swallowing

Swallowing evaluation and dysphagia testing

Of the 63 patients who underwent bedside swallowing evaluation, 32 (50.8%) were already on tube feeding on admission, and dysphagia testing was not performed in 30 (47.6%) because of consciousness. The remaining 2 (3.2%) patients showed dysphagia on bedside evaluation and underwent FEES, which confirmed dysphagia. Among 31 (49.2%) patients on oral feeding on admission, 24 (38.1%) subjects showed no dysphagia. Of the 7 (11.1%) patients who showed dysphagia on bedside evaluation, 3 (4.8%) performed safe swallowing on FEES, whereas dysphagia was confirmed in 4 (6.3%) individuals. Overall, dysphagia was not confirmed in 3 (33.3%) of 9 patients who underwent FEES because of a failed bedside swallowing evaluation.

Finally, 36 (57.1%) of 63 admissions to the PCU had dysphagia, whereas 27 (42.9%) patients had no dysphagia

Table 1. Characteristics of patients with and without dysphagia

	Total (n=63)	Dysphagia (n=36)	No dysphagia (n=27)	p
Age, years, median (IQR)	80.0 (14)	82.0 (13)	79.0 (18)	0.144
Age ≥65 years, n (%)	53 (84.1)	32 (89.9)	21 (77.8)	0.198
Age ≥75 years, n (%)	38 (60.3)	23 (63.9)	15 (55.6)	0.341
Sex, male, n (%)	34 (54.0)	18 (50.0)	16 (59.3)	0.461
Length of stay, median (IQR)	20.0 (12)	22.0 (11)	17.0 (10)	0.118
Rehospitalization, n (%)	15 (23.8)	10 (27.8)	5 (18.5)	0.292
Stroke, n (%)	33 (52.4)	21 (58.3)	12 (44.4)	0.201
Cancer, n (%)	14 (22.2)	6 (16.7)	8 (29.6)	0.179
Infection on admission, n (%)	25 (39.7)	20 (55.6)	5 (18.5)	**0.003**
Dementia, n (%)	30 (47.6)	21 (58.3)	9 (33.3)	**0.049**
Renal failure, n (%)	12 (19.0)	6 (16.7)	6 (22.2)	0.578
Hypertension, n (%)	36 (57.19	18 (50.0)	18 (66.7)	0.186
Diabetes mellitus, n (%)	16 (25.4)	8 (22.2)	8 (29.6)	0.504
Cardiovascular disease, n (%)	24 (38.1)	15 (41.7)	9 (33.3)	0.500
Malnutrition (at risk), n (%)	61 (96.8)	36 (100.0)	25 (92.6)	0.180
Mobility disability, n (%)	61 (96.8)	36 (100.0)	25 (92.6)	0.180
Pressure ulcer, n (%)	33 (52.4)	23 (63.9)	10 (37.0)	**0.031**
Permanent tracheostomy, n (%)	6 (9.5)	5 (13.9)	1 (3.7)	0.178
Sleep disturbance, n (%)	29 (46.0)	15 (41.7)	14 (51.9)	0.422
Antidepressant use, n (%)	5 (7.9)	1 (2.8)	4 (14.8)	0.101
Number of comorbidities, median (IQR)	4.0 (2)	4.0 (2)	3.0 (2)	**0.040**
Drug count, median (IQR)	8.0 (5)	9.0 (5)	8.0 (5)	0.398
APACHE-II score, median (IQR)	21.0 (8.3)	23.5 (11.3)	20.1 (7.6)	0.063
Primary cause of admission				
Dementia, n (%)	18 (28.6)	11 (30.6)	7 (25.9)	
Poststroke, n (%)	23 (36.5)	12 (33.3)	11 (40.7)	
Cancer, n (%)	10 (15.9)	4 (11.1)	6 (22.2)	
Pneumonia, n (%)	7 (11.1)	5 (13.9)	2 (7.4)	0.712
Heart failure, n (%)	3 (4.8)	2 (5.6)	1 (3.7)	
Pulmonary thrombotic disease, n (%)	1 (1.6)	1 (2.8)	0	
Crush injury, n (%)	1 (1.6)	1 (2.8)	0	
Outcomes				
Home discharge, n (%)	54 (85.7)	30 (83.3)	24 (88.9)	0.533
Transfer to ICU, n (%)	3 (4.8)	3 (8.3)	0	0.180
Transfer to other wards, n (%)	1 (1.6)	0	1 (3.7)	0.429
Died, n (%)	5 (7.9)	3 (8.3)	2 (7.4)	0.636

IQR: Interquartile range, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, ICU: Intensive care unit

(Table 1). Patients with on-admission dysphagia also had more infection, dementia, and pressure ulcer diagnoses and a higher number of comorbidities. Other variables and outcomes, however, did not significantly differ between the two groups.

Course of feeding route during PCU admission

The following admission, the route of feeding was not modified in 50.8% (n=32) patients (Table 2). The proportion of patients who returned to oral feeding was 30.2% (n=19). However, 6.3% (n=4) and 12.7% (n=8) of the sample underwent nasoenteral tube and PEG placement, respectively, after admission to the PCU.

Among the 32 patients who were on tube feeding on admission, 37.5% (n=12) of the patients returned to oral feeding during their PCU stay. Of them, 58.4% (n=7) who regained oral feeding were on nasoenteral tube feeding and 41.6% (n=5) were on PEG feeding on admission. Two patients on PEG feeding who showed dysphagia on both bedside testing and FEES returned to oral feeding following rehabilitation, whereas the other three PEG tube-fed patients did not show dysphagia on bedside testing or FEES on admission. All 7 patients on nasoenteral feeding on admission did not show signs of dysphagia on bedside testing or FEES; thus, they were given a trail of oral feeding. A total of 37 patients received swallowing rehabilitation.

Discussion

In this study on a heterogeneous PCP sample, half of the patients were on tube feeding on admission and most of them showed consciousness issues that did not allow assessment of swallowing. Dysphagia as assessed by the bedside swallowing test and FEES was recorded in over half of the sample. As the core finding, before discharge, almost one-third of the patients transitioned to oral eating. Of the tube-fed individuals on admission, over one-third returned to oral feeding before discharge. Nevertheless, one-fifth of the sample underwent new tube placement during their PCU stay. FEES did not confirm dysphagia in one-third of patients who failed the bedside swallowing test.

Swallowing problems can reach 79% in the PC setting (19). Our observation showed that 57.1% of the admissions to the PCU suffered from dysphagia, and most were older adults by 84%. Dysphagia is common in older age and is considered a geriatric syndrome (16). This is not only associated with the weakening of swallowing function with advancing age but also with a higher prevalence of significant comorbidities with advanced age, such as neurological diseases and head and neck cancers that ultimately cause dysphagia (20). On the other hand, the prevalence of dysphagia may also vary because of the measurement tool, disease type, or disease stage that was more clearly seen during the recent pandemic. In our sample, for instance, the group with dysphagia had a significantly higher number of comorbidities (Table 1), supporting the association between disease burden and swallowing dysfunction. On the other hand, despite the high prevalence of stroke and dementia (80% of the sample), over 40% of the patients showed intact swallowing function on admission, suggesting the potential for improvement or reestablishment of oral eating even in a far-end diseased population in the PC phase.

Table 2. Modification of the feeding route following PCU admission

Feeding route	n (%)
No modification	32 (50.8)
Oral feeding regained	19 (30.2)
Initiation of nasoenteral tube feeding	4 (6.3)
Initiation of PEG tube was feeding	8 (12.7)

PCU: Palliative care unit, PEG: Percutaneous endoscopic gastrostomy

The current findings contribute to the literature that recovery of oral eating in overall dysphagia or tube feeding is potentially possible in the PCU. We observed that 30.2% of all admissions to the PCU regained oral eating before discharge. In contrast to pediatric patients, recovery of oral intake in oropharyngeal dysphagia and tube feeding has not been studied effectively in complex adult patients other than stroke survivors. A Japanese study on 14 older adults with a mean age of 83.9 years reported a 50% recovery of oral eating among long-term tube-fed individuals (21). However, the participants were not PCU residents, limiting the comparisons with our study. Insufficient knowledge in the adult population may be associated with the absence of interventional evidence to re-establish oral intake in such patients. Buchholz proposed an algorithm for reinstituting oral feeding in adult tube-fed patients more than two decades ago (22). However, no well-grounded approach has yet been validated, even in an observational nature. Although transitioning to oral eating is possible in complex patients (11), choosing the correct patient is central to initiating the required practice. In this context, several authors have recently described potential predictors of swallowing recovery (23,24). Age, body mass index, cognitive status, presence of residue, higher risk of aspiration, aphasia, and larger white matter volume were linked to the achievement of oral intake (23,25,26). Moreover, several authors have developed prognostic models for recovery from enteral tube feeding to oral eating more than a decade ago (27). As a further step, Galovic et al. (28) introduced a model that included age, National Institutes of Health Stroke Scale score, stroke location, initial risk of aspiration, and initial impairment of oral intake, and performed successful validation that showed good agreement between predicted and observed outcomes in the short term. Subsequently, Lee et al. (29) developed a model for the prediction of 6-month swallowing recovery, which was validated using Bayesian network models. While the utility of such models awaits further research and accumulation

of clinical experience, well-conducted studies have focused almost entirely on stroke survivors as it is highly associated with dysphagia and aspiration (30), which increase LOS and mortality (31). Nevertheless, knowledge in other conditions such as cancer and chronic neurological diseases, as well as in different settings such as PCUs, is still lacking. In the current study, transitioning to oral eating was observed in nearly one-third of both all patients with dysphagia and the tube-fed subgroup, which is novel in the PC setting.

We performed FEES in patients with failure in bedside swallowing evaluation and identified that one-third of them had intact swallowing function when objectively evaluated. This confirmatory role of FEES in dysphagia is in agreement with previous reports (32,33). FEES is the gold standard tool (34) and aspiration detected by FEES predicts mortality in patients with oropharyngeal dysphagia (35). It allows rapid evaluation of swallowing function in critically ill patients, who are seldom available for bedside testing (36). The position paper by the Royal College of Speech and Language Therapists states that FEES may be safely practiced in other settings, including community hospitals, health centers, nursing homes, general practitioner surgeries, hospices, and rehabilitation facilities (37). To the best of our knowledge, our observation is the first to confirm the successful use of FEES to confirm dysphagia in a PCU setting. However, FEES was not routinely performed upon admission to the PCU in the current sample, but some patients underwent new tube placement during their PCU stay. It may be postulated that a routine FEES upon admission to the PCU might identify potential candidates for tube feeding in later days. However, the current study is limited by its low power to draw clear conclusions in this regard.

Study Limitations

This study has several limitations. First, the data were cross-sectional and retrospective without post-discharge follow-up observation. Second, because of the small sample size, our findings may not be generalizable to the broad range and diverse PC population. Third, we were unable to explore whether transitioning to oral eating was more likely in some subgroups of PCPs. Fourth, our analyses remained unadjusted for potential covariates because of several confounders but a low number of patients. Finally, the number of patients who received swallowing rehabilitation was low, and the procedures were performed on a routine basis, allowing only binary outcome information in the patient files. Several study strengths should also be acknowledged. First, the study was performed in a PCU connected to a tertiary referral center for complex medical and surgical patients, assuring data quality in the medical health records. Second, the displayed potential for the recovery of oral eating in the PC setting may encourage professionals in the field to adapt. Fourth, the findings may drive future research to identify who can benefit from a trial of transitioning to oral eating in the PCU. Fifth, as part of the routine, a 24-hour follow-up of patients in the study setting was likely to provide the patients with the best available care to recover or resume. Lastly, FEES and swallowing rehabilitation were performed by a single registered technician with sufficient experience.

Conclusion

In conclusion, tube feeding is not only uncomfortable and associated with mobility limitation but also associated with significant complications (38). This study showed a high rate of dysphagia on admission to the PCU. However, safe and adequate oral feeding could be re-initiated in almost one-third of patients with dysphagia on admission, including tube-fed individuals.

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Ethics

Ethics Committee Approval: The University of Health Sciences Türkiye Institutional Review Board approved the study protocol (date: 26.02.2019, decision no: 19/33).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: H.Z., V.Y., İ.T., Concept: H.Z., V.Y., İ.T., Design: H.Z., S.A., V.Y., İ.T., Data Collection or Processing: H.Z., N.E., C.E.Ü., S.A., Analysis or Interpretation: H.Z., N.E., C.E.Ü., S.A., Literature Search: H.Z., N.E., C.E.Ü., S.A., V.Y., İ.T., Writing: H.Z., N.E., C.E.Ü., S.A., V.Y., İ.T.

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Dextrose concentration for prolotherapy: A study on human neuroblastoma cells

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ABSTRACT

Aims: Prolotherapy is performed using a 15%-concentrated hypertonic dextrose solution that targets damaged soft tissues to induce a regenerative process. A novel technique pointing at perineural regions using 5%-concentrated dextrose is also gaining popularity in daily clinical practice. Animal and human studies have revealed the clinical and functional benefits of perineural prolotherapy; however, the cytological effects of dextrose solution are immature. This study compared the effects of dextrose solutions at different concentrations on neurons at the cytological level.

Methods: Cultures of human neuroblastoma cell lines that simulate neurons at the cytological level were prepared. The sample cultures consisted of the control group (CG), 5% dextrose-supplemented group (5DG), and 15% dextrose-supplemented group (15DG). The neuronal viability assay of the cultures before and 6 h after supplementation was compared between the groups.

Results: The pre-supplementation cell viability percentage of CG was presumed to be 100%. The post-supplementation cell viability percentages of CG, 5DG, and 15DG were 93.33%, 22.22%, and 0%, respectively. The decreases in cell viability in both dextrose groups were statistically significant when compared with CG ($p<0.001$). Moreover, the difference in cell viability between the 5DG and 15DG groups was also statistically significant ($p<0.001$).

Conclusions: Our findings suggest that dextrose solution has an acute, concentration-dependent toxic effect on cultured human neuroblastoma cells.

Introduction

Prolotherapy is the injection method of an irritant solution into a joint space, ligament, or tendon insertion site, mainly targeting chronic musculoskeletal pain (1). Clinical benefits of prolotherapy have been reported for osteoarthritis, tendinopathies, joint pains, and chondromalacia (2,3); thus, prolotherapy is becoming popular among physicians who deal with musculoskeletal system pathologies.

Prolotherapy acts as a trigger of local inflammation by inducing a regenerative response (4). Hypertonic dextrose is the most commonly used injection agent (5). The dextrose solution potentially contributes to the healing process in two ways: 1) by increasing the osmotic pressure of the extracellular space, thus causing the cells to lose water and destroy, consequently resulting in a temporary state of inflammation, and 2) by stimulating the proliferation of platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor, insulin-like growth factor, and connective tissue growth factor (4).

The most preferred concentration of dextrose for prolotherapy is 15% (6,7), which was first described by Hackett et al. (8) in the 1950s and subsequently modified by others not only for dextrose concentration but also for the injection sites (9). One of the modified versions is perineural injection therapy, which involves the injection of 5% dextrose solution into the peripheral

nerves and perineural location, resulting in considerable pain-relief effects (10). Perineural injection therapy was supported in 2005 by Maniquis-Smigel et al. (9,11) and declared to have therapeutic effects with neuromodulatory effects (4) on peripheral entrapment neuropathic (9) and neuropathic pain (11), probably by improving sensory and motor functions (9,12) and decreasing neurogenic inflammation (9), in comparison with 15% dextrose.

Less is known about the complications of dextrose prolotherapy (13). Except for general injection adverse reactions such as self-limited pain, bruising (14), inflammation, and hematoma (15), no significant side effects of prolotherapy, including either the concentration of hypertonic dextrose solution or the localization of injection, have been reported (14,15). Few clinical studies have revealed that 5% dextrose solution is a safe and effective choice and has no side effects on nerves, but the conclusions of these animal and human studies were based on ultrasonographic findings and electrophysiological parameters (9,10,12). To the best of our knowledge, the cellular effects of different concentrations of dextrose have been studied only on the cell cultures of human fibroblasts (16,17), which presented findings relatively in contrast. To the best of our knowledge, no cytological study has evaluated the “safe” or “side” effect of dextrose solution on neural cells (18). The cumulative effects of dextrose injection also remain unknown (9).

A variety of hypotheses have been stated about the mechanisms of action explaining the beneficial clinical effects of dextrose prolotherapy. Although the evaluation of the cytological changes may shed light on the mystery. Thus, we compared the effects of 5% and 15%-concentrated dextrose solutions on primary neuronal cultures *in vitro*, simulating the cytological level at the injection site. The obtained findings may also help to determine the optimal concentration of dextrose solution and the correct injection site for prolotherapy.

Methods

Neuroblastoma cell cultures

The human neuroblastoma cell line [SH-SY5YATCC® CRL-2266™], which mimics neurons in the cell culture, was obtained from the Cancer Research Center, Institute of Health Sciences, University of Health Sciences Türkiye. The SH-SY5Y neuroblastoma cell line has distinct functional characteristics with natural dopaminergic and adrenergic features (18,19). Cultures of the cell line were prepared in accordance with classical standards (19). The cell line was incubated in RPMI (Sigma Aldrich-R8758) medium containing 10% (v/v) foetal calf serum (Biochrom AG, Berlin, Germany) with 1% (v/v) penicillin and streptomycin (Biological Industries Ltd., Haemek, Israel) (37 °C, 5% CO_2) (Heraeus incubator, Hanau, Germany) for 24 h (19).

A total of nine Petri dishes of cell culture were used for the experiments to form three comparison groups: control group (CG), 5% dextrose-supplemented group (5DG), and 15% dextrose-supplemented group (15DG). Each comparison group comprised three Petri dishes of cell culture. The RPMI (Sigma Aldrich-R8758) Petri dishes were CGs themselves. 2 mL of 5% dextrose solution (prepared by dissolving 27.8 mM of Sigma-Aldrich dextrose, D9434) was directly added to the three RPMI (Sigma Aldrich-R8758) Petri dishes of 5DG. Likewise, 2 mL of 15% dextrose solution (prepared by dissolving 83.4 mM of Sigma-Aldrich dextrose, D9434) was directly added to the three RPMI (Sigma Aldrich-R8758) Petri dishes of 15DG on the next day. All cultures were incubated for 24 h (at 37 °C and 5% CO_2) and examined under a microscope (Zeiss Axio Vert. A1 inverted phase fluorescence microscope, Germany) immediately after the supplementation of dextrose solutions and every 6 h after that.

Neuronal viability assay

The viability of neuroblastoma cells was determined by the fixation of cells with an ethanol-formaldehyde-acetic acid (7:2:1) mixture and staining with trypan blue (Sigma Aldrich Co. 302643), because this method is simple, cheap, and allows rapid counting of cells with ruptured membranes under the microscope (20). Trypan blue was diluted to 0.8 mM with phosphate-buffered saline. The survival rates of neuroblastoma cells were calculated by counting the intact nuclei on a hemocytometer in five regions of view (19,21). A scanning microplate reader was used to measure the absorbance of the dye solution at 450 nm. The baseline viability of CG cell cultures was presumed to be 100%, and both dextrose solution-supplemented groups were calculated according to this approach (19,21).

This study was approved by the Gülhane Military Medical Academy Local Ethical Committee (decision/date no: session 05/04.07.2012). Because this study was conducted on a commercial cell line, no verbal or written consent was obtained.

Statistical Analysis

All data were recorded using nine different cell cultures from three independent experiments. The viable cell percentages of CG, 5DG, and 15DG were compared by one-way analysis of variance and Bonferroni post-hoc tests using Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 18.0 (SPSS Inc., Chicago, Ill., USA). P values of $p<0.05$ were considered statistically significant.

Results

The neuronal viability assay was planned to be performed by the end of incubation (and at the same time 24 h after the supplementation of dextrose solutions). However, the death of numerous neuroblastoma cells was observed in the 15DG at the first control and photographing stop, 6 h after the

supplementation (post-supplementation). We terminated the incubation process and proceeded to the neuronal viability assay stage.

The post-supplementation neuronal viability assay revealed that both 5% and 15% dextrose solutions caused a significant decrease in viable neuroblastoma cells in 5DG and 15DG (Table 1). In the 5DG and 15DG, the post-supplementation viability of neuroblastoma cells was significantly lower than CG ($p<0.001$). Moreover, the difference in post-supplementation viability of neuroblastoma cells was statistically significant between the 5DG and 15DG ($p<0.001$). The percentages of viable neuroblastoma cells were 93.33%, 22.22%, and 0% in CG, 5DG, and 15DG, respectively.

Discussion

This study aimed to compare the effects of 5% and 15% dextrose solutions on human neuroblastoma cell cultures and, thus, to foresee the real effects of perineural 5% dextrose injection therapy at the injection site. Both dextrose solutions were toxic to neuroblastomas in the first 6 h. Furthermore, 15% dextrose solution had a significantly more lethal effect than 5% dextrose. Our findings suggest that dextrose prolotherapy may have similar acute harmful effects on the perineural regions in a concentration-dependent manner.

Prolotherapy has been a widely used injection treatment; however, the mechanism of action of the therapeutic effects of prolotherapy is still barely known, and the limited knowledge prevents us from proposing a clear opinion. The suggested conclusions are the induction of inflammatory response, anabolic reactions (22), cellular proliferation, and vascular changes locally by the injection of dextrose solution (23).

The threshold concentration of dextrose is 10% to induce local inflammation. Concentrations of dextrose solution above 10%, especially the clinically preferred concentration of 15%, activate the inflammation cascade by increasing osmotic pressure (23). Some studies have revealed that the mechanism of action of dextrose solution on neuropathic pain might be the inhibition of transient receptor potential vanilloid receptor-1 (TRPV-1) (4,11,12,22), inactivating the secretion of substance P, calcitonin gene-related peptide (4,10), and nitric oxide (4), thereby alleviating neurogenic inflammation. In addition, Wu et al. (10) compared the effects of perineural injections of 5% dextrose with normal saline in patients with mild-to-moderate carpal tunnel syndrome and concluded that the neurogenic anti-inflammatory effects of 5% dextrose were significantly higher than those of normal saline. Similarly, Chen et al. (12) evaluated the neuropathic pain-relief effect of perineural 5% dextrose injection in a patient with radial nerve palsy. In another study, an epidural injection of 5% dextrose was compared with 0.9% saline in patients with moderate-to-severe low back pain, and pain relief was significantly higher in the 5% dextrose group (11). In addition to these pathways, non-inflammatory concentrations of dextrose solution contribute to the proliferation of musculoskeletal cells and influence the transport of amino acids and protein synthesis with no cellular toxicity (23). However, our findings indicated that 5% dextrose solution had lethal effects on cell cultures of neuroblastomas, simulating neurons at the perineural injection sites.

The only study evaluating the effects of dextrose solution on SH-SY5Y human neuroblastoma cell cultures compared the findings of different concentrations ranging between 0 and 25 mM with and without the existence of tumor necrosis factor-α (TNF-α) (18). Based on the hypothesis that dextrose would act like an anti-inflammatory molecule as a response to the TNF-α-induced inflammation, the authors concluded that a higher concentration of dextrose (i.e., 25 mM) decreased the neuronal impairment developed by inflammation and suggested this finding as a probable mechanism of the therapeutic effect of dextrose on neuropathic pain, in contrast with our observation. However, the studies of Güran et al. (16) and Woo et al. (17) have declared the final cytotoxic effect of dextrose solutions on fibroblasts despite the confusing findings concerning the available concentrations. Güran et al. (16) concluded that 15%, 20%, and 25%-concentrated dextrose solutions, but not 5% and 10%-concentrated dextrose solutions, led to significant fibroblast death, while Woo et al. (17) observed a significant decrease in fibroblast viability in 5% dextrose and complete death of almost all fibroblasts in 10% dextrose. The preferred concentrations of dextrose used in these two studies were in agreement with our method, which may be more conducive to clinical interpretation despite the difference in examined cells.

The pathophysiology of diabetic neuropathy shows similarities with the possible pathways that explain our findings. Hyperglycemia in diabetes mellitus contributes to neuropathy through increasing oxidative stress, generation of glycation end products, accumulation of polyols, and vasculopathy-

Table 1. Baseline and post-supplementation viable neuroblastoma cells

Groups	Baseline	Post-supplementation	p
Control, mean±SD	45.11±0.78	42.67±1.15	0.073
5% dextrose, mean±SD	44.66±1.15	9.33±0.58	**<0.001**
15% dextrose, mean±SD	44.66±0.58	0	**<0.001**

Bold data are statistically significant.
SD: Standard deviation

related nerve ischemia (24-27). Mohammadi-Farani et al. (28) evaluated the effects of glucose concentration on the PC12 cell line culture medium and reported that hyperglycemia had an unfavorable influence on neuronal viability by reducing the expression of cannabinoid-1 receptor and activating TRPV-1. Moreover, higher glucose concentrations were associated with more toxic effects through these receptor responses, confirming the concentration-dependent cellular death observed in our study. The same study also suggested that increased TRPV-1 expression facilitates neuronal damage due to long-term exposure to dextrose in the culture medium (28). However, we observed the neurotoxic effects of dextrose solution in the acute period, which is worth comprehensive research. In cultured neurons, glucose uptake is not dependent on insulin because of the absence of an interstitial barrier (25). This may explain the mechanism of our findings in part, but the concentration of the dextrose solution seems to have some influence.

The results of the studies regarding the effect of dextrose solutions at different concentrations on the morphology and function of nerves are contradictory. Yoshii et al. (23) studied New Zealand white rabbits by injecting 10% dextrose into the subsynovial connective tissue of one forepaw and 0.9% saline into the same location in the contralateral forepaw. The authors compared the electrophysiological, histological, and mechanical findings between the 10% dextrose and 0.9% saline groups and observed acute alterations in motor function of the median nerve in the 10% dextrose group, with no significant morphological difference. In another study testing the same hypothesis of a probable dose-response effect of hypertonic dextrose solution to experimentally induce carpal tunnel syndrome (29), the same authors compared the effects of saline, 10% dextrose, and 20% dextrose solutions with different numbers of injection sessions. They reported the development of an experimentally induced carpal tunnel syndrome and morphological flattening of the median nerve in 10% and 20% dextrose groups compared with saline, but no malfunction. In 2014, the same authors tested the effects of multiple once-a-week injections of 10% dextrose and saline to induce experimental carpal tunnel syndrome and reported the thinning of the myelin sheath and Wallerian degeneration in the 10% dextrose group compared with that in the saline group (30). They concluded that there was an association between larger doses of dextrose and the severity of neuropathy (30). Despite the controversy, the current knowledge supports the concentration-dependent neurotoxic effect of dextrose solution, which we observed in the current study. However, all were animal studies of experimental carpal tunnel syndrome. To the best of our knowledge, our study is the first to evaluate the neurotoxicity of dextrose prolotherapy on human cell culture and has indicated an acute concentration-dependent toxic effect on cultured human neuroblastoma cells. These findings led us to think that prolotherapy might be harmful rather than helpful, especially in perineural localizations.

Study Limitations

Despite the remarkable findings, this study has a few limitations. First, *in vitro* neurological disease models commonly use SH-SY5Y neuroblastoma cell cultures; however, these cells retain the properties of cancer cells. Second, we have not performed the advanced assay of neuronal viability, such as spectrophotometric, fluorometric, or caspase activity determinations. Our findings may be considered evocative but not sufficient to conclude a precise agreement on the negative effect of hypertonic dextrose prolotherapy. Not only *in vitro* designs but also *in vivo* studies evaluating the changes in cytokine levels in the localization of prolotherapy may enable us to obtain more accurate findings and clearly conclude.

Conclusion

This study revealed that 5% and 15% dextrose solutions have cytotoxic effects on neuroblastomas in the first 6 h. Thus, the concentration of dextrose and the injection site may need to be considered during prolotherapy indications. Whether these findings occurred due to a dextrose-induced mechanism of action or the dextrose itself remains unclear. Further studies evaluating these mechanisms and cytokine expression may explore the unknown aspects of the cellular effects of different dextrose solutions in prolotherapy.

Ethics

Ethics Committee Approval: This study was approved by the Gülhane Military Medical Academy Local Ethical Committee (decision/date no: session 05/04.07.2012).

Informed Consent: Because this study was conducted on a commercial cell line, no verbal or written consent was obtained.

Authorship Contributions

Surgical and Medical Practices: Ö.K., Concept: Ö.K., Z.D.Ç., Design: Ö.K., Z.D.Ç., Data Collection or Processing: Z.D.Ç., Analysis or Interpretation: Ö.K., Z.D.Ç., Literature Search: Ö.K., Writing: Ö.K.

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Association between plasma fluoride levels and pregnancy complications in women living in the rural and urban areas of Settat-Morocco

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ABSTRACT

Aims: This study aimed to investigate the relationship between plasma fluoride levels and pregnancy complications among rural and urban women living in the Settat province of Morocco, where fluorosis is endemic.

Methods: This cross-sectional study included pregnant women permanently residing in the province of Settat who visited the Provincial Hospital Hassan II. A close-ended questionnaire was completed to verify the presence or absence of pregnancy complications, including abortion, fetal and neonatal complications, diabetes mellitus, and pregnancy-induced hypertension. In addition, blood was collected from all participants and fluoride levels were determined.

Results: The sample included 511 rural (43.8%) and urban (56.2%) pregnant women with a mean age of 26.9±6.1 years. The prevalence of pregnancy complications was significantly higher in rural pregnant women (abortion=13.2%; fetal and neonatal complications=4.8%; pregnancy induced-hypertension=1.4%) than in urban areas (abortion=6.7%; fetal and neonatal complications=2.2%; pregnancy induced-hypertension=0.9%) ($p<0.05$). Plasma fluoride levels were also significantly higher in rural participants with a means of 0.036±0.009 mg/L than in urban areas with a means of 0.034±0.008 mg/L ($p<0.05$). However, in both rural and urban participants, the results showed that there was no correlation between plasma fluoride levels and abortion ($r=0.068$; $p=0.247$), fetal and neonatal complications ($r=-0.008$; $p=0.890$), diabetes mellitus ($r=-0.018$; $p=0.975$) and pregnancy induced-hypertension ($r=-0.063$; $p=0.285$).

Conclusions: Higher plasma fluoride levels were found in rural pregnant women than in urban ones. However, no significant correlation was found between plasma fluoride levels and the studied pregnancy complications, despite their higher prevalence in rural women.

Introduction

Fluoride is a chemical element belonging to the halogen family. It is widely distributed in the environment mainly in soil, air, and water (1). At the recommended doses, this element plays an essential role in increasing the structural stability of teeth and bones. It is also involved in the growth of both humans and animals (2). However, chronic exposure to high levels of fluoride can lead to chronic intoxication, such as dental and skeletal fluorosis (3). Furthermore, prolonged exposure to this halogen can induce other toxic effects in the reproductive, nervous, and immune systems, leading to non-skeletal fluorosis (4). In many parts of the world, fluorosis is a significant public health concern. It is endemic in more than 20 countries,

although it is most prevalent in tropical areas, with China and India among the worst-affected nations (5). Other regions, including the Middle East, South America, and Africa (Morocco, Tanzania, South Africa, Kenya, Ghana, and Sudan), are also poisoned (5). Moreover, it was documented that fluoride intake through groundwater is the major contributor to fluorosis and has become one of the most critical issues affecting human health (6). Normally, drinking water is considered contaminated when its fluoride level is between 1.1 and 2.5 ppm and toxic when its fluoride level is greater than 2.5 ppm (7). Hence, the World Health Organization reported that fluoride-rich drinking water exposes many countries to a high risk of being affected by high levels of fluoride (8).

The association between plasma fluoride levels and the prevalence of pregnancy complications is well established in women living in several endemic fluorosis areas (9-11). In these studies, a positive correlation between plasma and urinary fluoride levels and pregnancy complications was reported. In contrast, to the best of our knowledge, no study has focused on pregnancy complications in women living in Moroccan endemic fluorosis areas. In this country, fluorosis is considered a major problem because of its wide distribution (2). There are two types of fluorosis, the first is called hydrotelluric, where the contamination sources are soil and water; it is localized in phosphate areas. The second type is called industrial fluorosis, where the contamination source is air; it is localized in industrial areas (2). Additionally, it was reported that chronic consumption of high fluoride levels through groundwater is the major contributor to fluorosis in humans and several other species (12). Concerning Settat province, where fluorosis is hydrotelluric (13), it includes both urban and rural areas. Increasingly, the rural population consumes well water containing high fluoride levels (14), whereas the urban population consumes tap water that is filtered and controlled. This suggests that rural populations are more exposed to fluoride excess. Therefore, the present study aimed to investigate the relationship between plasma fluoride levels and pregnancy complications among rural and urban women living in the Settat province.

Methods

Study design

The current work was designed as a cross-sectional study that included rural and urban pregnant women aged between 18 and 45 years, who visited the Provincial Hospital Hassan II of Settat province, Morocco in 2019. This province is one of the Moroccan endemic fluorosis areas (14).

The current research was conducted according to the ethical principles of the Helsinki Declaration of the World Medical Association in 1964, amended by the General Assembly of Fortaleza, Brazil, October 2013. The authorization N°1295/18 from the Direction of Epidemiology and Diseases Control of the Ministry of Health of Morocco was obtained before conducting this study.

Inclusion and exclusion criteria

All pregnant women permanently residing in the province of Settat who visited the Provincial Hospital Hassan II during the study period were considered as the source population. After excluding unconfirmed pregnancies and temporary inhabitants in the province, the study sample was formed out of 511 participants.

General and medical information

To collect the general, medical, and obstetrical information of the population study, a close-ended questionnaire was developed and validated in the presence of the research team. Subsequently, it was tested in a similar population to ensure it was understandable. The questionnaire contained open and closed questions and focused on general information such as age, origin, educational level, medical and obstetric information through the verification of the presence or absence of dental stains, and pregnancy complications including abortion, fetal and neonatal complications, diabetes mellitus, and pregnancy-induced hypertension.

Determination of plasma fluoride levels

Blood samples were collected from venous blood using heparin tubes and centrifuged (3000 g for 15 minutes) to obtain plasma samples. Then, one volume of each sample was mixed with the same volume of total ionic strength adjustment buffer (TISAB II). Plasma fluoride levels were measured using a fluoride electrode (Thermo Scientific Orion 96-09, Orion Research, Cambridge, MA, USA) coupled to an analyzer ion (Star A214, Thermo Scientific Orion). The electrode was calibrated with standard fluoride solutions at concentrations of 0.025, 0.050, 0.075, and 0.1 mg/L and prepared with the same reagent used for the samples. Plasma fluoride levels in women without pregnancy complications and those with abortion, fetal and neonatal complications, diabetes mellitus, and pregnancy-induced hypertension were measured and expressed in mg/L.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to evaluate whether the data were normally distributed. The non-normally distributed numeric data were compared using the Mann-Whitney U test, and a p-value of 0.05 was considered statistically significant. The results were summarized as mean±standard deviation (SD), and the categorical data are presented with count (n) and percentage (%) values. We analyzed the correlations between plasma fluoride levels and

each complication in rural and urban women using Pearson's correlation test or Spearman's correlation test.

Results

Descriptive characteristics

The sample included 287/511 (43.8%) rural and 244/511 (56.2%) urban pregnant women with a mean±SD age of 26.9±6.1 years. Based on the questionnaire data, a remarkable variation in educational level was observed between rural and urban participants. Among rural women (n=287), 175 (61.0%), 98 (34.2%), and 7 (2.5%) subjects had primary, secondary, and higher educational levels, respectively. Among urban women (n=224), 70 (31.2%), 138 (61.6%), and 15 (6.7%) subjects had primary, secondary, and higher educational levels, respectively. The remaining 7 (2.5%) rural subjects versus 1 (0.4%) urban subjects were illiterate. Dental stains were significantly more common in rural participants than in urban participants (Table 1). Rural participants also showed poorer oral hygiene (Table 1). Regarding plasma fluoride levels, rural pregnant women had significantly higher plasma fluoride levels (0.036±0.009 mg/L) than their urban counterparts (0.034±0.008 mg/L) ($p<0.05$) (Table 1).

Prevalence of pregnancy complications

Regarding pregnancy complications, 79.8% of rural versus 88.9% of urban participants did not report any pregnancy complications (Table 2). Abortion and fetal and neonatal complications were significantly higher in rural pregnant women than in urban subjects ($p<0.05$). The prevalence of diabetes mellitus and pregnancy-induced hypertensive complications was not significantly different between rural and urban participants ($p>0.05$) (Table 2).

Relationship between plasma fluoride levels and pregnancy complications

In both rural and urban participants, plasma fluoride levels were significantly ($p<0.05$) higher in women with abortion, fetal and neonatal complications, diabetes mellitus, and pregnancy-induced hypertension than in those without these complications. When rural and urban women who reported the same complications were compared, we found significantly higher plasma fluoride levels only in pregnant women from rural areas with fetal and neonatal complications compared with those from urban areas ($p<0.05$). However, no significant difference was observed between rural and urban participants with abortion, diabetes mellitus, and pregnancy-induced hypertension ($p>0.05$) (Table 3). Additionally, in both rural and urban participants, the

Table 1. Dental stains, oral hygiene and plasma fluoride in rural and urban participants

Origin	Rural (n=287)	Urban (n=224)	p-value
Dental stains, n (%)	188 (65.5)	33 (14.7)	**0.001**
Poor oral hygiene, n (%)	181 (63.1)	69 (30.9)	**0.001**
Plasma fluoride (mg/L), mean±SD	0.036±0.009	0.034±0.008	**0.018**

The results are significant at the level of 0.05.
SD: Standard deviation

Table 2. Prevalence of studied pregnancy complications in rural and urban pregnant women

	Rural (n=287)	Urban (n=224)	p-value
Without complication, n (%)	229 (79.8)	199 (88.9)	**0.006**
Abortion, n (%)	38 (13.2)	15 (6.7)	**0.016**
Fetal and neonatal complications, n (%)	14 (4.8)	5 (2.2)	**0.047**
Diabetes mellitus, n (%)	2 (0.7)	3 (1.3)	0.146
Pregnancy induced-hypertension, n (%)	4 (1.4)	2 (0.9)	0.247

The results are significant at the level of 0.05

Table 3. Plasma fluoride levels in rural and urban participants according to their pregnancy complications

Origin	Rural (n=287)	Urban (n=224)	p-value
Without complication (mg/L), mean±SD	0.036±0.009	0.034±0.008	**0.005**
Abortion (mg/L), mean±SD	0.038±0.011	0.039±0.011	0.425
Fetal and neonatal complications (mg/L), mean±SD	0.038±0.003	0.035±0.008	**0.018**
Diabetes mellitus (mg/L), mean±SD	0.039±0.001	0.039±0.011	0.413
Pregnancy induced-hypertension (mg/L), mean±SD	0.041±0.001	0.039±0.004	0.125

The results are significant at the level of 0.05.
SD: Standard deviation

results showed that there was no correlation between plasma fluoride levels and abortion (r=0.068; p=0.247), fetal and neonatal complications (r=-0.008; p=0.890), diabetes mellitus (r=-0.018; p=0.975) and pregnancy induced-hypertension (r=-0.063; p=0.285) (Table 4).

Discussion

In Morocco, fluorosis extends to all phosphate areas (2,13). In these endemic areas, it has been reported that the weathering of phosphate rocks releases a large amount of fluoride, which contaminates groundwater and soil (2), causing hydrotelluric fluorosis (13). For instance, in Settat province (Morocco), a study revealed a high fluoride level in well water (14). Consequently, direct consumption of this contaminated water and/or the consumption of natural products (cereals, fruits, etc.) that grow in the contaminated soil leads to various toxic effects (15). After its intake, fluoride can lead to several adverse effects in different forms of fluorosis in humans (12,16). Recent studies have shown that fluoride can increase pregnancy complication risks (6,17). Conversely, low levels of awareness and prevailing dietary and behavioral practices may put populations at risk of high fluoride intake (18).

In the current study, it was revealed that the educational level was lower in rural participants than in those from urban areas. These results could be explained by family income and denial by the parents as these could be the most common reasons for school dropout in rural areas (19). Additionally, our findings showed that rural pregnant women had poorer oral hygiene than their urban counterparts. This may explain the higher prevalence of dental stains observed among rural participants. In this context, an association between oral health and several socioeconomic factors such as poverty, education, and health disparities between urban and rural areas has been documented previously (20).

Concerning the prevalence of pregnancy complications in both studied areas, abortion and fetal and neonatal complications were higher in rural pregnant women than in urban ones. In this sense, a previous study conducted in the province of British Columbia (Canada) reported that rural pregnant women had higher rates of severe maternal and neonatal morbidity (21). Another study conducted in Ethiopia found that rural women were more likely to experience adverse pregnancy outcomes, including abortion and neonatal deaths, compared with their urban counterparts (22). Given that blood is the main transporter of fluoride in the body, plasma fluoride level is an essential parameter for diagnosing the chronic toxic effects of fluoride, mainly in pregnant women (23,24). In this study, plasma fluoride concentration in pregnant women showed that its average was significantly higher in rural pregnant women compared with those of urban origin. This finding could be explained by the drinking water sources in each area, as the rural population consumes well water, which is contaminated by fluoride excess in phosphate rocks, whereas the urban population consumes tap water, which is filtered and controlled. Similarly, another study conducted in India revealed a positive correlation between plasma fluoride in pregnant women and fluoride in groundwater (25). To compare the values obtained in this study, it was revealed that they were lower than those obtained in pregnant Polish women (26) and higher than those obtained in Mexican pregnant women (27) and American pregnant women living in northern California (24). The current study contributes to the existing literature by providing new information on plasma fluoride concentrations in pregnant women in Morocco and highlighting the potential factors that may influence these concentrations.

Previous studies have investigated the passage of fluoride across the placenta during pregnancy (6,28). Exposure to a high fluoride level during this critical physiological stage can cause several pregnancy complications (6). These may be related to the high fluoride concentration, as reported in an Iranian study (29). In the present study, among both rural and urban participants, plasma fluoride levels were significantly higher in women with abortion, fetal and neonatal complications, diabetes mellitus, and pregnancy-induced hypertension than in those without these complications. This is in agreement with other studies that reported that fluoride consumption increased the risk of anemia (6), abortion (29), low birth weight, preterm delivery, and poor APGAR count (9,11,30). This suggests that there is a link between fluoride consumption and certain adverse pregnancy outcomes. For pregnant women, it is necessary to consider this potential risk factor when making decisions about prenatal care and fluoride intake. The finding that there was no correlation between plasma fluoride levels and abortion, fetal

Table 4. Correlation analysis of plasma fluoride levels with abortion, fetal and neonatal complications, diabetes and pregnancy induced-hypertension in rural and urban women

	Rural		Urban	
Parameter	r value	p-value	r value	p-value
Without complication	-0.038	0.515	0.042	0.525
Abortion	0.068	0.247	-0.060	0.371
Fetal and neonatal complications	-0.008	0.890	-0.012	0.854
Diabetes	0.0018	0.975	0.066	0.323
Pregnancy induced-hypertension	-0.063	0.285	-0.059	0.379

and neonatal complications, diabetes mellitus, and pregnancy-induced hypertension in both rural and urban participants may be attributed to several factors. First, because of the reduced number of participants in this study cohort studies on a large population are recommended to obtain clearer results. On the other hand, factors other than fluoride exposure could influence the prevalence of pregnancy complications. It was documented previously that genetics, immunological, and infectious factors could lead to abortion in women (31). Another study showed that maternal age, placental abruption, and pre-existing maternal pathologies (obesity, diabetes mellitus, hypertension) increased the risk of intrauterine fetal death (32). Furthermore, it was reported that maternal lifestyle factors such as obesity, physical activity, medication, poor nutrition, and coffee consumption are also associated with pregnancy complications (33,34). In summary, consistent with the literature, differences in plasma fluoride levels were found between rural and urban pregnant women.

Study Limitations

This study represents the first of its kind to be conducted in Morocco, focusing on the important topic of maternal and fetal health, which is a global priority. However, this study has several limitations. Most importantly, the sample size was small. Additionally, some information was unavailable due to incomplete or outdated health records. Finally, the results were not adjusted for potential covariates of fluoride intake.

Conclusion

In conclusion, this study showed that plasma fluoride levels were higher in rural pregnant women than in those from urban areas. However, despite the higher prevalence of abortion, fetal and neonatal complications, diabetes mellitus, and pregnancy-induced hypertension in women of rural origin, there was no correlation between plasma fluoride levels and pregnancy complications. Therefore, further studies in a large population are recommended to obtain clearer results.

Acknowledgments

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Ethics

Ethics Committee Approval: The study was approved by the Hassan First University of Settat, Faculty of Sciences and Techniques of Ethics Committee. The authorization N°1295/18 from the Direction of Epidemiology and Diseases Control of the Ministry of Health of Morocco was obtained before conducting this study.

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Concept: A.E., Design: A.E., Data Collection or Processing: L.A.K.M., Analysis or Interpretation: F.H., Literature Search: L.A.K.M., A.R., Writing: L.A.K.M., A.R., B.El.A., B.N.

Conflict of Interest: No conflict of interest was declared by the authors.

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Local anesthetic administration for frequent episodic tension-type headache in the elderly

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ABSTRACT

Aims: Although various symptomatic and prophylactic treatments exist for frequent episodic tension-type headaches (FETTH) in the elderly population, the constrained efficacy and associated complications and side effects of standard treatments necessitate a search for novel therapeutic options. This study aimed to assess the therapeutic efficacy of locally administered lidocaine to the pericranial muscles in elderly patients with FETTH.

Methods: This retrospective pilot study included elderly patients suffering from FETTH who received weekly bilateral injections of 2 mL 0.5% local lidocaine into the trapezius, masseter, frontal, temporal, splenius capitis, sternocleidomastoideus, and semispinalis capitis muscles over 5 weeks. Patients with infrequent episodic and chronic tension-type headaches were excluded. The monthly number of painful days, visual analog scale (VAS) score, and monthly number of analgesics used were recorded before (pre-treatment) and eight weeks after (post-treatment) the treatment.

Results: The study included 31 elderly individuals, with a mean age of 68.42 years (minimum-maximum: 65-81 years). In comparison to pre-treatment levels, the post-treatment observations demonstrated statistically significant reductions in the number of painful days [(5.8±1.7) vs. (4.6±1.9), p=0.003], VAS scores [(74.3±14.2) vs. (59.5±25.4), p=0.001)], and the number of analgesics used [(6.6±3.2) vs. (4.8±3.7), p=0.001].

Conclusions: This study suggests that local lidocaine administration to the pericranial muscles could serve as an efficient alternative therapeutic approach for FETTH in the elderly.

Introduction

The primary headaches commonly encountered include cluster headaches, migraines, and tension-type headaches (TTH). Notably, TTH is the most prevalent headache type across all age groups globally (1). In community-based studies, a population survey revealed a one-year prevalence of 38.3% for episodic tension-type headache (ETTHA) and 2.2% for chronic TTH (2). Population studies have also shown that TTH occurs more frequently in women than in men (3).

As per the International Classification of Headache Disorders, ETTHA manifests with frequent bilateral episodes of pressing or tightening headaches, characterized by mild to moderate intensity lasting from minutes to days (4). Remarkably, the pain associated with ETTHA does not exacerbate with routine physical activity and lacks a connection with nausea, although photophobia or phonophobia may be present (4).

TTH is acknowledged as one of the most prevalent and burdensome neurological conditions globally, affecting individuals across all age groups (4). It significantly hampers activities of daily living (5). Given the increasing aging population, exploring effective treatment options for TTH in the elderly is paramount.

Although numerous factors contribute to the pathogenesis of TTH, the precise mechanism remains elusive (6). Current understanding suggests that pain modulation is influenced by nociceptive impulses originating from the pericranial and

myofascial tissues. These impulses lead to sensitization in the region of the cervical spinal dorsal root trigeminal nucleus and stimulate supraspinal neurons, originating from areas such as the thalamus (7). Supporting this hypothesis, a separate study noted a reduction in gray matter volume associated with pain processing in individuals with chronic TTH (8). Peripheral abnormalities in TTH lack definitive evidence; however, muscle-related factors, particularly in ETTHA, may play a crucial role (9,10). Comparative studies between individuals with ETTHA and those without headaches have revealed increased latent and active trigger points, a lowered pain threshold in nerve trunks, and decreased neck mobility in patients with ETTHA (6,11). These results indicate that the activation of peripheral nociceptors and heightened muscle sensitivity are prominent and consistent features in individuals with TTH (12).

Treatment strategies for TTH are categorized into acute and preventive approaches based on headache frequency (4). For acute symptom relief, the options include simple analgesics, combined analgesics, parenteral analgesics, or antiemetics (4). Preventive treatment typically involves the use of antidepressant drugs (13). However, it is crucial to be aware of potential side effects and to consider the risk of dangerous drug interactions (14). Despite the effectiveness of pharmacological treatments, they may come with side effects (14). Additionally, non-pharmacological treatment methods, while having fewer side effects, require more scientific data to establish their effectiveness (15).

In the management of ETTHA, local anesthetics such as lidocaine have emerged as a potential therapeutic tool. Studies have explored the administration of these agents through various routes to assess their efficacy in alleviating pain and improving the overall well-being of individuals with TTH (16). Administering local anesthetics to the pericranial muscles presents a promising approach to minimize drug use in the elderly, mitigating potential drug-related side effects and interactions (16). Notably, there is a gap in the literature concerning frequent episodic tension-type headaches (FETTH), specifically in the elderly population. Recent administration of local anesthetics to the pericranial muscles has shown promise in treating TTH (16). Therefore, the primary objective of this study was to investigate the efficacy of local lidocaine administration on FETTH in elderly individuals.

Methods

A retrospective pilot study was conducted at the Neurology Headache Department of Gülhane Training and Research Hospital. Patients over 65 years of age with FETTH were enrolled based on the 2018 guidelines of the International Headache Society (4), which include the following criteria: i) at least 10 episodes of headache occurring on average 1-14 days/month for >3 months (≥12 and <180 days/year), fulfilling criteria ii-v; ii) headaches lasting from 30 minutes to 7 days; iii) at least two of the following four characteristics: bilateral location, pressing or tightening (non-pulsating) quality, mild or moderate intensity, not aggravated by routine physical activity such as walking or climbing stairs; iv) no nausea or vomiting and no more than one of photophobia or phonophobia; v) headaches not better accounted for by another the International Classification of Headache Disorders-3 diagnosis (4).

Eligible patients had been diagnosed with FETTH for at least 6 months. They were evaluated for other headache types, and those with alternative diagnoses were excluded. Additional exclusion criteria were current use of analgesics for more than two weeks, having undergone surgery (including cervical and cranial surgery), use of antidepressants, antipsychotic, and antiepileptic drugs in the last 12 weeks, having received botulinum toxin type A treatment, history of sensitivity to local anesthetics in the past 24 weeks, treatment without medication, having a neuromuscular disease, uncontrolled hypertension, anemia or bleeding disorder, a history of malignancy, psychiatric diseases, or hypothyroidism/hyperthyroidism. To eliminate potential secondary causes, complete blood count, routine biochemical parameters, vitamin B12, thyroid function tests, ferritin, and folic acid levels in the medical records were evaluated. The study received approval from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Local Ethics Committee (protocol no: 2023-234, date: 25.10.2023).

Procedures and outcomes

We included patients who received a weekly 2 mL injection of 0.5% lidocaine for 5 weeks. The injections were administered into the temporal, splenius capitis, sternocleidomastoid, frontal, masseter, semispinalis capitis, and trapezius muscles. Pre-treatment and 8-week post-treatment follow-up endpoints were the monthly number of painful days, visual analog scale (VAS) score, and monthly number of analgesics used.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences software, version 22.0 for Windows (IBM Corp., Armonk, NY: USA). Descriptive statistics included mean and standard deviation and percentages. Paired samples t-test was used to compare pre-and post-treatment measurements.

Results

The study involved 31 patients, with a median age of 73.2 [minimum-maximum (min-max): 65-81] years. There were 24 women [median age (min-max): 73.81 (66-81) years], while there were 7 male patients [median age (min-max): 72.65 (65-80) years].

The pre-and post-treatment results of the patients are summarized in Table 1. The mean number of painful days before

administering local anesthetic injections to the pericranial muscles in elderly patients with FETTH was 5.8±1.7. The following injections, the number of painful days decreased to 4.6±1.9, indicating a statistically significant reduction (p=0.003) (Figure 1). The mean pre-treatment VAS score of the patients was 74.3±14.2, it decreased to 59.5±25.4 following the local anesthetic administration, with a statistically significant difference (p=0.001) (Figure 2). The mean number of monthly analgesic use before local anesthetic injections was 6.6±3.2. Following local anesthetic injections, it decreased to 4.8±3.7, revealing a statistically significant reduction (p=0.001) (Figure 3).

Lidocaine injections were not associated with documented side effects in the medical records.

Table 1. Pre-and post-treatment number of painful days, pain intensity (VAS score) and number of monthly analgesics used

	Pre-treatment	Post-treatment	p value
Number of painful days, mean±SD	5.8±1.7	4.6±1.9	**0.003**
VAS score, mean±SD	74.3±14.2	59.5±25.4	**0.001**
Number of monthly analgesic use, mean±SD	6.6±3.2	4.8±3.7	**0.001**

SD: Standard deviation, VAS: Visual analog scale

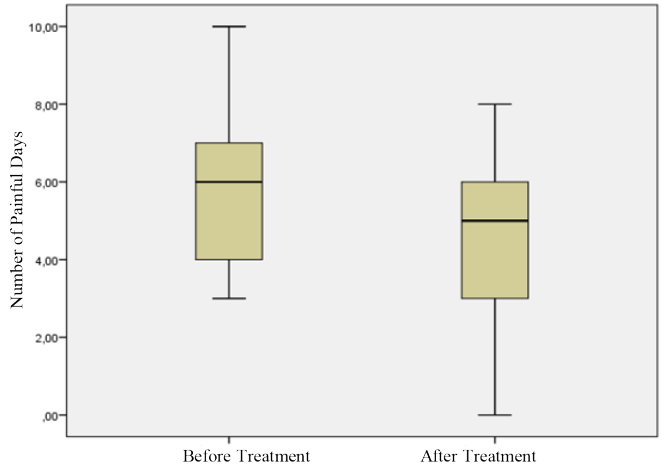

Figure 1. Comparison of pre-and post-treatment number of painful days

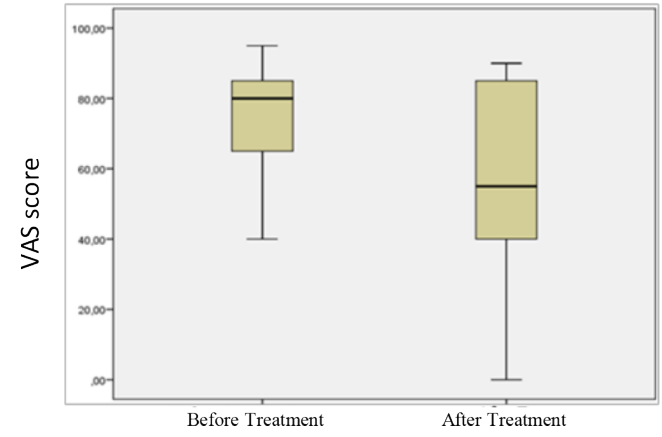

Figure 2. Comparison of pre-and post-treatment VAS scores
VAS: Visual analog scale

Discussion

This study assessed the efficacy of local administration of lidocaine at standard doses to the pericranial muscles in elderly patients with FETTH. The injections were performed once a week over a 5-week span. Upon the 2-month follow-up post-treatment, statistically significant improvements were observed compared with pre-treatment, specifically in the monthly number of painful days, pain intensity assessed by VAS, and monthly number of analgesics used.

Ongoing scientific research seeks to unravel the pathogenesis of TTH. The sensitivity of pericranial myofascial tissues can be influenced by psychological factors, potentially triggering increased peripheral nociceptive activity and subsequent pain attacks (17). While it has been suggested that central nociceptive mechanisms may contribute to chronic TTH (17), impulses originating from myofascial trigger points in the head and neck muscles, stimulated by C1-C3 or the trigeminal nerve, may also be involved (18). This could lead to heightened pain transmission and increased sensitivity of the central mechanisms, contributing to TTH (18). The administration of lidocaine to the pericranial muscles may be effective through this mechanism (19).

The efficacy of local anesthetics in managing ETTHA in the elderly involves intricate and multifaceted mechanisms (7). Lidocaine, the most extensively studied agent, is believed to act by blocking peripheral nociceptive signals and modulating central pain pathways (19). The effects of local anesthetics encompass three main aspects (19): i) Blockage of voltage-gated sodium channels. Local anesthetics such as lidocaine are recognized for their capacity to obstruct voltage-gated sodium channels in peripheral nerve endings. This action curtails the generation

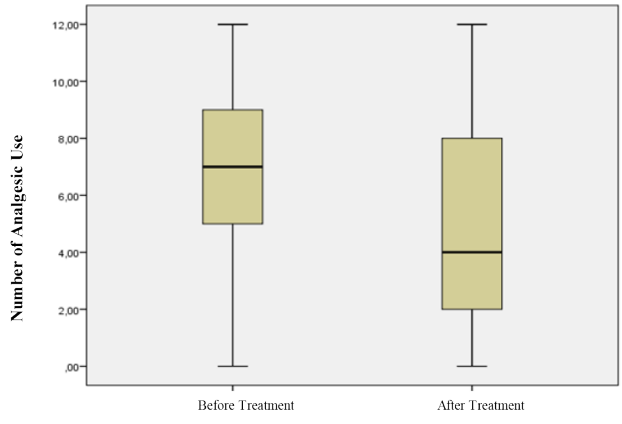

Figure 3. Comparison of pre-and post-treatment the number of analgesic use

and transmission of pain signals, effectively diminishing pain perception. Muscle relaxation at trigger points: TTH is often linked to heightened muscle tension, particularly in the neck and shoulder regions. Local anesthetics, when applied to trigger points, can induce muscle relaxation, mitigating the muscle tension that often precipitates TTH (19). iii) Modulation of central pain processing: beyond their peripheral effects, local anesthetics may also impact central pain processing. By diminishing incoming nociceptive signals, these agents can influence the central nervous system's perception of pain, offering additional relief to individuals experiencing TTH (20).

In a previous study, local lidocaine injections were administered three times to the pericranial muscles and trigeminal nerve exit points of 47 patients diagnosed with TTH (21). The results showed a decrease in the number of painful days, monthly analgesic use, and pain severity. Additionally, an improvement was noted in Hamilton's Depression and Anxiety Scale. However, the study did not evaluate the elderly population. Hence, the current study confirms the previous findings in a different age group.

Another study involved 108 patients diagnosed with TTH and administered 2 mL of 0.5% local lidocaine to the pericranial muscles (22). A reduction in pain intensity and the monthly number of painful days was observed in these patients (22). Our study shares similarities with that work concerning the administration procedure and findings. The primary distinction is that we focused on the elderly population. On the other hand, in contrast to our study, that study reported that lidocaine injections were effective for 6 months in patients with FETTH.

Venâncio et al. (23) conducted a comparative study assessing the effects of corticosteroids, dry needling, and lidocaine on myofascial trigger points in headaches. Their results indicated that the administration of lidocaine to myofascial trigger points was effective in alleviating headaches (23). In another study, they compared the use of botulinum neurotoxins type A, dry needling, and lidocaine on headaches, designating lidocaine as the primary option because of its cost-effectiveness (24). However, the authors focused on the adult population and included patients with TTH and migraine, introducing heterogeneity.

Different results have emerged from studies examining the use of local anesthetics in headaches. Karadaş et al. (25) similarly found that pericranial sensitivity served as a marker for a positive response to local lidocaine treatment in patients with episodic TTH associated with pericranial sensitivity. They concluded that pericranial sensitivity could influence the treatment outcomes of local lidocaine therapy. Their results also suggested that local lidocaine was both safe and effective in treating episodic TTH associated with pericranial sensitivity. On the other hand, in contrast to the current work, the patients were categorized based on pericranial muscle sensitivity. Furthermore, in contrast to our work, they administered 1% lidocaine injections every other day for 3 sessions to obtain improvements.

The evaluation of 25 patients aged 65 years diagnosed with TTH was also reported previously (26). Patients received local 0.5% lidocaine injections once a week for 4 sessions, resulting in a decrease in the number of painful days, pain intensity, and the number of analgesic use by the patients (26). Similarly, our study focused on the elderly population and employed a comparable treatment procedure. Both studies confirmed that local lidocaine administration to the pericranial muscles was an effective treatment option. The primary distinction was that we focused on a more specific group, namely, FETTH.

Variations among the studies so far may be attributed to factors such as the local anesthetic dose, research methodology, and duration of administration. Further well-designed clinical trials are essential to validate these results, establish standardized treatment protocols, and determine the long-term safety and efficacy of this approach.

Study Limitations

There are limitations of this study. Most importantly, the design was retrospective. The absence of a placebo group was also a significant limitation.

Conclusion

In conclusion, local lidocaine administration may be effective in treating FETTH in the elderly. The study demonstrated a reduction in the number of painful days, pain intensity, and the number of analgesics used due to headache.

Ethics

Ethics Committee Approval: The study received approval from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Local Ethics Committee (protocol no: 2023-234, date: 25.10.2023).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: U.B.Ş., Concept: U.B.Ş., Design: U.B.Ş., Data Collection or Processing: U.B.Ş., Analysis or Interpretation: M.T., Literature Search: M.T., Writing: U.B.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

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Colonic polyposis with underlying diffuse large B-cell lymphoma presenting with ileocolic intussusception

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Keywords: Colon, intussusception, lymphoma

ABSTRACT

Extranodal lymphoma rarely presents as a primary tumor in the gastrointestinal tract, particularly in the form of intussusception in adults. We report the case of a 30-year-old woman admitted with intermittent right lower abdominal pain for one month and a palpable mass on physical examination. Computed tomography revealed an ileocolic intussusception with rectal polypoid mass lesions. The patient underwent a right hemicolectomy because the lead point of the intussusception was identified as a terminal ileum polyp. Histopathological examination revealed diffuse large B-cell lymphoma. In the event of an abdominal mass with radiological evidence of colonic intussusception, surgery remains crucial to avoid unnecessary complications.

Introduction

Extranodal lymphoma is most common in the gastrointestinal tract, mainly in the stomach, followed by the small and large bowel. However, primary lymphoma arising in the gastrointestinal tract is rare. An uncommon sequelae of gastrointestinal lymphoma is intussusception, a condition in which a segment of the bowel telescopes into an adjacent distal segment. This condition mainly occurs in children, while adults comprise less than 6% of cases (1,2). They commonly occur because of intramural or intraluminal lesions that serve as the lead point of intussusception. Here we report an uncommon ileocolic intussusception in a woman with diffuse large B-cell lymphoma (DLBCL) and bowel involvement.

Case Presentation

A 30-year-old female was diagnosed with DLBCL through a biopsy of a nasopharyngeal lesion while being investigated for enlarged cervical lymph nodes. She was pregnant in her third trimester and defaulted chemotherapy after her first cycle because of concerns related to pregnancy complications.

In the fourth postpartum month, she was admitted to the hospital with intermittent right lower quadrant pain for one month. She reported no vomiting, abdominal distension, or blood-stained stool. Bowel-opening frequency and pattern before admission were normal. Abdominal examination revealed a right iliac fossa mass without peritonism. Biochemical tests were normal, with a hemoglobin level of 11.5 g/dL. Screening for human immunodeficiency virus was negative. Computed tomography (CT) revealed an ileocolic intussusception with rectal polypoid lesions (Figure 1A, 1B). Multiple enlarged mesenteric and para-aortic nodes were noticeable. There were no liver or lung lesions. Colonoscopy revealed multiple polyps with mucosal ulcerations in the large bowel (Figure 1C). Biopsy samples were taken from the appropriate lesions.

The patient subsequently underwent exploratory laparotomy, which confirmed the presence of ileocolic intussusception identified by CT (Figure 1D). Multiple polyps were palpable along the large and small bowels with enlarged, hard mesenteric lymph nodes. No stricture or stenosis was observed. A right hemicolectomy (Figure 1E) with ileocolic side-to-side stapled anastomosis was performed. The lead point of intussusception was identified as a terminal ileal polyp. Histopathological assessment of the resected specimen and biopsied polyps were similar to that the nasopharyngeal biopsy, revealing DLBCL, non-germinal subtype (Figure 2A-2D). A total of 12 of 16 lymph nodes were positive for tumor infiltration. The tumor cells were diffusely positive for CD20, BCL6 and MUM1 but negative for CD3, CD10 and BCL2 (Figure 2E, 2F). The Ki67 index was 70-80%. The patient recovered well and was discharged on postoperative day 4 with plans to resume chemotherapy. The patient is well with no recurrence of intussusception until a 6-month clinic follow-up.

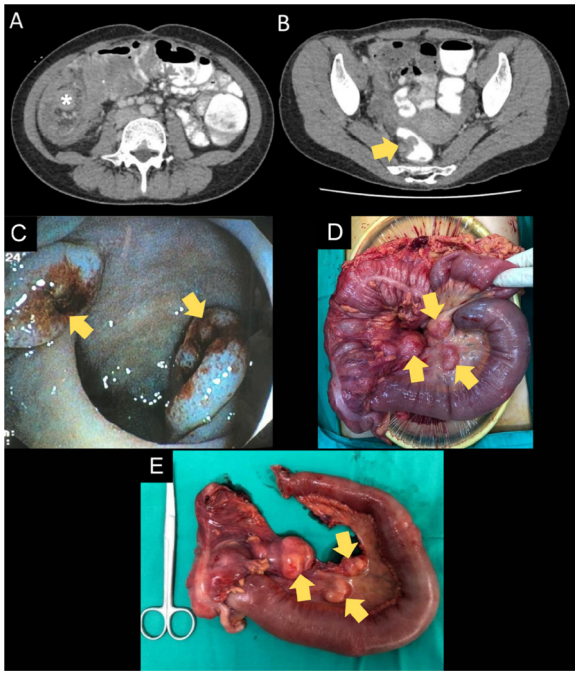

Figure 1. Computed tomography scan of the abdomen showing an ileocolic intussusception (white asterisks) (A) with rectal polypoid lesions (yellow arrow) (B). (C) A colonoscopic finding of multiple rectal polyps (yellow arrows) with mucosal ulceration. (D) Surgery finding of ileocecal intussusception with multiple enlarged mesenteric lymph nodes (yellow arrows). No stricture or stenosis was observed. Polypoidal nodules were palpable throughout the large and small bowel. (E) Post-limited right hemicolectomy specimen showing ileocecal intussusception with multiple enlarged mesenteric lymph nodes (yellow arrows)

Discussion

Intussusception is uncommon in adults. The clinical signs tend to be non-specific, including recurrent abdominal pain, nausea, vomiting, and weight loss. Abdominal pain is present in 95% and tends to be recurrent, frequently lasting longer than 1 week. Acute obstruction or blood-stained stool is less common. A palpable abdominal mass is also infrequent and reported in less than 15% of cases (3). As in our case, clinical course was stormy because her initial chemotherapy plan for DLBCL was affected by pregnancy. The patient then presented with intermittent right lower abdominal pain with a palpable mass during the postpartum period.

The anatomic location of intussusception can be enteroenteric, ileo-ileocolic, or colo-colic. Ileo-ileocolic

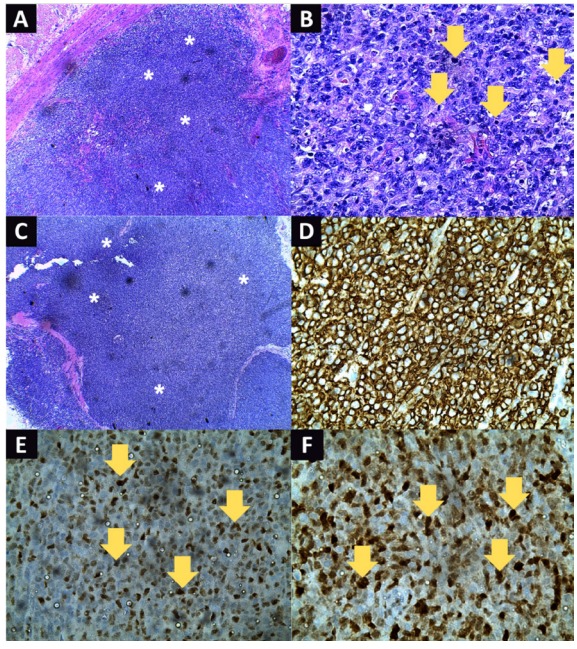

Figure 2. Lamina propria, submucosa, and subserosa (A) infiltrated by sheets of tumor cells (white asterisks) (hematoxylin and eosin, x4 magnification). The tumor cells are medium in size and show hyperchromatic nuclei, coarse chromatin pattern and irregular nuclear contour with mitosis (yellow arrows) (B) (hematoxylin and eosin, x40 magnification). A lymph node infiltrated by tumor cells of similar morphology (white asterisks) (C). Tumor cells are positive for CD20 (D), BCL 6 (yellow arrows) (E), and MUM-1 (yellow arrows) (F). The cells were negative for CD3, CD10, and BCL2 (immunohistochemistry, 40 magnification)

intussusception is the most common site because of its high density of lymphoid tissue (4-6). Endoscopic findings of lymphomatous polyps are variable. They appear as pseudopolypoid lesions, subcentimeter sessile polyps, larger broad-based polyps with ulcerated surfaces, and diffuse infiltration (7). Approximately 10% of cases have gastrointestinal polyposis (8,9). Our patient is interesting because colonoscopy showed synchronous polyps with mucosal ulcerations in the large bowel. The appearance of lymphomatous polyps under narrow-band imaging has not been well studied.

Because up to 63% of adult intussusception cases are related to malignancy (10), resecting the intussuscepted bowel is the mainstay of treatment. However, in the case of transient type, especially in the small bowel, intussusception can be conservatively managed when abdominal symptoms or complications are not found (11). Attempts to reduce the bowel may cause bowel perforation and subsequent tumor seeding in the peritoneum. Furthermore, endoscopic reduction of the intussusception puts the patient at risk of recurrence because the potentially malignant etiology is not excised (12). To the best of our knowledge, the available literature lacks information about recurrent intussusception following bowel resection in patients with multiple polyps. There is also no evidence to support the prophylactic resection of bowel polyps in such patients.

The standard treatment for DLBCL is a combination of cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP regime) or with rituximab (R-CHOP) (13). In patients with disseminated gastrointestinal DLBCL (Lugano stage 4), R-CHOP has been shown to improve 3-year overall survival (59%) compared with CHOP (29%), although the difference was not statistically significant (14). No benefit can be obtained in localized disease (14). In disseminated disease, the rate of complete response (CR) to chemotherapy is 46-52%, but the risk of relapse and progression remains high at 40-42%. However, in localized disease, surgery and chemotherapy result in a higher CR rate (85.3% vs. 64.4%, $p<0.001$) (13,14). For any patient who undergoes surgery for gastrointestinal lymphoma, to obtain a promising CR, adjuvant chemotherapy is mandatory. In this study, the patient opted for the CHOP regime during the postpartum period.

Conclusion

Adult intussusception is uncommon and frequently a sequelae of underlying intraluminal or intramural pathology. Even though the standard treatment for DLBCL is chemotherapy, in the event of an abdominal mass with radiological evidence of colonic intussusception, surgical resection remains crucial to avoid unnecessary complications.

Ethics

Informed Consent: The subject provided written consent, and patient anonymity was preserved.

Authorship Contributions

Surgical and Medical Practices: B.Z.Y.Y., D.E.Y.G., S.S., Concept: S.S., Design: N.A., F.H., Literature Search: D.E.Y.G., Writing: B.Z.Y.Y., F.H.

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T-cell acute lymphoblastic leukemia in an adolescent presenting with peripheral facial paralysis: A case report and literature review

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Keywords: Facial paralysis, leukemia, T-cell leukemia, steroids

ABSTRACT

A cause of visiting a pediatric emergency department is facial paralysis. Although it is mostly idiopathic, it may also be the first finding of malignancies such as leukemia. Corticosteroid therapy decreases blast counts and may postpone leukemia diagnosis. Before starting corticosteroid treatment for facial paralysis in pediatric patients, it is essential to perform a complete blood count and peripheral smear evaluation.

Introduction

A 17-year-old male patient who presented with peripheral facial paralysis and was diagnosed with T-cell acute lymphoblastic leukemia (T-ALL) was discussed in this report, and a literature review on pediatric patients who had no previously known malignancy and were diagnosed with leukemia after presenting with facial paralysis was performed.

Case Presentation

A 17-year-old male patient was admitted to the ear, nose, and throat diseases outpatient clinic with complaints of impaired vision, numbness, and decreased facial movement in the left half of his face. The child was referred to the pediatric neurology outpatient clinic for a systemic evaluation because of the appearance of new complaints and the lack of improvement after corticosteroid treatment for the diagnosis of peripheral facial paralysis. On physical examination, multiple lymphadenopathic and left peripheral facial paralysis were detected in the pediatric neurology clinic. The enlarged lymph nodes were 2 cm in the bilateral anterior and posterior cervical chains, 1.5 cm in the submandibular region, 1.5 cm in the bilateral axilla, and 1-1.5 cm in the bilateral inguinal region. He also had limited outward gazing, strabismus, and ptosis in the left eye.

Complete blood count (CBC), routine biochemistry tests, and other laboratory tests were within normal limits. There were no atypical cells/blasts in the peripheral smear. The patient was re-admitted two days later with complaints of shortness of breath and fever unresponsive to antipyretics. Oxygen saturation was

96%, body temperature was 38.6 °C, heart rate was 100 beats/min, and the lymph nodes were further enlarged compared with the previous admission. CBC and routine biochemistry tests were still within the normal range. However, immature cells were observed in the peripheral smear, and a bone marrow biopsy was planned.

Widening of the superior mediastinal shadow by a prominent thymus on chest X-ray (Figure 1) and soft tissue density filling the anterior mediastinum on contrast-enhanced thoracic tomography were consistent with an enlarged thymic tissue. The superior vena cava and left brachiocephalic veins were also compressed, causing mild to moderate stenosis in the superior vena cava and severe stenosis in the left brachiocephalic vein.

Following a true-cut biopsy of the anterior mediastinum and bone marrow aspiration and biopsy, the patient was diagnosed with T-ALL. Central nervous system imaging and cerebrospinal fluid examinations were within normal limits. The ALL Berlin-Frankfurt-Münster 2000 chemotherapy protocol was initiated. When the blast count in the peripheral blood examination exceeded 1000 cells/mm^3 on the eighth day of the protocol, the patient was considered to be at high risk. Because he did not have a fully compatible donor, the patient underwent haploidentity hematopoietic stem cell transplantation from his mother on the 72nd day.

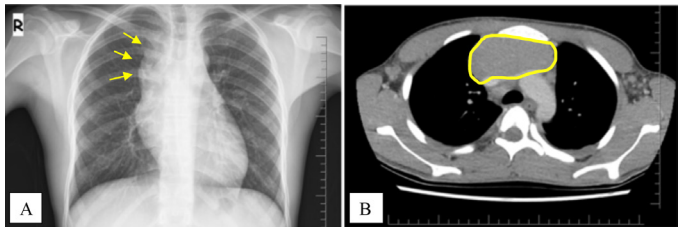

Figure 1. A) Increased thymus shadow on X-ray, B) Soft tissue density (enlarged thymic tissue) that completely fills the anterior mediastinum on computed tomography

Discussion

Facial paralysis is one of the causes of pediatric emergencies. Although most cases are idiopathic (e.g., Bell's palsy), the most prevalent underlying condition in children is acute otitis media, as well as Lyme disease in endemic areas. Malignancies are extremely rare causes of facial paralysis. Central nervous system tumors constitute the most common neoplastic cause of facial paralysis, followed by leukemias (1).

Facial paralysis as the first sign of leukemia is extremely rare. In a study by Babl et al. (2), the incidence of newly diagnosed leukemia in children with acute-onset peripheral facial paralysis was reported to be 0.6%.

It is thought that facial paralysis in leukemias develops because of a direct invasion of leukemic cells into the nerve or underlying infectious factors such as Epstein-Barr virus and human T-cell lymphotropic virus (3). Seventeen newly diagnosed pediatric leukemia patients with facial paralysis as a presentation finding were reported between 1984 and 2023. Eleven of them had acute myeloblastic leukemia, five had ALL, and one had chronic lymphoblastic leukemia. Only two pediatric cases of T-ALL have been reported before the current pediatric case having facial paralysis at the time of leukemia diagnosis (2-13) (Table 1).

Table 1. Our patient and seventeen newly diagnosed pediatric leukemia patients with facial paralysis as a presentation finding between 1984 and 2023

Reference number	Age	Additional finding	Diagnosis
Our patient	17 years	Periodontitis, lymphadenopathy	T-ALL
2	8 years	Exophthalmos	AML
2	7 years	Acute otitis media, pneumonia	T-ALL
2	5 years	Acute otitis media	Pre B-ALL
2	9 years	Blurred vision	T-ALL
3	8 months	Acute otitis media and anemia	AML
4	18 months	Acute otitis media	AML
5	10 months	Recurrent fever	AML
6	1 month	Fontanel strain	CML
7	32 months	Exophthalmos	AML
8	11 months	-	ALL
8	11 months	Ataxia, irritability, and hepatosplenomegaly	AML
8	6 years	Fever, pain	ALL
9	17 years	Paraplegia	AML
10	13 years	Mastoiditis	AML
11	6 years	Acute otitis media, mastoiditis, hearing loss	AML
12	5.5 years	Mastoiditis	AML
13	4 months	Hepatosplenomegaly, lymphadenopathy	AML

T-ALL: T-cell acute lymphoblastic leukemia, AML: Acute myeloblastic leukemia, CML: Chronic myeloblastic leukemia, Pre B-ALL: Precursor B-cell ALL

According to the 2013 American guidelines, laboratory examination is not recommended for the etiology of facial paralysis unless there is a history of residing in a Lyme-specific region (14). In a retrospective study by Babl et al. (2), it was recommended to evaluate CBC before starting corticosteroid therapy when investigating the etiology of pediatric patients, particularly those with idiopathic facial paralysis.

Glucocorticoid therapy is recommended in adult guidelines (14); however, its effectiveness for treating Bell's palsy in pediatric patients is debatable (15). Thus, more research is required to determine the effective corticosteroid dose, drug type, and duration of treatment.

On the other hand, while steroids are still used for the treatment of Bell's palsy, they are also included in the treatment protocols for ALL and most lymphoma types (2). In this context, patients on corticosteroid therapy can have normal CBC values and peripheral smears because corticosteroid treatment may delay leukemia and other oncological diagnoses. Furthermore, even in the absence of corticosteroid treatment, some patients may have normal CBC and peripheral smears at leukemia diagnosis (5).

Conclusion

T-ALL follows a more favorable course in pediatric patients than in adult patients, and the efficacy of corticosteroid therapy is debatable. Corticosteroid therapy may delay the diagnosis of leukemias by reducing the number of blasts in the peripheral blood and suppressing symptoms. In contrast, pediatric patients presenting with facial paralysis should have CBC and a peripheral smear.

Ethics

Informed Consent: Consent form was filled out by the participant.

Authorship Contributions

Concept: O.G., Design: O.G., Data Collection or Processing: B.Y., O.G., Analysis or Interpretation: B.Y., O.G., Literature Search: B.Y., Writing: B.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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