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EDITORIAL
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Possible impact of adopting extreme hypofractionation after FAST Forward trial publication

Gustavo Nader Marta[1,2]*

Breast cancer is the most common type of malignant tumor and the main cause of cancer mortality in women worldwide[1]. In general, a multidisciplinary therapeutic approach comprising surgical, medical, and radiation oncology is needed for the optimal management of breast cancer; this combination is correlated with improved overall survival rates[2]. After breast-conserving surgery or mastectomy, post-operative radiation therapy decreases cancer mortality and loco-regional relapse rates in most breast cancer patients[3,4].

Historically, conventionally-used radiation doses ranged from 50 to 50.4 Gy in fractions of 1.8 to 2.0 Gy over the course of 25 to 28 days. This dose was empirically confirmed based on the hypothesis this schedule was safe and effective. This idea was enhanced by studies that assessed early skills of the moderately hypofractionated whole breast irradiation practice; however, these reports used obsolete and incorrect radiobiological models and outmoded devices of treatment delivery and calculation, hence exhibiting unacceptably high rates of side effects[5,6].

Nonetheless, other groups posteriorly provided assessments of normal-tissue damage and fraction size in breast tumors developing the current protocols of moderately hypofractionated whole-breast irradiation which involved fraction ranges up to nearby 3 Gy pooled with an abridged total dose delivered over a shorter period of time (e.g., three weeks). This schedule attained radiobiological equivalence to the conventional radiation doses[7,8]. Long term follow-up in large clinical trials sustained the efficacy and safety of the moderately hypofractionated whole breast irradiation practices[9-11]. In fact, the all-purpose engagement of hypofractionation can serve to reduce the therapeutic period, decreasing the total number of fractions, and offering a more convenient treatment schedule for patients. Moreover, hypofractionation can also increase patients` access to oncology centers (particular importance for countries with limited resources with restricted radiation therapy assets), decrease indirect costs associated with work breaks and travel to the medical care center, and reduce treatment costs[12,13].

Now, the first tumor-results associated endpoint assessment from the FAST Forward trial was published, which offers a treatment extreme hypofractionated schedule of just five fractions in five consecutive days for patients with early breast cancer[14]. In this timely, multicenter, non-inferiority, prospective phase 3 randomised trial, 4,096 patients (pT1–3, pN0–1, M0) were randomly allocated into three groups to receive moderated hypofractionated RT (15x2.67 Gy; over three weeks) or two schedules of ultra-hypofractionated RT over one week (5x5.2 Gy – 26 Gy or 5x5.4 Gy – 27 Gy) directed to the whole breast or chest wall. No statistically significant difference in the 5-year cumulative incidence of breast tumor relapse among the groups was found (2.3% in moderated hypofractionated RT versus 2.0% in 27 Gy versus 1.5% in 26 Gy). Likewise, the acute and late adverse events were similar in the groups, apart from a higher late normal tissue effect in the 27 Gy RT arm. We would therefore certainly not consider the highlighted results for 26 Gy versus 40 Gy for breast distortion and breast/chest wall oedema clinically relevant. The other highlighted result, the one for breast induration outside the tumor bed, is statistically significant at $p<0.0001$; however, it is hard to maintain for clinical significance with the demonstrated 5-year moderate/marked events rates only 0.1% in 40 Gy and 1.9% in 26 Gy. In fact, the side effects are properly low across all of the endpoints, regardless the treatment schedule[14]. Hence, it should be recognized that the clinical outcomes of this trial could support the adoption of 26 Gy in 5 consecutive daily fractions as a treatment option for most of early breast cancer patients in the near future.

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Despite patients with ductal carcinoma in situ (DCIS) were not a formal inclusion criterion in the FAST Forward trial, extreme hypofractionated ones can also be considered, as there is no radiobiological concern regarding why five fractions are expected to be less effective in the DCIS setting[14]. This is coherent with a previous policy which adopted moderately hypofractionated irradiation for DCIS once the results of clinical trials that assessed patients with invasive breast cancer have been extrapolated to the context of in situ disease[15-18].

Along the same reasoning lines, extreme hypofractionated can be considered in patients who received both implant and autologous reconstructions as acute and late normal tissue site effects overall were similar in five fractions (26 Gy group) and 15 fractions in the FAST Forward trial, although numbers of reconstructions were small. In other words, the results of the FAST Forward trial[14] showed that most normal tissue adverse events that are frequently associated with radiation-related toxicities in implant and autologous breast reconstructions (e.g., fibrosis, skin retraction, and breast shrinkage) were similar in patients who underwent five fractions (26 Gy group) or 15 fractions. Additionally, no randomized phase III trial has yet validated the use of a conventional or moderately hypofractionated radiation doses after breast reconstruction. Historically, in empirical studies, the conventional dose has been used whenever breast-reconstruction techniques were described[19]. In recent decades, in clinical practice, once a treatment has been performed with conventional doses, there has been a simple incorporation of reconstructive surgeries.

When indicated, sequential boost can be added to 26 Gy in five fractions whole breast RT. In the FAST Forward trial, 25% of patients received a sequential boost of five to eight fractions of 2 Gy and were well tolerated. More will emerge with the FAST Forward nodal sub-study which is yet to report and where all patients are node-positive by definition. The adoption of an extreme hypofractionated schedule for higher risk breast cancer patients still need to be evaluated.

Since the majority of patients in the FAST Forward trial (14) are relatively low risk cases, we should be very careful when changing guidelines based on one clinical trial in particular for use in the higher risk patients. This is the reason why the UK group has a call-out for treatment de-escalation studies FAST Forward trial HIGH focusing on patients with high-risk disease, including those requiring internal mammary lymph nodes treatment.

Finally, the economic issues behind new ways of delivering radiation therapy need to be discussed. How to deal with the possible financial loss on reimbursement due to adopting extreme-hypofractionated radiation therapy schedules? While in countries like The Netherlands, Italy, and the UK (where reimbursement is largely independent from the number of fractions), moderate hypofractionated breast irradiation practice is used by the majority of centers, in the more reimbursement-driven models with payment-per-fraction countries, including Germany, France, Portugal and the USA, a lot of reluctance exists towards applying moderate hypofractionation in daily practice. The possible financial loss induced by the reduction in per-patient income due to fractionation-based reimbursement could be compensated by an evolution of the reimbursement model from a fee-for-service system to a bundled payment system based on quality parameters. It is important to encourage payers to abandon payment per fraction as the use of moderate radiation therapy and extreme-hypofractionation for breast cancer patients is a concrete reality.

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LETTER TO THE EDITOR
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Focusing on thyroid nodules in suspense: 10-15 mm with repeat cytology, Category III, the Bethesda System for Reporting Thyroid Cytopathology, TBSRTC

Ilker Sengul[1,2], Demet Sengul[3]*

Dear Editor,

Different management guidelines for thyroid nodules have been striving to enhance the diagnostic performance and to avoid unnecessary biopsy rates beyond surgery by determinants the different thyroid size thresholds, used as cutoff values, which are determinants for the scheduling of a thyroid nodule to undergo intervention of fine-needle aspiration (FNA). Ha et al.[1] stated that the high specificity and low rate of unnecessary biopsies in the 2017 American College of Radiology (ACR) guidelines arise from larger cutoffs (mildly suspicious nodules, 25 mm; moderately suspicious nodules, 15 mm), as compared to the 2015 American Thyroid Association (ATA) Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer and the 2016 Korean Thyroid Association/Korean Society of Thyroid Radiology (KTA/KSThR) guidelines (15 and 10 mm, respectively). In addition, they denoted that the ATA and KTA/KSThR guidelines were exhibiting high sensitivity, whereas the ACR guideline was less significant in terms of the diagnostic performance of ultrasonography (US)-guided FNA for the indicated thyroid nodules. Therefore, they pointed out in their respectable paper that the thyroid nodules with low or intermediate suspicion for the 2015 ATA and 2016 KTA/KSThR guidelines had undergone FNA at a cutoff of 25 and 15 mm, respectively, which could produce significant changes in the percentage of thyroid nodules that needed an FNA application.

However, we have recently investigated whether the cutoff points of 10 and 15 mm are effective in three diagnostic tools: SE, US-guided FNA (US-FNA) cytology, and histopathology. These current outcomes based on the size, both above 10 and 15 mm, revealed no significance regarding Tsukuba elasticity score (TES) 4&5 with the curves of the receiver operating characteristics (ROC), the areas under the curves (AUCs) of 0.531 and 0.623, respectively, the AUC of TES 4&5 above 15 was higher than above 10. Therefore, it may be alleged that the possible correlation between the size and the TES 4&5 becomes stronger when the cutoff was designated to have 15 mm. Likewise, the McNemar test was used to compare the nodules above 10 and 15 mm with malignant histopathology, and no significance was revealed with an additional calculation of the AUC of 0.509 and 0.515, respectively. Hence, it may be concluded that the association between the nodule size and the precise histopathology increased as the cutoff point was raised 15 mm[2], which is similar to the study of Ha et al[1].

Nevertheless, a cutoff of 10 mm could still not be an underestimating issue for the management of the thyroid nodules to date[3]. Therefore, some authorities have been set at a cutoff value of 10 mm on the size selection criteria for US-FNA till today. For instance, the American Association of Clinical Endocrinologists (AACE)/*Associazione Medici Endocrinologi* (Italian Association of Clinical Endocrinologists) (AME) recommended FNA for the ones above 10 mm, solid and hypoechoic in the US [Grade B; BEL (best evidence level) 3]. Besides, the novel European Thyroid Imaging and Reporting Data System (EU-TIRADS), based on a literature review and on the AACE, ATA, and Korean guidelines, comprising a thyroid US lexicon, proposed FNA for the nodules >10 mm of a high-risk category (EU-TIRADS 5). In addition, the Society of Radiologists in Ultrasound (SRU)[4] strongly suggested it for the ones ≥10 mm with microcalcifications. In addition, the 2009 ATA Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer recommended it for the ones ≥10 mm with microcalcifications and solid hypoechoic nodules, while the 2015 ATA Management Guidelines suggested the FNA for the nodules ≥10 mm with high-to-intermediate

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suspicion sonographic pattern (Recommendation 8I(A); Strong recommendation, Moderate-quality evidence, Recommendation 8I(B); Strong recommendation, Low-quality evidence, respectively).

The recommendation of the 2015 ATA Management Guidelines for the nodules ≥10 mm is obvious when stating that: it considers US-FNA for a high-to-intermediate suspicion sonographic pattern (Recommendation 8I(A); Strong recommendation, Moderate-quality evidence, Recommendation 8I(B); Strong recommendation, Low-quality evidence, respectively). However, the real challenging point is being able to decide as far as irreproachable management of the ones with controversial cytology, such as atypia of undetermined significance or follicular lesion of undetermined significance, AUS/FLUS, of indeterminate cytology. The 2015 ATA Management Guidelines recommend repeat FNA and molecular testing for a panel of mutations (*BRAF, NRAS, HRAS, KRAS, RET/PTC1, RET/PTC3, PAX8/PPARc*) to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery for (AUS/FLUS) indeterminate cytology (III of III, IV, and V, TBSRTC, 1st and 2nd ed) (Recommendation 15(A); Weak recommendation, Moderate-quality evidence). The last ATA Management Guidelines also suggest either surveillance or diagnostic surgical excision for an AUS/FLUS thyroid nodule, depending on the clinical risk factors, the sonographic pattern, and the patient's preference as repeated FNA cytology, molecular testing, or both, are not performed or inconclusive without specifying any cutoff size for nodules (Recommendation 15(B); Strong recommendation, Low-quality evidence).

Notably, the 2015 ATA Management Guidelines strongly recommended it, but with low-quality evidence. We have recently reported that the largest cutoff of 15 mm may strengthen its association with malignant histopathology, as compared to the lowest, of 10 mm[2], as shown by some authors[1]. Therefore, in this study, we would like to open a new window regarding the management of the nodules, between 10 and 15 mm in the largest diameter with Category III, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) of indeterminate cytology[4-7], as repeated FNA cytology, molecular testing[8], or both, are not performed or are inconclusive, and clinical risk factors, sonographic pattern[4], and patient's preference are controversial. We recommend surveillance for the management of the mentioned thyroid nodules, 10-15 mm with Category III of indeterminate cytology, TBSRTC, 1st and 2nd ed., harboring high-to-intermediate suspicion sonographic pattern, low clinical risk factors, repeatead FNA cytology, molecular testing, or both, are not performed or inconclusive, comparing the ones >15 mm. This issue deserves further investigation. We hope we might contribute and provide new insights into that challenging window in Thyroidology.

AUTHORS' CONTRIBUTIONS

IS: Conceptualization, Formal Analysis, Writing – Review & Editing. **DS:** Conceptualization, Formal Analysis, Investigation, Supervision Writing – Review & Editing.

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Refractory celiac disease type 2: how to diagnose and treat?

Jonathan Soldera[1]*, Karina Salgado[2], Karla Lais Pêgas[2,3]

SUMMARY

Refractory celiac disease is an uncommon condition which might be associated to poor prognosis. It is often treated with immunosuppressive medications, with poor results. It is divided in type 1 and type 2, the latter carrying a high risk for lymphoma and mortality. A case of a 41 year old female patient with refractory celiac disease type 2 is reported. She was treated with oral budesonide for six months, achieving histological remission.

KEYWORDS: Celiac disease. Budesonide. Diet, gluten-free.

INTRODUCTION

Celiac disease (CD) is defined as an immune-mediated disease, which affects genetically predisposed individuals. The immune response is triggered by the ingestion of gluten, which is present in cereals such as wheat, rye, and barley. In countries with high proportion of European descendants, it is estimated to affect 1% of the population as its incidence and prevalence has been rising[1-3]. Diagnosis of CD is made by an association of positive antibodies (generally, anti-transglutaminase for adults and anti-gliadin for children) and a duodenal biopsy demonstrating villous atrophy and an increase in intraepithelial lymphocytes (IELs) amount. The only treatment currently available is lifelong gluten-free diet (GFD), which normalizes antibodies and duodenal histology and improves symptoms[1-3]. Steroids are generally reserved at presentation only for celiac crisis[4].

Refractory celiac disease (RCD) is a severe and uncommon presentation of CD, occurring in <2% of celiac patients. It should be suspected in a patient who still has symptoms of malabsorption and villous atrophy on duodenal biopsies, even after at least 6 months of GFD[1]. It is paramount to confirm that GFD orientation is being strictly followed and to exclude malignancy and other causes of nonresponse to GFD.

The suspicion of RCD should prompt immunohistochemistry and/or flow cytometry of the duodenal biopsies, in order to stratify RCD into two types (Table 1). RCD type 1 is associated with normal CD3, CD4, and CD8 surface markers on lymphocytes, without clonal gene rearrangement of the gamma chain of the T-cell receptor (TCR). RCD type 2 is associated with aberrant clonal IELs that lack surface expression of CD3, CD4, and CD8 and is associated with TCR gamma gene rearrangement[2]. RCD type 2 carries a higher risk for T-cell lymphoma and mortality, and it is treated with strict GFD and the use of immunosuppressive medications, although with poor results[3].

The purpose of this study is to report a case of a female patient diagnosed with CD in an uncommon situation, during an investigation of chronic alteration of serum amylases. She was later diagnosed with RCD type 2 after 2 years of follow-up and went into remission with the use of oral budesonide, a novel treatment option for these cases.

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DESCRIPTION

A female patient, 41 years old, previously healthy, sought care due to unspecific abdominal pain, weight loss, and elevation of pancreatic enzymes for more than 1 year. Workup had shown normal abdominal magnetic resonance imaging (MRI) and computerized tomography, serum amylase level at 276 U/L (reference=120 U/L), lipase values of 16 U/L, and a negative in parasitological stool examination test. Anti-transglutaminase IgA was 128 U/mL (reference=10 U/mL), and anti-gliadin IgA was 212 U/mL (reference=30 U/mL). She underwent upper digestive endoscopy, which showed duodenum with severe atrophy, with MARSH IIIb in biopsies. The patient was oriented to start following GFD and showed some improvement of symptoms and normalization of serum amylases.

After 2 years of strict GFD with a follow-up by an experienced dietitian, she still presented daily mild abdominal pain, anemia, and occasional watery diarrhea, with normalization of anti-transglutaminase IgA and anti-gliadin IgA. Abdominal MRI was normal. Upper digestive endoscopy showed atrophic duodenum with MARH IIIa in biopsies (Figure 1A). Immunohistochemistry was performed, showing positive CD3 in the IELs and positive CD8 in 50% or less of IELs stained by CD3 (Figure 2). She was then diagnosed with RCD type 2. Her adherence to GFD was reviewed and confirmed again by another dietitian, and oral budesonide (OB) in the dose of 3 mg three times a day (Entocort®) was started, as stated in the study by Mukewar et al.: "Directions for taking OB were as follows:

1. first daily capsule: open the capsule, empty contents into applesauce and stir, grind the medicine between the teeth, rinse and swallow with a glass of water;
2. second daily capsule: open the capsule, empty contents into applesauce, stir, rinse and swallow with a glass of water;
3. third daily capsule: swallow the whole capsule"[1].

After 6 months of treatment with OB, upper digestive endoscopy showed recovery of duodenal atrophy (Figure 1B), with MARSH I on biopsies (Figure 3). She was successfully tapered off OB and remained asymptomatic on strict GFD after 2 years of follow-up.

DISCUSSION

In this case, summarized in Table 2, two important topics of CD are discussed: uncommon presentations, such as an unspecific

Table 1. Diagnostic findings for refractory celiac disease type 1 and 2 on duodenal biopsy immunochemistry or flow cytometry

RCD	Type 1	Type 2
IEL phenotype	IEL are CD3+/CD8+. CD3+/CD8– <40–50% by immunohistochemistry, and <20–25% by flow cytometry.	IEL mostly aberrant. IEL CD3+/CD8– >40–50% by immunohistochemistry, and >20–25% by flow cytometry.
TCR Gamma gene rearrangement polymerase chain reaction	Polyclonal TCR	Monoclonal TCR

RCD: refractory celiac disease; IEL: intra-epithelial lymphocyte; TCR: T-cell receptor.

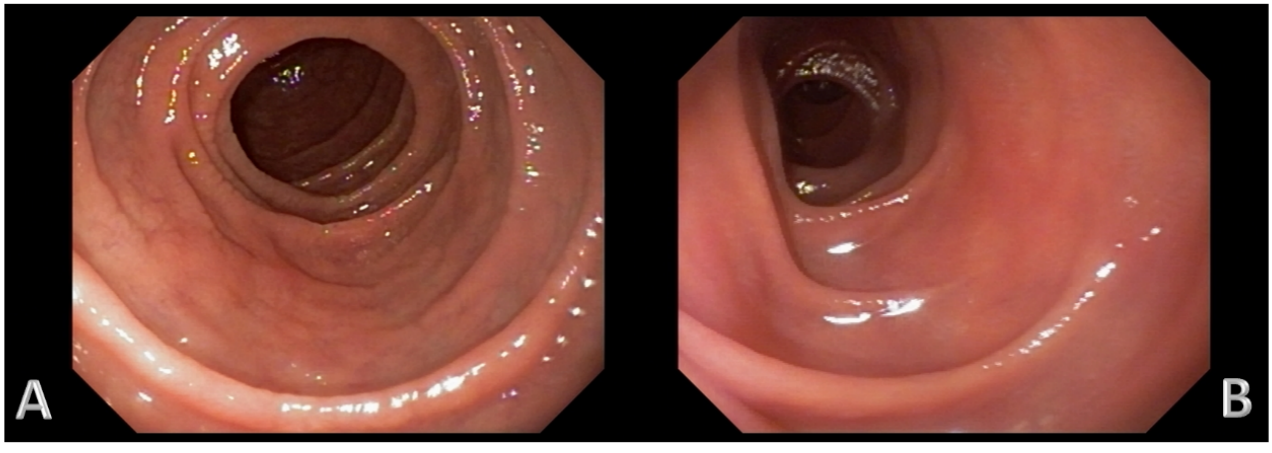

Figure 1. Upper digestive endoscopy (duodenum). (A) Duodenal atrophy and severe scalloping of duodenal folds; (B) Resolution of duodenal lesion.

abdominal pain associated with unspecific laboratory alterations, and RCD, which prevalence is bound to increase as CD prevalence continues to grow worldwide.

Most patients diagnosed with CD improve greatly with GFD, and complete clinical and histological response does not occur in every celiac patient. A small group of these patients will present persistent duodenal atrophy and recurrent symptoms (such as, anemia, diarrhea, abdominal pain, and weight loss), despite strict adherence to GFD[1,2]. RCD is defined by persistent or recurrent symptoms associated with villous atrophy and increased IELs despite 6–12 months of strict confirmed GFD, after the exclusion of malignancy and other causes of lack or loss of response to GFD[1,2]. Causes that should be considered in this step of differential diagnosis are: tropical sprue, autoimmune enteropathy, hypogammaglobulinemia, idiopathic AIDS enteropathy, eosinophilic gastroenteritis, Whipple disease, intestinal T-cell lymphoma, ulcerative jejunitis, collagenous sprue, giardiasis, other parasitic infections, and ischemic enteritis[2,4,5].

RCD is considered an uncommon condition, which might be related to a poor prognosis. In order to better stratify prognosis, it is divided into type 1 and type 2[2,3,6]. It is sub-classified by the detection of abnormal IEL phenotype, which helps clarify prognosis. The current methods can be done in fixed (double CD3/CD8 immunohistochemistry and TCR clonal rearrangement by PCR) or on fresh frozen intestinal tissue (flow cytometry). The abnormal phenotype is supported by the loss of normal surface markers CD3, CD4, and CD8 with preserved expression of intracytoplasmic CD3 in more than 50% of IELs as evaluated by immunohistochemistry[1] or more than 20–25% as determined by flow cytometry and detection of TCR chains clonal rearrangement by PCR[2,6].

Abnormal IELs are the hallmark of RCD type 2, which is associated with poor prognosis because of a higher risk of progression to T-cell lymphoma[1-3,5,6]. This was the major limitation of the reported case: due to the unavailability of flow cytometry, diagnosis of RCD type 2 was made using immunohistochemistry, which is a method that can reveal dominant aberrant

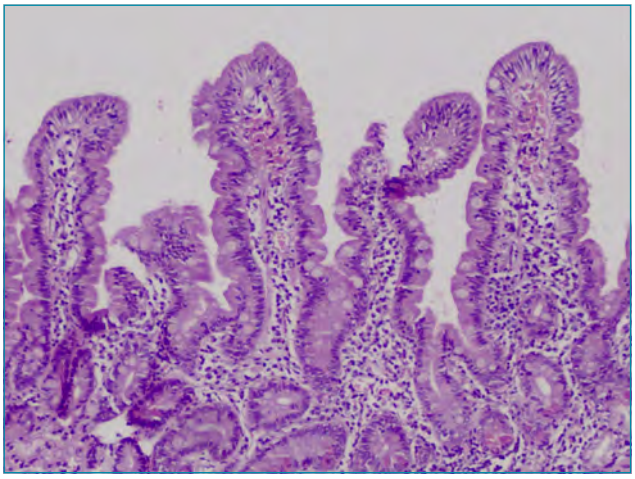

Figure 3. Duodenal biopsy (H.E, 100x). Recovery of the villous architecture, with discrete increase in IEL infiltrates (MARSH I).

Table 2. Summary of the reported case.

Clinical presentation	41 years old female patient. Abdominal pain. Elevation of serum amylases.
Diagnostic tests	Normal computed tomography. Celiac disease serology positive. Duodenal biopsy: MARSH IIIb.
Evolution	Persistent abdominal pain. Strict adherence to gluten-free diet. After 2 years of diagnosis: MARSH IIIa.
Final treatment	Immunohistochemistry: RCD type 2. Use of oral budesonide for 6-months. MARSH I at the end of treatment.

RCD: refractory celiac disease.

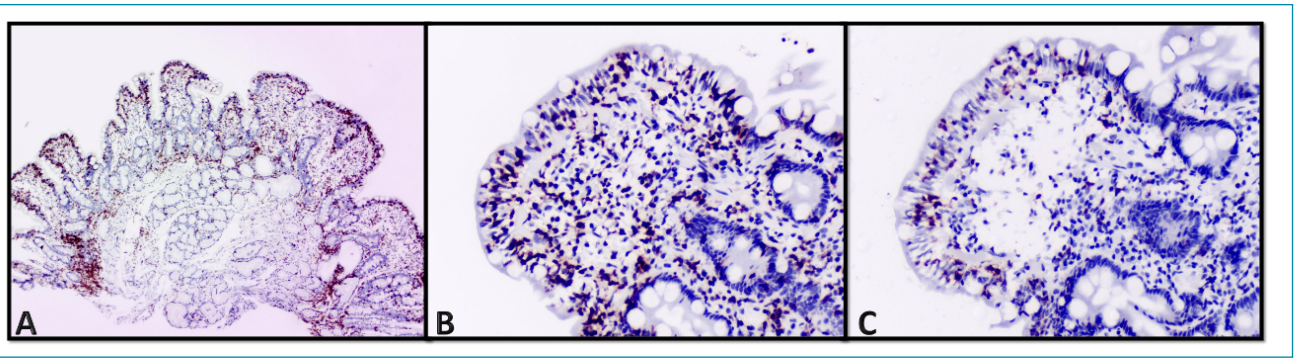

Figure 2. Duodenal biopsy and immunohistochemistry. (A) (anti-CD3 T lymphocytes, 100x): Villous with partial atrophy, fusion, and high number of IELs; (B) (anti-CD3 T Lymphocytes, 200x): Intraepithelial lymphocytosis (more than 30 lymphocytes/100 enterocytes); (C) (anti-CD8 T lymphocytes, 200x): Intraepithelial lymphocytosis in duodenal biopsy. The number of lymphocytes highlighted by antibody anti-CD8 is significantly lower than those stained with anti-CD3 (Figure 2A).

IEL populations only when they make up over 50% of IELs[7]. However, in patients with a moderate aberrant IEL population (between 20 and 50%), a significant number of patients will be undetected[8,9]. The lack of sensitivity of immunohistochemistry can be largely explained by the inability of this method to distinguish between cell surface and intracellular expression of CD3, the latter being the hallmark of aberrant IELs, whereas flow cytometric analysis can differentiate cytoplasmic from membranous CD3 expression. Therefore, it is a superior method to identify patients at a higher risk for T-cell lymphoma, since it can better analyze TCR clonality[5]. Immunohistochemistry also has two major problems: it lacks specificity and an underlying disease can be inadequately classified as RCD type 2[10]; and it has a higher inter-observer variability[11]. This lower sensitivity should be outweighed against the fact that this technique is easily applicable and readily available, whereas flow cytometric analysis of IELs requires fresh duodenal biopsies and skilled analysts[11].

CD is considered to be an uncommon cause of nonpancreatic elevation of amylases and lipases–this laboratorial finding is believed to occur in about 25% of celiac patients[12]. In a study comprised of 54 celiac patients, most of the elevated values were lower than twofold the reference values. After 12 months of GFD, serum amylase level was elevated in three cases and lipases in two cases, and these patients had not strictly adhered to the GFD[12]. It is reasonable, therefore, to screen patients with elevated serum amylases or lipases levels for CD, in the absence of signs of pancreatic disease.

Although GFD is being strictly followed by CD patients, mortality when compared to non-celiac controls is still higher, with a hazard ratio of 1.21. This higher risk of death is associated with cardiovascular disease, cancer, and respiratory disease[13]. This mortality increases even more in the presence of RCD. The 5-year survival rates described vary from 80% to 96% for RCD type 1 and 44% to 58% for RCD type 2[14]. Evolution to T-cell lymphoma in RCD type 2 generally occurs in patients with advanced age and if a late diagnosis of CD has been made. In this context, increased levels of lactate dehydrogenase and β-2-microglobulin might suggest that RCD has evolved to lymphoma, a life-threating complication of RCD type 2[15].

RCD is often treated with immunosuppressive medications. Prednisone (0.5–1 mg/kg/day) or a combination of prednisone and azathioprine (2 mg/kg/day) are clinically effective for most patients with RCD type 1. Clinical response to steroids is observed in the majority of patients with RCD type 2. However, mucosal recovery is infrequent, and progression to T-cell lymphoma might not be totally preventable[1,2,5,6]. Therefore, current immunosuppressive treatments for RCD type 2, such as azathioprine, achieve poor results and might increase the risk of T-cell lymphoma.

In a study developed at Mayo Clinic by Mukewar et al., 43 patients with RCD type 1 and 13 patients with RCD type 2 were enrolled. There were two deaths related to lymphoma: one patient with RCD type 1 and one with RCD type 2. All patients were treated with OB, with a response of 92% in both RCD type 1 and 2[1]. It is expected a recovery of duodenal mucosa with treatment with OB in a 6-month interval, although some patients might not be tapered off the medication[1].

In another study, published by Therrien et al., 42 patients with non-responsive CD treated with budesonide 9 mg daily were showed an inferior result compared with the study by Mukevar et al. It was found that, within 1 year of therapy, 25% of patients had clinical and mucosal recovery and 17% had both persistent symptoms and mucosal damage[16].

CONCLUSION

In conclusion, it is significant to screen celiac patients with persistent symptoms or villous atrophy for RCD. This screening should accompany an extensive review of the adherence to GFD. Afterwards, with the use of immunohistochemistry and/or flow cytometry, these patients should be stratified into RCD type 1 or type 2, with an objective of prognosticating the disease. If RCD type 2 is present, the physician should be aware of the higher risk of T-cell lymphoma. OB should then be considered as an effective treatment with a low rate of adverse effects.

AUTHORS' CONTRIBUTIONS

JS: Conceptualization, Writing – Original Draft, Writing – Review & Editing. **KS:** Data Curation, Writing – Original Draft, Writing – Review & Editing. **KLP:** Data Curation, Writing – Original Draft, Writing – Review & Editing.

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SHORT COMMUNICATION
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Telomere length: biological marker of cellular vitality, aging, and health-disease process

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SUMMARY

The aging process occurs due to the decline of vital physiological functions and adaptability of the body, being influenced by genetics and lifestyle. With advances in genetics, biological aging can be calculated by telomere length. Telomeres are regions at the ends of chromosomes that play a role in the maintenance and integrity of DNA. With biological aging, telomere shortening occurs, causing cellular senescence. Several studies show that shorter telomeres are associated with acute and chronic diseases, stress, addictions, and intoxications. Even in the current COVID-19 pandemic, telomere shortening is proposed as a marker of severity in individuals infected by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On the other hand, healthy lifestyle habits increase telomere length and balance of various cellular functions, preventing diseases.

KEYWORDS: Telomere. Telomere shortening. Telomere homeostasis. Telomerase. Biomarkers. Aging. Cellular senescence. Chronic disease.

INTRODUCTION

The irreversible aging process is marked by a decline in vital physiological functions and the adaptability of the body, being strongly influenced by genetics, environmental factors, and lifestyle. Currently, the aging process is divided into two main components, namely, chronological age and biological age, which may differ for the same individual. Biological aging can be calculated by telomere length (TL)[1,2] and DNA methylation levels (epigenetics)[3,4].

Telomeres are noncoding regions of the genome, located at the ends of chromosomes (functioning as protective covers of chromosomes), which consist of long series of short and repeated sequences formed by nitrogen bases 5′-TTAGGG-3′ and associated proteins, which play an important role in the maintenance and integrity of DNA. Telomere shortening may compromise the replicative potential of cells, contributing to the natural process of cellular senescence. To counteract this process, the telomerase enzyme promotes the maintenance of telomere length by synthesizing the repetitive sequences of lost telomeric DNA.

In 2009, Elizabeth H. Blackburn, Carol W. Greider, and Jack W. Szostak received the Nobel Prize in Physiology or Medicine for discovering the protective role of telomere and telomerase enzyme in chromosomes[5-7]. These extremely significant findings paved the way for researchers to further explore the role of telomere homeostasis in cell aging and chronic diseases in general.

Mechanism of action of Telomeres

During cell division or duplication, cells are unable to replicate approximately 50 pairs of nitrogen bases from the ends of chromosomes, as conventional DNA polymerase cannot reproduce the 3′ end of a linear molecule (end replication problem). This leads to progressive chromosome shortening along the divisions of a cell lineage, resulting in loss of replicative capacity and induction of cellular senescence. This mechanism of action is the main cause of cell aging and age-related chronic diseases[8-10].

To avoid this progressive telomere shortening that occurs at each cell division and the loss of respective genetic information,

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periodically, the lost DNA segments are recovered by the action of a ribonucleoprotein enzyme complex called telomerase. This complex has a small RNA component that is a template for the synthesis of the repetitive sequences, which make up the telomere. In the recovery of lost telomeric DNA, nucleotide bases are added individually and in the correct sequence, and telomerase progresses discontinuously, i.e., the RNA mold is positioned on the initiator DNA, several nucleotides are added to it, and finally the enzyme translocates to restart the process[10-12].

In neonatal period, telomerase activity is reduced or null, as evident from the absence in most somatic tissues of the body. Telomerase is active in early stages of human development (pluripotent embryonic cells), and throughout life, in blood stem cells, germ cells and cells of adult tissues undergoing continuous renewal, such as endometrial tissue[13]. Due to gradual loss of telomerase activity, in each cell division, the telomere terminals of these cells are shortened, reaching a minimum length that precludes cell division[14].

On the other hand, 90% of cancerous somatic cells, which reach "immortality," have high expressiveness of telomerase (increasing the telomere length). In these tumor cells, the reactivation of the telomerase silencer gene has been one of the mechanisms used to circumvent the natural system of cellular senescence and apoptosis, allowing cancer cells to promote continuous telomere elongation and replicate in an uncontrolled and uninterrupted manner[15,16].

Role of Telomeres in health disorders

Several studies show that shorter telomeres are associated with a number of chronic diseases: congenital dyskeratosis, aplastic anemia, idiopathic pulmonary fibrosis, and liver cirrhosis[17]; cardiovascular diseases in general[18,19], such as atherosclerosis[20], arterial hypertension[21], and stroke[22]; diabetes mellitus type 2[23-25]; autoimmune diseases, such as systemic lupus erythematosus[26] and rheumatoid arthritis[27]; psychiatric diseases[28]; and dementia[29,30], among other age-related diseases[31].

In cancer, telomere sizes play a dual role as follows: telomere shortening can lead to the induction of chromosomal instability and the onset of tumor formation (precancerous lesion); on the other hand, initiated tumors need to reactivate telomerase to stabilize chromosomes and gain "immortal" growth capacity[32,33].

The same telomere shortening is observed in other health disorders, addictions, and intoxications, namely: obesity[34]; inflammatory and oxidative processes[35]; smoking[36], alcoholism[37] and drug dependence[38]; and exposure to pollutants and mineral particles[39-41], among the others.

Even in acute diseases, such as the current coronavirus disease 2019 (COVID-19), telomere shortening is proposed as a marker of disease severity[42,43] identifying patients at risk of higher morbidity and mortality from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Studies suggest that T-cell lymphopoiesis may be discontinued in the infected individuals with short telomeres[44].

In cancer-surviving children, a study shows the decrease in telomere size associated with chronic health disorders as a result of the treatment received (radiotherapy and chemotherapy)[45]. Similarly, other treatments have demonstrated the same shortening effect on telomere (e.g., immunosuppressive drugs[46], proton pump inhibitors[47], and insulin[48]).

On the other hand, some therapies are being assessed to counteract telomere shortening and act on telomere diseases: sex hormones (aplastic anemia and idiopathic pulmonary fibrosis)[17,49], antidiabetic agents without acarbose (type 2 diabetes)[50] and lithium (bipolar disorder)[51], among the others. Similarly, natural compounds and their extracts have demonstrated to increase telomerase activation (*Astragalus membranaceus* or TA-65, *Centella asiatica*, *Euterpe oleracea,* oleanolic acid, maslinic acid, and multi-nutrient formulas)[17,52,53], which may be indicated in the treatment of diseases related to telomere shortening.

Given that in most cancer cells telomerase activity is higher, different anti-cancer approaches have been designed in the search for telomerase inhibitors: small-molecule inhibitors, antisense oligonucleotides (imetelstat), G-quadruplex stabilizers, immunotherapy, gene therapy using telomerase promoter-driven expression of a suicide gene, and chemicals that block telomerase biogenesis[17,54,55]. Among the natural compounds, anthraquinone[56] and wogonin (extract from *Scutellaria baicalensis*)[57] appear as promising anti-tumor agents.

Analogous to physical disorders, traumatic social exposures or lifelong psychoemotional disorders, such as chronic stress and childhood traumas (abuse, violence, racism, bulling, low socioeconomic status, maternal depression, family disorder, and institutionalization, etc.), also cause a decrease in telomere length[58-65].

Finally, in addition to natural and chronological aging, telomere shortening can be influenced by physical activity, body mass index, chronic inflammation, oxidative stress, hormone therapy, drugs, dietary antioxidants, and vitamins, among others. Studies show that individuals who follow a healthy lifestyle have longer telomeres[66].

CONCLUSION

Functioning as an important biomarker of cellular vitality or activity, longevity or aging, and the health–disease process, measuring the telomere length of leukocytes DNA extracted from peripheral blood[67] provides clinical and dynamic parameters of health and well-being and can be used as a diagnostic and prognostic method of the illness process[31,68-70], as well as

measuring the efficacy and effectiveness of various therapies employed, conventional[71] or nonconventional (e.g., homeopathy[72], acupuncture[73], and meditation[74]).

According to vitalist medical rationalities[75], such as homeopathy and acupuncture, cellular activity, physiological homeostasis, and the health–disease process would be related to vital force or chi (tsri), respectively; cellular senescence, physiological imbalance, and the disease manifestation would occur due to the disturbance of the body vitality. In order to approximate different rationalities, recent studies correlate the characteristics and properties of the homeopathic vital principle with those of the genome (exome *plus* epigenome), suggesting that the genome would be the biological representation or substrate of the organic vital force, according to biomedical episteme[76,77]. In this context, the telomere length could be used as an important biomarker of the effectiveness of homeopathic treatment in maintaining vitality, physiological balance, and health.

Current knowledge about telomeres and telomerase reiterates the importance that should be devoted to healthy lifestyle and health-promoting measures, such as regular physical activity, balanced diet, body weight control, spiritual and contemplative activities, and integrative and complementary practices in health, that increase telomere length and balance of various cellular functions, preventing diseases, and other somatic and psychic disorders.

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SHORT COMMUNICATION
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Ethical support to psychiatry residents: a report of a Brazilian ethics consultation group

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SUMMARY

OBJECTIVE: It is not uncommon for medical residents to deal with critically ill patients who frequently show several ethical and human dilemmas, highlighting the need for a consultation with ethical specialists. The objective of this article is to present a description of a Brazilian Ethics Consultation group designed to attend psychiatry residents.

METHODS: This article reports a case of a critically ill patient with Borderline Personality Disorder with multiple intervention failures and several ethical conflicts who was seen by a resident and supported by an ethics consultation group.

RESULTS: When medical residents and medical staff face severe and unusual ethical dilemmas, they might feel unprepared and have ones' mental health impaired. Thus, this article reports a successful ethics consultation and discusses its development in other academic institutions.

CONCLUSION: Medical educators and staff from academic hospitals should pay attention to the needs of the medical residents. The development and support of ethics consultation groups must be provided to fulfill the need of those residents who face serious ethical and human dilemmas.

KEYWORDS: Psychiatry. Ethics. Ethics consultation. Academic training.

INTRODUCTION

Dealing with critically mentally ill patients involves multiple aspects of subjective dimensions. This complexity raises a series of ethical, legal, and human dilemmas, which includes the patient (and his or her desires, dreams, mental capacity, etc.), the relatives of these patients, the attending physician, and other health professionals. In this context, mental health professionals are constantly exposed to and dealing with multiple ethical issues in their clinical practice[1,2]. Among these professionals, psychiatry residents, who are still under training, need special support when these ethical issues appear, since unsolved conflicts may impact their professionalism and mental health[3-5].

A study held in fourteen South Korean hospitals and including all residency specialties, showed that 77% of residents had faced serious ethical dilemmas during their training, and most of them didn't know how to manage them[6]. Surprisingly, most of these residents tried solving their ethical dilemmas by talking to a colleague instead of talking to a supervisor or an ethics specialist. Similar results were found in the US for psychiatry residents, where 76% of residents reported facing an ethical dilemma that they felt unprepared during their residency training[7].

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These alarming numbers highlight the need for ethical support in residency programs. An important tool to ensure an ethical environment, especially in academic and clinical settings, is the existence of ethics consultation groups. Ethics consultation groups are a team of ethical specialists who can access, evaluate and orientate physicians (either senior or residents) in any kind of ethical conflict that might emerge from a clinical encounter[8]. Resident physicians need education and special support when dealing with ethical conflicts and, the existence of clinical ethics consultation groups could be extremely helpful to them[9]. A previous study[10] evaluated the knowledge, use, and perceptions of resident physicians from different medical specialties regarding ethics consultation groups. They found that despite that the residents report awareness of ethics consultation services, most of them had never requested one. Thus, it is crucial the need of disseminating ethics consultation groups throughout academic hospitals, particularly for those who deal with medical residents.

This gap is more evident in psychiatry resident programs, where, to our knowledge, there are no studies that report the use or the description of ethics consultation services. As previously described, mental health professionals are vulnerable to several ethical issues and comprehensive support could impact their clinical practice and improve their patient care.

In an attempt to generate further evidence to this topic, the present article aims to discuss the support for psychiatry residents made by an ethics consultation group in Brazil. To illustrate the whole process, a case of a patient diagnosed with severe Borderline Personality Disorder will be used. This patient has been largely refractory for many types of pharmacological and non-pharmacological interventions, and several ethical conflicts have emerged from this complex multi-level approach. The approach includes efforts of a large and diverse clinical staff with different points of view and expectations regarding the patient outcome and the harsh intersection of wills, values, and expectations among physicians and patients, as well as the indent to develop leadership skills in psychiatry residents.

EDUCATIONAL CASE

This is a 29-year old female, single, childless, and a Jehovah's Witness. Currently unemployed but worked in several (approximately eight) different jobs. She lives with her mother, who herself is treated for depression. Her father divorced her mother when the patient was one year old, abandoning the family. The patient experienced several incidents during childhood and adolescence. Following her father's abandonment, she experienced a difficult relationship with her mother. As religion was very important to her family, she was expected by her family to display a strong religious commitment, which she was unable to do. Due to her smoking and self-injury habits, she was expelled from her religion, which caused her to withdraw from her family. A few years later, the patient initiated self-injurious behavior and mood instability culminating in her first suicide attempt (with rat poison), when she began outpatient care in our psychiatry hospital in specialized outpatient care.

At the start of treatment, the clinical staff reviewed the patient's symptoms and clinical history. The patient presented frantic efforts to avoid abandonment, in addition to demonstrating unstable relationships, impulsivity, suicidal behavior, affective instability, intense anger, delusion-like ideas, and severe dissociative symptoms, having the diagnosis of Borderline Personality Disorder (BPD). In 2018, she was involuntarily hospitalized at our institution, referred by the outpatient physician due to suicidal thoughts, dissociative episodes, and psychotic-like symptoms, for initiation of electroconvulsive therapy (ECT) justified by "multiple inefficacious treatments" and this being "the patient's final chance for treatment", which was immediately refused by the patient.

At admission, the resident physician responsible for her case felt under extreme pressure. The patient did not want ECT, but her psychopathology seems to impair her decision-making capacity[11]. The outpatient physician and her mother strongly supported it, sending several phone messages to the resident physician, sometimes threatening to sue him if he did not accept this indication, even though ECT is not a scientifically validated treatment for BPD patients[12,13]. At that time, the resident felt unable to make the best decision.

In this uncertain scenario, the resident was told that there was a clinical ethics consultant team at the hospital and the team was called to discuss the ethical dilemma and help the decision making. Then, a meeting was held, and the resident was told to present the case to a group of ethics specialists who were not involved with the case. The team urged the resident to discuss the best treatment options for the patient with all members of the clinical staff and the family. The ethics consultation group considered that the patient was, in fact, capable of making decisions regarding the ECT treatment (ability to communicate a choice, in addition to the ability of understanding, appreciation, and reasoning)[14].

Since the ECT was not performed, other treatment options were taken into consideration. Following multiple discussions, the re-introduction of Clozapine was selected as prudent drug therapy for this context (intermediary approach). This drug was the only medication that has been proven to control the patient's impulses and disorganization in the past, despite the risk of neutropenia and low evidence of efficacy[15], and despite the patient's refusal to be submitted to a weekly blood count,

which could help identify clozapine-induced neutropenia. The patient and her mother were advised of its risks and agreed with the joint decision (by impatient clinical staff and resident, clinical ethics consultant team, and outpatient clinical staff).

As a result, the resident involved in this case was able and felt prepared to deal with all these medical, legal, and ethical issues, feeling fully supported in this complex and multi-level ethical decision and improving his clinical skills.

ETHICS CONSULTATION GROUP FOR PSYCHIATRIC RESIDENTS

Figure 1 summarizes the framework of the Ethics Consultation Group of the Institute of Psychiatry of the University of São Paulo, the largest Brazilian university, located in the southwest of Brazil. The consultation group emerged from a forensic psychiatry group of the same institution called NUFOR (Forensic Psychology and Psychiatry Program). The model is based in six steps and will be discussed below, having as an example the case reported above.

First Step (Contact with the ethics consultation group): The first step is the promotion of the ethics consultation group to psychiatry residents. This is not an easy step, since some previous international studies showed that many residents are not aware of the existence of this type of support and also have a negative perception of it[10]. At our institution, dissemination of the objectives of the group was carried out through leaflets, banners, meetings, and grand rounds. Then, the first contact is available through the contact with a senior forensic resident, a fourth-year psychiatry resident who usually is a former resident of our institution and has close contact with other peers, or through direct contact with any NUFOR member, where a meeting is set up to present the case to the expert group.

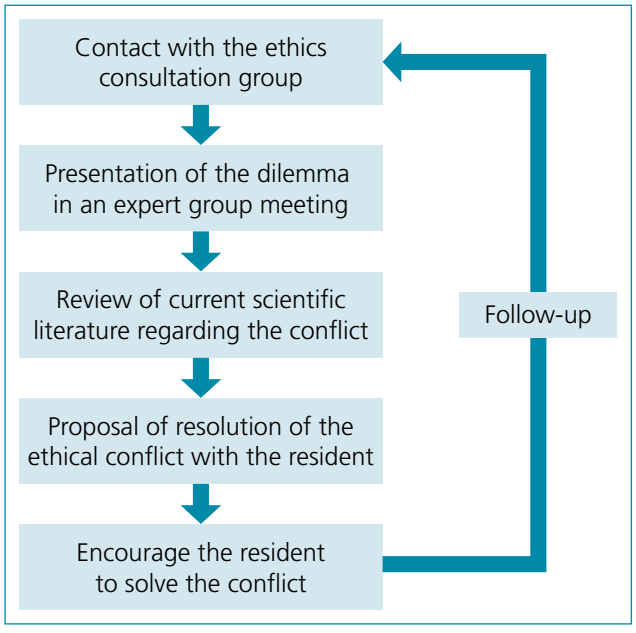

Figure 1. Framework of an ethical consultation group support program to psychiatry residents.

Second Step (Presentation of the dilemma to an expert group meeting): At this time, the resident is responsible to present the case in a meeting open to the scientific community of our institution, organized by NUFOR ethics experts' members. The case is presented and a subsequent discussion about any emerged ethical dilemma is done. NUFOR members use the moral deliberation method proposed by Gracia[16] as a theoretical framework. The author proposes that when dealing with an ethical dilemma the health professional should identify different courses of action (extreme, intermediary, and optimal), and always try to choose the more prudential one.

Third Step (Review of the current scientific literature regarding the conflict): The ethical consultants in collaboration with the responsible resident make a large literature review trying to identify similar cases where the specific ethical dilemma was reported. Furthermore, if needed, there is a revision of Brazilian laws to help the entire group in finding the best and the most suitable course of action based on the country's law, avoiding any kind of suing to the medical team.

Fourth Step (Proposal of resolution of the ethical conflict with the resident): Together with the resident and the clinical staff, the ethics consultant group proposes a resolution of the ethical conflict. For example, in the reported case above, there is an urgent need to conciliate all points of view to make the best decision. So, a meeting was proposed among all clinical staff to define a single course of action and thereof having an encounter with the family to explain all discussions and clinical prognosis that all the team (not only the medical resident) decided to the patient as the best course of action.

Fifth Step (Encourage the resident to solve the conflict): This is done throughout all steps. The ethics consultation member supports and encourages the resident to solve the conflict, developing the trainee's academic and personal skills, and if appropriate, present the whole case in a scientific encounter to incentive other mental health professionals to pay deeper attention to how ethical conflicts might emerge and some examples in how solving it appropriately.

Sixth Step (Follow-up): At all times (until the resolution of the conflict) a follow-up is done by the team, and there is an open channel to contact by the resident in any new emerged issue. Moreover, when a new and challenging conflict that deserves another presentation to the group emerges, a new encounter is set up and the steps start again.

CONCLUSION

This article discusses the importance of an ethics consultation group for supporting psychiatric residents while dealing with complex ethical and legal dilemmas. In our case scenario, the resident felt unprepared and overwhelmed by many different sources of opinions and information. The support from the ethics group was an important way to overcome these conflicts and helped the resident to further understand the family context, patient's autonomy, and the decision-making process, improving his training, professionalism, and healthcare.

We are aware that there are several pitfalls in implementing it, especially in non-tertiary care services. However, we strongly suggest that resident doctors should be trained to learn how to deal with and conduct this kind of dilemma, in special psychiatry residents, who can be an important tool to support other professionals and specialists dealing with complex ethical conflicts in primary and secondary settings. Furthermore, in the age of technology, online conferences between trained specialists and more junior doctors could be an important alternative to support doctors from distant and less-specialized areas.

AUTHORS' CONTRIBUTIONS

RFD: Conceptualization, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **TFS:** Conceptualization, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **GL:** Writing – Original Draft, Writing – Review & Editing. **EM:** Writing – Review & Editing. **JMK:** Writing – Review & Editing. **DMB:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **GBC:** Conceptualization, Writing – Original Draft, Writing – Review & Editing.

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SHORT COMMUNICATION
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New papulovesicular rash in the course of COVID-19 signaling viral reactivation

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SUMMARY

Cutaneous manifestations are considered an infrequent presentation of coronavirus disease 2019 (COVID-19) and are mostly described in outpatient settings. Its onset during the course of the severe COVID-19 disease has been poorly described in severe cases. Studies focused on dermatological manifestations mostly described maculopapular or pernio-like lesions and less frequently vesicular or varicella-like eruption. We described the occurrence of a vesiculopapular eruption in three laboratory-confirmed COVID-19 patients associated with severe lung injury in whom the skin findings preceded viral reactivation and recrudescence of hypoxemia. The potential mechanisms for COVID-19-related cutaneous manifestations include immune hypersensitivity, cytokine-release syndrome, deposition of microthrombi, and vasculitis.

KEYWORDS: Coronavirus infections. Severe acute respiratory syndrome. Dermatology. Exanthema. Recurrence.

The ongoing pandemic of coronavirus disease 2019 (COVID-19) is a global concern. The disease typically presents with symptoms resembling other viral infections, but in severe cases, it may develop acute respiratory distress syndrome with a high mortality rate[1]. Cutaneous manifestations are considered an infrequent presentation of COVID-19 infection but are probably under-recognized, although up to 44% of the patients had cutaneous findings at disease onset or during its course[2]. However, neither characteristics nor progression of the lesions were adequately documented[2,3]. Studies focused on dermatological manifestations have described different viral exanthems, of which vesicular eruptions appear in 9–11% cases[4,5]. The majority of cases were recognized in outpatient settings, and although described in some severe cases, its onset over the course of the disease has not been established.

We describe the occurrence of a vesiculopapular eruption in three laboratory-confirmed COVID-19 patients (Figure 1) associated with severe lung injury in whom the skin findings preceded viral reactivation and recrudescence of hypoxemia, as a second hit of viral injury.

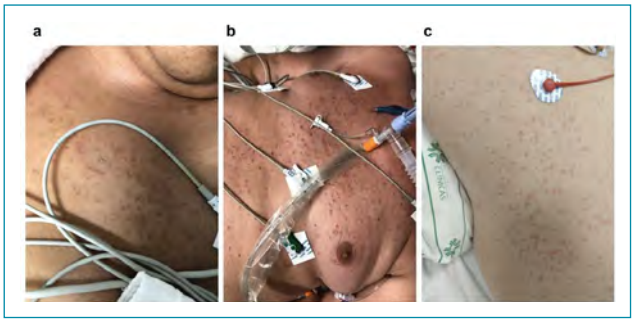

Figure 1. Papulovesicular eruptions before or concomitantly to second hit severe respiratory syndrome related to COVID-19 reactivation. (A) Case 1, (B) Case 2, and (C) Case 3.

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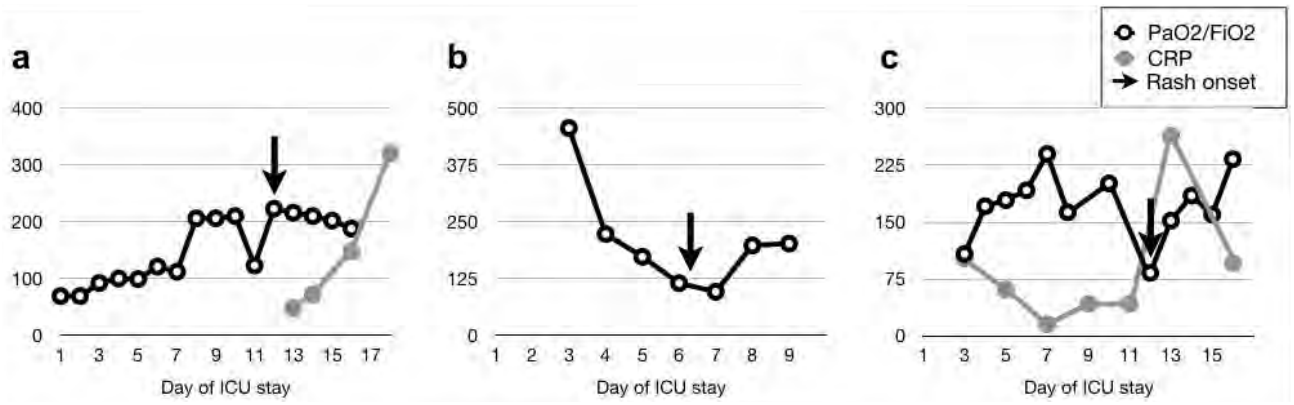

CRP, C-reactive protein; ICU, intensive care unit.

Figure 2. Graphs showing worsening in the PaO_2/FiO_2 ratio and raise of CRP after or concomitantly with the rash onset during the course of COVID-19 infection: (**A**) Case 1, (**B**) Case 2, and (**C**) Case 3.

CASE 1

A 58-year-old obese and diabetic female presented with 3-day COVID-19 symptoms and acute respiratory failure, requiring mechanical ventilation. Prone position was required for several days due to severe hypoxemia, with gradual improvement and progression to weaning. After 15 days, the patient developed a vesicular rash in the trunk, followed by new onset fever, severely worsening hypoxemia, and ascending C-reactive protein (CRP). Pulmonary embolism was ruled out, and no microbiological evidence of infection was found.

CASE 2

A 51-year-old male with diabetes history sought medical attention with symptoms of COVID-19 for 5 days, requiring invasive mechanical ventilation on the sixth day of hospital stay. One week later, the patient was in the process of weaning. He then developed a vesiculopapular rash followed by shock, fever, and loss of renal function, and over the following days, hypoxemia worsened. Blood and sputum cultures were negative. Computed tomography (CT) showed active viral lesions, with no evidence of pulmonary thromboembolism.

CASE 3

A 33-year-old obese male with ongoing viral symptoms for 1 week presented respiratory failure, requiring mechanical ventilation. After 11 days of intensive care, the patient showed signs of recovery and was weaned from the ventilator. The following day, he was reintubated facing worsening hypoxemia and simultaneously developed a vesicular rash affecting chest and back. CRP values increased significantly. Blood and sputum cultures were negative. The CT showed a small segmental pulmonary embolism and extensive ground-glass opacities compatible with viral activity.

In all three cases, the cutaneous eruption occurred either immediately before or concomitantly to second hit severe respiratory syndrome attributed to COVID-19 reactivation (Figure 2). Although a relation between vesicular skin eruption and disease recurrence has not yet been described, it is possible that inflammatory rebound and immune response phenomena could account for both features. The potential mechanisms for COVID-19-related cutaneous manifestations include immune hypersensitivity response to severe acute respiratory syndrome coronavirus 2 RNA, cytokine-release syndrome, deposition of microthrombi, and vasculitis[6]. Although further clinical data are warranted, new onset of papulovesicular rash might signal a more severe course of disease in patients with critical respiratory syndrome due to COVID-19.

AUTHORS' CONTRIBUTIONS

MFR: Conceptualization, Data Curation, Methodology, Writing – Original Draft, Writing – Review & Editing. **LCCF:** Conceptualization, Data Curation, Methodology, Writing – Original Draft, Writing – Review & Editing. **WLN:** Conceptualization, Data Curation, Methodology, Writing – Original Draft, Writing – Review & Editing.

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ORIGINAL ARTICLE
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The effect of mutation status, pathological features and tumor location on prognosis ın patients with colorectal cancer

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SUMMARY

OBJECTIVE: Colorectal cancer is the most common malignancy of the gastrointestinal tract. It is the third most common tumor in both genders and the second reason of cancer-related deaths. In recent years, tumor location has gained importance as a prognostic indicator. In this study, we aimed to analyze if there was a prognostic effect of tumor location, the pathological features, and the mutation status of patients on survival.

METHODS: Two-hundred and ten colorectal cancer patients aged 18 years and older were included into the study. One-hundred and forty-two patients had left-sided tumor and 68 patients had right-sided tumor. Patients who had other malignancies rather than squamous cell skin cancer and *in situ* cervical cancer were excluded. All statistical tests were carried out using two-sided process, and a $p \leq 0.05$ was considered statistically significant.

RESULTS: There were 140 men and 70 women in the study. The median age of the patients was 62 years old. There was no statistically significant difference according to tumor location and survival of patients. The overall survival of patients with right-sided tumors was 60.5 months and 47.2 months for left-sided tumors. Disease-free survival of patients was 63.7 months for right-sided tumors and 46 months for left-sided ones. Perineural invasion, grade and stage were crucial prognostic parameters. Disease-free survival was longer for female colorectal cancer patients.

CONCLUSION: According to our study, survival of patients was similar regardless of tumor location. This can be explained by the different sequencing of treatment strategies and divergent population genetics.

KEYWORDS: Colorectal neoplasms. Prognosis. Mutation.

INTRODUCTION

Colorectal cancer originating from either the colon or rectum is the third most common cancer diagnosed worldwide[1]. According to the World Health Organization Global Cancer Observatory (GLOBOCAN) database, colorectal cancer is the third most common cancer diagnosed in males and the second in females, with an estimated 1.8 million new cases and 861,000 deaths occurring in 2018 worldwide[2].

Colorectal cancer encompasses a heterogeneous group of diseases with complex genetic and epigenetic risk factors, such

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as tumor location, microsatellite instability, western lifestyle, physical inactivity, obesity, smoking and vitamin D deficiencies[3,4]. Studies in the literature have shown that prognostic status and survival rates differ between right- and left-sided colorectal tumors. However, the effect of location differences on survival does not show consistent variability in different tumor stages[5,6]. On the other hand, most studies revealed a poorer survival in right-sided primary tumor location[7-9].

Alongside tumor location, different pathological signs that affect colorectal cancer prognosis have also been identified. These pathological signs include variable factors, such as lymphovascular invasion, perineural invasion, tumor border configuration and host immune response to tumor[10,11]. In particular, "blood or lymphatic vessel invasion" in patients with colorectal cancer was reported by the College of American Pathologists (CAP) Consensus Statement as a prognostic status[12].

Colorectal cancer is a clinical entity that is rich in mutation diversity, in which genetic alterations are well defined in Oncology. At the same time, different genetic factors, such as K-RAS, N-RAS, BRAF and HER2, are known to affect the prognosis of colorectal cancer. Furthermore, microsatellite stabilization status is also as important as other prognostic factors[13,14]. Both these mutations and other prognostic signs observed in colorectal cancer progression affect the course of the disease, and this clinical process makes individualized treatment important for each patient.

In this study, we aimed to investigate the effect of tumor sidedness, pathological features and mutation status on survival in colorectal cancer patients.

METHODS

In this retrospective study, medical records of patients with histopathologically proven colorectal cancer between the dates of January 1, 2010 and December 31, 2016 were evaluated. Ethical approval was taken from our institution before the onset of study. There were 210 patients aged 18 years and older of whom 142 had left-sided tumors and 68 had right-sided tumors.

The exclusion criteria were as follows: any malignancy other than treated squamous cell skin cancer and *in situ* cervix carcinoma, not histopathologically proven colorectal cancer; any death other than colorectal cancer; and patients who lost their six-month follow-up at outpatient clinic. Age, gender, tumor location, histological tumor grade, disease stage, lymphovascular invasion, perineural invasion, comorbidities, such as type 2 diabetes, K-RAS, N-RAS and BRAF mutation status, overall survival, disease-free survival and progression-free survival of patients, were recorded. Right-sided tumors were defined as caecum and ascending colon; left-sided tumors were defined as descending colon, sigmoid colon, rectosigmoid region and the rectal region. Grade 1 and 2 tumors were defined as low grade, grade 3 tumors were defined as high grade. Grades 1 (well differentiated) and 2 (medium differentiated) were defined as low; grade 3 (poorly differentiated) was defined as high-grade tumor.

The staging of metastatic patients was done by using various imaging modalities, such as computed tomography, magnetic resonance imaging, and positron emission tomography/computed tomography scan. Patients were staged according to the International Union Against Cancer TNM classification.

Continuous variables were categorized using median values as the cutoff point. For group comparison of categorical variables, chi-square or one-way ANOVA tests were used; and for comparison of continuous variables, Mann-Whitney U test or Kruskall-Wallis tests were accomplished. Overall survival was calculated from the date of first admission to the clinics to disease-related death or date of last contact with the patient or any family member. Kaplan-Meier method was used for the estimation of survival distribution, and differences in overall survival was assessed by the log-rank statistics. All statistical tests were carried out using two-sided tests and a $p \leq 0.05$ was considered statistically significant. Statistical analysis used the SPPS 21.0 (SPSS Inc., Chicago, IL., USA) software.

RESULTS

There were 210 patients with histopathologically confirmed colorectal cancer of whom 142 (67.6%) had left-sided tumors and 68 (32.4%) had right-sided tumors. Seventy (33.3%) patients were female and 140 (66.7%) were male. The median age of the patients was 62 (range: 20–83) years. General characteristics of the patients were summarized in Table 1.

K-RAS mutation was positive in 33 (15.7%) patients, negative in 37 (17.6%) and unknown in 140 (66.7%). Thirty-four (16.2%) patients had type 2 diabetes mellitus as comorbidity. During their follow-up, 75 (35.7%) patients had progression. Eighty-six (41%) patients were not alive at the end of study.

There was no statistically significant difference according to the overall survival of patients with right- and left-sided tumors (60.5 months and 47.2 months, respectively, $p>0.05$), as seen in Figure 1. Patients with higher grade lived shorter than the ones with lower grade (overall survival=21 and 59.8 months respectively, $p<0.0001$), as in Figure 2. Stage was an independent surrogate of survival and patients with stage III–IV lived shorter (39.4–76 months, respectively, $p<0.0001$). RAS status had no effect on survival ($p=0.78$). Diabetes as comorbidity had no effect on survival ($p=0.13$ for overall survival and $p=0.09$ for progression-free survival/disease-free survival). There was no statistically significant difference in terms of overall survival

between males (47.4 months) and females (57.4 months), with p>0.05, but disease-free survival was higher in females (60.6 *versus* 48.8 months, p=0.02). Perineural invasion was considered to be important for disease-free survival (37.7 *versus* 63.8 months, respectively, p<0.0001).

DISCUSSION

Colorectal cancer survival rates are increasing due to sequential and good therapeutic management of patients. Disease stage, age, histological grade/tumor differentiation, lymphovascular invasion and perineural invasion are crucial prognostic parameters[15,16].

The prognostic impact of tumor sidedness is a crucial factor for colorectal cancer. Right-sided colorectal cancers are known to have higher mortality with shorter survival than the left-sided ones[17-20]. In our study, there was no statistically significant difference of overall survival and disease-free survival/progression-free survival according to tumor sidedness. In a study done by Liu et al.[19], in Chinese population, it was shown that right-sided tumors had a worse prognosis. In another prospective study done by Jess et al.[17], for Danish colorectal cancer patients, right-sided tumors had a higher mortality rate in the first two years of their follow-up. Hansen et al.[20] showed that right-sided tumors had worse prognosis than the left-sided ones.

For K-RAS wild type colorectal cancer, in which all patients had an anti-epidermal growth factor receptor antibody with chemotherapy, right-sided tumors had worse overall survival, progression-free survival and objective response rate[17]. Wolmark et al.[21] concluded that descending colon cancer patients had better prognosis than rectal and other localized ones. However, Sjo et al.[22] showed that descending colon and transverse colon cancers had worse prognosis. In a new study, rectal cancer was associated with worse Refeeding syndrome compared to right-sided colon cancer and left-sided colon cancer, however among patients with recurrence, rectal cancer was associated with better overall survival compared to right-sided colon cancer and worse overall survival compared to left-sided colon cancer[23]. A study performed

Table 1. General characteristics of the patients.

		Min-Max	Median	Mean±SD/n-%	
Age		20–83	62.0	61.0±10.9	
Gender	Female Male			70 140	33.3 66.7
Tumor location	Right Left			68 142	32.4 67.6
Grade	Low High			162 48	77.1 22.9
Stage at diagnosis	I II III IV			13 63 50 84	6.2 30.0 23.8 40.0
Lymphovascular invasion	No Yes Unknown			99 77 34	47.1 36.7 16.2
Perineural invasion	No Yes Unknown			133 43 34	63.3 20.5 16.2
K-RAS mutation	No Yes Unknown			37 33 140	17.6 15.7 66.7
Type 2 diabetes mellitus	No Yes			176 34	83.8 16.2
Recurrence or progression	No Yes			135 75	64.3 35.7
Patient status	Live Exitus			124 86	59.0 41.0

SD: standard deviation.

in the Turkish population has showed that tumor side has no effect on survival.[24]. It was similar to our findings. In 2020, new data demonstrated that there was no consensus with respect to the implications of tumor sidedness in second and subsequent lines of treatment, and the concept of tumor sidedness may not be true in this setting. There is certainly a need for a consensus statement in this space[25].

In literature, there is no gender diversity[24], but in our study, male/female ratio was 2/1. Türkoğlu et al.[24] found no prognostic impact of gender on survival. In our study, overall survival was the same for both males and females, but progression-free and disease-free survival were shorter in males than females.

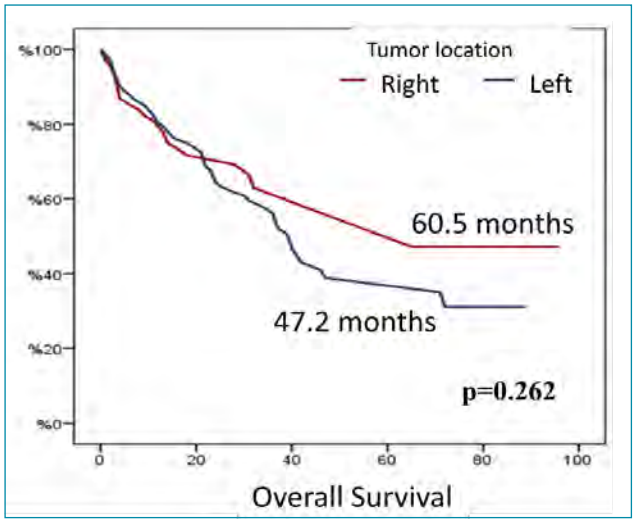

Figure 1. The effect of tumor location on overall survival.

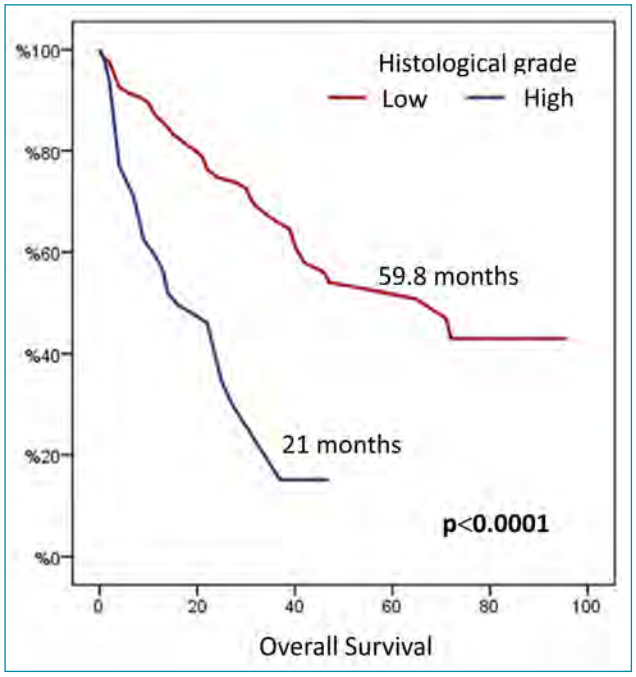

Figure 2. The effect of histological grade on overall survival.

In literature, lymphovascular invasion is considered to be a poor prognostic factor[16]. However, it was not a good surrogate of prognosis in our study. Perineural invasion is associated with tumor recurrence and it is a poor prognostic factor for colorectal cancer patients[15,16]. In our study, there was no difference on overall survival according to perineural invasion, thus progression-free and disease-free survival were shorter in patients with perineural invasion.

We found no prognostic effect of K-RAS status, but our sample size was too small to show the difference. N-RAS and BRAF mutations were also available in a limited number of patients. This is one of the study limitations. We should state that in our country RAS mutation test was not available in all hospitals and, for many years, patients with stage IV tumors had computed tomography with anti-vascular endothelial growth factor receptor antibodies at first line regardless of their tumor side. Nowadays, it is available and we start therapies with anti-epidermal growth factor receptor antibodies in patients with left-sided tumors and RAS wild type tumors. On the other hand, we still perform computed tomography with anti-vascular endothelial growth factor receptor to right-sided tumors regardless of their RAS status at first line. So all patients had computed tomography with anti-visual evoked flow response at first line regardless of their tumor side and RAS status. This might be a factor by which we found no statistically significant difference of survival due to tumor sidedness.

CONCLUSION

We have shown that perineural invasion, stage and grade were prognostic indicators for colorectal cancer patients. However, gender, age, RAS status and tumor side had no effect on survival in Turkish population. Larger and prospective studies are needed.

AUTHORS' CONTRIBUTIONS

IB: Conceptualization, Writing – Original Draft, Writing – Review & Editing. **HP:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **RUG:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **YB:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **AK:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **HB:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **SS:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **AC:** Conceptualization, Writing – Original Draft, Writing – Review & Editing.

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Dengzhan shengmai capsule combined with donepezil hydrochloride in the treatment of Alzheimer's disease: preliminary findings, randomized and controlled clinical trial

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SUMMARY

OBJECTIVE: To observe the effects of Dengzhan Shengmai capsule combined with donepezil hydrochloride on cognitive function, daily living ability, and safety in patients with Alzheimer's disease.

METHODS: A total of 294 patients with Alzheimer's disease were randomly divided into a treatment group and a control group, 147 cases each group. The control group was given oral donepezil hydrochloride 5 mg once a day, and the treatment group was given oral Dengzhan Shengmai capsule 0.36 g three times a day, based on the control group.

RESULTS: At 3 and 6 months of treatment, the ADAS-cog score of the treatment group was 48.69±6.23 and 44.24±5.53; for the control group, 45.48±5.94 and 41.57±5.10. The difference between the two groups is statistically significant ($p<0.05$). At 3 and 6 months of treatment, the NO level in the treatment group was (46.28±6.68) umol/l, (43.55±7.92) umol/l, and the control group was (42.95±7.92) umol/l, (38.89±5.93) umol/l. The differences between both groups were statistically significant ($p<0.05$). At 3 and 6 months of treatment, ET levels in the treatment group were (156.08±17.39) ng/l, (144.91±17.60) ng/l, and the control group was (150.48±22.94) ng/l, (135.04±10.08) ng/l. Correlation analysis showed that ADAS-cog score was negatively correlated with NO and ET ($p<0.001$).

CONCLUSIONS: Dengzhan Shengmai capsule combined with donepezil hydrochloride can improve cognitive function and the living capacity of patients with Alzheimer's disease, reduce the production of neurotoxic substances NO and ET, and provide higher safety.

KEYWORDS: Alzheimer's disease. Donepezil. Flavonoids. Medicine, traditional Chinese.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative condition of the central nervous system (CNS) characterized by progressive cognitive and behavioral impairment in older adults and presenile[1]. In China, the incidence of AD in people over 65 years of age is 3–7%, resulting in a total of 6–8 million AD patients in China[2]. Recently, studies have shown that oxidative stress, endothelial cell damage, and mitochondrial dysfunction are involved in the pathogenesis of AD. Donepezil hydrochloride is currently the only drug supported by clinical evidence for being effective in the treatment of AD. Dengzhan Shengmai Capsule is a traditional Chinese medicine that contains flavonoids, which regulate vascular endothelial cells and protect mitochondria. Recently, the efficacy of Dengzhan Shengmai Capsule combined with donepezil hydrochloride in the treatment of AD has been confirmed in our department.

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METHODS

Clinical information

A total of 294 patients with AD who were treated in the neurology clinics of our hospital or as inpatients, from July 2015 to November 2017, were selected for the study. Patients were randomly assigned to either the treatment or control groups. The treatment group consisted of 147 cases, of which 26 were male and 23 were female. Patients were aged 67–84, with an average age of 70.93±5.47 years. The control group consisted of 25 males and 24 females, aged between 66–81, with an average age of 70.48±6.56 years.

The inclusion criteria for the study were patients who meet the diagnostic criteria for AD[3]; patients >60 years of age; patients who could complete the study; and patients with brain CT or MRI who did not show signs of cerebral hemorrhage, cerebral infarction, and intracranial lesions. The exclusion criteria for the study were patients with other types of dementia, such as vascular dementia; patients with advanced Parkinson's disease; patients with intracranial infections, schizophrenia, or depression; patients with severe heart, liver, lung, and kidney dysfunction; patients previously treated for dementia; and patients allergic to Dengzhan Shengmai Capsule or donepezil hydrochloride.

The study was approved by the hospital ethics committee and all subjects were recruited under written informed consent. The Ethical Approval Number is 2012-10-003. No significant difference in the general patient characteristics was observed between both groups (p>0.05), as summarized in Table 1.

Table 1. Comparison of clinical data in two groups.

Item	Treatment (n=147)	Control (n=147)
Age (year)	69.12±5.28	69.31±5.41*
Sex (M/F)	75/72	74/73*
HR (Time/min)	78.52±15.18	71.48±12.30*
Weight (kg)	64.28±6.78	65.40±7.88*
BMI (kg/m^2)	22.40±1.92	22.89±2.06*
SBP (mmHg)	120.71±14.99	119.14±14.71*
DBP (mmHg)	74.42±10.51	73.57±12.25*
Education (year)	7.14±2.38	7.10±2.28*
Combined disease (n%)		
Hyperlipidemia	23(15.64)	25 (17.00)*
Hypertension	17(11.56)	16 (9.41)*
Diabetes	10(6.80)	11 (7.48)*

HR: heart rate; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. Compared to the treatment group, *p>0.05.

Treatment method

Patients in the control group received 5 mg of donepezil hydrochloride orally once a day (specification 5 mg/piece, Eisai (China) Pharmaceutical Co., Ltd., approval number: Sinopharm standard H20050978). The treatment group was based on the control group, who received 0.36 g Dengzhan Shengmai Capsule three times a day (specification: 0.18 g/capsule, Yunnan Biological Valley Dengzhanhua Pharmaceutical Co., Ltd., approval number: Sinopharm standard word H20054470). Patients in both groups were treated over six months.

Observation indicators

Cognitive function was measured with cognitive function scoring. A daily living capacity score and the adverse drug reactions of the two groups of patients before treatment and at three and six months of treatment were recorded. Cognitive function scores were assessed using the ADAS-cog scale, including structure, language, immediate recall and recognition of words, orientation, and the use of ideas. The total possible score was 70 points, in which a higher score indicated worse cognitive function. The daily living capacity score was assessed using the AD Collaborative Research Daily Ability Scale (ADCS-ADL) that consists of 19 ADCS-ADL scales with a total score of 54 points. A higher score indicated a better daily living capacity.

Fasting peripheral venous blood was collected in the early morning before treatment, three months and six months after treatment; the serum levels of nitric oxide and endothelin were measured using enzyme-linked immunosorbent assay after serum isolation. Nitric oxide (NO) detection kit was provided by the American Adlitteram Diagnostic Laboratorues Company (batch number: X01872); endothelin (ET) detection kit was provided by Shanghai Hengyuan Biotechnology Company (batch number: 20150219).

Statistical analysis

Statistical analysis was performed using SPSS17.0 software. Measurement data were expressed as mean ± standard deviation (x±s), comparison between groups was tested by t test; count data were compared by percentage (%), and comparison between groups was tested by chi-square (χ^2) test. p<0.05 indicates a statistically significant difference.

RESULTS

Clinical data

All participants from the treatment and control groups completed the study and were included in the analysis. The demographic characteristics, comorbid diseases, and laboratory tests

are shown in Table 1. All variables were not significantly different between the two groups. (p>0.05). There was no significant difference in ADAS-cog, ADCS-ADL scores between the two groups of patients before treatment (p>0.05). The scores of ADAS-cog and ADCS-ADL in the treatment group at three months and six months after treatment were significantly different from those in the control group (p<0.05) (Table 2). There was no significant difference in the levels of NO and ET between the two groups of patients before treatment (p>0.05). The differences between the treatment group and the control group at three and six months after treatment were statistically significant (p<0.05) (Table 3). The Spearman correlation method was used to analyze the correlation between ADAS-cog scores, and NO and ET levels in the treatment group. The results showed that ADAS-cog scores were significantly negatively correlated with NO and ET levels (r=-0.887, -0.608, p<0.05). During the six months of treatment, adverse drug reactions in the treatment group were as follows: three cases

Table 2. Comparison of Alzheimer's disease assessment scale-cognitive scores and Alzheimer's disease cooperative study – activities of daily living between the two groups (*x*±s).

Item	Treatment (n=147)			Control (n=147)		
	Before treatment	3 months	6 months	Before treatment	3 months	6 months
ADAS-cog	51.17±8.67	45.48±5.94	41.57±5.10	52.04±9.16*	48.69±6.23#	44.24±5.53#
ADCS-ADL	39.02±5.28	43.91±4.25	46.57±3.86	38.89±5.13*	40.77±4.44#	42.46±5.18#

ADAS-cog: Alzheimer's disease assessment scale-cognitive; ADCS-ADL: Alzheimer's disease cooperative study – activities of daily living. Compared to the treatment group, *p>0.05, #p<0.05

Table 3. Comparison of no and et levels between two groups(*x*±s).

Item	Treatment (n=147)			Control (n=147)		
	Before treatment	3 months	6 months	Before treatment	3 months	6 months
NO (umol/l)	49.25±10.97	42.95±7.92	38.89±5.93	49.17±9.18*	46.28±6.68#	43.55±7.92#
ET (ng/l)	158.59±24.13	150.48±22.94	135.04±10.08	159.95±20.16*	156.08±17.39#	144.91±17.60#

Compared to the control group, *p>0.05, #p<0.05.

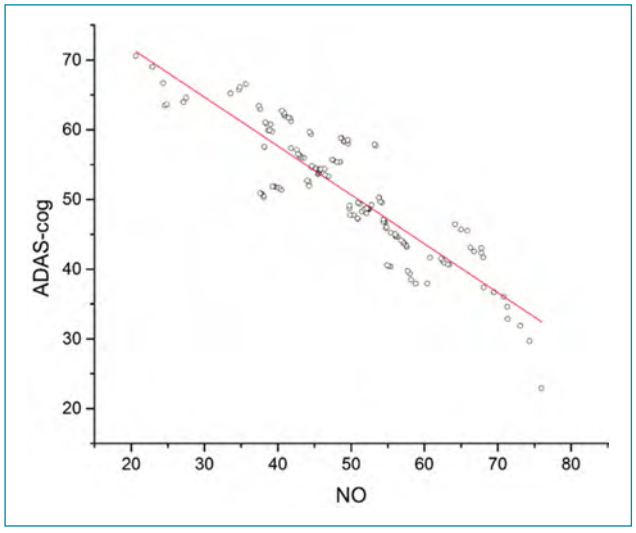

Figure 1. Correlation analysis between Alzheimer's disease assessment scale-cognitive with Nitric oxide.

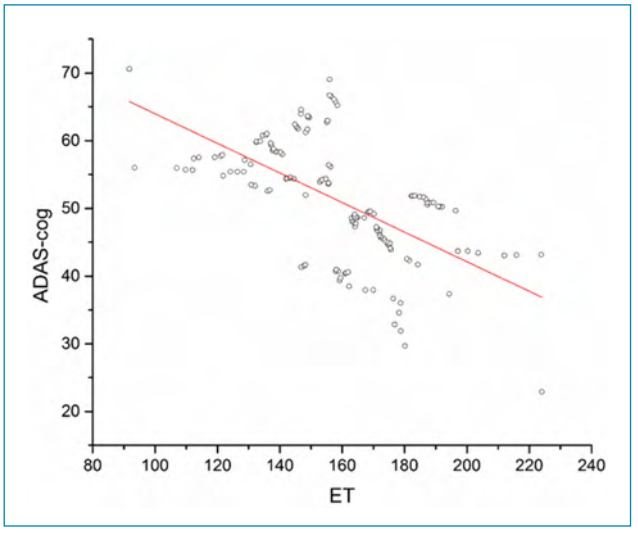

Figure 2. Correlation analysis between Alzheimer's disease assessment scale-cognitive with Endothelin.

of nausea, two cases of insomnia, one case of headache, two cases of dizziness, one case of diarrhea, one case of rash, and dynamic monitoring of liver and kidney function, and electrocardiogram during the whole course showed no significant dynamic changes. Adverse drug reactions in the control group were as follows: two cases of nausea, three cases of insomnia, two cases of rash, one case of fatigue, and dynamic monitoring of liver and kidney function, and electrocardiogram during the whole course showed no significant dynamic changes. The incidence of adverse reactions in the treatment group and the control group was 6.81 and 5.44%, and the difference was not statistically significant ($p>0.05$).

DISCUSSION

AD is one of the most common chronic diseases in older people and is a major global public health concern. Since ancient times, traditional Chinese medicine has provided a detailed description of the disease. "Qianjin Yaofang" refers to the disease as a person with more than 50 years of age and dysfunction. "Laziness" and "Medical Forest Corrects Errors" describes the fact that "in children with no memory, the brain is not full, and in those with advanced memory, the brain gradually declines".

Traditional Chinese medicine believes that the pathogenesis of AD involves "the lack of marrow sea and the use of a magic machine". Its pathological properties have been described as "the time when the essence is true, the essence is yin essence, qi and blood deficiency, and the essence is Qi, fire, Sputum and stasis are blocked in the brain". Therefore, "empty marrow sea, liver and kidney deficiency, heart and liver fire, qi stagnation and blood stasis, phlegm and turbidity" are the root causes of this disease[4].

For the treatment of AD, the principles of traditional Chinese medicine are Kaiyuzhutan, Huoxuetongqiao, Pingganxiuehuo treatment of its symptoms, tonicity, righteousness, and filling the brain. At present, the treatment of AD uses drugs. Donepezil hydrochloride in Western medicine can reversibly inhibit the hydrolysis of acetylcholine by acetylcholinesterase and can be used in the treatment of mild, moderate, and severe AD[5,6]. However, Western medicine often has more toxic side-effects compared to traditional Chinese medicine.

Dengzhan Shengmai Capsule is a traditional Chinese medicine preparation developed from several precious medicinal materials, such as Dengzhan Asarum, Ginseng, Schisandra, and Ophiopogon. The main component of the drug is Erigeron breviscapus, which is a flavonoid compound extracted from Erigeron breviscapus[7]. Schisandrol contained in Schisandra chinensis has been shown to have a protective effect on synapses and mitochondria. Also, basic research has shown that Schisandrolone can significantly improve the learning ability of AD model rats[8].

In this study, through the results of a randomized controlled clinical trial, we found that donepezil hydrochloride combined with the Dengzhan Shengmai Capsule significantly improved ADAS-cog, ADCS-ADL scores in patients with AD compared to donepezil hydrochloride alone.

There are currently many hypotheses concerning the pathogenesis of AD in Western medicine. The β-amyloid waterfall hypothesis suggests that imbalance in the production and clearance of Aβ is the initial event leading to neuronal degeneration and dementia[9]. The tau protein theory believes that over-phosphorylation of the tau protein affects the stability of neuron skeleton tubulin leading to the formation of neuron fiber tangles that disrupt the normal functions of neurons and synapses[10]. These two theories have been confirmed based on the histopathological manifestations of AD, which include neuritic and neuro-inflammatory plaques formed by beta-amyloid deposition, and neurofibrillary tangles formed by hyperphosphorylated tau protein.

Recently, oxidative stress has been implicated in the onset of AD[11]. Oxidative stress refers to the imbalance between the production and removal of oxygen free radicals in the body, resulting in the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Nitric oxide (NO) is one of the common species in the RNS family. Studies have shown that NO can regulate the synaptic plasticity of neurons and that excessive increases in NO can directly damage neuronal cells[12]. NO can also activate multiple intracellular signals to enhance the activity of P53, resulting in increased expression of pro-apoptotic caspases to promote neuronal cell apoptosis[13].

In this study, we showed that the expression of NO in the peripheral serum of patients with AD significantly increased. Correlation analysis showed that NO was negatively correlated with the ADS-cog score, which confirmed that NO is involved in the pathogenesis of AD[10,14,15]. Brain tissue hypoperfusion and microvascular endothelial changes have also been linked to the pathogenesis of AD[16,17], Endothelin-1 (ET-1) is currently the strongest known vasoconstrictor. A large amount of ET-1 production can cause intracranial vasoconstriction, resulting in chronic cerebral blood flow insufficiency, thus causing vascular and mitochondrial dysfunction, and increased production of ROS, leading to AD[17,18].

We showed that the level of ET-1 in AD patients was significantly higher than that in the normal population. Correlation analysis showed that ET-1 was negatively correlated with the ADAS-cog score, suggesting that ET-1 is involved in the pathogenesis of AD. Moreover, the levels of NO and ET-1 in the

experimental group were significantly lower than in the control group, in March and June, after treatment. These data suggest that Dengzhan Shengmai Capsules can reduce ET-1 by relaxing intracranial blood vessels in AD patients. The release of ET-1 can also protect mitochondria by reducing the production of the oxidative stress product NO.

CONCLUSIONS

Dengzhan Shengmai Capsule combined with donepezil hydrochloride can be used to effectively treat AD by significantly improving the ADAS-cog scores of AD patients. Reduced levels of neurotoxic substances (NO and ET) were also observed; patients showed an improved quality of life when compared to AD patients without adverse drug reactions.

AUTHORS' CONTRIBUTIONS

PH: Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **XH:** Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **MX:** Formal Analysis, Writing – Original Draft, Writing – Review & Editing.

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ORIGINAL ARTICLE
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Abdominal ultrasound augments the medical students' ability to identify free intraabdominal fluid

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SUMMARY

OBJECTIVE: Free intra-abdominal fluid describes an accumulation of free fluid in the peritoneal cavity. It has different etiologies, but it frequently constitutes a meaningful clinical sign. In this study, the authors interrogate whether abdominal ultrasound augments the medical students' ability to identify free intra-abdominal fluid.

METHODS: Thirty-one medical students without any previous formal ultrasound training were subjected to cognitive assessment before and after four and a half-hour of theoretical lecture and hands-on course about the diagnosis of free intra-abdominal fluid by physical examination and abdominal ultrasound. The hands-on sessions were done in healthy volunteers with a simulated peritoneal catheter and in patients treated with peritoneal dialysis with different amounts of dialysate in their cavity.

RESULTS: The cognitive assessment before and after the course increased from 6.7±2.3 to 11.6±1.1 points ($p<0.0001$). The sensitivity, specificity, and accuracy in the diagnosis of free intra-abdominal fluid were higher when students used abdominal ultrasound. The students agree with the inclusion of abdominal ultrasound in the diagnose of free intra-abdominal fluid in the undergraduate curriculum.

CONCLUSIONS: This study demonstrates that incorporating abdominal ultrasound is feasible and improves medical students' short-time competency in performing and interpreting the findings diagnostic of free intra-abdominal fluid.

KEYWORDS: Education, medical. Students, medical. Molecular Docking Simulation. Ultrasonography. Focused assessment with sonography for trauma.

INTRODUCTION

Thoracoabdominal trauma is the leading cause of death among young individuals[1]. The majority of traumatic injuries are blunt, followed by intra-peritoneal bleeds[2], and responsible for deaths secondary to hypovolemic shock[3]. Therefore, the quick diagnosis of free intra-abdominal fluid (FIAF) following thoracoabdominal trauma is paramount. Ideally, an ideal FIAF assessment should be rapid, accurate, and non-invasive[3].

Point-of-care ultrasonography (POCUS) is a safe and rapidly evolving diagnostic modality that has significantly impacted patients' evaluation and treatment in various conditions[4,5]. It has various advantages, including its bedside assessment, ease of

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use, non-invasiveness, no use of radiation or contrast agents, and is inexpensive[4,6].

Since the middle of the last decade, many medical schools in North America have been implementing POCUS in their undergraduate curricula to improve physical examination (PE) teaching[7-11]. Interestingly, FIAF was listed as one of the ninety core clinical milestones that all graduating medical students should obtain before graduation[12].

POCUS in the identification of FIAF is well-described among physicians, but so far, it is still little taught to medical students[6,13,14]. We hypothesized that short-term abdominal ultrasound (AUS) training would improve students' skills to identify FIAF. The objective of this study was to determine whether AUS augments medical students' ability to identify FIAF.

METHODS

Patients who expressed willingness to participate in the study were recruited and treated with automated peritoneal dialysis (APD) in the dialysis unit of the University Hospital of *Universidade Federal de Juiz de Fora*. Two APD male patients had been on treatment for less than 12 months, and one female patient had been under treatment for 20 years. Two healthy volunteers were included to demonstrate the normal ultrasonography anatomy of the abdomen and pelvic cavity. The exclusion criteria were: 1. Patients suspected with peritonitis; 2. Patients who were unable to assume the supine position.

Medical students of the 5th period of the medical course from the School of Medicine of *Universidade Federal de Juiz de Fora* and Faculty of Medical and Health Sciences of Juiz de Fora (SUPREMA), who had already attended the disciplines of anatomy and semiology and without prior hands-on experience with ultrasound were invited in their classrooms to participate. They were clarified about the study, and consent was implied if the participants, students, and volunteers, showed up on the day of the course and signed the written informed consent. The Ethics Committee approved the study (CAAE N°. 91487618.3.0000.5147).

Study Protocol

A questionnaire with 15 multiple-choice questions, each one with four options, was applied on day one. The test aimed to evaluate previous knowledge regarding the physical principles of ultrasound, ultrasound artifacts, types of transducers, image generation, and interpretation in the normal abdomen, presence of FIAF, and PE findings in healthy condition and ascites. Then, a four and a half hour course, including didactic lecture (one hour) and practical demonstration (three and a half hours), was held. The hands-on session was done on one health volunteer and an APD patient with two liters of peritoneal solution. Traditional bedside physical signs were reviewed. In the AUS training, the convex probe connected to an ultrasound equipment (Terason uSmart 3200T system, Burlington, MA) was used, initially with the probe placed in the right upper quadrant (RUQ) at the mid-axillary line using the coronal plane. The students were taught to identify the diaphragm, liver tip, hepatorenal interface, and the presence of FIAF, which appears as an anechoic stripe. In the left upper quadrant (LUQ), the students were trained to place the probe at the posterior-axillary line using coronal plane to identify the spleen, the diaphragm, and the left kidney. FIAF was captured beneath the diaphragm, spleen tip, splenorenal recess, and the inferior left kidney. Finally, in the pelvic cavity (PC), the medical students were trained to identify free fluid posterior/inferior or lateral to the bladder using sagittal and transverse planes. Two instructors (GC and MGB) supervised students individually (Figure 1).

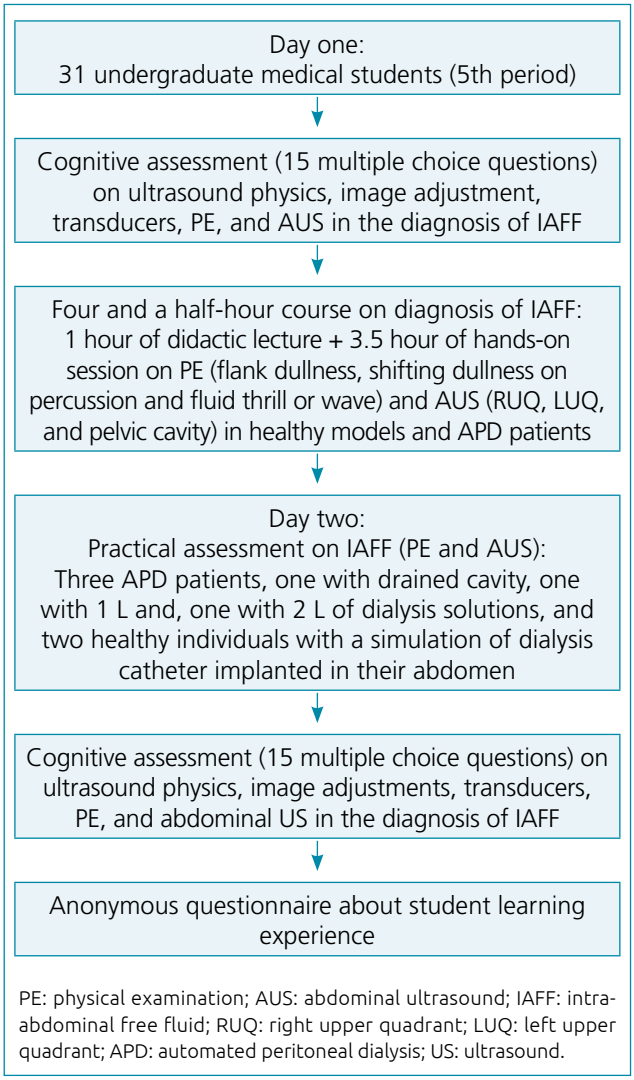


Figure 1. Work-flow chart

On day two, students were subjected to practical assessment of FIAF in two healthy volunteers with peritoneal catheter simulating insertion in the abdominal cavity, and in three APD patients, one with drained abdominal cavity, another with one liter of peritoneal solution, and a third patient with two liters of dialysate. Initially, one student at a time was instructed to use PE to answer yes or no to the question: "Is there FIAF"? After that, the same question was repeated with the student using AUS. Two researchers documented the students' responses. After the practical assessment, a second questionnaire with 15 multiple-choice questions, each with four options, and covering the same topics as those of the pre-course was applied. Finally, using a five-point Likert scale, the students completed an anonymous questionnaire about their learning experience with the course.

Statistics

A descriptive analysis was performed using means and percentages according to the characteristic of the variable. The cognitive assessment was compared before and after the course, using the Student's *t*-test for paired samples. FIAF by AUS was considered present if identified in one or more abdominal windows (RUQ and/or LUQ and/or pelvic cavity). Kappa statistics were used to assess the concordance between PE and AUS performed by the students. Additionally, compared to the radiologist's assessment, the sensibility and specificity of the PE and AUS were assessed using classic diagnostic test properties concepts. A 95% confidence interval was considered, using the software SPSS 17.0, Chicago, Illinois.

RESULTS

The study included 31 medical students (mean age 21.54±1.38 years; females 64.5%) and five human models. Cognitive assessment scores improved from before the course (6.7±2.3) to after its completion (11.6±1.1) p<0.0001.

In total, 372 abdomens were examined, 52 in healthy volunteers with a simulated peritoneal catheter, and 320 in APD patients. The abdominal cavity was examined with the PE 93 times and with the AUS 279 times. The study showed the diagnosis of FIAF by medical students using PE and AUS distributed in the absence (simulated peritoneal catheter) and presence (including drained abdominal cavity, abdomen with one litter, and two litter of dialysate) of FIAF. The agreement (*k*-value) between students was 0.88 for PE and 0.95 for AUS.

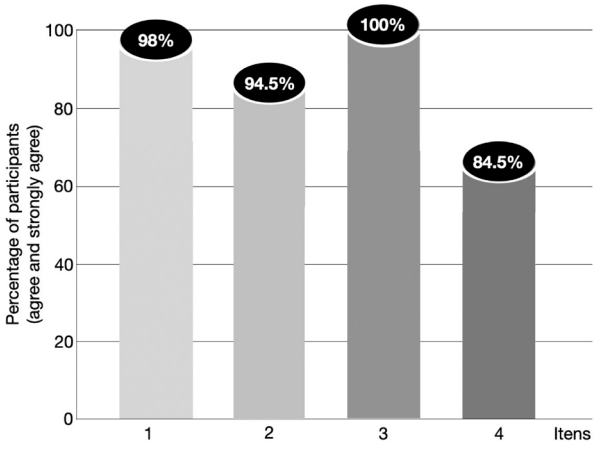
Compared to PE, AUS increased sensitivity (66.25 to 90.0%), specificity (7.69 to 82.05%), and accuracy (58.06 to 88.88%) for the diagnosis of FIAF.

Figure 2 shows students' anonymous responses about their opinion regarding the course. In general, the students approved the course.

DISCUSSION

Almost 15 years after the inclusion of ultrasound teaching to undergraduate students in North America, the vast majority of medical schools in Brasil has not yet integrated ultrasound in undergraduate medical education. Our study shows that students' ability to identify FIAF is much higher using AUS compared to PE of the abdomen.

Previous studies have shown that residents and faculty in radiology and emergency medicine can accurately diagnose FIAF using ultrasound[7,15-19]. Although FIAF assessment has been included as part of the final Core Medical Student Clinical Ultrasound Milestones that all graduating medical students should obtain before graduation, this training is not yet definitively proven feasible among undergraduate medical students[12]. Measurable benefits of teaching ultrasound at the undergraduate level include comparing cognitive improvement between pre- and post-training and psychomotor skill enhancement[16,20]. Our study suggests that a short training significantly improved the students' scores of the cognitive assessment, a success rate similarly described in other studies[21,22].



Items: 1. The hands-on session with abdominal ultrasound allows identify free intra-abdominal fluid; 2. The course about the ultrasound identification of free intra-abdominal fluid meets the pre-course expectation; 3. Abdominal ultrasound for the diagnosis of free intra-abdominal fluid should be a part of the undergraduate medical curriculum; and 4. Student's ability to diagnose free intra-abdominal fluid by abdominal ultrasound augmented after the course.

Figure 2. Anonymous questionnaire about student's experience with the course

AUS in the diagnosis of FIAF requires visual and psychomotor coordination. It is a skill that can be learned in a modest amount of ultrasound training. To train and assess the medical students' ability to identify FIAF correctly, we took advantage of a kind of treatment patients with end-stage renal failure are subjected to, the APD. As none of the three APD patients presented an empty abdominal cavity, a finding that the students were unaware of, two healthy volunteers with the simulated peritoneal catheter were included, thus, guaranteeing the absence of FIAF. As it was observed, our students' ability to exclude or confirm FIAF with AUS was superior compared to PE in sensitivity (66.25 to 90.0%), specificity (7.69 to 82.05%), and accuracy (58.06 to 88.88%). A comparison of these results with others is difficult since similar studies are scarce. One study noted that first-year medical students randomized to assess ascites with PE and AUS having a radiologist as a reference showed sensitivity, specificity, and accuracy similar to ours, but differed in the fact that there was no statistical difference between the groups[23].

In the APD patients, identification of FIAF by our students was more frequently diagnosed in Morrison's pouch (97.5%) when compared to the LUQ (93.7%) and pelvic cavity (78.75%). In supine patients, free fluid first collects in the most dependent portion of the abdominal cavity, the RUQ[22]. However, FIAF was mistakenly diagnosed in the RUQ (46%) in the two healthy volunteers with no free fluid in their abdominal cavities. One possible explanation for this percentage of incorrect diagnosis is that one volunteer had been fasting for almost 12 hours before the exam and presented a full gall bladder, which led students to have it mistaken by FIAF.

A common argument against implementing POCUS training at medical schools is the perceived significant time requirements for teaching it[24]. However, as previously shown in other publications, our study shows that ultrasound training consumes little time to be effectively taught[21,22]. Medical students able to perform POCUS efficiently at the end of their course have a higher chance of practicing and implementing it in the residency and later in their practice[24,25].

Some limitations are recognized in our study. First, the number of students included was small. Second, only short-term knowledge was assessed. Last, even though the volunteers used had free fluid in their cavity to simulate distinct conditions of FIAF, the study condition was quite favorable, different from the stressful clinical environment of the abdominal trauma, which can negatively affect the students' performance.

CONCLUSION

Results suggest that medical students' short-term training with AUS is an available, fast, and reliable test of high sensitivity, specificity, and accuracy in identifying FIAF. AUS is a diagnostic tool, and students enjoyed its learning and, therefore, should be an indispensable part of the undergraduate medical curriculum.

AUTHORS' CONTRIBUTION

GCT: Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing. **GBC:** Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing. **MGB:** Conceptualization, Methodology, Writing – Original Draft, Writing – Review & Editing. **FABC:** Data Curation, Methodology, Writing – Original Draft, Writing – Review & Editing. **NMSF:** Data Curation, Methodology, Writing – Original Draft, Writing – Review & Editing. **AS:** Data Curation, Methodology, Writing – Original Draft, Writing – Review & Editing.

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Comparison of cardiovascular risk calculators in patients with diabetes

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SUMMARY

OBJECTIVE: Cardiovascular risk stratification is an important clinical practice to estimate the severity of cardiovascular disease in patients with type 2 diabetes. This study aimed to compare the stratification of global cardiovascular risk with the specific risk stratification for patients with type 2 diabetes, seen at specialized outpatient clinics, and to evaluate possible differences in diagnoses and treatments.

METHODS: A total of 122 patients with type 2 diabetes treated at two specialized outpatient clinics, from 2017 to 2019, were studied. The cardiovascular risk stratification calculators, global risk score, Cardiovascular Risk Stratification Calculator, and *United Kingdom Prospective Diabetes Study-Risk Engine*, were used to calculate the risk of death from cardiovascular disease. The agreement between these calculators was analyzed using the kappa index. The indications for the use of statins and acetylsalicylic acid for the group studied were evaluated according to the Brazilian Diabetes Society Guideline.

RESULTS: There was a low degree of agreement among the three risk calculators. The global risk score calculator showed insignificant agreement with the Cardiovascular Risk Stratification Calculator (kappa=0.0816; p=0.0671). There was no agreement between the global risk score calculator and *United Kingdom Prospective Diabetes Study-Risk Engine* (kappa=-0.099), or between the Cardiovascular Risk Stratification Calculator and *United Kingdom Prospective Diabetes Study-Risk Engine* (kappa=-0.0095).

CONCLUSION: The substantial disagreements among the cardiovascular risk calculators may lead to different diagnoses and may consequently influence therapeutic strategies. The findings herein highlight the need for specific validated cardiovascular risk calculators for patients with DM2 that can reliably estimate risk in these individuals.

KEYWORDS: Diabetes mellitus. Cardiovascular diseases. Cardiovascular risk.

INTRODUCTION

Cardiovascular risk stratification is an important clinical practice to determine the severity of cardiovascular disease, especially in asymptomatic patients who are more susceptible to clinical complications, such as acute coronary syndromes, strokes, transient ischemic attacks, and peripheral arterial disease[1]. To help health professionals analyze their patients' risks quickly and easily to propose therapeutic measures, several cardiovascular risk calculators have been developed, all based on different risk factors. Some of these tools are used for the

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general population, whereas others are used for specific populations, such as those with diabetes mellitus.

Type 2 diabetes mellitus (DM2) is one of the main risk factors for cardiovascular diseases[2], increasing cardiovascular morbidity and mortality 2–4-fold in relation to individuals without DM2[3]. The diagnosis of DM2 was considered equivalent to a high cardiovascular risk[4] and, therefore, cardiovascular risk stratification guidelines and instruments traditionally used in clinical practice[5] automatically consider patients with DM2 to be at high cardiovascular risk. Nevertheless, not all patients with DM2 have the same degree of cardiovascular risk[6].

Stratification of cardiovascular risk appropriate to patients with diabetes can improve the accuracy of prediction of subclinical cardiovascular disease, silent ischemia, and future cardiovascular events. It can also prevent unnecessary use of aggressive treatment in low-risk patients that might otherwise increase the risk of adverse events and high treatment costs. For these reasons, cardiovascular prevention strategies must be individualized according to cardiovascular risk, whereas intensified treatment must be reserved for individuals at higher risk[7].

This article aims to compare global cardiovascular risk stratification with the specific cardiovascular risk stratification for patients with DM2, who attended two specialized outpatient clinics, and to evaluate possible differences in diagnoses and treatments. It also allows for the comparison of national calculators – global risk score (GRS) and cardiovascular risk stratification calculator (ER Calculator) – with the United Kingdom Prospective Diabetes Study–Risk Engine (UKPDS-RE) calculator and for an evaluation of the effectiveness of these calculators.

METHODS

Study design and population

This is a cross-sectional study derived from a cohort entitled HealthRise Vitória da Conquista, an intervention project designed to improve the control of diabetes and hypertension in primary care and specialized outpatient clinics in Vitória da Conquista City, Bahia State. The study included all patients with DM2 at two specialized outpatient clinics, referred by the city's primary health care facilities, from 2017 to 2019. Patients who had cardiovascular disease at the beginning of the study (high cardiovascular risk) were excluded.

Data collection

Patients examined between July 2018 and July 2019 were considered. The electronic medical records of patients examined at medical specialty clinics were reviewed. Patient data (variables of interest) selected from the electronic forms were recorded in an online questionnaire specially designed for this study.

Measures and definitions

The following variables were analyzed: a) Demographic: sex, age, marital status, religion, skin color, education, profession, and current economic situation; b) Anthropometric: weight, height, body mass index (BMI), waist circumference, hip circumference, and waist/hip ratio (WHR); c) Clinical: systolic blood pressure (SBP), diastolic blood pressure (DBP), ankle-brachial index (ABI), classic symptoms of diabetes (polyuria/urinary incontinence, urgency, polydipsia, polyphagia, and weight loss); d) Clinical features (hypoglycemia, ketoacidosis, hyperglycemia, and infection), time of diagnosis of DM and types of treatment, initial clinical presentation (diabetic ketoacidosis, hyperosmolar hyperglycemic state, asymptomatic laboratory findings); and e) Personal details and complications (retinopathy, nephropathy, peripheral neuropathy, autonomic neuropathy, infarction, stroke, carotid disease, diabetic foot, limb amputation, angina, atrial fibrillation, and metabolic syndrome), medications, and life habits (smoking).

Stratified cardiovascular risk

Age was stratified into four categories, and the following variables were dichotomized: sex, alcohol consumption, diagnosis, presence of treated systemic arterial hypertension (SAH), classic symptoms of DM, clinical complications, initial clinical presentation, comorbidities, use of acetyl salicylic acid and statins, clinical complications, presence of personal and family comorbidities, and family history of premature coronary artery disease, as defined by the Brazilian Diabetes Society[7].

Regarding smoking habits, patients were categorized as current smoking (defined when the last episode occurred less than a year before the moment of stratification), non-smoker, and former smoker[7].

The following continuous variables were studied: time of diagnosis of DM and SAH, body mass index (BMI), WHR, SBP, DBP, fasting glucose levels, postprandial glucose levels, HbA1c level, creatinine clearance, total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), and triglycerides. The stratified cardiovascular risk from the calculators was classified as low, intermediate, high, based on the risk score of each calculator used.

Cardiovascular risk scoring models

Risk stratification calculators were used to estimate the risk of death from coronary heart disease, non-fatal infarction, angina, fatal or non-fatal ischemic or hemorrhagic stroke, transient

ischemic attack, intermittent claudication, and heart failure over 10 years.

To calculate the cardiovascular risk (CVR) of patients with DM2, risk calculators were selected that derive from the Framingham models[1] and that use traditional risk factors: the GRS of the Brazilian Society of Cardiology (SBC)[8] and the UKPDS Risk Engine 2.0[9], specific for patients with DM. We also used the CVR stratification calculator (ER Calculator) prepared by the Department of Atherosclerosis of the Brazilian Society of Cardiology, which is based on the Update of the Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis 2017[10]. This score also uses non-traditional risk factors (RF).

Indications for the use of statins and acetylsalicylic acid (ASA) for the group studied were also evaluated from the CVR stratifications, according to the Brazilian Diabetes Society (*Sociedade Brasileira de Diabetes* – SBD) Directive[7]. Statin use and dose was based on LDL and n-HDL targets for each risk group. ASA use is indicated for DM patients considered high risk, without atherosclerotic disease, when they are older than 65 and present a low risk of bleeding; in very high-risk patients with atherosclerotic disease, it is indicated for secondary prevention.

These scoring models were selected because they have already been used in cohort studies with robust samples[8,9,11-14], including patients with DM.

Data analysis

CVR percentages were calculated considering the age groups for each calculator to estimate the development of cardiovascular disease (CVD) over 10 years. Patients with missing data were excluded when calculating the risk for each model. STATA 15.0 software was used for statistical analysis. P-value <0.05 was accepted as significant. Descriptive statistics were used for frequencies, means, and standard deviations. Pearson's χ^2, Mann–Whitney U, and Fisher's exact tests were used to assess demographic characteristics, risk factors, and 10-year risks. Pearson's χ^2 analysis was used to determine the relation between 10-year risk and age groups. The χ^2 test was performed, and the linear association value was considered when the expected values less than five in a column were greater than 25%. The Kruskal–Wallis test was used to assess the relationship between CVD risk, alcohol use, and physical activity habits.

To assess the degree of agreement among the SBC global risk score calculators, the kappa agreement coefficient was calculated.

Ethical considerations

The requirement for consent was waived because of the retrospective nature of the study. The work was approved by a local research ethics committee.

RESULTS

During the study period, 1,276 patients were followed up. Of these, 122 were eligible, 64 (52.46%) of whom were women. The median age was 59 years old, and those over 60 years old comprised 48.36% of the population. Regarding skin color, 47 (38.52%) declared themselves as *pardo* and 16 (13.11%) self-identified as black. In general, the subjects had low levels of education, with 11 (9.02%) reporting being illiterate. Regarding the economic situation, 44 (36.07%) patients were salaried, 11 (9.02%) were unemployed, and 44 (36.07%) were retired or received some pension.

When analyzing risk factors for CVD, stratified by sex, we found that the average time since diagnosis of DM in the overall population was five years; however, 50.41% of the population had been diagnosed within five years. Overall, 96 (79%) were hypertensive, 53 (55.21%) of whom were women.

Regarding DM treatment, 100 (82.64%) patients used oral antihyperglycemic agents and only 23 (18.85%) used insulins. Lipid-lower drugs (58.20%) and antiplatelet drugs (38.52%) showed a high frequency of use with little difference between genders. Peripheral neuropathy was the most frequent complication of DM in the study group, with 34 (27.87%) affected patients (Table 1).

When assessing the 10-year risk grouping on the selected calculators, stratified by age, the global risk score categorized high risk CVR in 105 (86.07%) patients. Of these, 57 (54.29%) older than 60 years ($p<0.05$). There was a high proportion of CVR of 47.62% among men and 52.38% for women; however, there was no significant difference for this calculator ($p=0.430$).

The calculator of the Brazilian Diabetes Society classified 111 (90.98%) patients with high cardiovascular risk, 55 (49.55%) of whom were over 60 years old. When stratified by sex, a high CVR was also observed between groups, in 55 (52.38%) women and 50 (47.62%) men. Stratification for both sex and age did not show a significant difference for this calculator ($p=0.940$).

The UKPDS–RE, unlike the other calculators, classified 77 (63.11%) and 30 (24.59%) patients, as low and intermediate CVR, respectively ($p<0.001$). Of the low CVR group, 34 (44.16%) were younger adults (31–50 years old). The proportions between men and women classified by this calculator were similar (Table 2).

There was a low level of agreement between risk calculators using the kappa index. When comparing GRS calculators with the ER Calculator, there was insignificant agreement (kappa=0.0816; $p=0.067$). No agreement was observed between the GRS calculator and UKPDS-RE (kappa=-0.099), or between the ER Calculator and UKPDS-RE (kappa=-0.0095).

Table 1. Risk factors for cardiovascular diseases and metabolic control parameters in people with type II diabetes, Vitória da Conquista City, Bahia State, 2017–2019.

	Total (n=122)	Women (n=64)	Men (n=58)	p-value
	n	n (%)	n (%)	
DM duration				
<5 years	61 (50.41)	29 (47.54)	32 (52.46)	0.558*
5–10 years	16 (13.22)	9 (56.25)	7 (43.75)	
10–20 years	27 (22.31)	17 (62.96)	10 (37.04)	
>20 years	17 (14.05)	8 (47.06)	9 (52.94)	
Presence of SAH				
No	26 (21.31)	11 (42.31)	15 (57.69)	0.243*
Yes	96 (78.69)	53 (55.21)	43 (44.79)	
SAH duration				
<5 years	26 (24.76)	7 (26.92)	19 (73.08)	0.001*
5–10 years	18 (17.14)	11 (61.11)	7 (38.89)	
10–20 years	33 (31.43)	26 (78.79)	7 (21.21)	
>20 years	28 (26.67)	15 (15.7)	13 (12.3)	
DM treatment				
ADO (%)	100 (82.64)	54 (54.00)	46 (46.00)	0.353*
Insulin	23 (18.85)	13 (56.52)	10 (43.48)	0.665*
ADO + Insulin	15 (12.40)	9 (60.0)	6 (40.0)	0.511*
Non-diabetic drugs				
Antihypertensives	106 (86.89)	59 (55.66)	47 (44.34)	0.068*
Lipid-lowering drugs	71 (58.20)	40 (56.34)	31(43.66)	0.311*
Antiplatelet	47 (38.52)	32 (68.09)	15 (31.91)	0.006*
Anti-arrhythmic	3 (2.46)	2 (66.7)	1 (33.33)	1.00**
DM complications				
Retinopathy	34 (27.87)	17 (50)	17 (50)	0.735*
Nephropathy	12 (9.84)	4 (33.33)	8 (33.33)	0.162*
Peripheral neuropathy	25 (20.66)	14 (56)	11 (44)	0.658*
Autonomic neuropathy	4 (3.28)	2 (50)	2 (50)	1.00**
Diabetic foot	8 (6.56)	3 (37.5)	5 (62.5)	0.476*
Amputation of limbs	4 (3.28)	1 (25)	3 (75)	0.345*
Angina	10 (8.20)	8 (80)	2 (20)	0.099**
AF	2 (1.64)	0 (0)	2 (100)	0.224**
Smoking[a]				
Non-smoker	89 (72.95)	47 (52.81)	42 (47.19)	0.833*
Former smoker	16 (13.11)	9 (56.25)	7 (43.75)	
Smoker	16 (13.11)	7 (43.75)	9 (56.25)	
Alcohol use[a]				
No ethanol	86 (70.49)	52 (60.47)	34 (39.53)	0.003*
Alcoholic	24 (19.67)	5 (20.83)	19 (79.17)	
Former alcoholic	11 (9.02)	6 (54.55)	5 (45.45)	
Physical Activity Practice[a]				
No	89 (72.95)	48 (55.93)	41 (46.07)	0.493*
Yes	32 (26.23)	15 (46.88)	17 (53.12)	
BMI[a]				
Underweight	5 (4.55)	3 (60)	2 (40)	0.05**
Normal	22 (20)	5 (22.73)	17 (77.27)	
Overweight	31 (28.18)	14 (45.16)	17 (54.84)	
Obesity	52 (47.27)	34 (65.38)	18 (34.62)	
SBP (average)	140 (±21.19)	150 (±27.82)	140 (±26.2)	0.2015***
DBP (average)	86 (±14.13)	85 (±14.46)	87 (±13.83)	0.8810***
GJ (average)	121.5 (±69.51)	124.5 (±67.37)	116 (±72.35)	0.374***
HBA1C (average)	7.5 (±1.98)	7.55 (±1.84)	7.1 (±2.14)	0.3363***
CT (average)	187.02(±43.33)	183.5 (±44.46)	183.5 (±41.62)	0.402***
HDL (average)	43.5 (±11.09)	45 (±9.47)	39 (±12.02)	0.0009***
LDL1 (average)	102 (±37.93)	101.5 (±38.21)	102.5 (±37.87)	0.8395***
TG (mean)	154 (±186.37)	140 (±117.05)	171.5 (±118.10)	0.4527***

DM: diabetes mellitus; SAH: systemic arterial hypertension; ADO: oral antidiabetics; AF: atrial fibrillation; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; GJ: fasting blood glucose; HBA1C: glycated hemoglobin; CT: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides. *Chi-square test; **Fisher's exact test; ***Mann–Whitney; [a]Variable with loss.

A second analysis was performed based on a grouping of the ER Calculator (ER1). The high-risk and very high-risk categories were grouped into only high risk. In this manner, better agreement was obtained between GRS and ER1 (kappa=0.1545), although it was still statistically insignificant.

Regarding the indication for medication use in the studied population, a large difference was noticed in the indications for statins and ASA. Whereas the GRS calculator and the ER Calculator indicate the use of statins in 105 and 118 patients, respectively, the UKPDS-RE calculator indicates their use only in 15 patients. For ASA, the number of patients referred by the GRS and ER calculators would be more than four times higher than by the UKPDS-RE calculator (Table 3).

DISCUSSION

Low levels of agreement were observed among the CVR calculators selected in this study. This observation is consistent with results of studies that used other types of calculators[15-17].

The GRS, recommended by the SBC and derived from the Framingham Heart Study equations[18], estimates the risk of CVD in 10 years. Using this tool, patients are categorized as low risk (<5%), intermediate risk (men with calculated risk ≥5 and ≤ 20%, and women with calculated risk ≥5 and ≤10%) and high risk (risk calculated >20% for men and >10% for women over 10 years). Patients classified in the low risk category and who have a family history of premature cardiovascular disease are reclassified as intermediate risk[18].

The recommendation is that the GRS be used in the initial assessment of individuals who were not included in high-risk conditions[18]. However, the study population in this study is composed exclusively of patients with DM2, which leads most patients to be classified as high CVR. This can lead to more aggressive therapeutic approaches and, consequently, to polypharmacy prescription[5].

The UKPDS risk engine is a specific risk calculator for type 2 diabetes, based on data from 53,000 patients in the UK Prospective Diabetes Study[10]. This tool provides risk estimates and 95% confidence intervals for individuals with

Table 3. Indication for the use of statins and acetylsalicylic acid, based on cardiovascular risk by cardiovascular risk calculator: GRS, UKPDS-RE, and ER Calculator, Vitória da Conquista City, Bahia State, 2017–2019.

	No n (%)	Yes n (%)
Statin Use		
GRS	17 (13.94)	105 (86.06)
UKPDS-RE	107 (87.7)	15 (12.3)
ER Calculator	4 (3.28)	118 (96.72)
ASA Use		
GRS	78 (63.93)	44 (36.07)
UKPDS-RE	112 (91.80)	10 (8.20)
ER Calculator	76 (62.30)	46 (37.70)

GRS: global risk score; ER Calculator: cardiovascular risk stratification calculator; UKPDS–RE: United Kingdom Prospective Diabetes Study-Risk Engine; ASA: acetylsalicylic acid.

Table 2. Grouping of 10-year risks according to models stratified by sex, Vitória da Conquista City, Bahia State, 2017-2019.

	Total n (%)	Women n (%)	Men n (%)	p-value
GRS				
Low	5	4 (80)	1 (20)	0.430**
Intermediate	12	5 (41.67)	7 (58.33)	
High	105	55 (52.38)	50 (47.62)	
ER Calculator				
Low	1	1(100)	0	0.940**
Intermediate	3	2 (66.67)	1 (33.33)	
Very High	111	58 (52.25)	53 (47.75)	
Extremely High	7	3 (42.86)	4 (57.14)	
UKPDS – RE				
Low	77	41 (53.25)	36 (46.75)	0.234*
Intermediate	30	18 (60)	12 (40)	
High	15	5 (33.33)	10 (66.67)	

GRS: global risk score; ER Calculator: cardiovascular risk stratification calculator; UKPDS–RE: United Kingdom Prospective Diabetes Study-Risk Engine. *Chi-square test; **Fisher's exact test

type 2 DM who do not have heart disease. The CVR can be calculated for all patients with DM2, regardless of the time of diagnosis. It uses the following risk factors: age; sex; ethnicity; smoking; presence or absence of atrial fibrillation; and levels of HbA1c, SBP, cholesterol total, and HDL cholesterol. Among the percentages referring to total risk, scores <10% indicate low risk, 10–19% indicates medium risk, and ≥20% indicates high risk[10].

The agreement between the global risk score and the UKPDS–RE calculators in this study was very low. This can be explained by the fact that these calculators use different risk factors and generate divergent classifications for the same patients. The overestimation of CVD risk that derive from the Framingham calculator compared to the UKPDS–RE demonstrates the importance of using glycated hemoglobin levels to estimate CVD risk in DM2[8].

The ER calculator[7] is valid for patients with DM onset after 18 years of age; therefore, it is well-suited for this study. Using this calculator, patients with DM were divided into four major categories of cardiovascular risk: low, intermediate, high, and very high, according to age, the presence of risk stratifiers (RS), subclinical atherosclerotic disease, or clinical atherosclerotic disease. The 10-year cardiovascular event rates for low, intermediate, high and very high risk were <10, 10–20, 20–30 and > 0%, respectively.

According to SBD[7], the ER calculator is derived from the UKPDS-RE risk score; however, the agreement according to the kappa index was insufficient, suggesting that the ER calculator is not ideal for risk stratification in patients with diabetes. The divergence in the stratification of CVR by these two calculators generated different indications for the use of ASA and statins for the studied group. Therefore, depending on the chosen risk calculator, different diagnostic and therapeutic approaches would be adopted.

The present study allowed us to understand the applicability of national non-specific CVR calculators for diabetic patients with a calculator already validated internationally for this population. It is suggested that, based on this study, other studies may address a larger number of patients to evaluate these calculators, as well as using other types of calculators produced nationwide.

Study limitations

The present study had a small study population derived from medical specialty clinics; therefore, they represent potentially more severely-affected patients. Nevertheless, the population was quite heterogeneous both in terms of demographics and clinical profiles. It is also important to note that the loss of information when reviewing medical records was substantial.

Another limitation was the use of statins prior to the study period, which might have underestimated cardiovascular risk, specifically in the UKPDS-RE calculator.

CONCLUSIONS

Important disagreements were observed between the CVR calculators; this can lead to different diagnoses and, consequently, can influence therapeutic strategies. The use of the UKPDS equation made it possible to identify those at high risk for CVD early. This may avoid polypharmacy prescription in patients considered to be at low risk. In this sense, according to our findings, this scale should be considered superior to the other calculators.

Incorporating DM2 as a categorical variable implies that diabetes increases the risk in a similar way, regardless of glycemic control or the duration of diabetes. This work, therefore, emphasizes the need to use specific and validated risk calculators for individuals with a diagnosis of DM2 that can reliably estimate the risk of CVD.

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AUTHORS' CONTRIBUTIONS

LKR: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft. **WWA:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft. **VMB:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft. **MGO:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft. **WSV:** Conceptualization, Data Curation. **ITAC:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **CNK:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **DSM:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **DAS:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **JAL:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **KOS:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **MLC:** Conceptualization, Writing – Original Draft, Writing – Review & Editing. **SM:** Conceptualization, Writing – Original Draft, Writing – Review & Editing.

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Evaluation of serum ghrelin, nesfatin-1, irisin, and vasoactive intestinal peptide levels in temporal lobe epilepsy patients with and without drug resistance: a cross-sectional study

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SUMMARY

OBJECTIVE: Epilepsy is a common disorder that affects the nervous systems of 1% of worldwide population. In epilepsy, one-third of patients are unresponsive to current drug therapies and develop drug-resistant epilepsy. Alterations in ghrelin, nesfatin-1, and irisin levels with epilepsy were reported in previous studies. Vasoactive intestinal peptide is among the most common neuropeptides in the hippocampus, which is the focus of the seizures in temporal lobe epilepsy. However, there is also lack of evidence of whether these four neuropeptide levels are altered with drug resistant temporal lobe epilepsy or not. The aim herein was the evaluation of the serum levels of nesfatin-1, ghrelin, irisin, and Vasoactive intestinal peptide in drug-resistant temporal lobe epilepsy patients and temporal lobe epilepsy (TLE) without drug resistance, and to compare them to healthy controls.

METHODS: This cross-sectional study group included 58 temporal lobe epilepsy patients (24 with drug resistant temporal lobe epilepsy and 34 with temporal lobe epilepsy who were not drug-resistant) and 28 healthy subjects. Nesfatin-1, ghrelin, irisin, and Vasoactive intestinal peptide serum levels were determined using enzyme-linked immunosorbent assay.

RESULTS: The serum ghrelin levels of patients with drug resistant temporal lobe epilepsy were seen to have significantly decreased when compared to those of the control group ($p<0.05$). Serum nesfatin-1, vasoactive intestinal peptide, and irisin levels were seen to have decreased in the drug resistant temporal lobe epilepsy group when compared to those of the control and temporal lobe epilepsy groups; however, the difference was non-significant ($p>0.05$).

CONCLUSIONS: The results herein suggested that ghrelin might contribute to the pathophysiology of drug resistant temporal lobe epilepsy. However, further studies are needed to confirm this hypothesis.

KEYWORDS: Ghrelin. Neuropeptides. Drug resistant epilepsy. Vasoactive intestinal peptide.

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INTRODUCTION

Epilepsy is a significantly prevalent neurological condition that affects about 50 million people worldwide[1]. Approximately 25% of epileptic patients have drug resistance[2]. Human temporal lobe epilepsy (TLE) is both the most prevalent seizure condition in adults[3] and the most frequent reason for drug-resistant (pharmacoresistant) seizures[4]. Pharmacoresistant epilepsy is associated with poor quality of life, injuries, psychosocial problems, premature mortality[5], and psychiatric problems[6]. Thus, finding new treatments is an urgent necessity[7], and there is an unmet need for finding new antiepileptic drugs with novel targets and different mechanisms[8]. Furthermore, drug-resistant epilepsy is the cause of 80% of the expenditure of epilepsy[4] and the mechanisms underlying pharmocoresistant epilepsy are not completely understood[5].Therefore, major attention has been directed towards elucidating the mechanisms underlying drug resistance.

There are numerous hypotheses explaining the mechanisms related with refractory epilepsy, including methylation, impaired mitochondrial function, neural network, intrinsic severity, transporter, and target hypothesis[9]. The intrinsic severity hypothesis assumes that drug resistance is the result of high excitatory neurotransmission, which leads to elevated intensity and frequency of seizures[9]. A deterioration in the balance of inhibitory and excitatory systems in the brain leads to seizures, which have been defined as aberrant, extreme, and synchronous neural activity[10]. Neuropeptides are significant in the field of epilepsy due to their modifying roles[11] on inhibitory or excitatory neurotransmitters[10]. Therefore, neuropeptides draw attention as drug or biomarker candidates in the field of epilepsy research[10].

Ghrelin is described as a new anticonvulsant[12], pleiotropic[13], and orexigenic peptide, known to be expressed in the brain[14]. Alterations in ghrelin levels have been reported both in clinical[15-17] and animal studies[18]. Irisin is defined as a myokine, which is produced in skeletal muscle with exercise[19]. In addition, FNDC5, the precursor of irisin, is present in the brain[19]. Significant alterations in serum levels of irisin[20] and FNDC5/irisin[18] have been reported. Nesfatin-1 is a recently identified neuropeptide, produced in different areas of the brain[21]. Increased nesfatin-1 levels have been reported in clinical[16] and animal studies[18]. Vasoactive intestinal peptide (VIP), a neuropeptide that contains 28 amino acids, is expressed in different areas of the brain[22]. VIP is among the neuropeptides in the hippocampus, which is the most common focus of seizures in TLE[22]. Although VIP can increase the electrical activity in various areas of the brain and may have a function in seizure pathology, VIP has not often been investigated in the field of epilepsy[10].

In summary, in previous studies, significant alterations were reported in ghrelin[15-18], irisin[20], FNDC5/irisin[18], and nesfatin-1[16,18] levels. Despite this, there is also lack of evidence of whether these four neuropeptide levels are altered with drug resistant temporal lobe epilepsy (DRTLE) or not. Therefore, the aim herein was the investigation of possible alterations of these peptides in TLE patients with or without drug resistance.

METHODS

Study design

This cross-sectional study was conducted during the period comprehending November 2018 to March 2020. All of the study protocols received approval from the local Medical Ethics Committee of Van Yuzuncu Yil University. A written informed consent was given to the subjects before participating in the study. Of the 116 eligible subjects, nine were excluded due to refusal to give blood and nine, due to non-fasting status at the time of sampling. Moreover, 13 other subjects were excluded due to a body mass index (BMI) over 30.0 kg/m^2, one was excluded with a newly diagnosis of ankylosing spondylitis. Some subjects have two excluded criteria. Finally, 86 subjects remained for this study. The cross-sectional study was carried out on 58 TLE patients (24 with DRTLE and 34 with TLE that was not drug- resistant) (who attended epilepsy outpatient clinic at Van Yuzuncu Yil University, Neurology department) and 28 healthy subjects. All of the patients had a body mass index (BMI) of less than 30.0 kg/m^2. The control group included age- and BMI-matched subjects who did not have any chronic illness and a BMI of less than 30.0 kg/m^2. The exclusion criteria are BMI greater than 30.0 kg/m^2, chronic illness except epilepsy. "*TLE is diagnosed by a history of characteristic partial seizure symptoms. The diagnosis is confirmed by the capture of a typical episode during an electroencephalogram (EEG) or video-EEG, with epileptiform activity over one or both temporal regions*"[23]. Despite significant advances involving both antiepileptic drugs and surgery in TLE treatment over recent decades, approximately one third of patients with this disease are only poorly controlled, or their seizures are resistant to drugs. "*Drug resistant epilepsy may be defined as failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom*"[24] by ILAE. This study has been reported in line with the STROBE criteria[25].

Ghrelin, nesfatin-1, irisin, and VIP assays

Blood was collected from the subjects at 08:00 and 12:00 h following one night of fasting. Centrifugation of the blood was performed for 5 min at 4,000 rpm. Storage of the serum was at -80°C until the testing. Serum ghrelin, nesfatin-1, irisin, and VIP

levels were measured using ELISA. Serum levels of the ghrelin (Cat No: YLA1024HU), VIP (Cat No: YLA0803HU), irisin (Cat No: YLA1361HU), and nesfatin-1 (Cat No: YLA0715HU) (all available commercially from YLbiont, Shanghai, China) were determined using ELISA kits.

Statistical analysis

In the study, ghrelin is considered for sample size calculation. From the previous studies[26], the standard deviation for ghrelin varies between 0.1 and 0.9. Thus, standard deviation was considered as 0.5. For the 95% of confidence coefficient and approximately 80% power value, Type I error is 0.05 (Z value is 1.96 for the 5% type I error), the effect size was defined by the researcher as 0.2. Based on this information, the necessary sample size was calculated by the equation "$n=Z^2 \times \sigma^2/d^2$"

According to this equation, minimum sample size in each group was found as 24 [$n=(1.96^2 \times 0.5^2/0.2^2 @ 24$].

For continuous variables, descriptive statistics were presented as the mean and standard error of the mean (SEM), whereas the categorical variables were presented as counts and percentages. One-way ANOVA was used for comparison of the means of groups. The Duncan multiple comparison test was used for identification of the different means of the groups, followed by ANOVA. To determine linear relations among the variables, the Pearson correlation analysis was performed. Additionally, the chi square test was used for determining relations between categorical variables. Statistical significance was defined as $p<0.05$, and SPSS v.13 (Chicago, IL, USA) was used for the statistical computations.

RESULTS

Table 1 presents the demographic characteristics of subjects. There were no statistically significant differences in terms of age and BMI between the groups.

Serum ghrelin levels in the DRTLE group decreased significantly when compared to the control group ($p<0.05$).

Table 1. Demographic characteristics of subjects.

	Age (Mean±SEM)	BMI (Mean±SEM)
Control	30.92±1.54	25.16±0.64
TLE	26.44±1.22	23.55±0.55
DRTLE	31.75±2.62	24.01±0.52

TLE: Temporal lobe epilepsy patients without drug resistance; DRTLE: drug-resistant temporal lobe epilepsy patients; BMI: body mass index. Data are presented as Mean±SEM (standard error of mean).

The difference between the TLE and DRTLE groups in terms of ghrelin was non-significant (Figure 1A). Serum nesfatin-1 levels had increased in the TLE group, whereas they had decreased in the DRTLE group when compared to the control; however, both were non-significant (Figure 1B).

No statistically significant difference was observed between the TLE, DRTLE, and control groups with regards to the serum VIP levels (Figure 1C). Serum irisin levels had decreased in the TLE and DRTLE groups when compared to the control, and they had decreased in DRTLE compared to TLE group; however, both were non-significant (Figure 1D).

DISCUSSION

Increased[15] or decreased[16,17] ghrelin levels have been reported in studies on epilepsy patients. In a previous study, significantly decreased ghrelin levels in the brain and serum were found in acute PTZ-induced seizures; and PTZ kindling models, in rats[18]. It was suggested that ghrelin has antiepileptic[27] and neuroprotective[28] properties. In the present study, serum ghrelin levels had decreased in the TLE and DRTLE groups when compared to the control. This decrease was non-significant in TLE group, whereas it was significant in the DRTLE group. Aydin et al.[17] suggested that the reason for the reduction of the ghrelin level may have been due to the high uptake of the neuropeptide by CNS for modulating epileptic discharges. Frago et al.[29] suggested that the anticonvulsant effects of ghrelin may be due to its actions on neuropeptide Y and gamma-aminobutyric acid (GABA). Therefore, in the present study, this decrease may be evaluated as a result of seizures; repetitive seizures may lead a decrease in the body's storage of ghrelin, which may have been responsible for the significant decrease in the serum levels of the DRTLE group.

Ghrelin has a role in a variety of neurophysiological process, including anti-inflammatory, neuroprotective, neurogenesis[30,31], anti-convulsant effects[30], learning and memory[31], and can cross blood brain barrier[30]. Ghrelin receptor GHSR1a is widely expressed in the body, involving prone areas in seizures, such as the hippocampus[30]. The mechanism underlying its anticonvulsant properties remains unknown[30].

In the present study, significant reduction was found in serum ghrelin levels of the DRTLE group compared to the control. The interactivity between ghrelin-NPY/GABA in hypothalamic circuitry was reported[12,31]. It was reported that the blockade of NPY receptors obstruct the anticonvulsant effects of ghrelin in rats' hippocampus[32]. Ghrelin supports the releasing of NPY presynaptically and, therefore, the releasing of GABA in the arcuate nucleus of hypotalamus[12]. Chronic seizures lead a change in expression of NPY receptors resulting an increase in

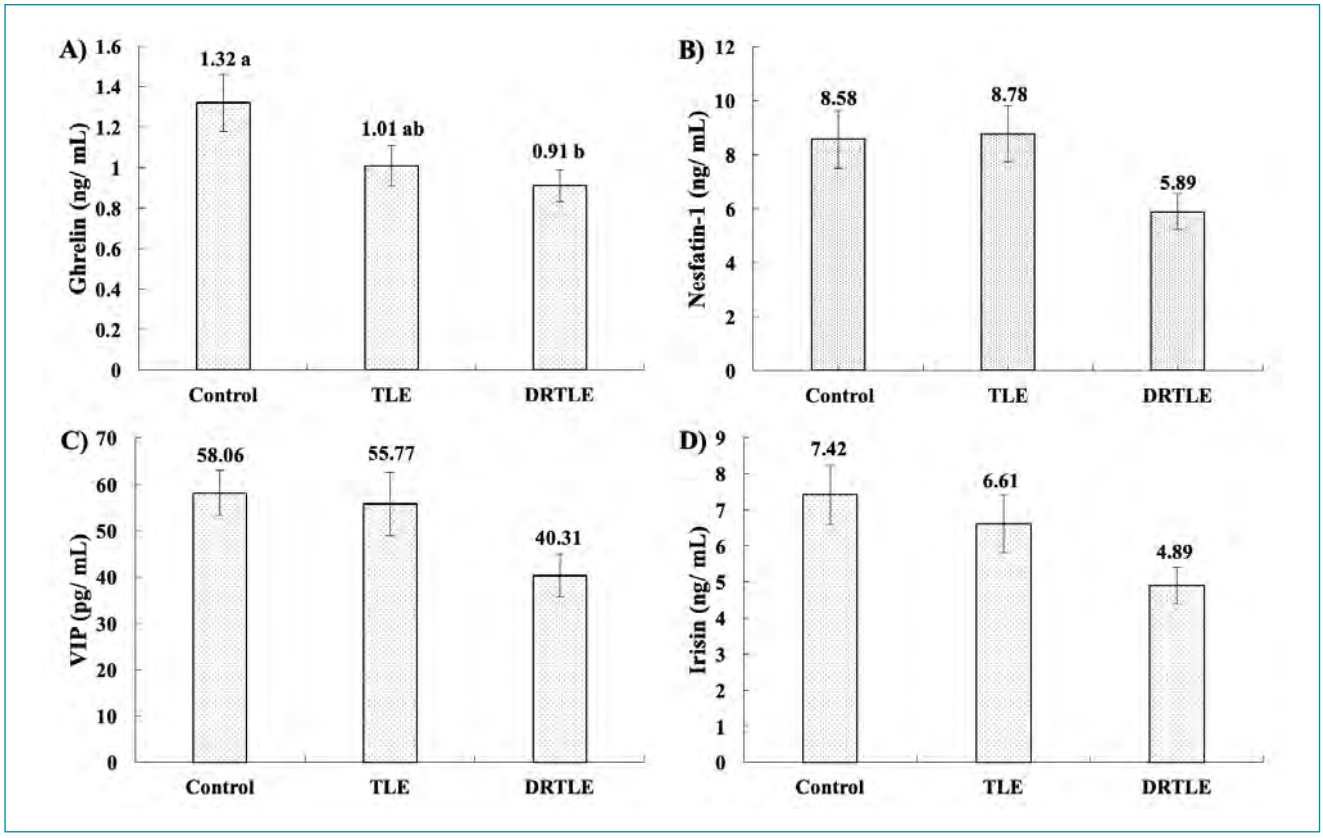

Figure 1. Serum neuropeptide levels of the control, TLE, and DRTLE groups.

Y2 and a decrease in Y1 receptors[10]. These changes in response to seizures may be a mechanism for dealing with hyper-excitability[10]. In the present study, the reduction of serum ghrelin levels might be due to the increased consumption of ghrelin to cope with chronic recurrent seizures which occurred in DRTLE.

Nesfatin-1, a neuropeptide, is expressed in many areas of the brain[21]. It induces satiety and is known for being a strong anorexigenic agent[21]. Its antiapoptotic and anti-inflammatory effects in the brain tissue of rats has been reported[33]. In a previous study, serum levels of nesfatin-1 increased significantly in acute PTZ and PTZ-kindling in rats[18]. However, the serum nesfatin-1 levels of rats that received valproate treatment were ameliorated and non-significant when compared to the control[18]. Herein, serum nesfatin- 1 levels had increased in the TLE group and decreased in the DRTLE group, but the differences were non-significant when compared to the control group. Antiepileptic drug treatment may ameliorate the increased nesfatin-1 levels of the serum. In a previous study, serum nesfatin-1 levels were reported to have increased, in newly diagnosed primary generalized epilepsy patients, approximately 160-fold higher than that of the control; however, this increase was decreased via treatment with antiepileptic drugs, but remained approximately 10-fold higher than that of the control[16]. In the present study, this decrease may have been due to the long duration of antiepileptic drug treatment.

In the present study, the serum VIP level was also evaluated. VIP is defined as a neuroprotective[34] neuropeptide. In the present study, serum VIP levels in the TLE and DRTLE groups were similar to that of the control. These results were in accordance with previous studies, in which no significant changes were reported in the VIP levels in the hippocampus of TLE patients[35] and brain tissue of PTZ-kindled rats[36]. Contrary to our results, increased serum and cerebrospinal fluid VIP levels were reported in children with seizure disorders[37]. These contrary results may have been due to the age of the subject population.

Irisin is secreted from skeletal muscle with exercise[38]. In recent years, its anti-inflammatory and antioxidative effects have drawn much attention from researchers[38]. However, its role in the central nervous system is not well known. There are limited studies present on irisin in the field of epilepsy. In a previous

study, serum and brain FNDC5/irisin levels were significantly increased in PTZ-kindling, and acute PTZ-induced seizure groups in rats without antiepileptic drug treatment[18]. Herein, differences between the serum irisin levels of the control, TLE, and DRTLE groups were non-significant. In a previous study, it was found that chronic antiepileptic drug treatment (valproate) decreased the PTZ-induced increase in serum and brain irisin levels in PTZ- kindling in rats[18]. Significantly increased serum levels of irisin were reported in children with idiopathic epilepsy[20]. These controversial results may have been associated with different factors; the present study conducted on adults and the subjects had therefore received longer antiepileptic drug therapy.

The strength of this study was that for the first time, to the best of our knowledge, the serum nesfatin-1, ghrelin, irisin, and VIP peptide levels in TLE and DRTLE patients were compared to healthy controls.

The limitations of our study are all patients being under antiepileptic drug therapy. Antiepileptic drug treatment and the age of epilepsy patients can influence the serum levels of ghrelin[31]. Second, the relation between these four peptide levels and the type of antiepileptic drug used did not investigate. In a previous study, it was reported that antiepileptic drug treatment could alter the serum levels of the ghrelin, FNDC5/irisin, and nesfatin-1 compared to the group who were not under drug treatment in PTZ treated rats[18]. In further studies, these peptides should be tested in the same way, but should be done with a larger sample group with subgroups (age groups, type of antiepileptic drug treatment groups).

CONCLUSIONS

In conclusion, the results herein demonstrated decreased serum ghrelin levels in DRTLE patients when compared to the control. Therefore, the results herein suggested that ghrelin might contribute to the pathophysiology of DRTLE. However, future studies are necessary to confirm this hypothesis.

AUTHORS' CONTRIBUTIONS

OEE: Conceptualization, Formal Analysis, Writing – Original Draft. **AM:** Data Curation, Formal Analysis, Writing – Review & Editing. **AUK:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing. **MK:** Conceptualization, Formal Analysis, Writing – Review & Editing. **ZH:** Conceptualization, Formal Analysis, Writing – Review & Editing. **SK:** Conceptualization, Formal Analysis,Writing – Review & Editing.

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Transcutaneous Electric Nerve Stimulation on ischemic rest pain in inpatients: randomised trial

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SUMMARY

OBJECTIVE: To investigate the efficacy of a short-term application of Transcutaneous Electric Nerve Stimulation to relieve rest pain in patients with chronic limb-threatening ischemia.

METHODS: In patients ³18 years old, with chronic limb-threatening ischemia and rest pain ³3 in the Visual Analogue Scale, without diabetic neuropathy were randomly assigned to 1) Transcutaneous Electric Nerve Stimulation (100 Hz, 200 μs) or 2) sham intervention, both during one or two 20 min treatment sessions. The primary outcome was pain intensity, assessed by the visual analogue scale (0–10 cm) and described by the McGill Pain Questionnaire. We used a t-test for difference of means.

RESULTS: A total of 169 patients were assessed, 23 met the study criteria and were randomized. Thirty-four applications were performed in two days: in the 17 Transcutaneous Nerve Stimulation and 17 sham. The within-group analysis indicated a pain decrease in both groups (Transcutaneous Electric Nerve Stimulation, from 7–3.9 cm, $p<0.0001$, and sham from 5.8–3.2 cm, $p<0.0001$). No statistically significant difference was verified between-groups ($p=0.5$).

CONCLUSIONS: Both groups showed a decrease in rest pain of 54 and 55%, respectively. However, there was no difference between short-term high-frequency Transcutaneous Electric Nerve Stimulation and sham intervention to relieve ischemic rest pain in chronic limb-threatening ischemia patients.

KEYWORDS: Peripheral arterial disease. Ischemia. TENS. Randomized controlled trial. Pain.

INTRODUCTION

Rest pain in individuals with chronic limb-threatening ischemia (CLTI) is caused by a chronic ischemic state in severe stages of peripheral arterial disease and incidence is higher in those with risk factors for atherosclerosis[1,2]. High doses of opiates and non-steroidal antiinflammatory are prescribed for CLTI patients, but have sparse or insufficient results[3]. However, prolonged use of analgesic drugs is related with risks[4,5].

Transcutaneous electric nerve stimulation (TENS) is an inexpensive, non-pharmacological and non-invasive electrical stimulation delivered through electrodes placed in the skin[4,6]. TENS delivered at high frequency (HF) stimulates large diameter myelinated fibers (afferent Aβ). Low frequency (LF) TENS activates descending pain modulating mechanisms deriving from the brain stem and is not recommended for opioid tolerant individuals[7,8].

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Studies have shown that TENS reduces pain intensity in limb ischemia[9-13]. Yet, they investigated pain relief in acutely induced ischemia on healthy adults[10,14]. Some authors concluded that HF TENS may be more effective for people using opioids[7]. Cuschieri et al. showed analgesia with TENS in rest pain through continuous >24 h intervention[4]. Therefore, the present study sought to investigate pain relief of a short-term stimulation of HF TENS on rest pain.

METHODS

Trial design

Randomized single-blinded, controlled trial. Ethical approval was obtained from the Ethics Committee of Universidade Federal de Minas Gerais, CAAE# 12954313.8.0000.5149 and registered at Rebec, RBR – 8jg3bk.

Eligibility

Consecutive individuals with CLTI and rest pain ≥3 rated in the 0 to 10 cm visual analogue scale (VAS), Rutherford graded II and III, admitted to the Vascular Surgery Unit at Hospital Risoleta Tolentino Neves, were screened for eligibility. Content specialists with active registry at medical board performed evaluations and CLTI diagnosis, and confirmed at least with one objective additional test, ankle-brachial index (ABI), duplex ultrasound or angiography.

Participants had to be able to respond to the researcher and have a cognitive status, assessed by the Mini Mental State Examination (MMSE), of ≥23 for literates, ≥18 for illiterates[15]. We did not include those who had been previously treated with TENS, those with VAS <3 and/or diabetic neuropathic foot. Detailed flow of participants is depicted in the CONSORT[16], Figure 1.

Randomization

Participants were randomly assigned (random.org) into the intervention groups: TENS (TG) or sham (SG) in a 1:1 fashion. The allocation schedule was generated by an external researcher. Researchers involved with data collection were trained by the same personnel. In a visit to the hospital site, the same examiner could be involved with data collection in both groups, which depended on the randomization schedule and the number of available participants. An examiner knew which group the participant would be assigned to only after they signed consent form.

Intervention and blinding

Each intervention took 20 minutes[17], and participants were positioned in a supine position. A two-channels Neurodyn Portable TENS/FES (IBRAMED Ltda., São Paulo, Brazil) was calibrated before each session. Two carbon rubber and oval, 4 x 6.4 cm electrodes (ValuTrode®, Fallbrook, CA, USA) were applied to both sides of the tibial tuberosity in the painful lower limb, in L4 and L5 dermatomes to cover the calves. TENS parameters were 200 µs, 100 Hz, in a 'continuous' pulse, delivered as biphasic asymmetrical waveform[11]. Participants were not told which group they were assigned to. In the SG, the machine was turned on and an alternative channel used, but it did not allow current to be delivered to their skin. This way, application procedure was similar in both groups. The TENS unit was not visible to participants throughout the session. Individuals of both groups were told that 'TENS can be effective even in non-perceptive intensities'[11], and frequently asked whether the intensity faded during session[12]. If response was positive, intensity was increased and maintained at a strong but comfortable submotor level in the TG[17], while we mocked an increase in the SG.

Considering the non-cumulative effect of TENS, a second and independent 20 min application of the same intervention was performed in participants who, after 24 h, were still at the nursery room with no change in their group allocation. For ethical reasons, drug therapy routine was clinically assigned by medical doctors and not changed during the study.

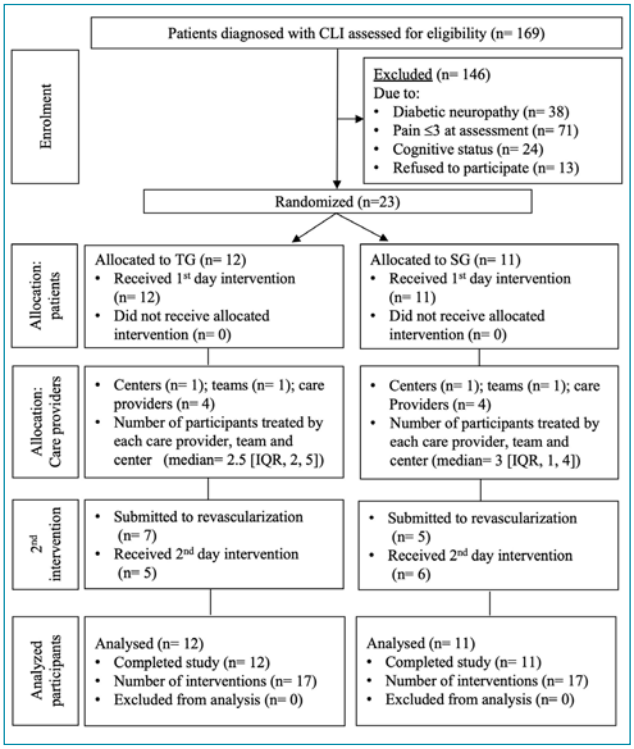

Figure 1. CONSORT flow diagram.

Pain description – experience

We assessed pain experience at the beginning of the study with the Brazilian version of the McGill Pain Questionnaire (MPQ)[18]. It consists of 68 adjectives distributed into five domains: sensorial, reactive, affective, evaluative, and miscellaneous[18]. To assess the number of words chosen (NWC), participants were encouraged to choose a single word from each category with a range from 0–20 possible words. Each word within these categories is assigned a scale value, which is intensity-dependent. The sensory pain-rating index (SPRI) is calculated based on the sum of scale value of each word chosen at the sensory category and ranges from 0–34. The reactive pain-rating index (RPRI) is the sum of rank values of all non-sensory categories and ranges from 0–34 points[18,19]. The total pain-rating index (TPRI) score is the sum of scale values of all categories, 0–68.

Demographic and clinical data

Demographic data were collected from medical records and included age, ABI, sex, comorbidities, presence of wound, medication in use, and duration of pain at the moment of hospital admission.

Primary outcome measure

The VAS was used to assess pain intensity[20]. Participants were encouraged to choose a value prior to and immediately after the application. They were explained that zero represents 'no pain' and 10, 'pain as bad as it could be'.

Sample size

Sample size was determined based on a previous study[10], which estimated a sample size of 17 participants in each group. The expected difference in VAS was 1.5 cm, or 30% improvement from baseline[21], considering a type 1 error of 0.05 and a type 2 error of 0.80.

Statistical analysis

Data were screened for normality with Shapiro-Wilk. Demographic characteristics expressed as means and standard deviation (SD). For the intragroup analysis, a paired t-test was used; independent sample t-test was adopted for the between-groups analysis. The significance level was set at p=0.05. The Levene's test for equality of variances was used with 2-tailed t-tests. An intention-to-treat approach was considered for data analysis with the last observation carried forward[22]. Subject was included in the analysis even when pain increased after intervention. The statistical analyses were performed with Stata/SE (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.).

RESULTS

A total of 169 individuals were screened for eligibility. Of them, 146 did not meet inclusion criteria or declined to participate. Then, 23 were randomized and completed the first intervention. Considering the second application, results shown in this section are for the analysis performed on the total number of interventions for each group, i.e., TG (17 interventions) and SG (17 interventions).

Participant characteristics and pain quality

There were no differences between groups regarding demographic characteristics (Table 1). Information on pain duration was found in the medical records of 18 participants. At the time of hospital admission, 27.8% were experiencing pain for less than seven days, 38.9% had it from eight to thirty days, and 33.3%, for more than 31 days. Both groups chose similar NWC, TPRI, SPRI, and RPRI scores (Table 1).

Table 1. Demographic and clinical characteristics at baseline. Data are expressed in mean (SD) or number of occurrence (%).

	Study Groups	
	TG (n=12)	SG (n=11)
Age, years old	62.9 (9.5)	65.6 (12.7)
Sex, female (%)	7 (58.3)	5 (45.5)
Current smoker (%)	6 (50)	5 (45.5)
Comorbidities		
Diabetes Mellitus (%)	5 (41.7)	4 (36.4)
SAH (%)	10 (83.3)	9 (81.8)
ABI	0.32 (0.29)	0.36 (0.27)
Open wound (%)	10 (83.3)	11 (100)
Medication in use		
Metamizole (Dypirone) (%)	12 (100)	9 (81.8)
Acetaminophene (Paracetamol) (%)	0 (0)	1 (9.1)
Opiates (%)	10 (83.3)	11 (100)
MMSE	23.6 (3.5)	23.1 (3)
McGill Pain Questionnaire Scores		
NWC	16.9 (2.9)	16.9 (2.8)
TPRI	38.8 (10.9)	41.1 (8.6)
SPRI	20.3 (4.9)	21.4 (5.6)
RPRI	18.5 (7)	19.7 (4.4)
VAS baseline	7 (1.9)	5.8 (1.4)

TG: TENS group; SG: sham group; SAH: systemic arterial hypertension; ABI: ankle-brachial index; MMSE: mini-mental state examination; NWC: number of words chosen; TPRI: total pain rating index; SPRI: sensory pain rating index; RPRI: reactive pain rating index; VAS: visual analogue scale score. *When differences were statistically significant at baseline.

Outcome measure

Prior to stimulation, the TG had a mean score of 7 cm (SD 1.9), whereas SG, of 5.8 cm (SD 1.4). Both showed similar baseline VAS, p=0.052 (Table 2).

The baseline VAS of both groups compared to its score after intervention showed they experienced a decrease in pain intensity. Despite the TG had a 54% and the SG a 55% decrease, no statistically significance was found between-groups, Table 2.

DISCUSSION

Short-term HF TENS at 100Hz did not provide greater pain relief in participants with rest pain when compared to a sham intervention. Ostelo et al. considered 30% the threshold of minimal clinically important difference in the VAS[22]. Both groups showed over 50% pain reduction after intervention.

Pain relief after sham intervention was demonstrated on several aspects of laboratory-induced ischemic pain and suggests involvement of affective mechanisms[10,12]. HF TENS efficacy has been reported in studies which used a tourniquet to induce ischemic pain[10,12]. However, induced ischemic models should be carefully extrapolated into CLTI chronic patients, given that induced ischemia elicits minimal tissue damage[10]. In our sample, 33.3% experienced rest pain for more than 31 days, different from acutely induced models. The MPQ scores confirm the difference in symptoms between our sample to those healthy individuals. Our participants had greater NWC, TPRI, SPRI, and RPRI scores than healthy individuals in induced ischemia studies, which indicates a more complex pain experience in our sample[9,12].

Peripheral nerve involvement, other than nociceptive C fibers, might corroborate to ischemic pain, which may be resistant to TENS. Chen & Johnson pointed that nerve fibers can experience refractory periods to electrical stimulation beyond 80 Hz of TENS stimulation, which might hinder transmission[10]. Seenan et al. demonstrated an increased walking distance with stimulation of 120Hz, but no reduction in pain intensity and quality[14]. However, our study shows a novel approach, as it included participants with rest pain.

Pain relief in rest pain was demonstrated by Cuschieri et al.[4] The average VAS score of our TG at baseline, i.e., 7 cm (SD 1.9), was analogous to their stimulated group, 72 mm, in a 100 mm scale. Our participants had similar CLTI staging compared to their study, as both included participants with very low ABI index[4]. In their report, pain relief was related to TENS intervention, despite analgesia in that group was only significantly greater than sham after 24h of continuous stimulation. However, our study found pain decrease after both TENS and sham stimulation; thus, we believe our short-term protocol is more feasible in the daily routine than their approach.

An important limitation of the present investigation is its single-blinded design. A triple-blind method should be the 'gold-standard'. Additionally, the TENS device did not have an amplitude display, so the intensity was individually set as a submotor level according to one's tolerance.

Despite this study's small sample size, we consider this a relevant contribution and alternative intervention to pharmacological resources and high opioid doses. However, further studies are required to investigate the effects of different parameters of high frequency (<80 Hz) of TENS stimulation in different aspects of pain, quality of life, and functionality in CLTI population.

CONCLUSIONS

This study showed a significant decrease in rest pain in inpatients diagnosed with CLTI in response to a short-term HF TENS and sham intervention of 54 and 55%, respectively. Despite HF TENS had no greater analgesic effect over sham, both therapies are safe and decreased rest pain.

AUTHORS' CONTRIBUTIONS

PEOG: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **MM:** Conceptualization, Writing – Review & Editing. **RLGF**: Conceptualization, Writing – Review & Editing. **TPN:** Conceptualization, Writing – Review & Editing. **CJM:** Conceptualization, Formal Analysis, Writing – Review & Editing. **LLC:** Conceptualization, Supervision, Formal Analysis, Writing – Original Draft, Writing – Review & Editing.

Table 2. Changes in rest pain intensity following interventions.

Study groups	Within-group change in VAS (95%CI)	p-value*	Between-groups change in VAS (95%CI)	p-value*
TENS	-3.1** (-4.6– -1.6)	<0.0001	0.5 (-2.1–1.1)	0.5
Sham	-2.6** (-3.3– -1.9)	<0.0001		

VAS: visual analogue scale; CI: confidence interval; TENS: Transcutaneous electric nerve stimulation. *t-test; **denotes statistically significant difference between pre and post intervention within-group. Significance was considered when p<0.05.

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ORIGINAL ARTICLE
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Quantitative evaluation of computed tomography findings in patients with pulmonary embolism: the link between D-Dimer level and thrombus volume

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SUMMARY

OBJECTIVE: To investigate the correlation of D-dimer levels and computed tomography properties of pulmonary embolism.

METHODS: A total of 58 treated patients with diagnosis of properties of pulmonary embolism were retrospectively studied. All patients underwent a D-dimer blood test. In computed tomography images, septal angle, interventricular septal thickness, and the diameters of all cardiac chambers and pulmonary arteries were measured. The thrombus volume (load) and density at all pulmonary arteries (main, right, left pulmonary arteries, and segmental arteries) were calculated.

RESULTS: A significant correlation was found between D-dimer and total thrombus volume (p=0.009, r=0.342). Total thrombus volume and total thrombus density were calculated with mean value of 23.40±60.63 ml and 66.16±38.48 hounsfield unit (HU), respectively. Right ventricle/left ventricle ratio showed positive correlation with the D-dimer level (p=0.02).

CONCLUSION: Increased D-dimer levels with RV/LV ratio and their correlation with total thrombus volume suggest that it may be a prognostic factor.

KEYWORDS: Thrombosis. D-dimer. Pulmonary embolism. X-Ray computed tomography. Quantitative analysis.

INTRODUCTION

Pulmonary embolus (PE) refers to obstruction of the pulmonary artery or one of its branches by material (*e.g.* thrombus, tumor, air, or fat) that originated elsewhere in the body. Acute PE is a common and fatal disease whose mortality rates can be as high as 31 to 58% when shock is present[1]. It increases the pressure of the pulmonary arterial system and right ventricle (RV), resulting in RV dysfunction, which may progress to right heart failure and circulatory collapse[2,3].

Difficulty may occur in diagnosis due to its clinical nonspecific nature and presentation with different clinical situations. Initiation of the treatment just after immediate diagnosis provides a decrease in morbidity and mortality.

Patients can be classified as massive and sub-massive according to their hemodynamic stability. For the confirmation of the probability of PE, D-dimer level is the most commonly used laboratory parameter in routine clinical practice. D-dimer is formed upon breaking down the cross-linked fibrin. In patients with suspected PE, blood D-dimer levels correlate with the probability of having PE[4,5].

As an imaging modality, computed tomographic pulmonary angiography (CTPA) is the frontline imaging modality in the patients with suspected acute PE[6,7]. CTPA is an essential component in commonly used clinical diagnostic algorithms with the highest sensitivity (83%) and specificity (96%)[8]. PE clot volume can be measured in CTPA setting using a semi-automated algorithm[9].

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To the best of our knowledge and the literature, there is no study correlating total thrombus volume (TTV: the thrombus load in the main and both pulmonary arteries including segmental branches) with D-dimer level.

The objective of this article was to investigate the correlation of D-dimer level and CT properties of thrombosis such as TTV and RV/left ventricle (LV) ratio.

METHODS

Study population

Between May 2016 and May 2019 in the hospital of the institution, in a total of 58 patients treated with the diagnosis of PE, were studied retrospectively. All patients had D-Dimer blood test. Patients with inadequate quality of CT images or insufficient breathing during CT scanning, patients with chronic diseases (malignancy, cardiac insufficiency or chronic cardiac diseases), and those under 18 years of age were excluded from the study.

Patients between 18-65 years of age with optimum CTPA image quality and breathing, patients clinically and radiologically diagnosed with PE, were enrolled in the study.

The study protocol was approved by the institutional ethics review committees (approval number: 2019.191.10.12).

Computed tomography pulmonary angiography acquisition

A 128-row multi-detector CT scanner (Aquilion™ Prime; Canon Medical Systems) was used for CTPA scanning. Field of view (FOV) of the whole chest was scanned from the lung apex to the diaphragm with a single breath-hold.

Computed tomography protocol

CT acquisition was done by the following parameters: the current of 100–250 mAs modulated by personal body mass index dose; tube voltage of 100–140 kV and collimation of 0.5 mm x 80, gantry rotation time of 0.35 sec, pitch factor of 0.813, FOV: 20x20 cm, slice thickness of 1 mm, and slice interval of 0.8 mm. For intravenous bolus injection of non-ionic contrast material (350 mg/100 mL, Iohexol, Omnipaque®; GE Healthcare, Cork, Ireland), a mechanical injector was used at a flow rate of 4.5–5.0 mL/sec. The automatic bolus-tracking method was used with the ROI (region of interest) positioned at the level of the main pulmonary artery with a pre-defined threshold of 100 HU, and a fixed delay of 5 sec was used for data acquisition. Electrocardiogram (ECG)-gating technique was not performed. Patients with good quality of images, without artifacts, and those the largest ventricular diameters were enrolled in the study. Cardiac measurements were made in the phase where the largest ventricle diameters were thought to be in the diastolic phase.

Imaging review

An eight-year-experienced radiologist assessed the patients for the presence of PE and analyzed the distribution within the main pulmonary vasculature and segmental arteries bilaterally.

After acquisition, the obtained CTPA images were transferred to Picture archiving and communication system (PACS) (Sectra 7.0, Sectra AB, Linköping, Sweden) and were analyzed at the workstation (Vitrea 2 workstation; Vital Images, Minnetonka, MN, USA).

Interventricular septal angle, thickness, and the diameters of all cardiac chambers in a four-chamber plane (4CH) (Figure 1); and the diameters of main, right and left pulmonary arteries were measured on the axial CT images (Figure 2). The thrombus volume (load) and density at all pulmonary arteries (main, right, left pulmonary arteries, and segmental arteries) were calculated (Figure 3). Determination of the boundaries of the thrombus (seen as an intraluminal filling defect in CTPA) was made using semi-automated (a pixel-based image segmentation method known as region growing algorithm) and manual

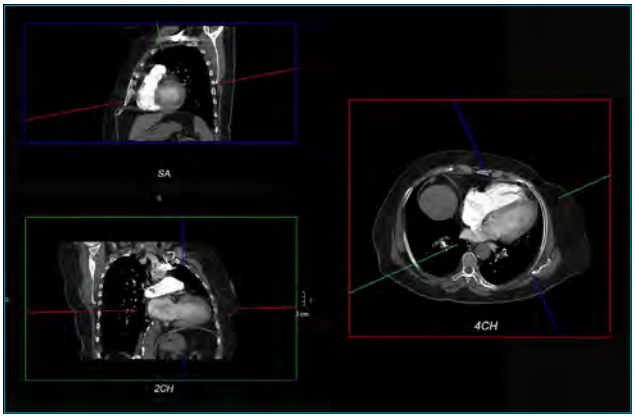

Figure 1. Shows cardiac planes including four-chamber (4CH; including both atria and ventricles), two-chambers (2CH, one atrium and ventricle) and short axis (SA, just both atria or ventricles).

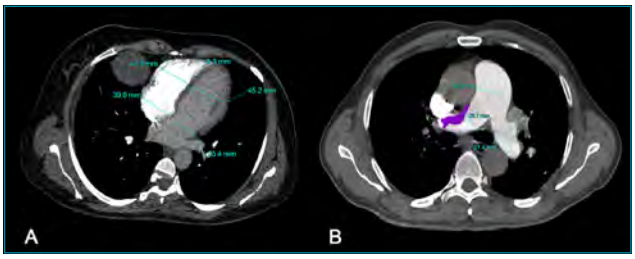

Figure 2. The measurements of cardiac chambers at 4CH plane (A) and pulmonary arteries (B) are shown. The thrombus area at right pulmonary artery (B).

drawing tools. The measurements of thrombus volume were categorized into five subgroups including the main pulmonary artery (PA), right PA, left PA, right lung thrombus volume (upper, middle, and lower lobe segmental arteries), and left lung thrombus volume (upper and lower lobe segmental arteries).

The diameter of cardiac chambers was performed between the two endocardia. Interventricular septal angle measurement due to calculation method defined by Tang et al. was done in axial CT images[10]. The angle between the connecting line from the midpoint of the sternum to the thoracic vertebral spinous process and interventricular septum (Figure 4).

Laboratory tests

In all patients, complete blood count (hemoglobin, hematocrit, neutrophil, leukocyte, and platelet), C-reactive protein, and D-dimer level were analyzed.

Statistical analysis

All data were analyzed using a statistical package program (SPSS version 17.0; SPSS, Inc., Chicago, IL, USA). The variables were investigated using visual (histograms and probability plots) and analytical methods to determine whether they are normally or not normally distributed. Investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. A 5% type-1 error level was used to infer statistical significance.

RESULTS

In a total of 58 cases, 22 were males (37.9%) and 36 females (62.1%), with a mean age of 60.14 years (minimum 20 years, maximum 93 years).

There was a significant correlation between D-dimer and TTV in the Pearson correlation test (p=0.009, r=0.342). D-dimer level was ranging between 0.16 and 22.94 (mean value 6.16±5.04). TTV and total thrombus density (TTD) were calculated with a mean value of 23.40±60.63 ml (min: 1.46 mL, max: 404.12 ml) and 66.16±38.48 HU (min: -67.70 HU, max: 213.50 HU), respectively (Figure 5). TTD was automatically calculated at the workstation (Vitrea 2 workstation; Vital Images, Minnetonka, MN, USA) when the volume was measured (Figure 1). Demographic properties and CT measurement values of the study population are shown in Table 1.

The thickness of the interventricular septum was measured with the mean value of 13.46±11.64 mm.

Interventricular septal angles were measured as 47.88±22.00 degrees. There was no statistically significant correlation between angles and TTV.

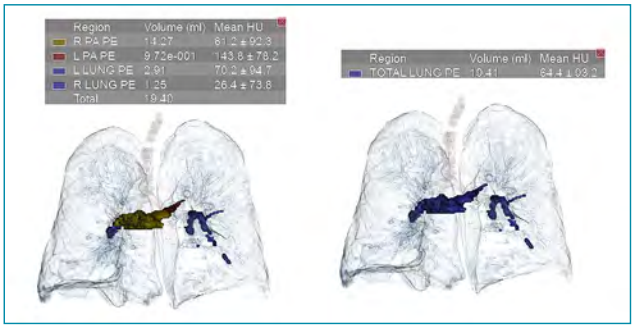

Figure 3. The measurement of thrombus volume (load) and density at main, right, left pulmonary arteries are shown. The thrombus volume of segmental arteries in both lungs is defined as lung PE (A). Total thrombus volume and mean density of total thrombus volume (B).

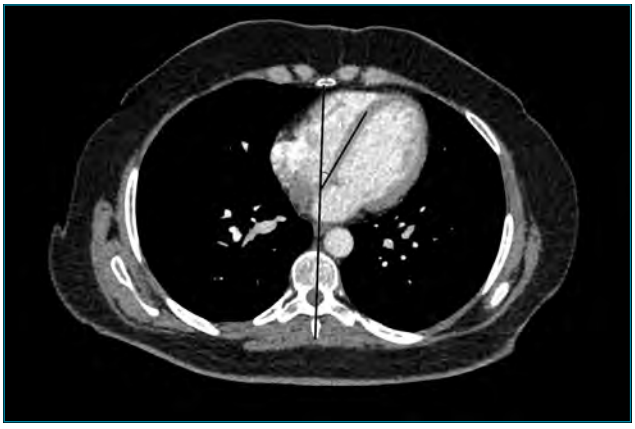

Figure 4. The angle between the connecting line from the midpoint of the sternum to the thoracic vertebral spinous process and interventricular septum.

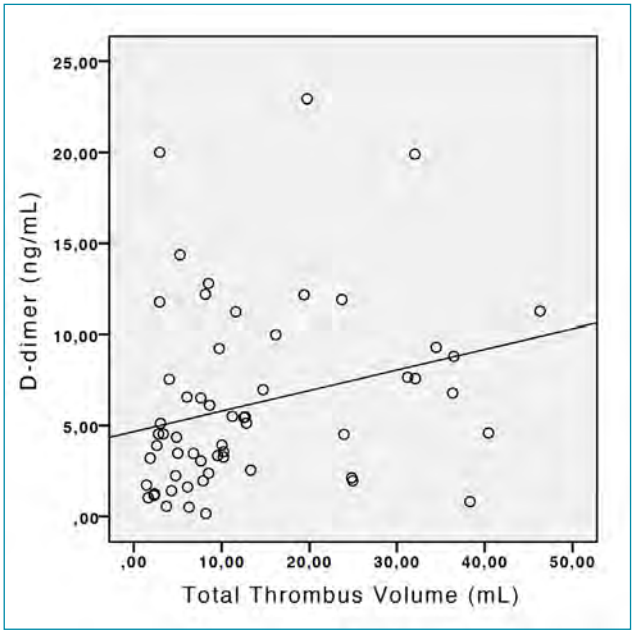

Figure 5. Shows a significant correlation between D-dimer and total thrombus volume (p=0.009, r=0.342).

In the non-parametric Spearman test, there was a significant correlation between D-dimer and TTV (p=0.009). A significant correlation was found between TTV and the hematocrit level (p=0.034) as well as C-reactive protein (CRP) (p=0.044).

Interventricular septal angle, and left atrium (LA) and right atrium (RA) diameter showed a significant correlation with TTLV (p=0.023, p=0.037, and p=0.002, respectively).

RV/LV ratio showed positive correlation with the D-dimer level (p=0.02).

DISCUSSION

In PE, although the diagnosis is based on clinical findings, laboratory and imaging findings are crucial. The amount, the location of the thrombus, and the degree of obstruction affect survival[11,12]. In our study, RV/LV ratio showed positive correlation with D-dimer level and a significant correlation was found between D-dimer and TTV.

In some studies, thrombus load has been shown to be predictive of mortality and determination of hemodynamic severity in patients with acute PE[13,14]. By using measurement parameters (including vascular obstruction, RV/LV ratio, minimum LV diameter, and diameter of the central PA), the thrombus load can be evaluated in the determination of hemodynamic severity[14]. Also, quantitative cardiac CT measurements obtained on axial CT images (RV short axis, LV short axis, and particularly the RV/LV short axes ratio) have shown a significant positive (RV short axis, RV/LV diameter ratio) or negative (LV short axis) correlation with the severity of PE[14] or with fatal outcomes[15,16].

Table 1. Demographic properties and CT measurement of the study population.

	Unit	Value
Age	years	60.14±17.92
Female	n (%)	36 (62.1)
White blood cell count	x10^9/L	9.4±4.4
D-dimer	µg/mL	6.11±5.05
CRP	mg/dL	63.06±88.94
Platelet	x10^3/mL	251.8±101.01
PDW	%	15.15±3.28
TTV	mL	23.40±60.64
TTD	HU	66.17±38.49
Interventricular septum thickness	mm	13.47±11.65
Septal angle	degree	151.88±22.00

TTV: total thrombus volume, TTD: total thrombus density, CRP: C-reactive protein, PDW: platelet distribution width.

The results of the studies of patients with PE revealed that signs of RV strain at CTPA (RV/LV diameter ratio >1, leftward septal bowing) had a sensitivity of 78–92%, specificity of 100%, and positive predictive value of 100% comparing to echocardiographic findings for the detection of RV dysfunction. Other studies predicted a severe PE when the RV/LV diameter ratio was more than 1.5[14-16]. In the current study, the diameter ratio of RV/LV in 29 of 58 patients (50%) with mean value was calculated as 1.17±0.48. RV/LV ratio also correlated with D-dimer level. Therefore, increased D-dimer level may indicate an increase in right ventricular tension.

An RV/LV diameter ratio calculation on 4CH CT images greater than 0.9 has been shown to have association with a sensitivity of 83% and specificity of 49% for predicting the occurrence of adverse clinical events (for example 30-day mortality or the need for cardiopulmonary resuscitation, mechanical ventilation, vasopressors, thrombolysis, or embolectomy)[17]. Schoepf et al. reported a higher mortality rate in patients with increased RV/LV diameter ratio of more than 0.9 compared to not increased or equal to 0.9, calculated in PE patients on 4CH images. RV enlargement had a sensitivity, specificity, positive predictive value, and negative predictive value of 78.2, 38, 15.6, and 92.3%, respectively, for prediction of 30-day mortality[18].

Similarly, to our study, Kaufman et al. evaluated the reproducibility of PE clot volume quantification using CTPA in a multicenter setting. They used anonymized CTPA data of 23 patients acquired from 23 scanners of 18 imaging centers. Two independent analysts measured PE volumes using a semi-automated region growing algorithm. TTV was calculated per patient as the primary endpoint and individual thrombus volume (ITV), Qanadli score, and modified Qanadli score per patient as secondary endpoints. Inter- and intra-observer reproducibility were evaluated using intra-class correlation coefficient (ICC) and Bland- Altman analysis. As results, clot volumes ranged from 0.0041–47.34 mL (mean±SD, 5.93±10.15 mL), found by Analyst 1. On the second read, analyst 1 found the same number and distribution of emboli with a range of volumes for read 2 from 0.0041–45.52 mL (mean±SD, 5.42±9.53 mL). For Analyst 2, thrombus volumes ranged from 0.00459–46.29 mL (mean±SD, 5.91±10.06 mL). Inter- and intra-observer variability measurements indicated excellent reproducibility of the semi-automated approach for quantifying PE volume load. ICC for all endpoints was greater than 0.95 for inter- and intra-observer analysis. No significant biases were indicated by Bland-Altman. They concluded that, the semi-automated region growing algorithm for quantifying PE was a suitable method for image analysis in multicentered clinical trials and was reproducible using data from multiple scanners[9]. By the methodology used in the study,

they mentioned that the changes in absolute thrombus volume greater than about 2 mL or 5% regardless of the starting size of the clots, should be pick up. This could be helpful to estimate sample size requirements for clinical trials using clot burden quantification as an endpoint in PE treatment studies. In the present study, the mean value of TTV was measured as 23.40±60.63 mL.

The current study was designed as a retrospective study and the patients enrolled were those with acute onset PE. Measurements were only performed on CTPA images statically, no dynamic investigation method was used in the current study such as wall motion abnormality and ejection fraction calculation in ECG or pulmonary wedge pressure at pulmonary catheter angiography. The acquisitions were not done with ECG-gating method.

D-dimer becomes active after 24–48 hours and then decreases. It may decrease after the patient applies to the hospital; thus, a low D-dimer level might have been analyzed. In this retrospective study, although TTV correlates with D-dimer level, larger prospective studies are needed.

There are some limitations in the in the current study. Firstly, the only diametric measurements of the cardiac chambers were performed in 4CH plane and the ratio of RV/LV was calculated. Measurements of the thrombus volume and determination of its borders were made in contrast enhanced images using semi-automated method and manual drawing option; the thrombus volume in the right, left, and total lungs were calculated, though not separately in segmental branches by lobe. Therefore, the measured thrombus could show variations in density. Secondly, due to the retrospective design nature of the study in the emergency condition, the acquisitions were not performed by ECG-gating method and the cardiac measurements were done at a single cardiac phase. The images with the largest ventricle diameter at diastolic phase were selected for the evaluation. However, it could be better to do it with ECG-triggering technique.

CONCLUSIONS

Many studies showed thrombus volume to not be a significant determinant of outcomes; rather, the right heart strain is the best predictor. On the other hand, the current study showed TTV and RV/LV ratio had a positive correlation with increasing D-dimer level. Therefore, increased D-dimer levels with RV/LV ratio and their correlation with TTV suggest that it may be a prognostic factor, but larger prospective studies are needed to reveal this more clearly.

AUTHORS' CONTRIBUTIONS

HS: Conceptualization, Data Curation, Writing – Original Draft, Writing – Review & Editing. **LCM:** Conceptualization, Formal Analysis, Writing – Review & Editing.

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ORIGINAL ARTICLE
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Lower LDL-cholesterol levels associated with increased inflammatory burden in patients with acute ST-segment elevation myocardial infarction

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SUMMARY

OBJECTIVE: Association of low-density lipoprotein cholesterol and highly sensitive C-reactive protein in ST-elevation myocardial infarction patients was assessed in this study.

METHODS: 591 consecutive patients who were hospitalized with a diagnosis of ST-elevation myocardial infarction were enrolled and assigned into tertiles according to their serum low-density lipoprotein cholesterol levels. Differences in highly sensitive C-reactive protein among low-density lipoprotein cholesterol tertiles and correlations between highly sensitive C-reactive protein and low-density lipoprotein cholesterol were assessed.

RESULTS: Highly sensitive C-reactive protein levels differed significantly among the groups (p<0.001) and found to be highest in the low-density lipoprotein cholesterol tertile 1 and lowest in the low-density lipoprotein cholesterol tertile 3 (post-hoc p-values: tertile 1 vs. 2 <0.001; tertile 1 vs. 3 <0.001; tertile 2 vs. 3=0.019). There was a negative correlation between hs-CRP and both low-density lipoprotein cholesterol (r=-0.332, p<0.001) and total cholesterol (r=-0.326, p<0.001). There was also a negative correlation between highly sensitive C-reactive protein and high-density lipoprotein cholesterol, though the strength of this relationship was weak (r=-0.103, p=0.014).

CONCLUSION: Lower low-density lipoprotein cholesterol levels are associated with higher inflammatory burden in patients with acute STEMI. Further studies are required to elucidate the significance of low-density lipoprotein cholesterol levels in ST-elevation myocardial infarction settings.

KEYWORDS: ST elevation myocardial infarction. Cholesterol, LDL. Hs-CRP.

INTRODUCTION

Elevated low-density lipoprotein cholesterol (LDL) is one of the most emphasized risk factors for cardiovascular disease (CVD). Results of the studies evaluating the effect of statins on LDL reduction led up to the motto "the lower the better" for prevention of CVD[1,2]. Inversely, lower LDL levels were noticed in acute myocardial infarction (AMI) patients for more than 50 years[3,4]. Subsequent studies revealed that serum cholesterol levels fall rapidly after an AMI such that serum LDL decreased 48% below baseline on the 7th day of AMI[5-8]. Moreover, several studies showed worse clinical outcomes in AMI and heart failure patients with lower total cholesterol (TC) and LDL levels indicated a "cholesterol paradox"[9-14]. Previously, several clinical studies demonstrated

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the acute phase reactant properties of serum lipoproteins in bacterial and viral infections in humans[15,16]. Alterations in LDL levels in heart failure and its prognostic impact were also largely explained by acute phase reactant properties of serum lipoproteins[10]. Thus, LDL levels may have the potential to be an inflammatory marker in AMI. However, association of LDL and inflammatory burden in AMI patients were not assessed before. Thus, the relationship of serum LDL with highly sensitive C-reactive protein (hs-CRP) in patients with ST-segment elevation myocardial infarction (STEMI) was investigated.

METHODS

We retrospectively enrolled 591 consecutive patients who were hospitalized with a diagnosis of STEMI. Patients were enrolled into the study if their hs-CRP levels and fasting lipid profiles were available within 24 hours after the onset of symptoms. Medical history of the patients were obtained from medical records. Forty six patients were excluded from the study due to the use of lipid-lowering medications. In order to perform analysis, the patients were assigned into tertiles according to their serum LDL levels. STEMI was defined as ≥1 mm ST-segment elevation in at least two contiguous electrocardiogram leads, except V2-V3, which required 1.5 mm for female patients, 2 mm for male patients >40 years of age, and 2.5 mm for male patients <40 years old or new onset left bundle-branch block in the presence of ischemia symptoms. A transthoracic echocardiography was performed 48 to 72 hours after admission using a Vivid 7 system (GE Medical Systems, Milwaukee, WI, USA) and the left ventricular ejection fraction was calculated by the modified Simpson method. Serum levels of hs-CRP were measured using the latex enhanced immune-turbidimetric method (Cardio-Phase High Sensitivity C-Reactive Protein; Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA). Serum TC, triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations were analyzed by a BM-Hitachi-747 auto-analyzer (Boehringer Mannheim GmbH, Mannheim, Germany). Serum LDL-C values were estimated by the formula of Friedewald et al. or directly measured if TG>400 mg/dL[17]. The study protocol was approved by the Local Ethics Committee.

SPSS Statistics, version 17.0 (SPSS Inc, Chicago, IL), was used for statistical analysis. Kolmogorov-Smirnov test was used to determine the distribution patterns. Data were presented as mean and standard deviation, median and interquartile range, or proportions as appropriate. The one-way ANOVA was used to compare data with normal distribution and Kruskal-Wallis test was used to compare the data without normal distribution. Bonferroni correction was used for multiple comparisons. Pearson's correlation analysis was used to assess the correlation between hs-CRP and serum lipid parameters. Categorical variables were compared with the χ^2 test. A two-tailed p-value<0.05 was considered to be statistically significant.

RESULTS

Every LDL tertile contains 197 patients. Mean age of the participants was 61.71. There was no difference in regard to demographic characteristics among the LDL tertiles. Time to lipid measurement was also similar. Mean LDL level was 75.59±17.207 for tertile 1, 115.79±9.487 for tertile 2, and 161.61±25.968 for tertile 3 (Table 1). hs-CRP levels differed significantly among the groups (p<0.001) and found to be highest in tertile 1 and lowest in tertile 3 (post-hoc p-values: tertile 1 *vs.* 2 <0.001; tertile 1 *vs.* 3 <0.001; tertile 2 *vs.* 3 =0.019). Mean troponin T level was 25.3±14.7 in tertile 1, 19.5±15.5 in tertile 2, and 16.6±12.2 in tertile 3 (p=0.01). There was a negative correlation between hs-CRP and both LDL (r=-0.332, p<0.001) and TC (r=-0.326, p<0.001). There was also a negative correlation between hs-CRP and HDL, though the strength of this relationship was weaker (r=-0.103, p=0.014). There was no correlation between hs-CRP and TG. (Figure 1)

DISCUSSION

In the present study, the association of serum LDL with hs-CRP in STEMI patients was association and it was found that hs-CRP levels are higher in the lowest LDL tertile and lower in the highest LDL tertile. In addition, there was a negative correlation between LDL and hs-CRP in this patient group. Moreover, hs-CRP was negatively correlated with both TC and HDL. Troponin T levels were also higher in the lowest LDL tertile and lower in the highest LDL tertile. To the best of our knowledge, this the first study in the literature revealing the inverse association of hs-CRP with serum LDL in STEMI patients.

Previous studies showed that marked changes in serum lipoproteins occur during the course of AMI including reductions in TC, LDL, and HDL and increases in TG[4-8]. Infarction size is probably important in reducing LDL, as shown in the study of Rott et al., which suggested that LDL levels decrease significantly after an AMI and the reduction is correlated with cardiac troponin T levels[18]. Several mechanisms were proposed to explain the lipoprotein alterations after AMI. First, it was shown that acute phase response causes up-regulation of LDL receptor

Table 1. Demographic, clinical, and laboratory characteristics of study participants.

	Tertile 1, n=197	Tertile 2, n=197	Tertile 3, n=197	p-value
Age (years)	62.93±11.62	61.79±13.05	60.43±11.39	0.09
Male gender – n (%)	154 (78.2)	136 (69.90)	133 (67.5)	0.055
Body mass index (kg/m^2)	28.0±4.6	28.0±4.3	28.0±4.4	0.319
Hypertension – n (%)	80 (40.6)	88 (44.7)	83 (42.1)	0.712
Diabetes – n (%)	77 (39.1)	62 (31.5)	62 (31.5)	0.183
Smoking – n (%)	71 (36.0)	93 (47.2)	100 (50.8)	0.009
Prior MI – n (%)	19 (9.6)	8 (4.1)	14 (7.1)	0.092
Prior stroke – n (%)	8 (4.1)	5 (2.5)	6 (3.0)	0.600
Glucose (mg/dL)	160.5±83.9	153.6±81.5	143.6±72.5	0.107
Creatinine (mg/dL)	1.12±26	1.09±25	1.03±21	0.001
CK-MB (mg/dL)	75.14±39.76	77.88±39.41	76.71±39.11	0.955
Troponin T	25.3±14.7	19.5±15.5	16.6±12.2	0.010
Cholesterol (mg/dL)	143.0±23.6	187.8±17.8	241.8±41.9	<0.001
LDL-C (mg/dL)	75.59±17.2	115.79±9.5	161.61±25.9	<0.001
HDL-C (mg/dL)	38.33±9.922	41.45±9.312	42.84±9.613	<0.001
Triglyceride (mg/dL)	142.1±78.3	152.6±76.6	185.7±110.4	<0.001
Hs-CRP (mg/L)	7.19±3.38	5.93±2.98	5.09±2.76	<0.001
Hemoglobin (g/dL)	14.16±1.53	14.43±1.46	14.54±1.44	0.061
WBC (x10,000/mL)	10.89±3.50	10.85±3.42	10.59±3.33	0.639
Platelets (x1,000/mL)	222.29±65.48	240.46±62.38	243.05±59.47	0.002
LVEF (%)	46.20±11.78	48.44±9.44	48.26±9.93	0.080
Pulse rate (min^{-1})	79.93±15.54	79.89±14.83	79.16±13.56	0.842
Systolic BP (mmHg)	124.97±22.01	131.39±24.03	132.32±26.94	0.005
Diastolic BP (mmHg)	76.43±13.073	79.17±13.425	79.71±15.062	0.044
Syntax Score	16.69±10.51	15.36±9.25	15.08±9.01	0.210
Time to lipid measurement (h)	6 (4-8)	7 (4-8)	6 (4-8)	0.374
Initial Treatment				
Medical-Fibrinolytic	6 (3.0)	4 (2.0)	9 (4.5)	0.356
PCI-stent	162 (82.2)	172 (87.3)	168 (85.3)	
CABG	29 (14.8)	21 (10.7)	20 (10.2)	

MI: myocardial infarction; CK-MB: creatinine kinase myocardial band; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; hs-CRP: high sensitivity C-reactive protein; WBC: white blood cell; LVEF: left ventricular ejection fraction; BP: blood pressure; PCI: percutaneous coronary intervention; CABG: coronary artery by-pass graft operation.

activity and reduction in some HDL regulatory proteins[19,20]. In addition, myocardial necrosis facilitates adrenergic-mediated adipocyte lipolysis leading to free fatty acid mobilization, increased hepatic very low density lipoprotein (VLDL) secretion, TG elevation, and alteration in LDL and HDL particle composition[21,22]. Other possible contributors to lipid changes after AMI include in-hospital therapy and lifestyle changes such as heparin, which causes lipoprotein lipase-mediated TG hydrolysis, beta-blockers which suppress hormone-sensitive lipase, postural effects and reduction of saturated fat intake[23-28].

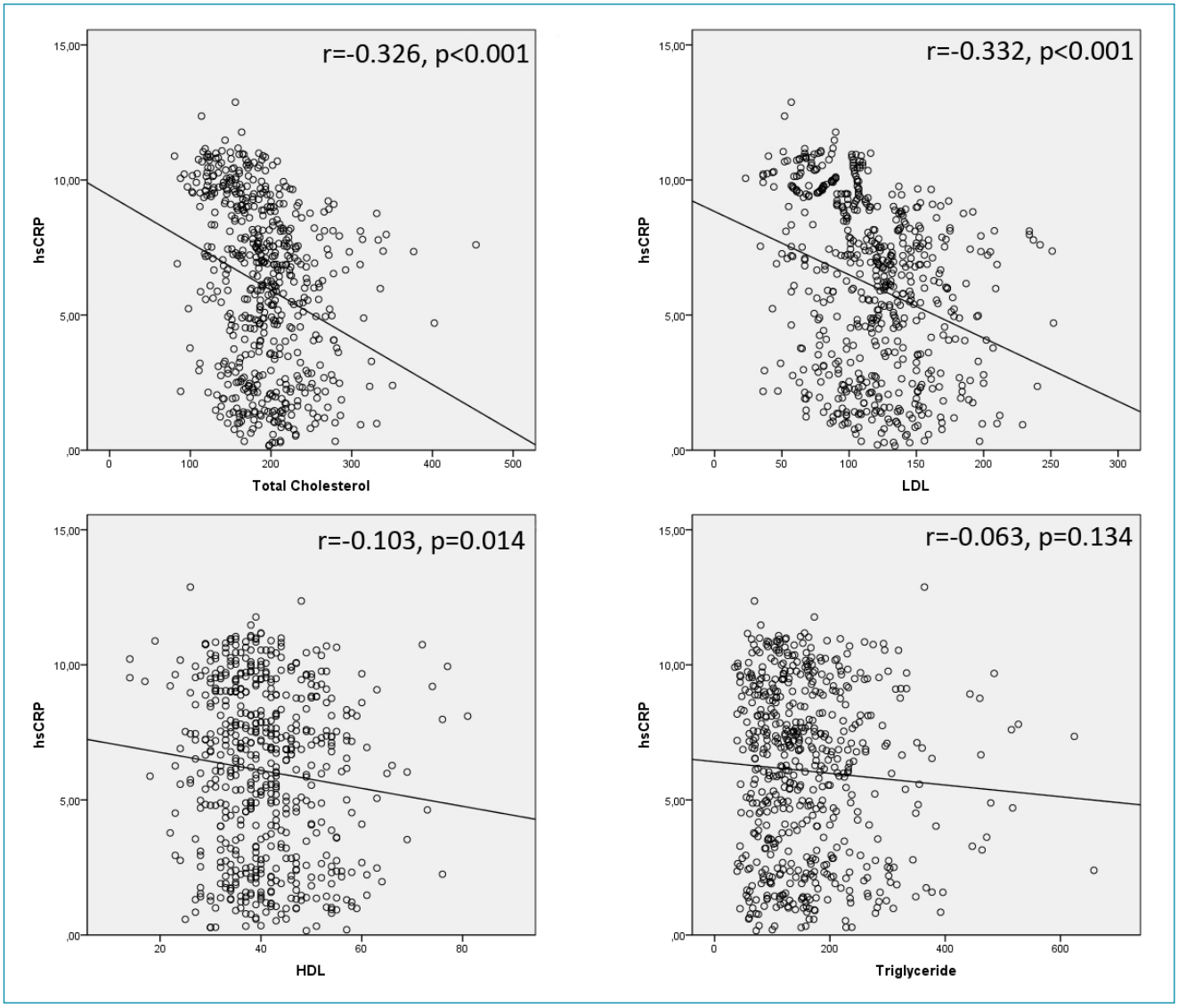

Figure 1. Scatterplots summarizing the correlations between hs-CRP and serum lipoproteins.

Impact of the low cholesterol levels on the prognosis of patients with CVD was investigated in some studies previously. In one of them, Richartz et al. found out that lower cholesterol levels were associated with poor survival in patients with advanced heart failure on mechanical support[9]. In another, Rauchhaus et al. demonstrated that lower cholesterol levels are associated with poor clinical outcome in patients with chronic heart failure regardless of the heart failure etiology. In addition, negative correlation of serum cholesterol and tumor necrosis factor alpha was revealed in this study[10]. A similar result was found in the study by Norwich et al., which showed that low serum TC, LDL, HDL, and TG are related to increased mortality in advanced heart failure[11]. In two studies about low LDL levels in myocardial infarction, Al-Mallah et al. found out that low LDL levels are associated with increased 3-year mortality in non STEMI and Reddy at al. demonstrated that low LDL levels are associated with higher in-hospital mortality after AMI[12-14].

Results of the present study revealing the relationship of hs-CRP and lipoprotein levels, support the hypothesis that AMI patients with lower LDL have higher inflammatory burden and acute phase response is responsible for lipoprotein changes after AMI. Cho et al. demonstrated that life-saving medications, including lipid-lowering drugs, were underused in MI patients with lower admission LDL levels[13]. Considering these patients have higher hs-CRP levels, hesitation to initiate adequate doses of lipid-lowering therapy due to low LDL levels may contribute to a worse prognosis.

The present study has several limitations that should be taken into account when interpreting its results. First, it is a retrospective and single center study. However, all consecutive cases in a period of time were enrolled in order to eliminate selection bias. In addition, preadmission serum lipoprotein levels of the patients were not available and serial measurements of LDL were not performed. Thus, exact changes in serum lipoproteins due to AMI were not available.

CONCLUSIONS

Results of the present study suggest that acute STEMI patients with lower LDL levels have higher inflammatory burden reflected by higher hs-CRP levels. Further prospective studies are needed to confirm this hypothesis and elucidate the clinical significance of the findings of this study.

AUTHORS' CONTRIBUTIONS

EA: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **SKA:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **BY:** Conceptualization, Data Curation, Writing – Original Draft, Writing – Review & Editing. **AK:** Conceptualization, Formal Analysis, Writing – Original Draft, Writing – Review & Editing.

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ORIGINAL ARTICLE
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Parathyroid hormone levels after parathyroidectomy for secondary hyperparathyroidism

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SUMMARY

OBJECTIVE: The parathormone level after parathyroidectomy in dialysis patients are of interest. Low levels may require cryopreserved tissue implantation; however, the resection is necessary in case of recurrence. We analyzed post parathyroidectomy parathormone levels in renal hyperparathyroidism.

METHODS: Prospective observation of postoperative parathormone levels over defined periods in a cohort of dialysis patients that underwent total parathyroidectomy and immediate forearm autograft from 2008 to 2010, at a single tertiary care hospital.

RESULTS: Of 33 patients, parathormone levels until 36 months could be divided into four patterns. Patients with stable function (Pattern 1) show relatively constant levels after two months (67% of the cases). Early function and later failure (Pattern 2) were an initial function with marked parathormone reduction before one year (18%). Graft recurrence (Pattern 3) showed a progressive increase of parathormone in four cases (12%). Complete graft failure (Pattern 4) was a nonfunctioning implant at any period, which was observed in one patient (3%). Parathormone levels of Pattern 3 became statistically different of Pattern 1 at 36 months.

CONCLUSIONS: Patients that underwent the total parathyroidectomy and autograft present four different graft function patterns with a possible varied therapeutic management.

KEYWORDS: Parathyroid glands. Hyperparathyroidism. Hyperparathyroidism, secondary. Parathyroidectomy. Parathyroid hormone.

INTRODUCTION

Secondary hyperparathyroidism in dialysis patients (2HPT) may require parathyroidectomy, especially in developing countries, where 10.7% of patients under dialysis have parathyroid hormone (PTH) levels greater than 1,000 pg/mL[1]. Parathyroidectomy is effective, and its costs are lower than the use of cinacalcet in the long-term[2]. Surgical treatment may improve the long-term survival in dialysis patients with advanced 2HPT[3].

In general, these patients are seen by nephrologists after surgery with great relief of bone pain and other symptoms[4].

Recently, the Kidney Disease: Improving Global Outcome (KDIGO) guidelines suggested that PTH should be maintained between two and nine times the upper limit of the method[5]. The best postoperative PTH levels have not been defined yet[6,7].

We performed a prospective observational study of a cohort of patients with severe 2HPT to observe the PTH levels after parathyroidectomy. A classification of patients in four different patterns could be suggested, with possible helpful clinical implications.

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METHODS

We included dialysis patients operated for 2HPT from January 2008 to February 2010 and followed up for 36 months in a single tertiary university institution. The Institutional Review Board approved the study. All participants provided their informed consent.

Intended total parathyroidectomies and immediate heterotopic forearm parathyroid autografts were performed. Pieces of the less diseased parathyroid were selected macroscopically and autografted in the forearm, with multiple or single pockets, as described in previous papers[8].

The analysis included the age at parathyroidectomy, gender, parathyroid graft non-function, graft recurrence (defined here as graft-dependent non-suppressible elevated levels of PTH six months after the parathyroidectomy), and implantation of cryopreserved tissue. We studied the following biochemical parameters before and after the operation: total calcium (tCa, reference range 8.6–10.2 mg/dL), ionized calcium (iCa, 4.6–5.3 mg/dL), phosphorus (P, 2.7–4.5 mg/dL), and PTH (16–87 pg/mL). Preoperative alkaline phosphatase (AP, 35–104 U/L, for women and 40–129 U/L for men) and 25-hydroxy-vitamin D3 (Vitamin D, 30–100 ng/mL) were also included, when available.

Individual systemic levels of PTH (sPTH) were considered before the operation, until the first 15 days and, 1, 2, 3, 4, 6, 9, 12, 18, 24 and 36 months after the operation. We initially searched for possible different patterns of autograft profile for graft non-function or hypofunction (low or undetectable PTH), adequate function (PTH in normal or target levels, with long-standing stability), and graft recurrence (high non-suppressible sPTH)

RESULTS

There were 33 patients (19 males and 14 females) who were followed-up properly during the study period. Their ages ranged from 24 to 62 years (mean=45.2, SD=10.1). Preoperative laboratory results are presented in Table 1.

Some different patterns of sPTH levels attributable to implant secretion were observed until 36 months of follow up (Table 2).

In 22 cases (67%), there was a relatively stability in systemic PTH (Pattern 1). Interestingly, two cases had a significant decrease of sPTH when iatrogenic hypercalcemia ensued (values of 0 and 3 pg/mL). After normalization of calcium, PTH levels were 98 and 104 pg/mL. These two events occurred more than 12 months postoperatively. Conversely, in 15 cases, isolated or repetitive episodes of hypocalcemia did not increase sPTH (median values at 6, 12, 24 and 36 months were, respectively, 42, 40.5, 30, 30). In two cases, the attempt to improve PTH levels with cryopreserved parathyroid tissue had no impact in the PTH levels in one of them (21 months of storage) and a possible function in the other one (23 months of storage).

Six patients (18%) presented initial good implant function. However, very low or undetected levels of systemic PTH ensued after 12 months, despite significant hypocalcemic stimulus (Pattern 2). Of these, two had cryopreserved parathyroid autografting at nine and 23 months postoperatively. Only the latter implant showed evidence of good function. After repeated undetectable sPTH, there was detectable graft and sPTH six months after transplantation: 1672 and 17pg/mL, respectively. There was a sustained systemic level at least after 16 months of the implant. This patient had a biopsy of the failed graft during cryopreserved transplantation. There was interstitial fibrosis.

There was a progressive elevation of sPTH levels in four cases (Pattern 3). In two of these patients, PTH levels were still acceptable according to KDIGO recommendations (248 and 185 pg/mL), and they were under observation before a possible graft excision. In one patient, high PTH was considered clinically significant because of sustained hypercalcemia and this graft was eventually excised at the 38th month after surgery, when PTH was 471 pg/mL and tCa was 10.7 mg/dL. The last case is still under investigation: despite sPTH level as high as 1,006 pg/mL (simultaneous graft value of 2,063 pg/mL), marked and sustained hypocalcemia (tCa=7.8 mg/dL and iCa=3.84 mg/dL) is still present. Bone biopsy of this patient at the 33rd month was compatible with osteoporosis and mixed bone disease.

In only one patient, all postoperative PTH values were zero, even after cryopreserved tissue grafting at the 18th month (Pattern 4). This patient had a preoperative PTH of 1,507 pg/mL.

Patients with progressive increase of PTH levels (tendency to recurrence) had a non-significant higher level of PTH when compared to stable patients until 24 months, except at the 18th month (p=0.04, Mann-Whitney's test). Figure 1 shows that at the 36th month, the difference became clearly significant (p=0.003, Mann-Whitney's test).

The highest ratios between PTH measured in the autograft arm and sPTH at 6, 12, 24 and 36 months were 146, 74, 89, and 74. In Pattern 3, the highest ratios were 15, 3, 6, and 2, respectively. In Pattern 1, the respective ratios were 146, 74, 89, and 74. In Pattern 2, ratios were 109, 12, 16, and 73.

Altogether, seven patients underwent renal transplantation after the parathyroidectomy (21.2%). The mean time from the parathyroid operation to kidney transplantation was 20 months (SD=8.3; 95%CI 12.3–27.7). One male patient lost the kidney graft a few days after receiving it, and he restarted dialysis (he is in Pattern 1 group). All the others are doing well. Of these successful cases, five were in Pattern 1 and one in Pattern 4. The exclusion of these cases from the analysis did not affect the results of previous comparisons of Patterns 1, 2, and 3.

DISCUSSION

In the present study, we showed that autograft parathyroid tissue in 2HPT has a stable tendency in most patients, at least until 36 months after the operation. This tissue seems to be sensitive to calcium, with marked decrease in PTH secretion during periods of unintentional iatrogenic hypercalcemia. Even though uremic milieu is considered a constant stimulation to parathyroid hyperplasia, these implants are only capable of secreting a maximum quantity of PTH. One possible explanation for these observations is that the implanted tissue is still not autonomous and its response is physiological, as noted in other studies[9]. In these cases, the upper limit of secretion is most likely determined by the autografted tissue mass. Interestingly, this tissue seems to be rather "resistant" to continuous stimulation of uremia. Consequently, letting patients hypocalcemic will not elevate the PTH significantly. In that case, the only possibility to elevate sPTH to target levels of KDIGO would be cryopreserved tissue grafting. A cryopreserved tissue may present function even after long periods of storage in some patients[10]. Possibly, a long delay from harvesting until cryopreservation processing affected that result[11].

Uremic stimulation is commonly considered to lead to a progressive increase in the PTH levels over time, although it occurs in few cases, only 12% in our series. This observation requires caution when considering total parathyroidectomy without immediate autograft in all patients under dialysis[12]. Low levels of PTH may have deleterious effects[13].

Another fact described herein and in previous papers suggest that despite good initial function, late failure is possible[14]. The frequency of this problem seems to be clinically relevant, affecting almost one in five patients. The cause is unclear, but progressive fibrosis encircling the graft may be questioned. It seems wise to keep cryopreserved parathyroid tissue for periods not lower than one year[15].

Some patients presented a considerably higher rate of PTH secretion by the graft, which is not affected by unintentional iatrogenic hypercalcemia, during the early periods of parathyroidectomy (data not shown). In these cases, there may be a significant number of autonomous cells in the autografted tissue, as demonstrated by others[9]. In some cases, sPTH shows a progressive increase earlier, but the recurrence may not be clear until long-term follow-up. The PTH should be measured at least once a year to identify the recurrence before any symptom or consequence occurs.

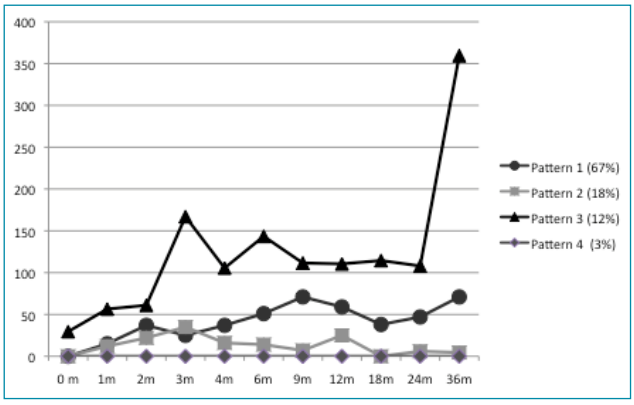

Figure 1. Patterns of implant function after the parathyroidectomy. Each point represents the median value at different periods.

Table 1. Preoperative biochemical parameters of patients undergoing parathyroidectomy.

	tCa (mg/dL)	iCa (mg/dL)	P (mg/dL)	PTH (pg/mL)	tCa X P (mg^2/dL^2)	AP (U/L)	Vit D (ng/mL)
minimum	7.6	4.1	2.0	788	20.4	66	7.8
maximum	12.4	5.7	9.4	5439	102.5	1694	50.1
mean (SD)	10.0 (0.9)	5.1 (0.4)	5.8 (1.6)	2036* (1528–3056)	58.8 (18.6)	432* (221–636.5)	24.7 (11.4)

*Median and interquartile range for non-parametric distributions by Kolmogorov-Smirnov Test. tCa: total calcium; iCa: ionized calcium; P: phosphorus; PTH: parathyroid hormone; tCa X P: calcium-phosphorus product; AP: alkaline phosphatase; Vit D: vitamin D.

Table 2. Median PTH values and interquartile range (pg/mL) before and after the parathyroidectomy. Time in months after the operation (m).

	Preoperative	0 m	1 m	2 m	6 m	12 m	24 m	36 m
Pattern 1 n=22	2026 1458–4098	0 0–40.8	15 0–39.5	37 10–85	51 25.5–89.2	59 28.5–94	47 23.5–113.5	71 30–100
Pattern 2 n=6	2138 2003-2421	0 0–14.2	19 4.5–311.8	26 13.5–107.3	28.5 9.5–94.8	21 8.2–59	3 0.0–15.8	4.5 0.8–10.5
Pattern 3 n=4	2075 1092–2492	29.50 0–166.3	56.5 7.5–123.5	61 47.25–277.3	143 2.75–297.8	110.5 58.75–646.8	108 53.75–772.8	359.5 200.8–872.3

Another useful piece of information is that PTH levels in graft arm vary widely during the follow-up. The specific site of puncture or different time of day may drastically affect the results. The simultaneous determination of the PTH levels in each forearm seems to be the most reliable method to obtain direct and useful evidence of graft function to predict recurrence[16,17]. Based on our present experience, graft arm PTH only indicates some graft function as the possible source of sPTH. Any decisions regarding autograft excision should be based only on repeated sPTH levels with or without suppression of graft arm secretion[18].

Despite rare, total failure of the graft is a significant problem, as absence of PTH is associated with adynamic bone disease[19]. The cause is still unclear. In a previous study of parathyroid ultrastructure before cryopreservation, tissue showed signs of irreversible cellular damage right after harvesting in one of 11 cases (9%)[11]. In some cases, the surgical approach may trigger irreversible cell damage.

It is hard to explain why a patient with very high levels of PTH (close to 1,000 pg/mL) presents hypocalcemia more than two years after parathyroidectomy. At this time, hungry bone is not expected. Perhaps, a large amount of 7–84 PTH fragments is present in this patient. These fragments are not able to improve calcemia or even may induce the patient's hypocalcemia[20]. The study of different circulating forms of PTH in similar cases may give interesting clues to parathyroid cell and PTH function.

In a prospective study, graft-dependent recurrence was 2.85%[21]. If we consider that only one case had graft excision up to the conclusion of this paper, we have a similar rate (3%). Interestingly, the same study has a graphic of PTH levels at different postoperative periods. Apparently, at 150 weeks (37 months approximately) after the operation, there were 5 out of 35 initial cases (14%) of increasing PTH[18]. This seems quite comparable to our observation of 12% of Pattern C, with levels markedly different at 36 months. The true comparison is difficult as individual data are not available[21].

Recurrence after parathyroid autograft in type 1 multiple endocrine neoplasia is apparently less frequent and occurs later than that observed in renal hyperparathyroidism[22]. This indicates that different parathyroid disease mechanisms affect graft function, and they are not comparable.

CONCLUSIONS

Patients that undergo total parathyroidectomy and autograft present four different patterns of graft function with possible different therapeutic management. Most patients evolved with stable parathyroid hormone levels.

AUTHORS' CONTRIBUTION

CPNJ: Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – Original Draft, Writing – Review & Editing. **SSA**: Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Review & Editing. **MRC:** Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Review & Editing. **LMMN:** Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Review & Editing. **MDGB:** Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Review & Editing. **RMAM:** Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Review & Editing. **FLMM:** Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – Review & Editing. **VJ:** Conceptualization, Formal Analysis, Investigation, Methodology, Project Administration, Supervision, Validation, Writing – Review & Editing.

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Combined effects of nutritional status on long-term mortality in patients with non-st segment elevation myocardial infarction undergoing percutaneous coronary intervention

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the performance of controlling nutritional status (CONUT) index, geriatric nutritional risk index (GNRI), and prognostic nutritional index (PNI) scores in predicting the long-term prognosis of patients with non-ST-elevated myocardial infarction (NSTEMI) who underwent percutaneous coronary intervention (PCI).

METHODS: A total of 915 patients with NSTEMI (female: 48.4%; mean age: 73.1±9.0 years) who underwent PCI at Adana Numune Training and Research Hospital, Cardiology Clinic between January 2014 and January 2015 were included in this cross-sectional and retrospective study. CONUT, GNRI, and PNI scores were calculated based on the admission data derived from samples of peripheral venous blood. The mean follow-up duration was 64.5±15.4 months.

RESULTS: During follow-up (mean 64.5±15.4 months), 179 patients (19.6%) died. The mean GNRI and PNI scores were significantly lower in the nonsurvivor group; however, the median CONUT score was significantly higher in the nonsurvivor group compared with the survivor group. The receiver operating characteristic (ROC) curve analyses have shown that GNRI score has similar performance to the CONUT score and has better performance than PNI score in predicting 5-year mortality. The Kaplan–Meier curve analysis has shown that patients with lower PNI or GNRI had higher cumulative mortality than the patients with higher PNI or GNRI. Also, the patients with higher CONUT scores had higher cumulative mortality compared with those with lower scores. The multivariate analyses have shown that GNRI (HR: 0.973), PNI (HR: 0.967), CONUT score (HR: 1.527), and body mass index (BMI) (HR: 0.818) were independent predictors of the 5-year mortality in patients with NSTEMI.

CONCLUSION: In this study, we have shown that CONUT score, GNRI, and PNI values were associated with the long-term mortality in patients with NSTEMI who underwent PCI, and GNRI yielded similar results to CONUT score but was better than PNI.

KEYWORDS: Controlling Nutritional Status, Geriatric Nutritional Risk Index, Prognostic Nutritional Index, Non-ST-Elevated Myocardial Infarction, Percutaneous Coronary Intervention

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INTRODUCTION

Despite the evolving pharmacological treatments and reperfusion strategies, cardiovascular diseases still remain to be the leading cause of overall morbidity and mortality in the world. The majority of deaths linked to cardiovascular diseases is caused by acute coronary syndromes categorized into three conditions, namely, unstable angina pectoris, non-ST-elevated myocardial infarction (NSTEMI), and ST-elevated myocardial infarction (STEMI). Although patients with STEMI have higher in-hospital mortality, the long-term follow-up studies showed that the mortality rates increased significantly in NSTEMI cases over time[1,2]. Comprehensively designed studies that include the nutritional status indicate that the coexistence of cardiovascular diseases and malnutrition results in higher mortality rates[3]. In earlier studies, it has been shown that the malnutrition rate in hospitalized patients ranges 20–50% in developed countries and is higher in some geographical regions[4,5]. Controlling nutritional status (CONUT) index, geriatric nutritional risk index (GNRI), and prognostic nutritional index (PNI) are reliable and easily calculated nutritional indicators and have shown to have prognostic values in multiple chronic conditions[6,7]. In various studies, these indicators have demonstrated a strong association with the prognosis of patients with cardiovascular diseases and conditions, including coronary artery disease and congestive heart failure[8,9]. Among these studies, the researchers focused exclusively on acute myocardial infarction (AMI) or STEMI cases[10,11]. The clinical characteristics, risk factors, and prognosis of the NSTEMI differ significantly than the STEMI. The main differences are the increased mean age and the number of comorbid chronic diseases[12]. There are reports assessing the association of these nutritional indicators with the prognosis of NSTEMI cases exclusively. Given the noticeable differences compared with STEMI cases, the lack of a study including an analysis of CONUT score, GNRI, and PNI together may have foreclosed valuable information. The aim of this study was to investigate the performance of CONUT score, GNRI, and PNI in predicting the long-term prognosis of the patients with NSTEMI who underwent percutaneous coronary intervention (PCI).

METHODS

A total of 915 NSTEMI patients who underwent PCI at Adana Numune Training and Research Hospital, Cardiology Clinic between January 2014 and January 2015 were included in this cross-sectional and retrospective study. The diagnosis of NSTEMI was made according to the global myocardial infarction guide[13]. The data at the time of hospitalization, including age, gender, height, weight, body mass index (BMI), smoking, diabetes mellitus (DM), coronary artery disease, hypertension (HT), stroke, and cerebrovascular disease were collected from the patient profiles. The patients with congestive heart failure, malignancy, chronic kidney failure, nephrotic syndrome, liver failure, hematological disease, autoimmune disease, and rheumatological disease were excluded.

According to the standard criteria, admission values of patients with DM, HT, hyperlipidemia, and smoking were considered for selection. The subjects with HbA1c >6.5%, fasting blood glucose >126 mg/dL, or being on antidiabetic medication were considered as DM patients. The patients with arterial blood pressure >140/90 mmHg or being on antihypertensive medication were considered having HT. The patients with serum total cholesterol levels >200 mg/dL or on antilipidemic medicines were regarded having hyperlipidemia. The CONUT, GNRI, and PNI scores were calculated based on the admission data derived from samples of peripheral venous blood. GNRI=14.89×albumin (g/dL) + 41.7×body weight (kg)/ideal body weight (kg). The ideal body weight was calculated as follows: body height–100–[(body height–150)/4] for males and body height–100–[(body height–150)/2.5] for females. The CONUT scores (0–10, varying from nourishment to malnutrition) were calculated using serum albumin level and lymphocyte count. CONUT: serum albumin ≥3.5 g/dL=0 points, 3.0–3.4 g/dL=2 points, 2.5–2.9 g/dL=4 points, and <2.5 g/dL=6 points; total cholesterol ≥180 mg/dL=0 points, 140–179 mg/dL=1 point, 100–139 mg/dL=2 points, and <100 mg/dL=3 points; and total lymphocyte count ≥1600/mL=0 points, 1200–1599/mL=1 point, 800–1199/mL=2 points, and <800/mL=3 points (Table 1). PNI: 10×serum albumin value (g/dL) + 0.005 × total lymphocyte count (per mm^3). BMI: weight (in kg)/(height)^2 (in m). As the primary end point in this study, nonsurvivor details

Table 1. Controlling nutritional status (CONUT) score calculation.

Score	0	2	4	6
Serum albumin (g/mL)	≥3.5	3.0–3.49	2.5–2.99	<2.50
Score	0	1	2	3
Total cholesterol (mg/dL)	≥180	140–179	100–139	<100
Score	0	1	2	3
Lymphocytes (count/mL)	≥1600	1200–1599	800–1199	<800

were collected from the National Health Insurance System in July 2020, including the date of death, and all causes of death were accepted.

Statistical analyses

The continuous variables were represented as mean (± standard deviation) or median (25th–75th quartile). The distribution was analyzed with the Kolmogorov–Smirnov test. The Student's *t*-test was used to analyze the normally distributed variables, and the Mann–Whitney *U* test was used for the non-normal distributions. The variables with normal distribution were represented as mean (± standard deviation) and with non-normal distribution as median (25th–75th quartile).

The categorical variables were summarized as percentages and number. Categorical variables between the groups were compared by using the chi-squared test or Fisher's exact test. To demonstrate the sensitivity and specificity of the GNRI, PNI, and CONUT scores and their cut-off values for predicting the long-term mortality, the receiver operating characteristic (ROC) curves were used. The DeLong's method was used to compare area under the curve (AUC) of these nutritional indexes. The Kaplan–Meier analysis and log-rank test were performed to determine whether the nutritional indexes could help predict the long-term mortality. The multivariate Cox proportional hazards model was created to calculate the hazard ratios for all-cause mortality. Variables with $p \leq 0.1$ in the univariate analysis were included in the multivariate Cox proportional hazards model.

The statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS 20.0) for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc 15 statistical software (Ostend, Belgium). A $p<0.05$ was considered statistically significant.

RESULTS

Totally, 915 patients (female: 48.4%; mean age: 73.1±9.0 years) were included in this study. The mean follow-up duration was 64.5±15.4 months. During the follow-up period, 179 patients (19.6%) died. The baseline demographic and clinical characteristics of the patients were summarized in Table 2. Despite the

Table 2. Baseline characteristics of the study population.

	Survivor Group (n=736)	Nonsurvivor Group (n=179)	p
Age, year (mean ± SD)	72.8±9.4	74.1±7.1	0.084
Sex/female, % (n)	47.8 (352)	51.4 (92)	0.391
BMI (kg/m^2)	25.6±2.6	23.7±2.0	<0.001
Weight (kg)	71.4±8.2	70.1±7.4	0.055
Height (m)	1.67±7.2	1.77±8.1	<0.001
SBP (mmHg)	125±18	131±19	<0.001
DBP (mmHg)	78±10	78±11	0.638
Hypertension, % (n)	51.0 (375)	55.9 (100)	0.238
Diabetes mellitus, % (n)	32.3 (238)	33.0 (59)	0.873
Hyperlipidemia, % (n)	33.2 (244)	31.8 (47)	0.738
Stroke, % (n)	3.0 (22)	4.5 (8)	0.319
COPD, % (n)	7.2 (53)	3.9 (7)	0.130
Smoker, % (n)	36.0 (265)	34.6 (62)	0.732
Family history, % (n)	30.7 (226)	26.3 (47)	0.243
Previous myocardial infarction, % (n)	22.3 (164)	201. (36)	0.529
AF, % (n)	7.2 (52)	7.3 (13)	0.977
ASA, % (n)	20.1 (148)	22.9 (41)	0.407
ACEI/ARB, % (n)	32.1 (236)	28.5 (51)	0.355
Beta-blockers, % (n)	17.1 (126)	19 (34)	0.544
Diuretics, % (n)	5.2 (38)	7.8 (14)	0.168
Statins, % (n)	18.1 (133)	21.2 (38)	0.331

ACEI: angiotensin-converting enzyme inhibitors; ASA: acetylsalicylic acid; ARB: angiotensin receptor blockers; BMI: body mass index; CONUT: controlling nutritional status; GNRI: geriatric nutritional risk index; PNI: prognostic nutritional index; COPD: chronic obstructive pulmonary disease; AF: atrial fibrillation; SBP: systolic blood pressure; DBP: diastolic blood pressure. Statistically significant values are given in bold.

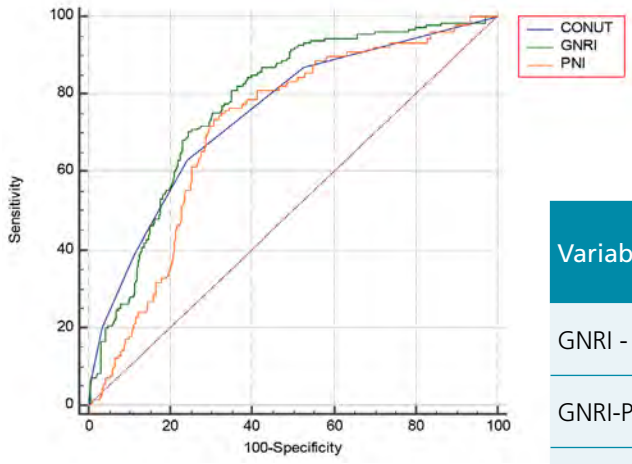
significantly higher mean age, height, and systolic blood pressure values in the nonsurvivor group, the survivor group displayed a higher mean BMI. The laboratory parameters of the two groups were summarized in Table 3. The median total cholesterol and mean albumin levels were significantly lower in the nonsurvivor group than the survivor group. The mean GNRI and PNI values were significantly lower in the nonsurvivor group; however, the median CONUT scores were significantly higher in the nonsurvivor group compared with the survivor group (Table 3). Figure 1 shows the ROC curve analyses of the scores. To predict mortality, the cut-off value for the PNI was ≤50.65, with 73.7% sensitivity and 69.4% specificity (AUC 0.714; 95%CI 0.684–0.744; p<0.001), the cut-off value for the GNRI was ≤114.77, with 81.0% sensitivity and 65.27% specificity (AUC 0.778; 95%CI 0.750–0.805; p<0.001), and the cut-off value for the CONUT score was >3, with 63.1% sensitivity and 76.0% specificity (AUC 0.751; 95%CI 0.722–0.779; p<0.001). The comparison of the ROC curve analyses shown that GNRI has similar performance to the CONUT score and has better performance than PNI in predicting 5 years mortality. Also, in predicting 5 years mortality, the prognostic values of the PNI and the CONUT scores were found similar (Figure 1).

The Kaplan–Meier curve analysis was performed for each nutritional index according to the cut-off value for all-cause mortality (Figure 2). Patients with lower PNI or GNRI had higher cumulative mortality than those with higher PNI or GNRI (37.0 *vs.* 8.4%, p<0.001; 36.2 *vs.* 6.6%, p<0.001, respectively). Patients with higher CONUT scores had higher cumulative mortality compared with those with lower scores (10.6 *vs.* 39.0%, p<0.001). Moreover, the cumulative 5-year mortality was higher in patients with the lowest quartile of GNRI (≤107.4) or PNI (≤46.43) than in patients with the top quartile of GNRI (≥125.1) or PNI (57.36). Furthermore, the cumulative 5-year mortality was higher in patients with the top quartile of CONUT score (≥4.0) than in patients with the lowest quartile (≤2.0) (Figure 3). The univariate and multivariate Cox proportional hazard analyses of predictors on mortality were summarized in Table 4. The multivariate analyses have shown that GNRI (HR: 0.973; 95%CI 0.964–0.982; p<0.001), PNI (HR 0.967; 95%CI 0.947–0.988; p<0.001), CONUT score (HR 1.527; 95%CI 1.404–1.661; p<0.001), and BMI (HR 0.818; 95%CI 0.767–0.872; p<0.001) were independent predictors of 5-year mortality in patients with NSTEMI.

Table 3. Baseline laboratory and echocardiography parameters of the study population.

	Survivor Group (n=736)	Nonsurvivor Group (n=179)	p
Albumin (g/mL)	4.1±0.58	3.9±0.32	<0.001
Total protein (g/mL)	7.6±0.6	7.5±0.30	0.104
Total cholesterol (mg/dL) median (25th–75th)	183 (155–210)	182 (163–207)	0.072
LDL-C (mg/dL) median (25th–75th)	123 (99–145)	122 (106–138)	0.602
HDL-C (mg/dL) median (25th–75th)	39 (34.2–47)	40.0 (35.0–45.0)	0.476
White blood count (×10³)	8.2±3.2	9.2±2.7	0.458
Platelet count (×10³)	245±65	233±91	0.053
Hemoglobin (mg/dL)	13.8±1.7	13.7±2.1	0.322
CRP (mg/dL) median (25th–75th)	0.7 (0.3–1.50)	1.0 (0.6–1.60)	0.558
Creatine median (25th–75th)	0.8 (0.7–1.0)	0.9 (0.7–1.1)	0.581
Urea (mg/dL) median (25th–75th)	29.9 (23.5–36.4)	38.5 (29.9–51.3)	0.604
LVEF, %	49.7±6.1	48.7±9.3	0.059
PNI, mean	53.2±7.4	47.9±6.7	<0.001
GNRI, mean	117±13.8	104±15.2	<0.001
CONUT score, median (25th–75th)	3.0 (2.0–3.0)	4.0 (3.0–5.0)	<0.001

LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CRP: C reactive protein; LVEF: left ventricular ejection fraction; PNI: prognostic nutritional index; GNRI: geriatric nutritional risk index; CONUT: controlling nutritional status. Statistically significant values are given in bold.

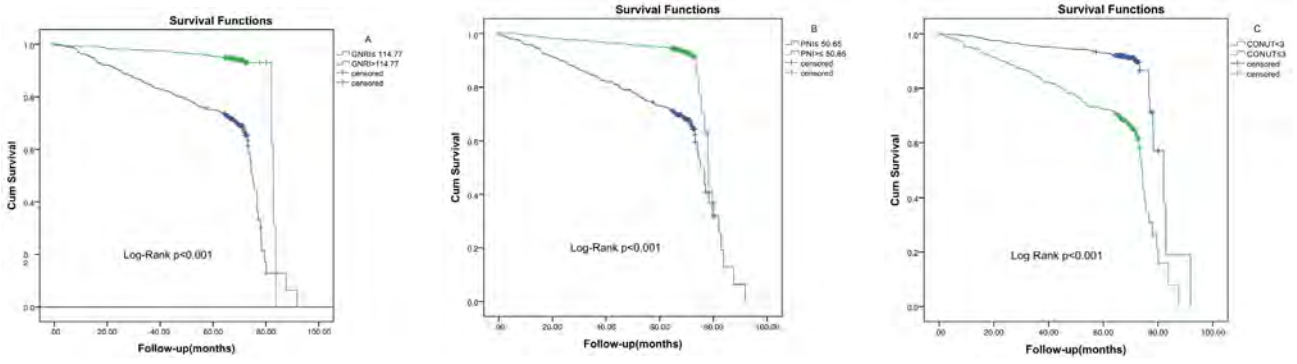


Variable	AUC	SE	95%CI
GNRI	0.778	0.018	0.750-0.805
CONUT	0.751	0.019	0.722-0.779
PNI	0.714	0.020	0.684-0.744

Variable	Differences between areas	SE	95%CI	Z statistic	P
GNRI - CONUT	0.027	0.0272	-0.0264-0.0804	0.992	0.321
GNRI-PNI	0.0635	0.0238	0.0169-0.1100	2.672	0.0075
PNI-CONUT	0.0365	0.0200	-.0002-0.0756	1.827	0.0677

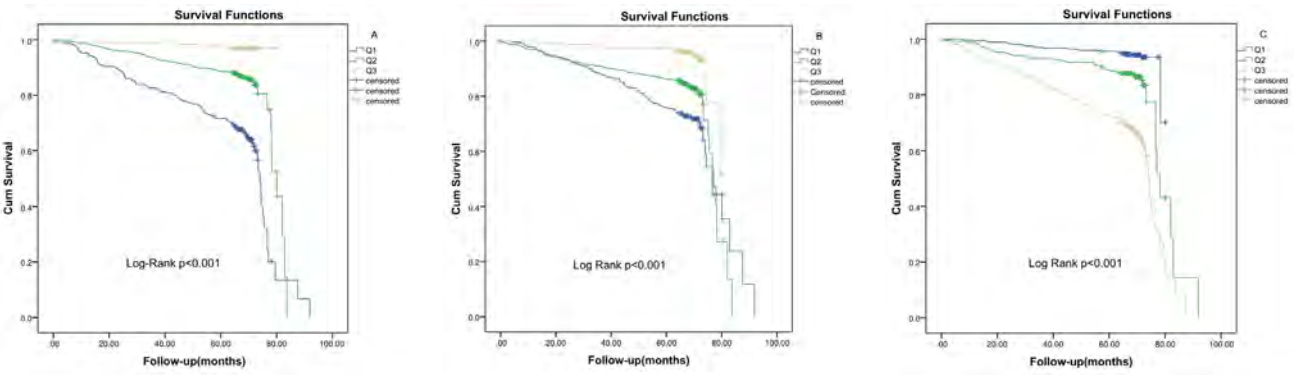
AUC: area under the curve; CI: confidence interval; CONUT: controlling nutritional status; GNRI: geriatric nutritional risk index; PNI: prognostic nutritional index; SE: standard error.

Figure 1. Comparison of receiver operating characteristic (ROC) curves for all-cause mortality.



(A) GNRI, geriatric nutritional index. (B) PNI, prognostic nutritional index. (C) CONUT, controlling nutritional status score.

Figure 2. The Kaplan–Meier analysis for all-cause mortality, according to the cut-off values of (A) GNRI, (B) PNI, and (C) CONUT scores.



(A) GNRI, geriatric nutritional index. (B) PNI, prognostic nutritional index. (C) CONUT, controlling nutritional status score.

Figure 3. The Kaplan-Meier analysis for all-cause mortality, according to interquartile of (A) GNRI, (B) PNI, and (C) CONUT scores.

Table 4. Univariate and multivariate Cox proportional hazard analysis of all-cause mortality.

Analysis	Univariate		Multivariate	
	p	HR [95%CI]	p	HR [95%CI]
Age	0.212	1.011 (0.994–1.029)		
Height	<0.001	1.134 (1.090–1.179)		
Weight	0.131	0.985 (0.966–1.004)		
BMI	<0.001	0.787 (739–839)	<0.001	0.818 (0.767–0.872)
Albumin	0.001	0.639 (0.495–0.825)		
SBP	0.003	1.012 (1.004–1.019)		
Total cholesterol	0.050	1.003 (1.00-1.006)		
Total protein	0.079	0.800 (0.624–1.026)		
LVEF	0.089	0.982 (0.963–1.003)		
PNI	<0.001	0.927 (0.910–0.945)	0.002	0.967 (0.947–0.988)
CONUT score	<0.001	1.494 (1.380–1.619)	<0.001	1.527 (1.404–1.661)
GNRI	<0.001	0.968 (0.962–0.973)	<0.001	0.973 (0.964–0.982)

BMI: body mass index; CI: confidential interval; CONUT: controlling nutritional status; EDV: end-diastolic volume, ESV: end-systolic volume; HR: hazard ratio; GNRI: geriatric nutritional risk index; PNI: prognostic nutritional index; LVEF: left ventricular ejection fraction; SBP: systolic blood pressure. Statistically significant values are given in bold.

DISCUSSION

This study was the first to examine the association of the CONUT, GNRI, and PNI scores with the long-term mortality in patients with NSTEMI who underwent PCI. It was noted that all the three indexes were significantly associated with the long-term all-cause mortality in patients involved in this study. Besides, in the comparison of predicting mortality, GNRI yielded similar results to CONUT score but was better than PNI. Malnutrition is a frequent and significant problem and is seen especially in hospitalized elderly patients. Earlier reports suggest that malnutrition is closely related to poor prognosis and mortality in terminal kidney failure, malignancy, and hematological diseases[6-8]. In search of a valuable marker, various nutritional indicators have been identified, including lymphocyte count, serum albumin levels, serum cholesterol levels, Mini-Nutritional Assessment (MNA)[14], and Subjective Global Assessment (SGA) in addition to the CONUT scores, GNRI, and PNI. Among these indicators, the ones with additional parameters put by the healthcare professionals, such as MNA and SGA, although expected to be more accurate, may be considered as potentially biased. On the other hand, CONUT scores, GNRI, and PNI include the objective quantitative data of the patient. In the calculation of the GNRI, only the height, weight, and serum albumin data of the patient are required. Similarly, the calculation of the PNI needs only the serum albumin level and the lymphocyte count of the patient. The calculation of the CONUT score requires the serum cholesterol level of the patient in addition to their serum albumin level and the lymphocyte count. These three indexes use different methods and variables and therefore have unique advantages and disadvantages. Nevertheless, serum albumin is the only common parameter, and its low levels alter the results of all the three indicators. A recent study suggested that in patients who underwent PCI, low serum albumin levels, independent of the traditional risk factors, were associated with major adverse cardiac event (MACE) development[15]. The association of the CONUT scores, GNRI, and PNI with the mortality in patients with AMI[16], cardiac failure[17], and chronic coronary syndrome who underwent elective PCI[18] was reported in earlier studies. In a retrospective study, including patients who underwent PCI due to stable coronary disease, the mean follow-up duration was 7.4 years and a high CONUT score was found to be associated with all-cause mortality and nonfatal MI in the long-term follow-up[19]. A research conducted in 802 patients who received elective PCI to the *de novo* lesions due to stable angina pectoris or objective ischemia showed a significant association between GNRI and poor cardiac prognosis following PCI[20]. Furthermore, the majority of the patients with chronic coronary syndrome were on an antilipidemic medication; the use of CONUT score on this particular group may seem erroneous. In this study, a low percentage of the patients were on statins, and the difference between the two groups was insignificant. Therefore, the CONUT scores in the study might be considered optimal[20]. In a recent report conducted on 2853 patients with first PCI procedure, 849 had acute coronary syndrome,

suggesting that low GNRI scores were independent predictors of all-cause mortality[21].

Moreover, these indicators have also been shown to yield different associations with poor prognosis and mortality in similar disease groups. In a study examining the prognostic values of CONUT score and PNI, conducted in 945 elderly STEMI patients who underwent PCI, at the end of a 2-year follow-up, CONUT scores were found to be associated with the increase in all-cause mortality rates, whereas PNI scores failed to present a predictive value[16]. Conversely, in a research carried out in 345 STEMI patients who underwent primary PCI assessing PNI predictivity only, it was found that PNI was an independent predictor of mortality in this group[22]. Similarly, in a study of 1823 STEMI cases who underwent primary PCI, it was proposed that low PNI values were associated with both in-hospital and 3-year long-term mortality[18]. In a study of 2251 patients, 975 had STEMI and 1276 had NSTEMI, and low GNRI scores were found to be significantly associated with post-MI complications and in-hospital mortality[23].

In the present study, low GNRI and PNI and high CONUT values were shown to predict the long-term mortality in NSTEMI patients. The multivariate analysis revealed that all the three indicators had independent prognostic values for mortality. Moreover, GNRI was demonstrated to have similar value compared with CONUT score and better than PNI. Since the serum albumin level is the common parameter, the different results were based on serum cholesterol levels and weight data. Lowering the cholesterol levels of the patients is one of the primary goals in coronary heart disease[24]. Low cholesterol levels before treatment in a patient attach particular importance, since the nutritional assessment will result in low nutrition status. According to the guidelines, patients with NSTEMI should receive antilipidemics even with low cholesterol levels[24]. However, the possibility of a poorer prognosis of the patients with low cholesterol levels at the time of the index event should always be noted. In the same manner, this study has revealed that low weight was associated with the long-term outcomes. Weight lower than the ideal is associated with fragility, which was reported as closely linked with the long-term poor prognosis in patients with cardiovascular diseases[25]. In patients with coronary heart diseases, overweight or underweight, the latter pointing fragility, both are undesirable. In this study, it was shown that the patients with low GNRI levels, indicating more fragility, were reported to have a higher mortality rate. In NSTEMI cases, the possibility of experiencing the long-term poor prognosis for patients with low weight at the time of index event should be considered.

Limitation

There were significant limitations to this study. This study was a single-centered retrospective study. Moreover, all-cause mortality was set as the primary end point and the effect of the indicators on cardiovascular-related mortality was not assessed exclusively. Moreover, not all AMI cases were included in this study. The aim of not involving STEMI patients was to form a relatively homogeneous study group due to the differences in the mean age, risk factors, and the comorbidities of the STEMI patients compared with the NSTEMI population. Larger study populations, including all AMI patients assessing the nutritional indicators, are required.

CONCLUSION

In this study, it was shown that CONUT score, GNRI, and PNI were associated with the long-term mortality in NSTEMI patients who underwent PCI. Furthermore, in the comparison of predicting mortality, GNRI yielded similar results to CONUT score but was better than PNI.

ETHICAL APPROVAL

All the procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all the individual participants included in this study.

AUTHORS' CONTRIBUTIONS

AY: Conceptualization, Investigation, Writing – Review & Editing. **MK:** Data Curation, Writing – Original Draft. **NYK:** Formal Analysis, Software. **YC:** Methodology, Validation. **MCB:** Project Administration, Visualization. **SK:** Resources, Supervision.

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Comparison of two endoscopic spine surgical techniques

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SUMMARY

OBJECTIVE: The objective of this study is to compare the clinical outcome among patients who are surgically treated for lumbar disc herniation by transforaminal and interlaminar endoscopy techniques.

METHODS: For the treatment of lumbar disc herniation, 31 patients were assigned to undergo the interlaminar technique and 24 patients the transforaminal technique. They were evaluated using visual analog scale and Oswestry disability index in the preoperative period, in the first postoperative period, and in the 12th month after the procedure. The clinical results between the two techniques were then compared.

RESULTS: Overall, 89.1% of the patients obtained good results, with 12.5% complications in the transforaminal technique and 9.6% in the interlaminar technique.

CONCLUSION: Although both the endoscopic techniques, compared in this study, are safe and effective for the surgical treatment of lumbar herniated disc, the interlaminar technique presented significantly better results and lower rates of complications than the transforaminal technique.

KEYWORDS: Spine. Intervertebral disk displacement. Endoscopy. Decompression, Surgical. Sciatica.

INTRODUCTION

Herniated disc is a disabling pathology. In most cases, its treatment is conservative and good results are obtained, but when this treatment fails, the best option is the surgical treatment[1]. This treatment has varied over time, from laminectomy and discectomy to the most recent percutaneous surgeries. With the popularization of minimally invasive spine surgeries, percutaneous endoscopic lumbar discectomy has increasingly become an alternative for the treatment of herniated discs, due to the advantages of this procedure. Two techniques that are mostly used in the endoscopic spine procedures are: the transforaminal and the interlaminar routes.

In the transforaminal technique, the patient is positioned in the ventral decubitus position; the midline and the lower and upper vertebral plateaus of the desired level are marked under visualization of the image intensifier, and lateral markings are the midline of 8, 10, and 12 cm, which will be the possible entry points. The patient is submitted to light sedation, and at the entry point, an infiltration is performed with a local anesthetic without vasoconstrictor. The sedation must be light, because the patient should be conscious enough to alert if any nerve root is stimulated during the procedure. After this stage, the intervertebral disc is punctured, and discography with methylene blue or indigo carmine, associated with non-ionic contrast, is performed. Through the guides, the endoscope is inserted in the intervertebral disc and an indirect decompression of the intervertebral disc is performed (inside-out technique), followed by a thermal nucleoplasty. The entire procedure takes place through Kambin's safety triangle. It is indicated for the treatment of hernias located in the foraminal or extraforaminal

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region, mainly at the L2 to L5 lumbar spine. Generally, this approach is made difficult at the distal L5–S1 level by the anatomical interposition of the iliac crest[2-4] (Figure 1).

In the interlaminar endoscopic discectomy, the patient is positioned in the ventral decubitus position, on a radiotransparent table, under general anesthesia. In this technique, general anesthesia is used because it is necessary to remove the neural root, which generates discomfort to the patient. The interlaminar window at the desired level is marked on the skin with the aid of the image intensifier, and a longitudinal access of 1 cm is made near the midline. An initial dilator is positioned in the interlaminar space, and the endoscope is introduced. First, the multifidus musculature is dissected into the yellow ligament, which is opened for exp osure of the descending root and perineal fat. The nerve root is removed and protected with the aid of a beveled cannula. The intervertebral disc is perforated and decompressed. At the end of the procedure, a thermal nucleoplasty is performed. In general, this technique is used for the levels L4/L5 and L5/S1, where a wide interlaminar interval is observed, which provides more working space. This procedure is indicated for central and central-lateral hernias[3-5] (Figure 2).

The endoscopic surgical techniques evaluated in this study are not widely known to spine surgeons, and its practice in Brazil is still restricted to some reference centers. The presentation and comparison of results between the two main endoscopic approaches may help in the indication for each case.

The objective of this study is to present two minimally invasive surgical techniques in the spine and compare the results obtained in the treatment of lumbar disc hernias.

METHODS

This longitudinal, observational, and prospective study was approved by the Research Ethics Committee under number CAAE 50750515.2.0000.5225. We grouped 55 patients who underwent the surgical treatment for lumbar disc herniation in a tertiary hospital, a reference in the surgical treatment of the spine, by the percutaneous endoscopic lumbar discectomy technique. Inclusion criteria for this study were as follows: patients of both genders and aged between 18 and 99 years, who had not been submitted to the previous procedures in the spine and had failed to undergo the conservative treatment of lumbar disc herniation, administered for at least 3 months, and who presented herniated disc in at most two levels in the lumbar region. These patients were subdivided into two groups: the endoscopic transforaminal technique and the interlaminar technique. Clinical evaluation was performed, and the Oswestry 2.0 questionnaire and the visual analog pain scale were used to quantify the results, applied on the following dates: the day before surgery, the first day after, and at 1 year after the surgery. The epidemiological data such as gender and age were also evaluated, as well as the postoperative complications such as surgical site infection, neurological alteration

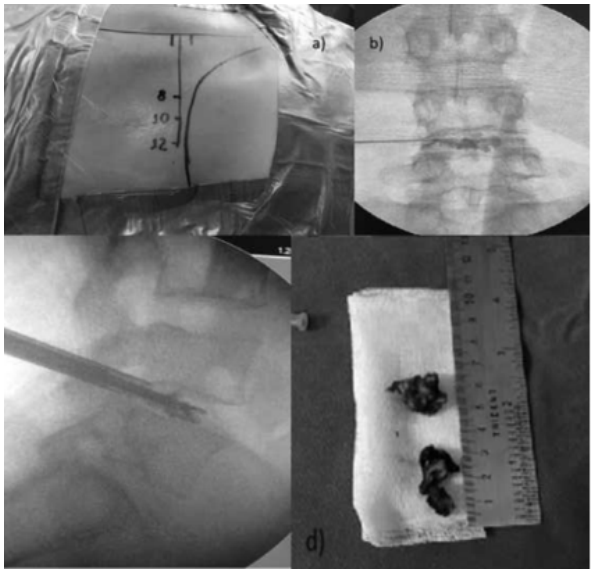

Figure 1. Percutaneous endoscopic transforaminal discectomy. (a) Skin markings, (b) anteroposterior view discography of radioscopy, (c) radioscopy profile view with demonstration of inside-out discectomy technique, and (d) disc material removed.

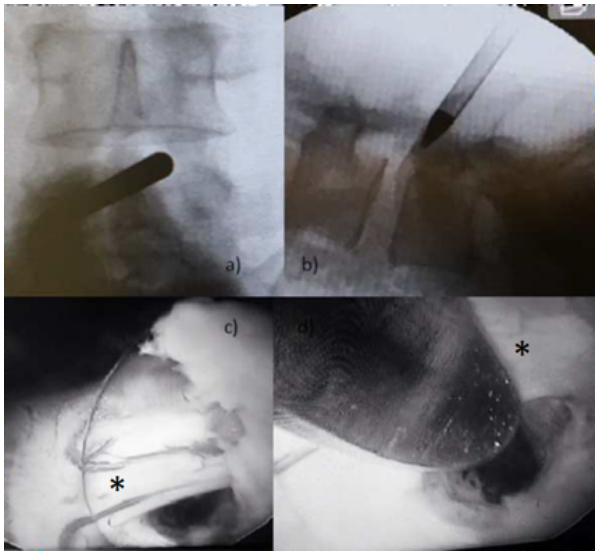

Figure 2. Interlaminar percutaneous endoscopic discectomy. (a) Point of entry into anteroposterior vision of radioscopy, (b) point of entry into radioscopy profile view, (c) yellow ligament opening and neural root being evidenced, and (d) probe by removing the root after discectomy. *Marking the neural root.

(paresis, paraesthesias), neural lesions, iatrogenic durotomy with or without lichen fistula, and relapse of herniated disc. The clinical outcome was compared separately between males and females in search of statistically significant differences between the sexes.

Patients who underwent the surgical treatment for lumbar disc herniation by open surgical technique, those over 80 years of age or under 18 years of age, and nonconsenting individuals were excluded.

The study was conducted from December 2014 to November 2015, totaling 1 year of endoscopic surgical procedures, and followed prospectively for 12 months postoperatively.

The material used for the procedures was the Vertebris Richard Wolf endoscopes®.

Two different endoscopic surgical techniques were used: transforaminal and interlaminar. Patients with central and central-lateral disc hernias were submitted to the interlaminar technique. On the other hand, those with foraminal and extraforaminal herniations were submitted to the transforaminal technique. When the herniation was foraminal or extraforaminal at the L5/S1 level, the transforaminal technique was performed only in cases where there was no anatomical barrier of the iliac bone in the surgical access. Patients with this anatomic limitation were excluded because they were submitted to non-endoscopic surgical technique. The procedures were performed by four different surgeons of the Spine Group, all of whom had the same training and previous experience in performing the endoscopic surgical technique.

The R software (R Core Team, 2015, version 3.2.3) was used for data analysis. As a statistical method, a multivariate analysis was conducted with a regression model for the longitudinal data. A significance level of 5% was adopted, and considered significant if $p<0.05$.

RESULTS

All the 55 patients included in this study underwent the surgical treatment of lumbar disc herniation by lumbar endoscopic technique. There were 29 female patients (52.8%) with a mean age of 37.8 years and 26 male patients (47.2%) with a mean age of 42.9 years. Of them, 24 (43.7%) underwent transforaminal technique and 31 (56.3%) interlaminar technique. The predominant operated levels were L4/L5 (20 cases) and L5/S1 (19 cases). Only 4 patients were operated at the L3/L4 level. Of this, 12 patients had a two-level approach in surgery.

The average score of the Oswestry scale in the preoperative period was 26.2, which was dropped to 5.5 on the first day after the procedure and 12 months after the procedure, it was 5.3, regardless of the surgical technique used. In the transforaminal technique, the mean preoperative rate was 27.5, which dropped to 6.3 on the first day after the procedure and 12 months after the procedure, it was 8.0; in the interlaminar technique, the values were 25.1, 4.8, and 3.1, respectively. The mean preoperative pain scaling, independent of the technique, was 8.4; on the first postoperative day, it was 2.3; and at the 12th month, it was 1.9. In the transforaminal technique, the preoperative mean was 8.3, which was 2.9 on the first day, and 3.2 after 12 months; in the interlaminar technique, the values were 8.5, 1.7, and 1, respectively. Both were statistically significant with $p<0.0001$. There was no statistically significant difference between males and females when using a $p<0.05$.

There was an incidence of 10.9% of complications in general, with three complications in each group, representing 12.5% rate of complications in the transforaminal group and 9.6% in the interlaminar group. The complications evaluated were as follows: neurological alteration, surgical site infection, durotomy with or without lichen fistula, and relapse of herniated disc. No patient had surgical site infection. Two durotomies occurred during the procedures (3.6%), both in patients submitted to the interlaminar technique, one being asymptomatic without clinical repercussions and the other studied with lichen fistula associated with postural headache with spontaneous resolution after 48 h of rest and without surgical reintervention. There were no cases of complete neural injury, but three patients (5.4%) had paresthesia in the lower limbs, two (8.3%) underwent transforaminal technique, and one (3.2%), interlaminar technique. Only two patients were submitted to surgical reintervention due to any of these complications, representing 3.6% of the cases. One of the patients was reoperated due to relapse of the disc herniation (transforaminal group, 4.1%), and the other due to technical difficulty in removing all the disc herniation which was calcified (interlaminar group, 3.2%). This last patient evolved with paresthesia of the interlaminar group (Figure 3).

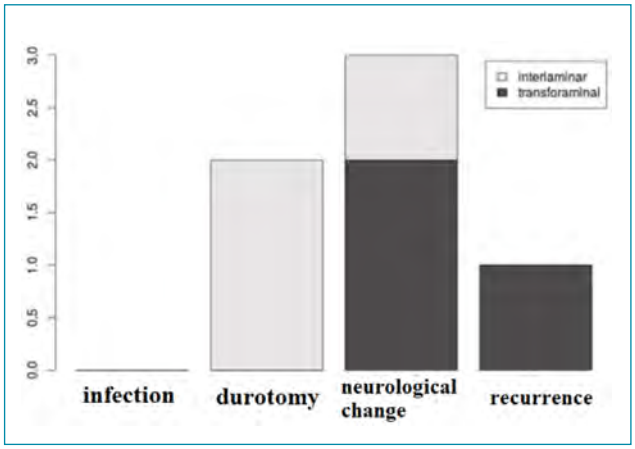

Figure 3. Complications between the two study groups.

DISCUSSION

Among the endoscopic techniques of the spine, the most used are transforaminal and interlaminar[9,10]. We observed a slight predominance of the interlaminar technique (56.3% of the cases), considering the higher incidence of centro-lateral disc hernias in the studied population. In this study, the patients were evaluated preoperatively, on the first day after the procedure, and after 12 months. Oswestry disability index (ODI) and visual analog scale (VAS) were evaluated and showed that the patients achieved a statistically significant improvement ($p<0.0001$) with the endoscopic treatment of lumbar disc herniation, and 89.1% of the patients had a good response to the surgical treatment. These values are higher than the success rates of the gold standard, which varies from 70 to 84% of good results, according to the study of Dohrmann et al., who analyzed 39,000 patients with lumbar disc herniation[11]. Separating the surgical techniques, the transforaminal technique had a slightly lower result than interlaminar, mainly in the evaluation done after 12 months of the procedure ($p<0.05$). On the first postoperative day, the improvement of pain complaints assessed by VAS was greater in the interlaminar group. The rate of complications was 12.5% in the transforaminal technique and 9.5% in the interlaminar, in which the values were comparable to the gold standard, according to the study of Shriver et al. The incidence of durotomy in our study was similar to that in the literature for the gold standard, which is approximately 3.1%[12,13]. There was a higher incidence of postoperative paresthesia in patients who underwent the transforaminal technique (8.3 *vs.* 3.2%). A possible explanation for the higher incidence of postoperative pain complaints in patients submitted to transforaminal technique may be due to stimulation of the dorsal root ganglion during the insertion of the instrument. The literature shows a possibility that the dorsal root ganglion may present within the triangular safety zone described in the endoscopic procedures[14]. Stimulation could be associated with the most incident neuropathic complaints in patients undergoing the transforaminal technique.

The literature reoperation rate for open microdiscectomy according to the study by Soliman et al. was 18.5%[15]. Aichmair et al., however, showed a higher rate, reaching 25%[16]. Both presented a follow-up of at least 5 years. Our reoperation rate was only 3.6% (two patients), and in the transforaminal technique, it was 4.1% (one patient) and in the interlaminar technique, it was 3.2% (one patient). In the transforaminal group, the reoperation was occurred due to the herniated disc recurrence. This patient presented a herniated disc which occupied more than 50% of the vertebral canal in the preoperative period. The literature shows that these cases are the most difficult for the complete removal of the hernia by endoscopic route. However, this patient was reoperated by the same surgical technique and presented satisfactory improvement of symptoms after the second procedure[17]. In the interlaminar approach, the reoperation occurred due to a calcified hernia, which made the procedure difficult by the endoscopic route, being converted to open microdiscectomy[18].

Complications are the very important factors in assessing the safety of a surgical procedure. A recent meta-analysis compared the clinical outcomes between open microdiscectomy and endoscopic microdiscectomy and showed that the rate of complications was similar in both groups, suggesting that the minimally invasive procedure is safe for the surgical treatment of lumbar herniated disc[19-22].

As a limitation of this study, the postoperative follow-up of only 12 months and a limited number of patients in our casuistry should be taken into consideration. The literature review shows that the main postoperative complications only occur during the 1st year after the procedure, so most of them were contemplated in this follow-up period. Another limiting factor is that we compared the surgical techniques at different disc levels. It is known that the neurological symptoms are different in each vertebral segment. However, the indications are described and enshrined in the literature. This study is a part of a research line intended to present the updated data in future publications[23,24]. The level of evidence in this study is five. Spine endoscopy is a promising spine technique; however, its learning curve is long and not all the specialized services have access to the method. Another limiting factor is its high cost, making it difficult to expand in the medical environment, and increasing healthcare costs.

In the future, it is expected that spinal endoscopy will have its reach extended and its benefits will bring better clinical results to patients. Studies in this sense, with larger samples and in different centers, need to be conducted to reinforce the findings of this study.

CONCLUSIONS

Spine endoscopy for the treatment of lumbar disc herniation showed approximately 90% of good results, regardless of the technique used. Both the endoscopic techniques performed are safe and effective for the surgical treatment of lumbar disc herniation, but the interlaminar technique presented significantly better results and lower rates of complications than the transforaminal technique.

AUTHORS' CONTRIBUTIONS

ALS: Conceptualization, Data Curation, Investigation, Methodology, Writing – Original Draft, Writing – Review and Editing. **ALK:** Conceptualization, Formal Analysis, Methodology, Writing – Original Draft, Writing – Review and Editing. **PGS:** Investigation, Writing – Original Draft, Writing – Review and Editing. **MLB:** Conceptualization, Investigation, Writing – Original Draft, Writing – Review and Editing. **XSG:** Investigation, Project Administration, Writing – Review and Editing.

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ORIGINAL ARTICLE
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Prediction of impacts on liver enzymes from the exposure of low-dose medical radiations through artificial intelligence algorithms

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SUMMARY

OBJECTIVES: This study aimed to develop artificial intelligence and machine learning-based models to predict alterations in liver enzymes from the exposure of low annual average effective doses in radiology and nuclear medicine personnel of Institute of Nuclear Medicine and Oncology Hospital.

METHODS: Ninety workers from the Radiology and Nuclear Medicine departments were included. A high-capacity thermoluminescent was used for annual average effective radiation dose measurements. The liver function tests were conducted for all subjects and controls. Three supervised learning models (multilayer precentron; logistic regression; and random forest) were applied and cross-validated to predict any alteration in liver enzymes. The t-test was applied to see if subjects and controls were significantly different in liver function tests.

RESULTS: The annual average effective doses were in the range of 0.07–1.15 mSv. Alanine transaminase was 50% high and aspartate transaminase was 20% high in radiation workers. There existed a significant difference (p=0.0008) in Alanine-aminotransferase between radiation-exposed and radiation-unexposed workers. Random forest model achieved 90–96.6% accuracies in Alanine-aminotransferase and Aspartate-aminotransferase predictions. The second best classifier model was the Multilayer perceptron (65.5–80% accuracies).

CONCLUSION: As there is a need of regular monitoring of hepatic function in radiation-exposed people, our artificial intelligence-based predicting model random forest is proved accurate in prediagnosing alterations in liver enzymes.

KEYWORDS: Aspartate aminotransferase. Alkaline phosphatase. Bilirubin. Alanine aminotransferase. Radiation dosages. Artificial intelligence. Machine learning.

INTRODUCTION

Ionizing radiation (IR) is a cancer-causing agent that can alter several biological effects via oxidative stress[1-4]. Oxidative stress in the body can develop a liver injury, which can lead to liver diseases[5]. The liver is a radiosensitive organ[6], and there is a need that the hepatic function should be monitored in medical radiation-exposed personnel. The current study was conducted to examine the hepatic function in medical radiation workers who are exposed to low doses of medical radiation from the Radiology and Nuclear Medicine departments of Institute of Nuclear Medicine and Oncology (INMOL) Hospital, Pakistan during 2014–2020. For comparisons, radiation-unexposed workers (n=30) of the same institute as controls were also included. The selection of a powerful predictive bio-computational tool

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is always a challenge. This study was focused to develop artificial intelligence (AI)-based models to predict alterations in liver enzymes with the following cofactors: age, gender, and exposure to radiation doses. Therefore, three supervised learning models (multilayer precentron, MLP; logistic regression, LR; and random forest, RF) were trained, applied to data, and cross-validated on the samples (n=90) of radiation-exposed medical workers. We further compared the accuracies and errors of these models and suggested the best. There is an extensive use of X-ray machines, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), intensity-modulated radiotherapy (IMRT), cardiac catheterization, fluoroscopic interventions, intensity-modulated proton therapy (IMPT), conformal therapy (CRT), three-dimensional conformal radiation therapy (3D-CRT), etc. in hospitals for the diagnosis and the treatment of various diseases and cancers. The radiotherapy units and diagnostic instruments are handled by the technicians and physicians, which include a linear particle accelerator (LINAC), the cobalt-60 teletherapy units, brachytherapy units, gamma cameras, mammography units, etc. The Nuclear Medicine department workers handle various radionuclides, such as Tc-99m, F-18, I-131, TI-201, and P-32. Occupational radiation workers, especially from medical procedures and equipment are being chronically exposed to low doses of IRs[7-10]. Low-dose radiation-induced (few mSv) late health effects, including cancers, are evident from various studies[11-13]. The liver function test (LFT) has been found effective in diagnosing the elevation or alteration in radiation-induced liver damages[14]. We considered the following liver enzymes to assess: aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (ALP), and bilirubin for both radiation-exposed workers and controls (radiation-unexposed workers). A study had reported that a low-dose gamma radiation can impact liver function[15]. Irradiation of the body can lead to a protein oxidation, which can cause DNA damage. Irradiation of a liver can initiate the oxidation of liver enzymes[16]. To diagnose a potential problem in the liver, the ALT and AST are more important from LFT. Their high levels are the indication of specific problems in the liver[17]. The health-risk assessments induced from the exposure to IR were prompted from the calculation and observations from the studies of atomic bomb survivors of Japan. These observations are reported by Nuclear Regulatory Commission, International Commission on Radiological Protection (ICRP) , and United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). The documents of the Radiation Protection Division of the Health Protection Agency are also available as the guidelines to assess the risk of chronic low doses for workers exposed to radiation. It was reported that out of 28 cancers, including liver cancer, the estimated excess relative risk per Sv was significant[4]. It has been observed that liver enzymes can be influenced from the exposure of X-rays. The BEIR-V Committee of National Research Council has mentioned that a long-term exposure to radiation can induce a liver cancer[18]. It is known that there was a liver cancer-related mortality risk existed from the exposure of plutonium in Mayak nuclear facility workers[19-21]. Although the liver irradiation risks are reported in patients who were treated with radiotherapies, these risks are not much evaluated in those personnel who are involved in treatments, such as single photon emission computed tomography (SPECT), CT, and 3D-CRT. The radiation-induced liver disease (RILD) has been reported in patients with intrahepatic cancer who were exposed to the radiation dose of 45–84 Gy[22]. Cao et al. (2008)[22] mentioned that considerable variations are reported on the sensitivity of the liver in radiation treatment, which can be measured by liver perfusion during the treatment of dynamic contrast-enhanced CT (DCE-CT) scanning.

AI and machine learning (ML) are extensively being in use to build models and make predictions for useful decisions and outcomes in clinical medicine. The real-time problems can be approximated and solved analytically with such models[23,24]. ML is considered a method of AI, in which the system learns the given patterns from the data and then with the learning and training functions, the model build-up[25]. Artificial neural networks (ANNs) mimicked the neural networks of human brain. AI and ML can help decide the diagnosis, treatment choices, postprocessing and other calculations through different functions and algorithms[25]. An MLP is a generic ANN model called feed-forward network, which can be adapted to training via learning algorithms. ANNs are the learning methods, which can provide a robust tool to solve real- and discrete-valued functions. ANNs are the interconnected units (neurons), which take real values for the inputs to produce an output[26]. ML algorithms can be used in predictive quantitative models from clinical symptoms and risks, which might be useful in diagnosing earliest risks involved and afterwards, in the selection of the treatment. ML models are characterized by making a few preassumptions, learning mechanisms, and then mine the structured knowledge from the data provided. There are supervised learning methods, such as neural network (NN) algorithms and support vector machines (SVM). There are unsupervised learning methods for clustering and other statistical configurations. For modeling, there should be some available features vs. target variables[26,27]. MLP is a "finite acyclic graph composed of nodes with neurons in logistic activation." The network consists of the input neurons and output neurons in layers. The number of output neurons depends on the target value of each training pattern[26,28]. The MLP algorithms support regression, classification, and prediction problems. ANN-based MLP is a biological

model mimicking the neurons of the brain, which are formulated into a specific function[29]. The basic random forest (RF) algorithm[30-32] is a nonparametric general purpose ensemble ML algorithm[26,33]. An ML-based logistic regression (LR) is a simple, rapid tool, which is effective in solving many problems through training, learning, and achieving specific coefficients[26,29].

METHODS

Study design and setting

A cross-sectional study was conducted in the year 2020 to examine the hepatic function in radiation-exposed medical workers with low dose in two departments (i.e., Radiology [RDG] and Nuclear Medicine [NMD] of INMOL Hospital, Pakistan).

Sample size and data collection

The low-dose radiation-exposed workers were included as volunteers through informed consents. The average service time for all the included (n=90) INMOL radiation-exposed workers was consecutive 5 years (2014–2020). The radiation-unexposed workers (n=30) as controls were also included, who were age-matched and with the same socio-economic background. The control group individuals were also from the same institute. They were scientists, nurses, ward attendants, supervisors, accounts officers, medical assistants, security guards, engineers, technicians, among others. The background/clinical data were collected from all subjects.

Measurement of annual average effective dose in millisieverts

Thermoluminescent (TLD) dosimeter reader was used to assess the whole-body AAEDs in mSv. The radiation doses in the Radiology and Nuclear Medicine departments were measured by Radiation Dosimetry Laboratory (RDL)[35-36]. The RDL, Pakistan Nuclear Regularity Authority (PNRA) uses a software RaDLab to calculate, assess, and keep record of the TLD received doses, according to the guidelines of ICRP[37]. Few people of the nuclear medicine department were also working with radiopharmaceuticals (Tc-99m and I-131) in Hot and Synthesizer Laboratories.

Blood sampling and background information

Blood samples were collected from the volunteers (n=120) with informed consents from RDG and NMD radiation-exposed personnel and other unexposed employees of the INMOL hospital. The general background information was recorded on a proforma from each participant.

Liver function test

The LFTs were conducted for radiation-exposed (n=90) and radiation-unexposed workers (n=30) in the biochemistry lab of the INMOL hospital. AST in U/L, ALT in U/L, alkaline phosphatase (AP) in U/L, and bilirubin in mg/dl were recorded. The following normal ranges were considered: AP, 115–539 (U/L); ALT, up to 40 (U/L); AST, up to 35 (U/L); and bilirubin, 0.3–1.2 (mg/dL).

ARTIFICIAL INTELLEGENCE MODELS

This study was focused to develop AI-based models to predict alterations in liver enzymes with the following cofactors: age, gender, and exposure to radiation doses (i.e., AAED in mSv). For this purpose, three supervised learning models (MLP, LR, and RF) were trained, applied to data, and cross-validated (fivefold) on the samples (n=90) of radiation-exposed medical workers. All the model buildings were done in Waikato Environment for Knowledge Analysis (WEKA ver. 3.8.3) developed by The University of Waikato Hamilton, New Zealand. Figure 1 shows the flow diagram for the model processing. These models were compared for their accuracies (i.e., kappa statistics, correctly classified instances, TP rate, FP rate, precision, recall, *F*-measure, Matthews correlation coefficient (MCC), receiver operating characteristic (ROC) area, and precision-recall curve (PRC) area and errors (absolute error, root mean squared error, relative absolute error, and root relative squared error). The kappa statistics is a mean to evaluate the prediction performance of the classifiers. Two classes of liver enzymes (ALT and AST) were made according to 'above-the-range normal values' in each. Class A in ALT consisted of the values greater than 40 U/L, whereas class B consisted of the values lesser than B. Similarly, class A in AST consisted of the values greater than 35 U/L, whereas class B consisted of the values lesser than B.

Artificial neural network based multilayer precentron

An MLP is a function for classification, which includes a back-propagation algorithm. This classifier can be optimized during learning and training phases with certain numbers of epochs. Usually, a Sigmoid function is included in the network of MLP. This model gives the following options for model building: seed, momentum, hidden layers, learning rates, momentum, epochs, batch sizes, training times, etc. The seed is used to initialize the generation of random numbers. The momentum is applied to the weight updates. The hidden layers are used to add where specifically required[34].

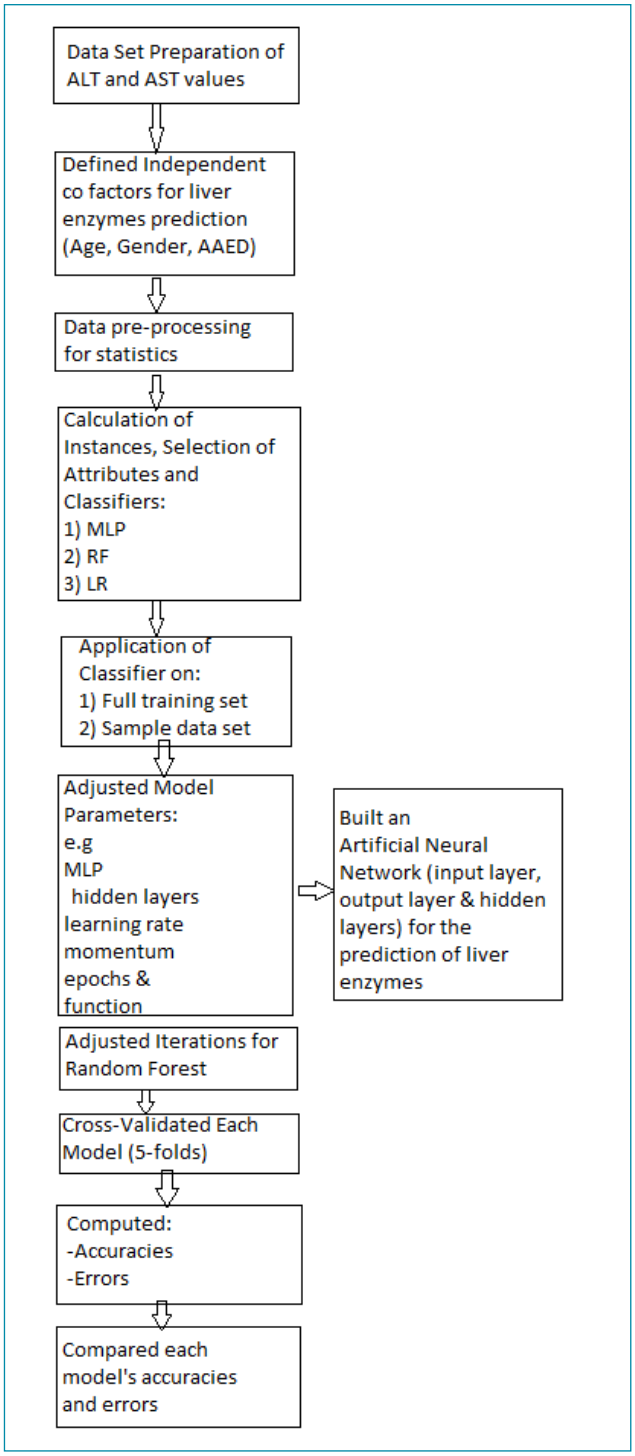

Figure 1. Flow diagram showing all process steps of the models.

Logistic regression

Logistic is a classifier function used for constructing a multinomial logistic regression (LR) model with a ridge estimator[73]. We used a modified LR from the original to handle the weights of the instances[72]. It gives the option of changing batch size, debug, ridge (in the log-likelihood), maximum number of iterations, or using conjugate gradient descent instead of Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm[34].

Machine Learning-based random forest

A RF is a classifier tree for constructing a forest of random trees[30]. It gives the options to change in seed, the number of execution slots to construct the ensemble, bag-size percentage, batch sizes, number of iterations, debug, maximum depth, number of randomly chosen attributes, etc.

Statistical analyses

The statistical calculations and analyses were done in SPSS version 25. A *t*-test (unpaired) was applied to the mean values of the following: AST (U/L), ALT (U/L), AP (U/L) and bilirubin (mg/dl) to discover a difference of significance (at $p<0.05$) between radiation-exposed and radiation-unexposed workers. A *p*-value <0.050 was considered significant.

RESULTS

Background information and annual average effective dose assessment

Out of 90 medical workers exposed to radiation, 78 (86.7%) of them were male and 12 (13.3%) were female. The mean age of the radiation-exposed workers was 42.7±12.08 years with a range 21–59 years. There were 19 males (63.3%) and 11 (36.7%) females who were radiation-unexposed workers. The mean age of unexposed workers was 44.46±10.43 years with a range 27–58 years. All participants were not found with any hepatic disease during their lifetime.

The mean value of AAED was 0.2550±0.27516 (mSv). The personnel of RDG and NMD were exposed to low AAEDs (whole body) in the range of 0.07–1.15 mSv during 2014–2019, which is well below (<20 mSv) the limit implied by UNCEAR.

Mean values of liver function test parameters

The AP was normal in both radiation-exposed and radiation-unexposed personnel. There were high values reported in ALT and AST enzymes with a mean value of 61.6 U/L (n=45; 50%) and 38.83 (n=18; 20%), respectively, in radiation-exposed workers. There were also high values reported in ALT and AST enzymes with a mean value of 51 U/L (n=5; 16.7%) and 45.8 (n=516.7%), respectively, in radiation-unexposed workers. Low values in bilirubin were reported in 6 (6.7%) (mean=0.23 mg/dL) radiation-exposed workers and in 3 (10%) (mean=0.27 mg/dL) radiation-unexposed workers. The details are given in Table 1.

Comparison of classifier models

The AI-based prediction classifier models were developed to anticipate the alteration in the liver enzymes, ALT and AST, with three cofactors, i.e., age, gender of the radiation-exposed worker, and AAED in the range of 0.07–1.15 mSv, using MLP, LR, and RF on cross-validation (fivefold) over 90 samples. Tables 2 and 3 describe the detailed comparisons between these three models along with the their characteristic features.

Table 1. Mean, Min and Max. values of Liver Function Test parameters.

LFT parameter	Mean±SD	Min/Max	High/Low Values (mean)	Normal Range
Radiation Exposed Personnel (n=90)				
Alkaline Phosphatase (AP)	222.1667±42.62701	157.00/320.00	None	115–359 (U/L)
Alanine Transaminase (ALT %)	44.8000±22.02460	18.00/102.00	High 61.6 (n=45; 50)	Up to 40 (U/L)
Aspartate Transaminase (AST %)	28.9000±6.48256	16.00/43.00	High 38.83 (n=18; 20)	Up to 35 (U/L)
Bilirubin (%)	0.5717±0.30208	0.22/1.80	Low 0.23 (n=6; 6.7)	0.3–1.2 (mg/dL)
Radiation Unexposed Personnel (n=30)				
Alkaline Phosphatase (AP)	232.2667±51.54906	150.00/348.00	None	115–359 (U/L)
Alanine Transaminase (ALT %)	30.1667±12.86825	14.00/80.00	High 51 (n=5; 16.7)	Up to 40 (U/L)
Aspartate Transaminase (AST %)	28.7333±10.17412	15.00/60.00	High 45.8 (n=5; 16.7)	Up to 35 (U/L)
Bilirubin (%)	0.5367±0.18907	0.26/0.98	Low 0.27 (n=3; 10)	0.3–1.2 (mg/dL)

Table 2. Comparisons of AI Models (on Five-Fold Cross-Validation) for the Prediction of Alterations in Liver Enzymes (ALT/AST) in Medical Radiation-Exposed Personnel.

Model: Multilayer Perceptron (MLP) Classifier Hidden Layers: 1 (nodes=2); Learning Rate: 0.4; Momentum: 0.3; Epochs: 500; Batch Size=100; Function: Sigmoid					
Correctly Classified Instances (%)	Kappa Statistics	Mean Absolute Error	Root Mean Squared Error	Relative Absolute Error (%)	Root Relative Squared Error (%)
ALT					
65.5556	0.3192	0.393	0.4455	78.8807	89.2278
AST					
80	0	0.2847	0.3829	87.5395	95.5839
Model: Logistic Regression (LR) Classifier Ridge Parameter of 1.0E−8					
ALT					
48.8889	−0.036	0.4998	0.5149	100.3204	103.1264
AST					
78.8889	0.1441	0.2873	0.3821	88.3471	95.3939
Model: Random Tree (RF) Classifier Bagging with 100 iterations and base learner; Seed=1					
ALT					
90	0.7982	0.1696	0.2526	34.0503	50.5894
AST					
96.6667	0.898	0.0908	0.1618	27.9296	40.3842

ALT: alanine transaminase; AST: aspartate aminotransferase.

Machine Learning-based Random Forest

According to the results, the best model was the RF, which achieved 90% and 96.6% accuracies in ALT and AST predictions, respectively, with the defined cofactors. RF model achieved a reduced number of errors and good kappa statistics (i.e., 79% and 89%) (Tables 2 and 3).

Artificial Neural Network-based Multilayer Perception

The second best classifier model was the MLP with respect to the accuracies and errors for both ALT and AST. The MLP model was tested and trained on different learning rates (LR), momentum, number of hidden layers, epochs, and the hidden layers. The best accuracy was found with one hidden layer of two nodes, LR=0.4, momentum=0.3, and epcohs=500. Figure 2A and B show the ANN of MLP with and without hidden layers.

Machine Learning-based logistic regression

This model worked well for AST prediction with 78% accuracy as compared to 48% accuracy in predicting ALT. The odds ratios in class A (values more than 40 U/L) of the ALT model were as follows: age=1.0342; gender: female=1.3057; and AAED=0.5493. The odds ratios in class A (values more than 35 U/L) of the AST model were as follows: age=1.0619; gender: female=0; and AAED=0.1614.

Comparisons based on t-test

The *t*-test was applied to see if both groups (radiation-exposed and radiation-unexposed workers) were significantly different from each other in LFT parameters. There existed a significant difference (p=0.0008; 95%CI t=3.445; df=118; 6.22–23.05) in the mean values of ALT between radiation-exposed and radiation-unexposed workers. There existed a nonsignificant difference in the mean values of AP, AST, and bilirubin with the following p-values: 0.2890, 0.9169, and 0.554, respectively.

DISCUSSION

Liver is a radiosensitive organ[6], and the long-term low-dose radiation effects must be regularly monitored in occupational workers. It has been reported that a radiation exposure can induce hepatic toxicity and can increase the risk of hepatic

Table 3. Comparisons of AI models: accuracy details by class.

Class	TP Rate	FP Rate	Precision	Recall	F-Measure	MCC	ROC Area	PRC Area
Model: Multilayer Perceptron (MLP) Classifier								
ALT								
A	0.762	0.438	0.604	0.762	0.674	0.329	0.696	0.694
B	0.563	0.238	0.730	0.563	0.635	0.329	0.696	0.767
AST								
A	1.000	1.000	0.800	1.000	0.889	–	0.700	0.909
B	0.000	0.000	–	0.000	–	–	0.700	0.366
Model: Logistic Regression (LR) Classifier								
ALT								
A	0.381	0.417	0.444	0.381	0.410	-0.036	0.494	0.456
B	0.583	0.619	0.519	0.583	0.549	-0.036	0.494	0.549
AST								
A	0.944	0.833	0.819	0.944	0.877	0.166	0.723	0.922
B	0.167	0.056	0.429	0.167	0.240	0.166	0.723	0.355
Model: Random Forest (RF) Classifier								
ALT								
A	0.857	0.063	0.923	0.857	0.889	0.800	0.976	0.974
B	0.938	0.143	0.882	0.938	0.909	0.800	0.976	0.980
AST								
A	0.972	0.056	0.986	0.972	0.979	0.898	0.997	0.999
B	0.944	0.028	0.895	0.944	0.919	0.898	0.997	0.983

TP: true positive; FP: false positive; MCC: Matthews correlation coefficient; ROC: receiver operating characteristic; PRC: precision-recall curve; ALT: alanine transaminase; AST: aspartate aminotransferase.

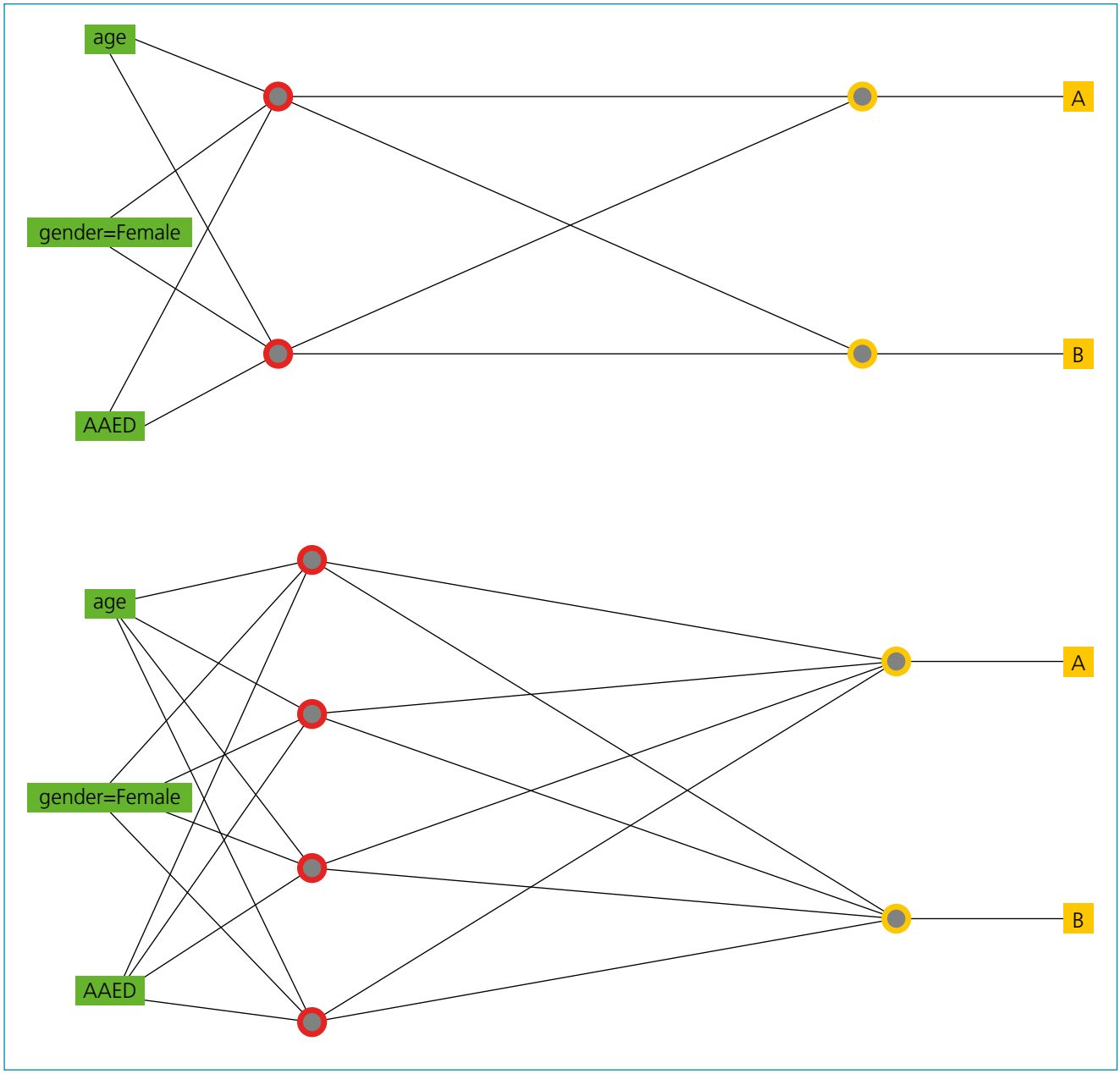

Figure 2. **(A)** Top: Neural network of liver enzymes with one hidden layer (nodes: 2); **(B)** bottom: Neural network of liver enzymes with one hidden layer (nodes; 4).

cancers[19]. Liver cancer has been reported[12] in medical radiation workers who were exposed from low doses. A study reported that the X-ray workers were found with high risks of leukemia, lung, liver, and breast cancers from the chronic exposure of radiations[38]. The workers of the Mayak nuclear production facility have been diagnosed with high risks of mortality from liver, lung, and bone cancers[20-40]. Similarly, the mortalities were also reported from liver cancer in the workers of the Sellafield nuclear plant in Britain[41]. We figured out a best predicting model from AI and ML-based algorithms for the impact on liver enzymes with the following cofactors: age, gender, and low doses of AAEDs (mSv) in radiology and nuclear medicine workers of the INMOL hospital. Among three supervised learning models (MLP, LR, and RF), the ML-based classifier RF achieved high accuracies (90–96%) in predicting altered levels of liver enzymes, AST and ALT. The ML-based decision tree models have been used for the detection or diagnosis of the diseases[42-46]. ML tools are now considered more powerful to assist in the decision making for problems in medical science[46]. ML is a branch of AI that has been found best in its

implications in nonlinear biological systems with complex measurements[46]. The ML-based RFs or the random decision forests are the ensembling learning methods for the classification and regression by building a multitude of decision trees via training. The RF consists of many individual decision trees, with each tree splits on a class prediction, and the class which is most opted, becomes the model's prediction[47].

According to the study conducted by Boice et al.[48], the cancer risks were evaluated in the employees of Rocketdyne (Atomic International), who were having an intake of radionuclides. They reported that the lung cancer and other cancers of liver, bone, esophagus, and kidney were not reported from the average dose of the external radiation of 13.5 mSv. However, cardiovascular disease, diabetes, the cirrhosis of the liver and other respiratory diseases were reported with significant deficits. Guha and Kavanagh[49] reported the RILD within 4 months in patients receiving hepatic radiation therapy. They reported higher levels of AP, but normal levels of bilirubin and ammonia through LFT. Lian et al.[50] assessed the severity and risk factors of liver radiation tolerance in more than 100 primary liver cancer patients who were treated with 3D-CRT. Although, they found that the mean dose of 23 Gy was tolerable for a normal liver, however, they did not asses the hepatic radiation tolerance from the dosimetry calculations. They suggested that the most important risk factor for RILD was related to the liver cirrhosis. Wang et al (2013)[51] has mentioned that during radiotherapy, the 99mTc-labeled immindodiacetic acid (IDA) SPECT obtained can be employed to assess the hepatic function, which can help to anticipate any post radiotherapy liver function alteration. Therefore, an optimized radiation treatment plan can be decided to avoid RILD in patients[51]. Howe et al. and Azizova & Muirhead[52,53] had mentioned that the chronic use of radiation can induce some changes in liver metabolism in many occupational radiation-exposed groups. When a liver receives radiation doses via whole-body exposure, the "upregulation in the genes of main proinflammatory chemokines occurs from the activity of proinflammatory cytokines"[54,55]. "An exposure of radiation induces the oxidative stress and this in turn can impact the liver through increase in concentrations of thiobarbituric acid-reactive substances (TBARS), decrease in superoxide dismutase, glutathione peroxidase activity[55,56], reduced glutathione concentration (GSH), and hence an activation of the stress-inducible haemoxygenase-1 (HO-1) gene"[57]. It is known that the reduced levels of GSH can lead to the increased stress induced oxidation[58]. The HO-1 gene has a protective function as anti-inflammation and antioxidant and has a role in the production of bilirubin[59,60]. However, some researchers did not report any change in TBARS levels from the radiation exposure[61,62]. The elevation in hepatocyte growth factor was observed with the exposure of total body irradiation[55-63]. A whole-body irradiation can impact other body organs, including the liver. Nwokocha et al.[55] conducted a study in which they evaluated the impacts of total-body radiation (1.27 Gy/min for 5 days), which leads to the alterations in liver enzymes in rats. They found that the levels of ALT and AST were significantly increased with the increase in the radiation doses. The decreased serum total protein and albumin levels were also reported from radiation exposures, mentioned by Holten and Christiansen, Moulder et al, and Wheeler and Bernard [64-66]. An increase in cholesterol and lipid levels were also reported from the radiation injury, due to the increased inflammatory actions[38,39]. Nwokocha et al.[55] reported that with the radiation exposure, the levels of bilirubin varied within the normal range, and the high levels were not significant.

LFT is the first helpful screening to find out any dysfunctioning in the hepatic system[69]. "Overproduction and leakage in blood are the basis of abnormality in AP levels. Leakage from the damaged tissue is a basis of normality in ALT/AST levels. The elevated levels of ALT/AST are used to mark in hepatitis, autoimmune diseases, toxicity, and ischemic conditions. Mild high levels of AST can be an indication of a liver disease; whereas, its moderate levels can be the indication of extrahepatic biliary atresia (EHBA), IHBA (intrahepatic biliary hypoplasia), infiltrating disorders or granulomatous hepatitis. The basis of normality in bilirubin is related to the decreased hepatic clearance. Its mild increased levels can indicate physiological jaundice, inherited hyperbilirubinemia; whereas, its moderate high levels can indicate EHBA, IHBA, drug toxicity, viral hepatitis, or inherited hyperbilirubinemia"[69]. There existed a significant difference (p=0.0008) in ALT between radiation-exposed and radiation-unexposed workers. None of the radiation-exposed or radiation-unexposed people of INMOL were having an abnormal value of AP. The major change was observed in ALT, which was high in 50% radiation-exposed workers. The AST was high in 20% radiation-exposed workers. Only 6.7% lower levels were found in bilirubin in radiation-exposed workers. "It is known that the extremely high levels of ALT/AST are found in viral hepatitis, drug toxicity induced hepatic necrosis, and circulatory shock. Moderate-high levels of ALT/AST are found in patients with acute/chronic hepatitis, autoimmune hepatitis, drug-induced hepatitis, alcoholic hepatitis, and acute biliary tract obstructions. In chronic liver diseases, the ALT can frequently increase. The mild high levels of ALT/AST are seen in EHBA, fatty liver, liver cirrhosis, nonalcoholic steato hepatitis (NASH), drug toxicity, myositis, Duchenne muscular dystrophy or after strenuous exercises. The lower levels of bilirubin may be reported from the side effects of certain drugs, such as sulphonamides and salicylates"[69]. Abnormalities in liver enzymes

are commonly reported in elderly people[70]. The elevated levels of ALT/AST can be observed in short duration and may not point towards any significant damage to the liver. A chronic intake of antidepressants, pain relief medicines, antibiotics, or muscle relaxants can temporarily raise liver enzymes. Barshishat-Kupper et al.[16] reported a hepatic metabolic alteration with the radiations of 8.5 Gy, which also led to the radiation-induced carbonylation of associated liver enzymes. A study had reported that the altered levels of AST, AP, and bilirubin were significantly linked with the radioactivity of thorium in occupational workers[71]. Moreover, high levels of AST, AP, bilirubin, and albumin were significantly associated with the alpha-radiation (50 μCi) emission from the radium industry in female workers[72].

Recommendations

There is a need to evaluate the same models on large data. There should be some planning in implementing these models in hospitals for the health and safety of the radiation workers. The practical implications could provide the real testing to solve for the errors and other limitations. More AI and ML-based models can also be tested with more specific cofactors for their robustness and validations. There should also be more consideration of different learning methods and more data for the training samples. Moreover, the developed AI models can be further helpful in diagnosing any intial health abnormality in patients who receive radiotherapies.

Limitations and strengths

This was a single-center based pilot study and was conducted to test the validity of AI and ML predictive models for the prediagnosis of biochemistry alterations. Although, few specific models were successfully validated, there is a need to test more AI models on larger data. The accuracy in the results of the tested models indicates that they can help clinicians to prediagnose any abnormality in the biochemistry of popultation who are being exposed to environmental toxics.

CONCLUSION

A radiation-induced injury can occur in the medical radiation workers from low doses. Therefore, there is a need to monitor the hepatic function of radiation-exposed people on a regular basis. The RF achieved the highest accuracy in predicting the altered levels of liver enzymes. The application of ML-based models can provide us fast monitoring and assessment of biochemistry to point out an earliest risk in case of any alterations.

AUTHORS' CONTRIBUTIONS

SS: Conceptualization, Formal Analysis, Writing – Original Draft. **KM:** Data Curation, Writing – Review & Editing. **AWK:** Data Curation, Writing – Review & Editing.

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ORIGINAL ARTICLE
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Anxiety in candidates for radical prostatectomy in a university hospital

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SUMMARY

OBJECTIVE: Metabolic changes caused by anxiety can interfere in both the surgery itself and the recovery process. One way to reassure the patient is to clarify how the procedure will be performed and discuss the possible complications. This study aimed to investigate the anxiety level of candidates for radical prostatectomy at a university hospital.

METHODS: Thirty-four patients with a diagnosis of prostate cancer were studied prospectively. Data collection involved the administration of the Hospital Anxiety and Depression Scale and a radical prostatectomy knowledge test.

RESULTS: The results showed that 94.1% of the patients reported having received clarifications from the physician or healthcare team regarding the surgery and 23.5% reported having received information on the probability of a medical error during surgery. The most cited postoperative complications were sexual impotence and urinary incontinence. A significant association was found between the total Hospital Anxiety and Depression Scale score and the complications cited (p=0.0004); patients who marked a larger number of possible complications had a higher Hospital Anxiety and Depression Scale score.

CONCLUSION: The present study demonstrates that the explanations given by the multidisciplinary health team are not achieving their maximum potential in terms of lowering patient anxiety.

KEYWORDS: Anxiet. Prostatic neoplasms. Prostatectomy.

INTRODUCTION

Prostate cancer is the most prevalent neoplasm in men and is considered the second most common type of cancer in the male population throughout the world[1]. The most recommended treatment option is radical prostatectomy, which consists of the complete resection of the prostate gland, including the prostatic urethra, seminal vesicles, and ductus deferens, and may also include bilateral lymphadenectomy[2], which is the gold standard for the treatment of localized prostate cancer[3]. This surgery can have undesirable effects in the postoperative period that exert a negative impact on quality of life[4]. Such consequences include erectile dysfunction, infertility, and urinary incontinence; moreover, the condition can be fatal or may recur[1]. Facing this danger, patients elected for this surgical procedure often anticipate a vague, unknown threat of the possible negative consequences due to a lack of information[5], which can generate potentially negative feelings[6], such as an objectless fear that is manifested in the form of anxiety.

Anxiety is an emotional reaction that is essential to survival, as it prepares an individual for either fight or flight[7]. This feeling stimulates the central nervous system to secrete neurotransmitters (catecholamines)[8] that have several metabolic effects.

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Anxiety has been associated with physiopathological responses, such as hypertension, arrhythmia, an increased heart rate, a diminished immune response, diminished tissue healing, and other consequences that are favorable to an individual in danger, but can increase the occurrence of morbidity in the perioperative period[9] by promoting more intense bleeding during surgery or possible infection at the incision site. Therefore, the expectations of patients awaiting surgery, such as the fear of anesthesia, disability, and even death, can interfere with the course of the surgery itself as well as the recovery process[5].

Considering the impact of anxiety on surgical procedures, such as prostatectomy, it is fundamental to know the main anxiogenic triggers in order to prevent such factors and avoid surgical complications. One of the triggering factors most widely known in the scientific community is the lack of clarification patients have regarding the procedures their bodies will undergo during hospitalization[6-10]. It is therefore common for healthcare providers to offer a brief explanation to the patients regarding the disease and how the surgery will be performed. The purpose is to quell doubts and diminish negative expectations related to the procedure[11]. However, there is a lack of empirical studies and quantitative data on this issue. What the patient grasps from conversations with the physician, the extent to which healthcare providers efficiently transmit their knowledge, and how much a patient needs to know about the surgery are issues that have not been duly investigated. Therefore, this study aimed to investigate the level of anxiety in candidates for radical prostatectomy at a university hospital and the knowledge they have regarding the procedure.

METHODS

A quasi-randomized (order of arrival), prospective, cross-sectional study was conducted involving 34 patients with a diagnosis of prostate cancer scheduled for surgical treatment at a urologic oncology service of a tertiary hospital in the State of São Paulo, Brazil, between August 2019 and May 2020. The exclusion criterion was a lack of understanding of the subject.

This study received approval from the Human Research Ethics Committee of the institution (certificate number: 1247119.0.00005415). All patients received clarifications regarding the objectives and importance of the study and those who agreed to participate signed a statement of informed consent. Data collection involved the administration of the Hospital Anxiety and Depression Scale (HADS) and a radical prostatectomy knowledge test.

The HADS was developed to be applied to non-psychiatric patients at a general hospital[12,13] and was used in the present study to determine the degree of patient anxiety. One day before the surgical procedure, the patient was instructed to answer the questions using the previous week as a reference. The HADS has 14 multiple-choice questions and two subscales – one addressing anxiety and one addressing depression. The score of each subscale ranges from 0 to 21 points. Patients with a score of 0 to 7 points were classified as "not anxious", those with a score of 7 to 12 points were classified as "possible anxious", and those with a score of 12 to 21 points were classified as "probably anxious". The anxiety subscale of the HADS has 93.7% sensitivity and 84.6% specificity[12,13].

The purpose of the radical prostatectomy knowledge test was to ascertain the patient's general knowledge regarding the procedure to which he would be submitted. This questionnaire was developed by the authors of the present study and has 14 multiple-choice questions.

Statistical analysis

Data analysis was performed with the aid of the Excel 2010 and Biostat 5.1 computational programs. The data were described using means and frequencies (absolute and relative). Pearson's and Spearman's correlation coefficients were calculated for the correlation analysis. The chi-square test was used to determine associations between the state of anxiety (HADS score) and questions on the radical prostatectomy knowledge test. The level of significance was set to 5% ($p<0.05$).

RESULTS

Thirty-four men scheduled for the surgical treatment of prostate cancer participated in the present study. Based on the results of the HADS questionnaire, 14.7% expressed being fearful; 20.6% spent most of the time with worrying thoughts; 32.4% reported feeling restless; and, 23.5% reported feeling tense or wound up most of the time in the previous week.

Regarding knowledge of the procedure, 94.1% of the patients reported having received clarifications from the physician or healthcare team regarding the surgery; 91.2% reported that the prostate would be removed and 8.8% responded that the prostate and seminal vesicles would be removed. The majority (64.7%) reported that the surgical incision would be made in the lower portion of the abdomen. Regarding for whom such surgery is indicated, the majority (62.5%) stated that the procedure is suggested for patients with localized cancer and a life expectancy of more than 10 years, 9.4% stated that the surgery was indicated for individuals in a very advanced stage of the disease, and 3.1% did not offer an answer. A total of 61.8% stated that they would not be awake during surgery and 8.8% stated that they would be awake. Regarding the duration of surgery, 61.8% believed that it would last two to three hours.

A total of 23.5% reported having received information on the probability of a medical error during surgery. Regarding postoperative complications, 47.1% of the patients stated the possibility of sexual impotence. When asked about the possibility of urinary incontinence, 38.2% stated that the likelihood was low and 35.3% stated that the likelihood was moderate (Figure 1). Regarding urinating normally after surgery, 50% stated that this would occur in around two weeks, 11.8% stated that they would not need a catheter and would urinate normally immediately after the removal of the urinary catheter. When asked to mark the possible complications related to surgery, 55.9% marked hemorrhage as a possible danger and 44.1% marked sexual impotence. Regarding what would occur if surgery were not performed on someone with prostate cancer elected for radical prostatectomy, 64.7% answered that their cancer would continue to exist, could attack other sites, and, placed the general health of the patient at risk and 8.8% stated that their cancer would disappear on its own at some point. A total of 38.2% reported that recurrence is possible.

In the present study, 73.5% of the patients exhibited manifestations of anxiety and 47% demonstrated signs of panic one week before radical prostatectomy (Figure 2).

Approximately one-third of the interviewees stated that there is no chance of the surgeon making a mistake during the medical procedure (Figure 3).

A moderate, directly proportional correlation (rs=0.5713) was found between the total HADS score and complications cited, as individuals who marked a greater number of complications had higher HADS scores. This correlation was considered statistically significant (p=0.0004). A weak inversely proportional correlation was found between the score on the radical prostatectomy knowledge test and the score on the anxiety subscale of the HADS (r=-0.4057, Pearson's correlation). Despite being weak, this correlation was statistically significant (p=0.0261).

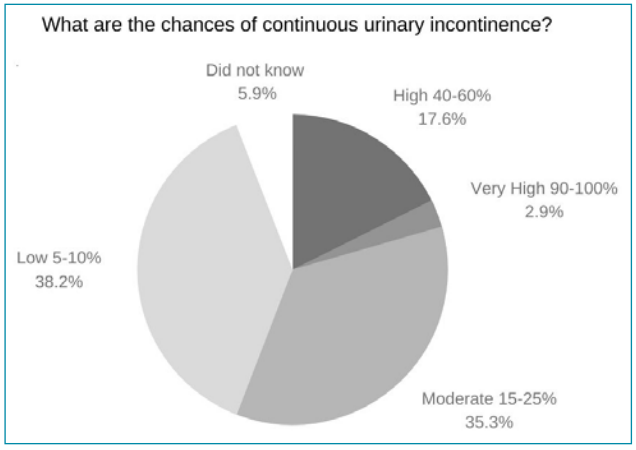

Figure 1. Chances of continuous urinary incontinence.

DISCUSSION

In the present study, the majority of the patients exhibited manifestations of anxiety and demonstrated signs of panic one week before radical prostatectomy. The findings suggest that this surgery is terrifying for most patients. Moreover, 75% exhibited some degree of fear and 75% reported worry related to the surgical procedure or the recovery process.

No significant association was found between the technical details of the surgical procedure and the level of anxiety. This finding diverges from data described in previous studies[14-17], in which receiving information regarding the surgery was able to diminish stress and the level of anxiety by making the procedure less worrisome to the patient[18]. This divergence may be related to the sample size in the present investigation.

Approximately one-third of the participants informed that there is no chance of the surgeon making a mistake during the surgical procedure. This finding suggests a certain degree of naivety on the part of the patients but reveals the level of

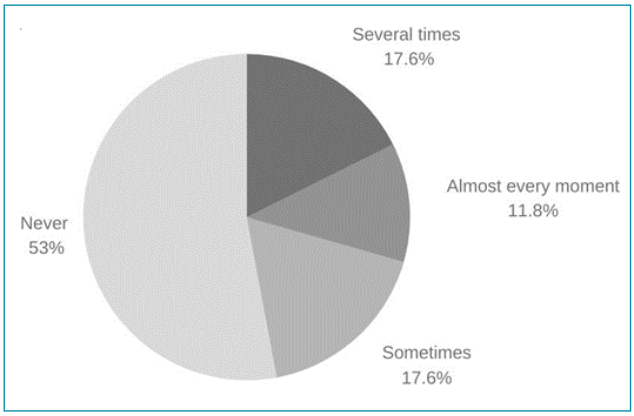

Figure 2. Signs of panic one week prior to radical prostatectomy.

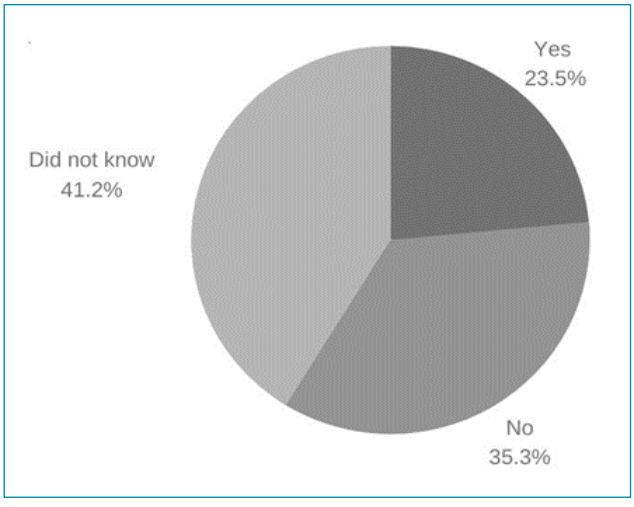

Figure 3. Chance of the surgeon making a mistake during the surgery.

confidence they have in the surgeon and the surgical procedure. Indeed, the amount of knowledge that patients believed to have regarding the operation was important to diminishing anxiety[19], despite the low correlation. Thus, the confidence patients have in the procedure, the medical team, and themselves diminished the degree of stress related to the surgery.

Postoperative complications generated greater concern. Urinary incontinence is one of the most feared sequelae of radical prostatectomy. Although the incidence of urinary incontinence related to this surgical procedure is low[4], approximately two-thirds of patients believed that the likelihood of its occurrence was moderate to very high, which is inconsistent with the literature[20]. Another feared complication is sexual impotence, mentioned by one-third of the patients as highly to very highly probable. These findings suggest miscommunication between the healthcare team and patients, as patients may not be receiving or grasping information that they actually would like to know[21].

The strongest correlation found in the present was the degree of anxiety and the number of perioperative complications cited by the patients, as patients with greater knowledge on the complications involving surgery had a higher HADS score. It is possible that this association may occur because previously anxious patients are prone to misunderstand information offered by the healthcare team[22]. Thus, rather than understand that a given complication is possible, the individual internalizes the information as if the complication will actually occur[23,24]. It is therefore important for the healthcare team to identify anxious patients in the preoperative period before offering details regarding the procedure to quell doubts and enable the information to be truly beneficial.

CONCLUSIONS

The present study demonstrated that explanations given by the healthcare team of the university hospital to patients are not achieving their maximum potential in terms of lowering patient anxiety. Thus, such information should be complemented with other forms of communication that can contribute towards greater clarification regarding the radical prostatectomy, thereby reassuring the patient.

AUTHORS' CONTRIBUTIONS

MDNSOF: Conceptualization, Data Curation, Writing – Original Draft, Writing – Review & Editing. **LCFS:** Conceptualization, Data Curation, Writing – Original Draft, Writing – Review & Editing. **FNFJ:** Conceptualization, Data Curation, Writing – Original Draft, Writing – Review & Editing.

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ORIGINAL ARTICLE
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Immunohistochemical and clinicopathologic features of estrogen receptor-negative, progesterone receptor-positive, HER-2 negative breast carcinomas

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SUMMARY

OBJECTIVE: Currently, there is an ongoing debate whether progesterone receptor positive and estrogen receptor negative breast carcinomas represent a true distinct subtype of tumor or a mere immunohistochemical artifact. In this study, we conducted an immunohistochemistry panel with the antibodies TFF1, EGFR, and CK5 to reclassify this phenotype in a luminal or basal-like subtype.

METHODS: Tumors estrogen receptor -/progesterone receptor +, Her-2 – from a large population of breast cancer patients were selected to be studied. Immunohistochemistry with the antibodies TFF1, EGFR, and CK5 was performed. Tumors showing positivity for TFF1, regardless of EGFR and CK5 results, were classified as luminal-like carcinomas. Those lesions that were negative for TFF1, but were positive for EGFR and/or CK5, were classified as basal-like triple-negative carcinomas. When the three markers were negative, tumors were classified as undetermined. Clinical pathologic characteristics of patients and tumor recurrence were evaluated.

RESULTS: Out of 1188 breast carcinomas investigated, 30 cases (2.5%) presented the estrogen receptor -/progesterone receptor +/HER2- phenotype. Of them, 27 tumors (90%) were classified as basal-like triple-negative carcinomas, one as luminal-like (3.3%), and two as undetermined tumors (6.7%). The mean follow-up for the study group was 27.7 (2.7 to 50) months. Out of the 26 patients, 6 had cancer recurrence: 2 local and 4 systemic recurrences. The average time for recurrence was 17 (8 to 38) months.

CONCLUSION: Estrogen receptor -/progesterone receptor +/tumors exhibit aggressive behavior, similar to triple-negative tumors. An appropriate categorization of these tumors should be made to improve their therapeutic management.

KEYWORDS: Breast neoplasms. Immunohistochemistry. Receptors, estrogen. Receptors, progesterone. Carcinoma, basal cell.

INTRODUCTION

Breast cancers are currently classified into different subtypes based on gene expression signatures, but gene profiling still has a limited role in clinical practice[1]. Immunohistochemical surrogate markers have been used, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), to identify tumors with distinct clinicopathological characteristics, therapeutic responsiveness, and oncological outcomes[1-4].

ER is a well-defined prognostic factor, and its expression is related to a higher chance of a favorable response to anti-estrogen hormonal therapy. In contrast, the role of PR as a prognostic factor is still unknown. PR is positively regulated by the ER, and the presence of PR indicates a more intact ER pathway.

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Tumors with ER expression are classified as luminal-like and have a better prognosis than tumors without ER expression, such as pure HER2 positive and triple-negative tumors[4,5].

The existence of the ER-/PR+ carcinomas remains under debate between authors[6,7]. This subtype represents 3.4 to 7% of all cases. Some suggest this phenotype does not exist and may merely represent technical artifacts, resulting from a failure of the IHC[6]. In contrast, others support that ER-/PR+ tumors, although rare, represent a true distinct phenotype, with more aggressive biological behavior and worse oncological prognosis than double-hormone receptor-positive carcinomas[7]. A study conducted by Itoh et al. demonstrated that 65% of ER-negative and PR-positive tumors were really basal triple-negative, and only 20% were luminal according to genetic expression[8].

More recently, Yu et al.[9] also evaluated the molecular essence and clinical characteristics of ER–/PR+/HER2 negative breast tumors. They revealed that these tumors' clinicopathologic features and survival outcomes fell in between ER+/PR+ and ER–/PR– phenotypes, being similar to the ER–/PR– phenotype. Among the ER–/PR+ tumors, 30% were luminal-like, and 60% were basal-like carcinomas (BLC). For the first time in this subtype, the authors performed an immunohistochemical analysis combining three markers: trefoil factor 1 (TFF1), EGFR, and CK5. They demonstrated that this immunohistochemical method could be used as a surrogate of genetic testing.

These three markers have already been used to categorize basal-like breast tumors, with an IHC sensitivity of 76% and specificity of 100%[10,11]. TFF1 immunohistochemical expression of 10% or more is significantly associated with a luminal-like molecular profile, whereas CK5 and/or EGFR expression was associated with the basal-like subtype[9]. TFF1 is an estrogen-dependent protein, and its expression in breast tumor cells is related to ER expression and response to hormone therapy[12]. EGFR (or HER1) is a member of the family of transmembrane receptors of the epidermal growth factor, and it is involved in several cellular functions such as proliferation, differentiation, motility, survival, and tissue development. CK5 is part of the high molecular weight cytokeratins found in basal breast cells[13].

The present study is the first to reproduce the immunohistochemical panel proposed by Yu et al.[9] to determined IHC and clinicopathologic features of ER–/PR+/HER2– tumors in a large population of breast cancer patients.

METHODS

We performed a cross-sectional study, including all cases with histopathologic diagnosis of invasive breast carcinoma managed at our institution between 2012 and 2016. Tumors with the phenotype ER-/PR+/HER2 – were selected to be submitted to additional IHC for the proteins TFF1(pS2), CK5, and EGFR.

Immunohistochemical reactions were performed on the automation platform Benchmark® ULTRA (Ventana Medical Systems, Tucson, Arizona). The samples were cut into 3µm, and each slide received a positive control. Deparaffinization was performed with the equipment using reagent EZ PREP. The antigenic recovery was made with CC1 cap (cell conditioning 1), alkaline pH, for 64 minutes, at 95°C. The blocking of peroxidase was accomplished using reagent Ultra View Universal DAB Inhibitor, from the detection system.

The sections were incubated for 4 minutes at 36°C in the ready-to-use anti-PR monoclonal antibody (clone 1E2, Ventana). In the ready-to-use anti-ER antibody (clone SP1, Roche), the incubation occurred for 16 minutes at 37°C. The cuts remained for 32 minutes at 37°C in the TFF1 antibody (clone EPR3972, Abcam) in a 1:800 dilution. As for the CK5 antibody (clone EP1601Y, Cell Marque) in a dilution of 1:50, it stayed for 32 minutes at 42°C, and in the ready-to-use EGFR antibody (clone 5B7, Roche), the slides remained for 16 minutes at 37°C.

Reactions were detected with the Ultra View Universal DAB detection kit, using the diaminobenzidine (DAB) chromogen from the kit. The slides were counterstained with Mayer hematoxylin, differentiated with bluing reagent (Li2CO3+Na2CO3), and examined after dehydration and assembly.

In ER-/PR+ tumors, the nuclear staining was considered positive for ER and/or PR in at least 1% of tumor cells of any intensity[14]. The cut-off point for TFF1 positivity was 10% of staining observed in the neoplastic cellular cytoplasm, as described by Yu et al.[9]. The reaction was considered positive for CK5 and EGFR if ≥1% weak, moderate, or strong staining on the cell membrane and/or cytoplasm was observed[13]. The reading of the slides was made by a pathologist specialized in breast pathology. Tumors that were positive for TFF1, regardless of EGFR and CK5 results, were classified as luminal-like. Those without positive staining for TFF1, but showing positivity for EGFR and/or CK5, were considered triple negative BLC. When the three markers were negative, the results were classified as undetermined.

Clinicopathological features, such as age, race, histological type, tumor grade, lymphovascular invasion, tumor size, axillary involvement, percentage of Ki67, type of treatment, and disease-free survival were correlated with tumor subtype. The study began after the approval of the Committee of Ethics in Research from the HCPA (Approval Number: 1464984 - Date of approval: 03/26/2016).

The quantitative variables were described by the mean and standard deviation or median and range between percentiles, and categorical variables by absolute and relative frequencies. The relation between categorical variables was assessed with Fisher's exact test. Medians were compared using the Mann-Whitney test. To assess the association between numeric and ordinal variables, Spearman's correlation test was applied. Recurrence-free survival curves were estimated with the Kaplan-Meier method. Poisson regression analysis was applied to control confounding factors. Relative Risk (RR) and a confidence interval of 95% were used as the effect measure. The significance level adopted was 5% ($p \leq 0.05$), and the analyses were run in the SPSS program version 21.0.

RESULTS

Out of a total of 1188 breast carcinoma, 38 (3.19%) presented the ER-/PR+/HER2 – phenotype. Eight cases were excluded due to the unavailability of paraffin blocks for IHC. The 30 (2.5%) remaining available cases were submitted to additional IHC for the proteins TFF1 (pS2), CK5, and EGRF.

After analyses, 27 cases (90%) of the ER–/PgR+/HER2– tumors were classified as BLC, one as luminal-like (3.3%), and two as undetermined carcinomas (6.7%). Among the BLC cases, EGFR was positive in 24 (88.9%), and CK was positive in three (11.1%) cases. There was only a case considered positive for TFF1 with also presented positivity for EGFR (85% weak) and negativity for CK5. Two cases were negative for all three markers.

The patients' clinicopathological characteristics and the treatment regimen patients underwent are shown in Table 1 and 2, respectively.

The mean follow-up of the study group was 27.7 (2.7 to 50) months. Two patients lost follow-up right after diagnosis, and two lost follow-up at 2.7 and 7 months, respectively, with no tumor recurrence observed. Out of the 26 patients remaining, six had cancer recurrence: two local and four systemic recurrences. The average time for recurrence was 17 (8 to 38) months. The probability of disease-free survival at 3.1 years was 64%. Three (7.4%) patients died during the follow-up, two deaths were related to breast cancer recurrence.

The recurrences were correlated with different breast cancer subtypes. Two cases classified as inconclusive, presented systemic recurrences (RR 6,9; CI95% 2.4–14; p=0.046). Positivity for EGFR and CK5 was not related to prognosis (p=0.87 and p=0.64, respectively). Patients with tumors showing a higher number of cells with positive staining for EGFR and CK5 presented more recurrence than tumors without EGFR or CHK5 staining, but these data were not statistically significant (p=0.1 and p=0.058 respectively).

Table 1. Clinicopathological characteristics of the patients.

Characteristics	n=30
Age (years) – mean ± SD	56.7±12.5
Tumor Size (cm) – median	2,4 (1.8–3.8)
Lymph nodes	
N0 (%)	16 (57.1)
N1(%)	8 (25)
N2(%)	4 (10.7)
N3(%)	2 (7.1)
Histological subtype	
Invasive ductal carcinoma (%)	27 (83.3)
Inflamatory (%)	2 (6.7)
Medullar (%)	2 (6.7)
Metaplastic (%)	1 (3.3)
Lymphovascular invasion	
Yes (%)	12 (40)
No (%)	18 (60)
Histological grade	
2 (%)	11 (36.7)
3 (%)	19 (63.3)
Ki-67 (%)	
<14	1 (3.3)
≥14	29 (96.7)

SD: standard deviation.

Recurrence were no statistically associate with age (p=0.18), tumor size (p=0.35), lymph node involvement (p=0.61), tumor grade (p=1.00) and lymphovascular invasion (ILV) (p=0.16). Like radiotherapy, hormone therapy, and chemotherapy, none therapy modality was associated with recurrence (p>0.4).

A higher rate of complete pathologic response was observed in the basal-like subtype (44.4%).

DISCUSSION

We analyzed a large series of invasive breast carcinoma and identified 3.2% of the tumor phenotype ER-/PR+/HER2-. Although this subtype is rare, our results are consistent with other literature data, which described frequencies ranging from 1 to 5%[6-18].

Our study is the first to reproduce the immunohistochemical panel using TFF1, EGFR, and CK5 antibodies proposed by Yu et al.[9] In line with those authors, we identified the basal-like phenotype in most ER-/PR+/HER2- tumors. However, our

Table 2. Types of treatment.

Treatment	n=30 (%)
Type of surgery (n=29) – n(%)	
n°	3 (10.3)
BCS + SLN	9 (31.0)
BCS + ALND	6 (20.7)
Mastectomy + SLN	5 (17.3)
Mastectomy + ALND	6 (20.7)
Radiotherapy (n=28) – n(%)	
Yes	23 (82.1)
No	5 (17.9)
Chemotherapy (n=28) – n(%)	
Yes	20 (71.4)
Adjuvant	8 (40)
Neoadjuvant	11 (55)
Palliative	1 (5)
No	8 (28.6)
pCR (n=11) – n (%)	
Yes	4 (36.4)
No	7 (63.4)
Hormonal Therapy (n=28) – n (%)	
Yes	17 (60.7)
Tamoxifen	9 (52,9)
Anastrozole	8 (47.1)
No	11 (39.3)

SD: standard deviation; P: percentile; IDC: invasive ductal carcinoma; BCS: breast-conserving surgery; SLN: sentinel lymph node; ALND: axillary lymph node D: dissection; pCR: pathological complete response.

prevalence of BLC (90%) was even higher than previously reported (60%). This difference probably reflects biological variations in breast cancer between distinct populations[19].

It has been demonstrated that EGFR and CK5 had good accuracy in classifying triple-negative tumors as basal-like tumors[11]. The third antibody of the IHC panel was TFF1. It is an estrogen-sensitivity marker and, whenever positive, indicates that hormone therapy can be prescribed as effective adjuvant therapy[12]. Although only one tumor was positive for TFF1 in our series, 60% of our patients received either tamoxifen or anastrozole as part of their treatment. Therefore, the utility of these compounds in this specific clinical situation might be called into question and deserves further investigation. Almost 89.0% of the tumors were positive for EGFR, and more than half (55.6%) were positive for CK5. Previous studies show that both EGFR and CK5 are factors for poor prognosis[20,21]. In our study, patients with early disease recurrence had tumors with high expression of EGFR and CK5; however, the association was not statistically significant (p=0.4).

Basal-like carcinomas are known to be a very aggressive subgroup of tumors. They tend to develop distant metastases, particularly within the first five years after disease diagnosis, associated with poor oncological prognosis and high mortality[10]. Despite the limited length of follow-up in our study, cancer recurrence was detected in up to 23% of our patients, confirming the aggressive biological behavior of the ER-/PR+/HER2- tumors.

Our data seems to be following the study conducted by Fan et al.[16] They reviewed 3,966 breast cancers operated in China, between January 2005 to May 2008, finding 240 (6%) cases of ER-/PgR+/HER2- and 348 (8.8%) cases of triple-negative tumors. Although ER-/PgR+/HER2-carcinomas had a smaller tumor size (p=0.036) than triple-negative carcinomas, no significant differences were found between the two tumor groups in terms of relapse-free survival and overall survival. The authors concluded that ER-/PgR+/HER2- tumors should be regarded as a biologically and clinically distinct group of breast cancers, presenting an aggressive biological behavior.

Out of the 27 basal-like cases, 24 were invasive ductal carcinomas, two were medullar carcinomas, and one was metaplastic. The most common histological type in BLC is invasive ductal carcinoma, medullary, and metaplastic carcinomas[22,23]. All cases presented very high Ki67 values, with only one case presenting a <14% value. Sixty percent of cases presented grade 3, and the remainder were classified as grade 2 (40%), which reflected the association of BLC with high grade and high mitotic index tumors[10]. Lymph node involvement was identified in 38.5% of the cases, and tumor size had a median of 2.3 cm (0.6 to 5.8 cm), similar to a study conducted in 2017 by Li et al.[24], that demonstrated an association between advanced cancer stages and BLC tumors.

In our study, we identified a pCR rate of 44.4% in patients with the basal-like profile. Tumors with basal-like phenotype showed higher rates of pCR when compared to tumors with luminal phenotype. Chou et al.[25] demonstrated a response rate of 78% to chemotherapy with anthracyclines and taxanes in triple-negative breast carcinomas and a higher rate of pCR related with this subtype compared to luminal (45% versus 8%, respectively).

The median time for disease recurrence in our study was 17.8 months. Early disease recurrences are frequently observed in patients with tumors with the BLC phenotype. Li et al. described early disease recurrence, between one to three years after diagnosis, in patients with triple-negative tumors[25]. Also, the

rate of disease-free survival (64% in 3.1 years) was similar to the rate described in previous studies for basal-like tumors[22,24].

CONCLUSIONS

In conclusion, we showed that most ER-/PR+/HER2 breast tumors, which represent a rare and frequently misdiagnosed phenotype, can be classified as BLC. Further studies are warranted to elucidate the role of IHC in the management of this subgroup of tumors.

AUTHORS' CONTRIBUTIONS

RPN: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **DU:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **APD:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **JVB:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **G.R:** Writing – Review & Editing.

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ORIGINAL ARTICLE
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Factors associated with the perceived benefits and barriers to physical activity in liver cirrhosis

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SUMMARY

OBJECTIVE: To analyze the sociodemographic and clinical factors associated with the perceived benefits and barriers to physical activity (PA) in subjects with liver cirrhosis.

METHODS: This cross-sectional study assessed 102 outpatients with liver cirrhosis regarding the clinical and sociodemographic profile and the perceived benefits and barriers to PA by the Exercise Benefits and Barriers Scale and muscle strength. A Generalized Step-Forward linear regression analysis was used to identify the factors associated.

RESULTS: The participants were 59±10 years and 60.8% were men. Around 29.4% had ascites decompensation. Perceived benefits and barriers were associated with the presence of ascites (95%CI -0.079 – 0.03; p=0.06 and 95%CI 0.003 – 0.217; p=0.045, respectively). In the group with ascites, both benefits and barriers were associated with muscle strength. In the group without ascites, benefits were associated with cardiovascular risks and no association was observed with barriers to physical activity.

CONCLUSIONS: Perceived benefits and barriers to physical activity are associated with intrinsic factors such as the presence of ascites and cardiovascular risk in individuals with liver cirrhosis. The results of this study highlight key elements that must be considered for increasing physical activity in this population.

KEYWORDS: Health promotion. Liver cirrhosis. Physical activity.

INTRODUCTION

Cirrhosis is a chronic disease characterized by destruction and abnormal regeneration of the liver parenchyma[1]. The disease accounts for approximately 2 million deaths, making it the 11th most common cause of death worldwide[2]. The main causes are harmful alcohol consumption, viral hepatitis B and C, and metabolic syndromes related to overweight and obesity[3].

Two distinct stages of cirrhosis with different prognostic implications have been defined: compensated cirrhosis a stage 4 fibrosis with or without esophageal varicose veins; and decompensated cirrhosis, including variceal bleeding, hepatic encephalopathy, ascites, spontaneous bacterial peritonitis, and/or hepatorenal syndrome[4]. Chronic cirrhosis also affects the musculoskeletal system and leads to a reduction in peripheral muscle mass and

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sarcopenia, with consequent exercise intolerance, reduction in functional capacity, and quality of life[5,6].

Regular physical activity has been recommended in the management of chronic diseases and is associated with health benefits and control of disease progression[7]. Although limited data are available on the effect of regular physical activity on liver cirrhosis, recent studies have reported that exercise may preserve muscle mass and reverse sarcopenia, resulting in reduced fatigue, improved physical activity levels, and quality of life[6,8]. A recent systematic review of the literature, including a study in which lifestyle intervention with exercise and diet reduced the hepatic venous pressure gradient, physical exercise did not increase the frequency of adverse events[9]. Although the benefits of physical activity have been documented in patients with liver cirrhosis, adherence to a more active lifestyle is still low in this patient population.

Benefits of and barriers to physical activity have been described in other clinical populations, but it is still scarce on liver cirrhosis. To increase adherence to physical activity in cirrhosis patients, it is essential to understand their perceptions and behaviors towards physical activity[10].

The present study aims to identify the sociodemographic and clinical factors associated with perceived benefits of and barriers to physical activity in patients with liver cirrhosis.

METHODS

This is an observational, cross-sectional study conducted from January to July 2018. A convenience sample of outpatients diagnosed with liver cirrhosis who attended the Hepatology Outpatient Clinic of the Department of Gastroenterology of the University of Juiz de Fora was invited to participate. The study was approved by the institutional Ethical Committee (number 2.494.069). All patients signed a freely informed consent term.

Inclusion criteria were the previous diagnosis of liver cirrhosis made by clinical, laboratory, or histology methods, age ≥18 years and under 70 years. Exclusion criteria were inability to answer the questions, hepatocellular carcinoma, and malignancy, other chronic diseases as immunodeficiency syndromes, end-stage renal disease, neuromuscular diseases, cardiopulmonary disease (except diabetes and controlled systemic arterial hypertension), obesity grade II, or any other condition that presented physical limitations, not due to cirrhosis.

Information sociodemographic were collected from medical records. All respondents were asked about complications related to the disease including ascites decompensation, presence of esophageal varicose veins, and variceal bleeding during the last year, as well as cardiovascular risks (hypertension and diabetes). Additionally, laboratory exams regarding the Child-Pugh model[11] and classification of the disease were accessed from medical records within three months of the interview or according to the responsible physician.

The perceived benefits of and barriers to physical activity were assessed with the EBBS Brazil, a 42-item questionnaire: 14 belonging to the Barrier Scale ($EBBS_{BAR}$) and 28 to the Benefits Scale ($EBBS_{BEN}$). $EBBS_{BEN}$ score was calculated by the sum of 28 items into five domains: biological aspects, physical performance, psychological aspects, social interaction, and preventive health. $EBBS_{BAR}$ score was calculated by the sum of 14 items into four domains: time expenditure, physical exertion, exercise milieu, and family discouragement. Higher values indicated greater benefits or perceived barriers[12,13].

Handgrip strength (HGS) was assessed using the Jamar® dynamometer this is a valid method for the diagnosis of malnutrition and sarcopenia in patients with cirrhosis[14]. Sarcopenia may be a factor that is associated with barriers to physical activity in this population. Guidelines of the American Society of Hand Therapists were followed[15]. The highest value among the three measurements was considered for analysis.

Statistical analysis

Data were analyzed using SPSS Statistics version 19.0. Kolmogorov-Smirnov test was used to test the normality of the data. A descriptive analysis was performed, where continuous variables were summarized as mean±SD and categorical variables as frequency or percentage. Demographic variables, sex, and age were recorded; clinical variables, such as laboratory tests for total bilirubin, albumin, and INR referred to the Child-Pugh model[20]; complications related to the disease in the last year included ascites decompensation, presence of esophageal varicose veins, and variceal bleeding; associated cardiovascular risks (diabetes/hypertension); and handgrip strength. A generalized linear regression analysis using gamma distribution and logarithmic linking functions was used to examine the association between $EBBS_{BEN}$ and $EBBS_{BAR}$ and independent variables. The level of significance for univariate analysis was set at $p<0.10$. For multivariate analysis, variables with $p<0.05$ remained in the model and were included following the *step-forward* method. The percentage of individuals who strongly agreed or agreed with the barrier/benefit item was analyzed for each question as well as for each domain. Comparisons were made using Student's t-test and Mann-Whitney test, and statistical significance was accepted at $p<0.05$.

RESULTS

This study included 102 outpatients. The mean age of the study group was 59.2 years (SD 10.58), 60.8% were men, 57.8% were diagnosed with CP-A. The most common disease etiologies were alcohol (38.2%), hepatitis C (30.4%), nonalcoholic fatty

liver disease (21.6%), and others (9.8%). In the sample, 48% had esophageal varicose veins and 29.4% had ascites decompensation during the last year. At the time of the evaluation, seven patients (6% of all samples) presented ascites, which were classified as Child C. Diabetes and systemic arterial hypertension were present in 43.1 and 57.8%, respectively (Table 1).

EBBS Brazil had good reliability. The reported internal consistency (Cronbach's alpha) for the total, $EBBS_{BEN,}$ and $EBBS_{BAR}$ were 0.87, 0.90, and 0.78, respectively. Using generalized linear regression analysis, it was identified a significant association between ascites, and $EBBS_{BEN}$ and $EBBS_{BAR}$ (Table 2). Patients were divided into two groups: one group involving patients with had ascites and the other one without ascites in the last year. Then, we analyzed the relationship between benefits of and barriers to physical activity in these groups separately (Table 3). In the group with ascites, the $EBBS_{BEN}$ (95%CI 0.001–0.009, β=0.005, p=0.019) and $EBBS_{BAR}$ (95%CI -0.027– -0.003, β=0.109, p=0.016) was associated with handgrip strength. In the group with non-ascitic patients, there was an association between $EBBS_{BEN}$ and the presence of cardiovascular risks (95%CI 0.005–0.096, β=0.05, p=0.03) with no association with $EBBS_{BAR}$.

Considering EBBS responses, both groups presented high perceived benefits for physical activity. Mean $EBBS_{BEN}$ was 96.53 (SD 9.15) in the group with ascites, and the domains with the highest agreement percentage (≥95%) were biological aspects, physical performance, and psychological aspects. Mean $EBBS_{BEN}$ was 100.29 (SD 9.2) in the group without ascites, and the domains with the highest agreement percentage (≥95%) were biological aspects and physical performance. Individuals without decompensation had a higher agreement percentage (≥80%) in the preventive health domain. When compared, the

Table 1. Baseline demographic and clinical characteristics of patients with liver cirrhosis.

	n=102
Age, years*	59.20±10.58
Gender, F/M (%)	62:40
Disease severity	
Child-Pugh A, n (%)	59 (57.8)
Child-Pugh B/C, n (%)	43 (42.2)
Etiology	
Alcohol, n (%)	39 (38.2)
HCV, n (%)	31 (30.4)
NAFLD, n (%)	22 (21.6)
Others, n (%)	10 (9.8)
Esophageal variceal bleeding in the last year, n (%)	53 (52.0)
Ascites in the last year, n (%)	72 (70.6)
Bilirubin (mg/dL)*	1.68±1.31
Albumin (g/dL)*	3.67±0.65
INR*	1.56±2.59
Cardiovascular risk	
Diabetes, n (%)	58 (56.9)
Hypertension, n (%)	43 (42.2)
Handgrip strength (kgf)*	28.21±10.12

*Mean±standard deviation. HCV: hepatitis C virus; NAFLD: nonalcoholic fatty liver disease; INR: international normalized ratio.

Table 2. Generalized linear regression analysis of factors associated with EBBS Benefits and EBBS Barriers.

	EBBS Benefits	EBBS Barriers	
	Univariate	Univariate	Multivariate
	β (95%CI)	β (95%CI)	β (95%CI)
Age	-0.001 (-0.002–0.001)	-0.001 (-0.006–0.004)	–
Sex (female)	-0.008 (-0.047–0.030)	0.057 (-0.044–0.159)	–
Bilirubin	0.000 (-0.015–0.014)	-0.034 (-0.071–0.004)*	–
Albumin	0.014 (-0.016–0.044)	0.035 (-0.054–0.124)	–
INR	-0.022 (-0.066–0.021)	0.081 (-0.032–0.194)	–
HGS	0.000 (-0.002–0.002)	-0.004 (-0.009–0.001)*	–
Ascites	-0.038 (-0.079–0.003)*	0.110 (0.003–0.217)*	0.110 (0.003–0.217)*
Esophageal variceal bleeding	-0.030 (-0.067–0.008)	0.065 (-0.034–0.164)	–
Cardiovascular risks	0.022 (-0.018–0.062)	0.059 (-0.047–0.165)	–

CI: confidence interval; INR: international normalized ratio; HGS: handgrip strength. *p<0.05 (univariate) and *p<0.10 (multivariate).

Table 3. Sociodemographic and clinical characteristics of the groups with and without ascites.

Variable	Without ascites (n=72)	With ascites (n=30)
Age	58.76±11.12	60.23±9.27
Gender		
Male (%)	45 (62.5)	17 (56.7)
Female (%)	27 (37.5)	13 (43.3)
Child-Pugh A (%)	52 (72.0)	7 (23.0)
Child-Pugh B/C (%)	20 (28.0)	23 (77.0)
Bilirubin (mg/dL)	1.66±1.27	1,74±1.42
INR	1.58±3	1,53±0.64*
Handgrip strength	30.46±10.32	22,83±7.3*
Esophageal variceal bleeding (%)	29 (40.3)	20 (66.7)
Cardiovascular risks (%)	49 (68.0)	21 (70.0)

Data represented as mean±standard deviation. INR: international normalized ratio; *p<0.05.

perception of benefits in biological aspects and physical performance domains were higher in the group without ascites.

$EBBS_{BAR}$ mean was 31.47 (SD 7.83) in the group with ascites and 28 (SD 7) without ascites. For both, the highest perception was the physical effort domain, with an agreement greater than 60%. Regarding $EBBS_{BAR}$ affirmatives, "exercise tires me" showed 80% agreement in the group with ascites and 68% in the group without ascites. In the statement "I am fatigued by exercise" there was 73% agreement in the group with ascites and 70% in the group without ascites. When comparing barriers perception in the exercise and environment domains, it was higher in the group with ascites.

When mean scores were compared, patients with ascites decompensation perceived greater barriers to physical activity (28.5±7 *versus* 31.4±7; p=0.04), while those without ascites decompensation perceived greater benefits to physical activity (96.5±9 *versus* 100±9; p=0.04).

DISCUSSION

To our knowledge, this is the first study to address factors associated with perceived benefits of and barriers to physical activity in patients with liver cirrhosis. The main study findings show that patients who had ascites in the last year had different perceived benefits of and barriers to physical activity than those who did not.

The natural history of liver cirrhosis is marked by a phase termed "compensated", evidenced by being asymptomatic, where portal pressure may be normal or below the threshold level identified for the development of varicose veins or ascites. There is a second phase termed "decompensated cirrhosis" in which portal pressure increases and liver function decreases with the progression of the disease, resulting in several complications[16]. Ascites occur at the rate of 6–7% annually so that after a decade, 60–70% of cirrhosis patients will have developed ascites[17]. Besides ascites, other complications are expected with the progress of the disease. The incidence of malnutrition increases with the progression of liver failure and is reported in approximately 20% of individuals with compensated cirrhosis and in more than 80% in decompensated cirrhosis[18]. A reduction in muscle mass, strength, and function, called "sarcopenia", is an important adverse clinical consequence observed in liver cirrhosis[19].

Our results showed that the group with ascites presented reduced HGS (22.83±7.3) compared to the group without ascites (30.45±10.32). The degree of malnutrition is frequently masked by the presence of ascites in cirrhosis patients[20]. Measurement of HGS is a valid, simple, rapid, inexpensive method for investigating nutritional status. The Japan Society of Hepatology proposed that is one of the key criteria for evaluating liver cirrhosis[21]. Although there are no pre-established benchmarks for cirrhosis, some recent studies have reported values that help in this evaluation[21,22]. In this group, patients with lower handgrip strength perceived greater barriers to physical activity, while those with higher strength perceived greater benefits. Alterations in the musculoskeletal system lead to reduced physical performance, disability, increased risk of related injuries, and frailty[23]. Thus, it is understandable that perceived benefits of and barriers to physical activity have been related to greater or lesser muscle strength.

The findings of Naseer et al. (2019) suggest that changes in muscle mass and strength are potentially modifiable in cirrhosis with exercise[24]. Other trials also related exercise to improved functional capacity, quality of life, fatigue, and reductions in hepatic venous portal gradient, without adverse events[25]. Considering that muscle strength in patients with ascites was associated with perceived benefits and barriers, we suggest that, in addition to the guidelines on the importance of physical activity and participation in physical training programs, these individuals should also be followed up for their longitudinal strength measure. Since force is a marker for comorbidity and mortality, this measure should not be neglected and should be a routine approach in clinical practice. In addition, interventions such as increased physical activity to reduce the symptoms and fragility of these individuals imply an improvement in the quality of life and reduced symptoms of decompensated

cirrhosis, tending to decrease the financial burden of the patient, the caregivers, and the health system[20].

Greater benefits of physical activity were still perceived by those who had diabetes and/or associated hypertension in the group without ascites. These individuals also had a higher agreement in the preventive health domain, which includes prevention of heart problems, prevention of high blood pressure, and longevity with the practice of physical activity, demonstrating greater perception of morbidity and mortality. For several chronic diseases, accumulated evidence has led to the development of exercise-based rehabilitation programs with government funding[25]. It is possible, that these patients may have better-perceived benefits because they are assisted by a multidisciplinary team due to the presence of cardiovascular risks. Although there are few guidelines for physical activity in liver cirrhosis, this is a consolidated recommendation for the clinical treatment of hypertension and diabetes[7].

Some limitations were present in the study: *i)* it was an observational study, requiring longitudinal studies that demonstrate that complications cause negative behaviors and perceptions related to physical activity; *ii)* although this was not the objective of the study, there was no qualitative assessment of barriers and benefits reported by the patients; and *iii)* a relatively small number of patients in the group that presented ascites in the last year leading to the possibility of a type II error for the studied variables, this limitation was due to the complexity to find outpatients with more severe cirrhosis.

We identified an influence of previous or current ascites decompensation concerning the perceived benefits of and barriers to physical activity. Patients with ascites, but with greater handgrip strength, identified more benefits perceived to physical activity. Individuals without ascites in the last year and the presence of cardiovascular risks were also associated with the perception of greater benefits for physical activity. The recognition of factors associated with perceived benefits of and perceived barriers to physical activity in patients with liver cirrhosis points to alternatives for interventions that allow changes in lifestyle and management of these patients.

CONCLUSION

In conclusion, the results of our study show that the EBBS Brazil presented good reliability in liver cirrhosis patients, and for the patients with ascites, the perception of the benefits of physical activity was positively associated with muscle strength while the perception of barriers was negatively associated with muscle strength. In the group without ascites the perception of the benefits of physical activity was associated with the presence of cardiovascular risks. These findings are important elements that should be considered to encourage increased physical activity in this population.

AUTHOR'S CONTRIBUTION

MRS: Data Curation, Investigation, Project Administration, Writing – Original Draft. **FHLP:** Conceptualization, Funding, Resources, Formal Analysis, Visualization. **TML:** Data Curation, Investigation, Project Administration. **DMNH:** Data Curation, Investigation, Project Administration. **PACM:** Conceptualization, Methodology, Funding, Resources. **TMDO:** Data Curation, Investigation, Project Administration. **CCO:** Investigation, Project Administration, Conceptualization, Resources, Supervision, Visualization, Writing – Review & Editing. **ASA:** Conceptualization, Funding Acquisition, Project Administration, Resources, Supervision, Visualization, Writing – Review & Editing. **CM:** Conceptualization, Funding Acquisition, Project Administration, Resources, Supervision, Visualization, Writing – Review & Editing.

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Exercise training program in children with lower-limb amputation

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SUMMARY

OBJECTIVE: Few physical exercise programs for children with limb loss have been described in detail recently. We provided information regarding the characteristics and effectiveness of an alternative rehabilitation exercise developed for children with lower-limb amputation.

METHODS: An 8-year-old boy with a below-knee amputation and a 9-year-old bilateral amputee girl performed an exercise program of one 2-h session per week for 20 weeks, aimed at developing muscular strength and coordination. Walking ability and walking speed were assessed by using the L-test of functional mobility and 10-m walk test, respectively. Mechanical and neuromuscular muscle function was assessed by using tensiomyography.

RESULTS: In case 1, a decrement of 9.5% and 10.5% was found in the L-test (42 s vs. 38 s) and in the 10-m test (19 s vs. 17 s) scores, respectively. In case 2, walking ability remained unchanged (L-test score: 38 s), while a 5.2% reduction in walking speed was observed (10-m test score: 19 s vs. 18 s). No relevant changes were observed in the muscular tone in both cases.

CONCLUSIONS: Practitioners should be aware that, contrary to what could be expected, a multidisciplinary training program held once per week for 5 months had a minimal impact on the gait pattern and neuromuscular function of two children with lower-limb amputation.

KEYWORDS: Amputees. Child. Exercise. Gait. Muscle tonus.

INTRODUCTION

Children with lower-limb amputation must adopt specific compensation strategies to overcome the limitations associated with amputation in their daily activities, mainly due to the loss of muscle mass and proprioception functions[1]. In fact, scientific evidence has shown that in children, the amputation of a lower limb can result in several biomechanical (i.e., changes on the level of muscular co-contraction, gait asymmetry, and increased loading of both lower limbs)[1] and physiological limitations (i.e., increased oxygen demand and higher heart rate while walking)[2,3]. Similarly, kinematic and spatiotemporal lower limb asymmetries can be present because of loss of muscle function[4]. As a result, children with lower-limb amputation show impaired balance, decreased postural control, slower walking patterns, and increased physiological energy costs[4,5]. In addition, it has been suggested that some of these limitations could lead to degenerative joint diseases in the long term[6,7].

Therefore, rehabilitation strategies are needed for this population, especially those aimed at normalizing gait patterns and restoring muscle function. In this regard, physical exercise has been confirmed as a useful therapeutic approach in lower-limb amputees given that its performance can lead to significant improvements in their fitness level and locomotor skills[8,9]. However, the vast majority of studies that provide

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information about the feasibility, characteristics, and effects of physical exercise training programs in people with lower-limb amputation have been focused on adult populations, and there are very few reports describing this rehabilitation process in pediatric populations. This lack of research is a matter of concern for several reasons. First, according to the authors' knowledge, there is no clear consensus on the best practice guideline regarding the physical therapy process that should be carried out by a child following lower-limb amputation. Second, children have complex and specific rehabilitation needs that are distinguishable from those of adults (i.e., expected skeletal growth, functional demand on the locomotor system and prosthesis, appositional bone stump overgrowth, and psychological challenges)[10], implying that they require tailored rehabilitation programs that are different from the ones usually carried out with adult populations[11]. Finally, it is difficult to engage children in prescribed exercise rehabilitation programs, due to the inclusion of repetitive and often tedious tasks during the exercises, which suggests the need for exploring the efficacy of other dynamic and more attractive therapy approaches for younger amputees[12]. Under these circumstances, this case study aims at providing information regarding the effectiveness of an alternative rehabilitation exercise intervention on walking ability and mechanical and neuromuscular functions of two children with lower-limb amputation.

METHODS

Participants

Two children with lower-limb amputation who were referred to rehabilitation agreed to take part in this study. Both children were established walkers who used their prostheses every day for more than 4 years. Case 1 was a boy (age: 8 years, height: 131 cm, and weight: 31 kg), with a below-knee amputation, who met with an accident with a motor vehicle when he was 2 years old. Case 2 was a bilateral amputee girl (age: 9 years, height: 139 cm, and weight: 33 kg), due to meningitis that she suffered at the age of 5. She had a lower-limb amputation at the transtibial (left) and knee disarticulation levels (right). Written informed consent was obtained from both the parents and the children, who consented to the experimental measurements. The protocol of this study was approved by the Local Ethics Committee, and this study was conducted in accordance with the Declaration of Helsinki.

Procedures

Both children took part in an exercise training program performed once a week for 20 weeks. The sessions were 2-h-long and included exercises aimed at developing muscular strength and coordination. The first part of the session was based on the performance of core training (CT) exercises. Initially, the children performed one set of six exercises, for 20 s each. As the intervention progressed, the number of exercises increased as well as the number of sets. The children ended the program by performing 3 sets of 10 exercises, with a 2-min resting interval between them. The second part of the session was focused on coordination exercises and lower-limb strengthening exercises. The children progressed from performing 1 set of 10 exercises to 3 sets, with a 1-min resting interval between them. Drills involved in a typical training session were as follows: (a) CT: (i) forearm plank with toes tucked under (3×20 s with 2 min recovery), (ii) bent-knee forearm side plank (3 × 20 s each side with 2 min recovery), (iii) Bosu V-sit and pectoralis activation with ring (3×20 s with 2 min recovery), and (iv) bridging on a mat with the pelvis raised off the mat (3×20 s with 2 min recovery); (b) coordination: (i) stepping over the mini-hurdles, the prosthetic lower limb is not abducted (5×10 mini-hurdles with 2 min recovery) and (ii) stepping up and down in a forward direction (3×10 reps each lower limb with 2 min recovery); and (c) strength: (i) lateral squat with hands at the waist (3×10 reps with 2 min recovery), (ii) lateral squat walk (3×15 m with 2 min recovery), (iii) 1 kg medicine ball half squat throw (3×10 reps with 1 min recovery), and (iv) standing leg abduction with resistance band (3×10 reps with 1 min recovery).

All sessions were performed on a sport facility and were designed and supervised by an exercise and rehabilitation specialist with experience in the prescription of physical exercise for people with lower-limb amputations.

Measurements

Walking ability and walking speed were assessed by the L-test of functional mobility and 10-m walk test (10MWT)[13], respectively. Mechanical and neuromuscular muscle functions were assessed by tensiomyography (TMG), a noninvasive method to measure radial muscle belly displacement of the biceps femoris (BF) and rectus femoris (RF) in the lower limbs of both children. The assessment followed the protocol suggested by Simunic et al.[14] Each measurement involved recording the following parameters of involuntary isometric contraction produced by the electrical stimulus: Dm is the maximum radial muscle belly displacement of the muscle measured in mm, Tc is the time measured in ms from 10% to 90% of Dm of the muscle, and radial displacement velocity (Vrd) is the rate (mm/s) between the radial displacement occurring during the time period of Tc (Dm80) and Tc (Dm80/Tc).

RESULTS

Both children completed a total of 20 sessions. TMG results are presented in Table 1 for both participants. In case 1, RF showed a decrease in muscle tone in both lower limbs, being greater in the amputated lower limb (an increase of 20.8% of the Dm). In the BF of the amputated lower limb, a reduction in muscle tone was also observed (an increase of 62.5% of the Dm), which was increased in the opposite lower limb. In case 2, an increase in muscle tone was observed in the RF of the knee-disarticulated limb (right) and a reduction in the RF of the transtibial amputated limb. No changes were observed in the BF of both lower limbs.

Moreover, in case 1, there was an increase in Vrd in the muscles tested in both lower limbs, except for BF in the nonamputated side. In case 2, the Vrd tested in the RF decreased in the knee-disarticulated limb and increased in the transtibial amputated limb. No relevant changes were observed in the Vrd of the BF.

Regarding walking ability and walking speed, the observed changes were of little relevance in both children. In case 1, a decrement of 9.5% and 10.5% was found in the L-test (42 vs. 38 s) and in the 10-m test (19 vs. 17 s) scores, respectively. In case 2, walking ability remained unchanged (L-test score: 38 s), while a 5.2% reduction in walking speed was observed (10-m test score: 19 vs. 18 s).

DISCUSSION

In this study, a training program based on the performance of strengthening and coordination exercises has shown to be feasible for being carried out by two children with lower-limb amputation. However, its effects on the walking ability and walking speed were modest at best. One explanation for this lack of effects could be the characteristics of the training program, in which specific balance exercises were underrepresented. In this intervention, CT was expected to lead to a better walking ability and walking speed through balance improvements. In people with reduced mobility, it has been suggested that CT can reduce balance impairment through improvements in trunk muscle strength on the basis that a stable core facilitates the transfer of torque and angular momentum between the lower and upper extremities, providing proximal stability for distal mobility[15]. In fact, previous study has shown that the performance of CT has resulted in balance improvements in people with impaired gait performance[16]. According to the present findings, it seems that CT does not have these effects on these two children with lower-limb amputation. Finally, it should be noted that the impact of the program on walking ability and walking speed was slightly different since a positive trend was observed in the boy who had suffered an amputation below the knee, while no changes were observed in the girl with transtibial amputation. These findings could be related to the different

Table 1. Assessments of descriptive tensiomyography parameters of preintervention and postintervention.

Case 1							
Parameters	Side	RF pre	RF post	Dif%	BF pre	BF post	Dif%
Tc	R	27.8 ms	24.5 ms	–11.8	26.2 ms	35.4 ms	35.1
	L	52.8 ms	43.7 ms	–17.2	33.1 ms	26.4 ms	–20.2
Dm	R	4.8 mm	5.8 mm	20.8	7.2 mm	11.7 mm	2.5
	L	8.6 mm	9.3 mm	8.1	8.2 mm	5.5 mm	–32.9
Vrd	R	138.1 mm/s	189.3 mm/s	27	219.8 mm/s	264.4 mm/s	16.8
	L	130.3 mm/s	170.2 mm/s	23.4	198.1 mm/s	166.6 mm/s	–18.9
Case 2							
Parameters	Side	RF pre	RF post	Dif%	BF pre	BF post	Dif%
Tc	R	94.2 ms	48.4 ms	–48.6	45.6 ms	51.8 ms	13.5
	L	22.0 ms	22.1 ms	0	47.1 ms	49.3 ms	4.6
Dm	R	3.1 mm	1.2 mm	–61.2	3.4 mm	3.5 mm	2.9
	L	3.0 mm	4.3 mm	43.3	6.4 mm	6.3 mm	–1.5
Vrd	R	26.3 mm/s	19.8 mm/s	–32.8	59.6 mm/s	54.0 mm/s	–10.2
	L	109.0 mm/s	155.6 mm/s	29.9	108.7 mm/s	102.2 mm/s	–6.3

Dm: maximum radial muscle belly displacement of the muscle; Tc: time from 10% to 90% of Dm of the muscle; Vrd: radial displacement velocity; RF: rectus femoris; BF: biceps femoris; Dif%: difference percentage; R: right; L: left.

gait patterns observed between children with an amputation distal to the knee or above the knee[17] and imply that rehabilitation strategies for children with lower-limb amputation could differ according to the amputation level.

After the intervention, a decrease in muscle tone was observed in the amputated lower limb in case 1, which can be considered as a positive effect of the intervention. However, in case 2, this effect was only evident in the RF of the transtibial amputated limb. Also, in case 1, a noticeable increase in the Vrd was observed in both lower limbs. The variable Vrd was sensitive to control training effects on sprinting speed in elite soccer players[18]. In this study, this increase could also be considered a positive effect. In relation to the Tc, previously carried out research with 9-year old children found that the lower this parameter was, the greater the running speed. However, no similar trend was observed in our study, once the training program was over. However, in relation to the Tc, although this parameter has been useful to differentiate the fastest 9-year-old children due to a lower Tc[19], no clear trend is evident for the Tc in our two cases as a result of the training program.

This study showed the results of an original rehabilitation approach. The description of the proposed training program and the obtained results could help rehabilitation professionals to develop new physical therapy procedures for children with lower-limb amputation. In this regard, it should be noted that although exercises were designed to improve strength and coordination, and in some ways, a very slight change in gait pattern was expected. The low frequency of training may help explain the relative improvement obtained with the training program.

CONCLUSIONS

In conclusion, a multidisciplinary training program held once a week for 5 months has shown to be feasible for being carried out in two children with lower-limb amputation. However, its performance had a minimal impact on their walking speed, walking ability, and neuromuscular function.

AUTHORS' CONTRIBUTIONS

OG and SM: Data curation and formal analysis. **DS:** Supervision, writing – original draft, and writing – review and editing. **CA:** Conceptualization and writing – original draft.

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Effect of pulsed electromagnetic field therapy in patients with supraspinatus tendon tear

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SUMMARY

OBJECTIVE: The aim of this study was to compare the effect of transcutaneous electrical nerve stimulation (TENS), ultrasound (US), and pulsed electromagnetic field (PEMF) combination with TENS and US therapy alone in patients with supraspinatus tear.

METHODS: Forty patients were included in this study. The patients were randomly divided into two groups as follows: PEMF (n=20) and Sham (n=20) groups. PEMF was applied to the first group at a frequency of 50 Hz, 25 G intensity, and 20 min/session. The device was turned off while PEMF was applied to the second group. Diathermy (US) and electrotherapy (TENS) were applied to both groups for 10 sessions. Numerical Rating Scale (NRS), University of California–Los Angeles (UCLA) Shoulder Scale, and Shoulder Pain and Disability Index (SPADI) were used as outcome measures.

RESULTS: In both groups, there was a significant improvement in the NRS, UCLA Shoulder Scale, and SPADI scores after treatment compared with pretreatment (p<0.05). In the comparison of the difference between the pretreatment and posttreatment measurement values between the groups, no significant difference was found between PEMF and Sham groups according to the NRS (p=0.165), UCLA Shoulder Scale (p=0.141), and SPADI (p=0.839) scores.

CONCLUSIONS: In our study, a combination of PEMF therapy with conventional physical therapy modalities was not found to be superior to the conventional therapy alone, and adding it to the routine treatment of symptomatic supraspinatus tear would not provide any additional benefit.

KEYWORDS: Diathermy. Rotator cuff. Magnetic field therapy. Transcutaneous electric nerve stimulation.

INTRODUCTION

Shoulder pain is an important cause of disability and morbidity in individuals' daily life activities causing limitedness and loss of workforce[1]. Rotator cuff pathologies lead by up to 70% among the causes of shoulder pain[2]. It is observed that supraspinatus tendon is mostly affected by the background of subacromial impingement syndrome. Chronic tears are seen later in life as a result of chronic overuse and degeneration[3].

Degenerative tears, which make up most of rotator cuff tears, can be partial or complete. It can be accompanied by symptoms such as pain and loss of function, but it may remain asymptomatic. Conservative treatment should be preferred in partial rotator cuff tears. The surgical indication is limited to symptomatic cases that cause loss of function in full-thickness tears. The purpose of conservative treatment is to reduce pain, eliminate joint motion limitation, improve shoulder functions, and improve muscle strength. Physical therapy modalities and exercise are becoming increasingly important due to the side effects of analgesic drugs. Heat application with therapeutic ultrasound (US) and transcutaneous electrical nerve stimulation (TENS) are the physical therapy modalities that have been widely used in rehabilitation clinics for a long time. The pulsed

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electromagnetic field (PEMF), which is among the physical therapy methods, has started to be used in the treatment of many musculoskeletal diseases. Magnetic field therapy increases the local cellular activity, provides the organization of collagen fibers, increases the use of oxygen in the tissues, and accelerates the circulation by increasing the vasodilation of blood vessels without increasing local temperature[4]. There are insufficient data on its evidence-based efficacy and superiority to physical therapy agents.

This study aims to compare the effect of TENS, US, and PEMF combination on pain and functional status in patients diagnosed with supraspinatus tear with TENS and US therapy alone.

METHODS

This study was performed according to the Declaration of Helsinki and with permission from the local ethics committee (No. 2018/04). All patients included in this study were informed in detail about this study, and written informed consent forms were obtained.

This study was a prospective randomized study. A total of 40 patients who were diagnosed as supraspinatus tear by MRI and who had fulfilled the inclusion criteria were included in this study. The patients were randomly divided into two groups as follows: the patients who received PEMF therapy (Group 1, n=20) and those who received Sham–PEMF therapy (Group 2, n=20). A sealed envelope method was used for randomization. The patients were treated with PEMF in Group 1, and the device was set to 0 to prevent from current flow when applying to the patients in Group 2. Diathermy application (US) and TENS treatments were applied to all patients within the scope of the standard physical therapy program.

Treatments were done by a physiotherapist, and the results were followed up by experienced physiatrists observationally. Information about the cases deemed eligible to participate in the study was recorded in the patient assessment form prepared at the first evaluation session. All patients were evaluated twice routinely with shoulder pain and functionality measurements, at the beginning and the end of the treatment program.

All patients were included in the physical therapy program, i.e., one session a day, 5 days/week for a period of 2 weeks. Diathermy application was performed with a therapeutic US device (Chattanooga, Intelect Advanced, United Kingdom) for 10 min to the relevant shoulder at a dose of 1 W/cm^2. The TENS device (Fizyomed Fizyotens, Turkey) with two electrodes surrounding tendon was performed for 30 min at a frequency of 100 Hz for current application. The intensity of current was applied in the amplitude, which does not create muscle contraction and creates a feeling of numbness and tingling.

In the PEMF application, with the PEMF device (Roland HC, Pagani Elettronica, Italy), two solenoid applicators were placed at the anterior and posterior positions in the patient's shoulders and applied for 25 min at 25 G intensity at a frequency of 50 Hz.

All cases were evaluated clinically and radiographically. For a definitive diagnosis, anamnesis, locomotor system examination, biochemical tests, and shoulder MRI images were used. Numerical Rating Scale (NRS), University of California–Los Angeles (UCLA) Shoulder Scale, and Shoulder Pain and Disability Index (SPADI) were used before and after treatment to assess pain and functional status.

Statistical analysis

All statistical analyses were performed using SPSS software for Windows version 24.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test the normal distribution of continuous variables. The Wilcoxon signed-rank test was used to determine the change of values within the groups before and after treatment scores. The Mann–Whitney *U*-test was used to evaluate the difference between patients' pretreatment and posttreatment scores by groups. A p-value <0.05 was considered statistically significant.

RESULTS

Out of 40 patients, 13 were males and 27 were females with a median age of 66 (range: 45–85) years. Regarding the type of tear, the complete tear was detected in 5 (12.5%) of all patients and the partial tear in 35 (87.5%) patients. There was no significant difference between the two groups in terms of age, gender, and impaired shoulder (Table 1).

When the NRS, UCLA Shoulder Scale, and SPADI scores of the PEMF (Group 1) and Sham (Group 2) groups were compared between the first and the last measurements of within-group values, a significant decrease was found in the NRS, UCLA Shoulder Scale, and SPADI values in both groups (p<0.001; Table 2).

When comparing the difference between the pretreatment and posttreatment values between the groups, there was no statistically significant difference between Group 1 and Group 2 according to the NRS, UCLA Shoulder Scale, and SPADI scores (p-values are 0.146, 0.141, and 0.839, respectively; Table 3).

DISCUSSION

Rotator cuff syndrome is the most common cause of shoulder pain seen on the background of subacromial impingement syndrome, which can be seen with different settings from tendinitis to complete tear in the rotator cuff. It can be partial or full thickness according to the affected tendon region. In the classification of Neer's subacromial impingement syndrome, rotator cuff tears are included in Stage 3, whereas reversible edema and tendinitis develop in Stage 1, tendinosis develops in Stage 2, and tear occurs in Stage 3[5]. In our study, patients with chronic rotator cuff tear (Stage 3) were treated. There may be symptoms, such as pain and loss of function in rotator cuff tears, and asymptomatic tears may also occur. Partial tears become more symptomatic due to the tension in the intact muscle fibers and increase the admissions to the hospitals[6]. The partial tear dominance in our study may be related to this situation.

In cases other than acute traumatic tear, the conservative approach is recommended for the treatment. In addition to medical therapy, physical therapy agents such as electrotherapy, superficial and deep heaters, and their combination with exercise practices are often used. The conservative treatment applications in patients with full-thickness tear help reduce inflammation, control pain, and maintain muscle strength. Moosmayer et al.[7] investigated the results of surgical and conservative (i.e., physiotherapy) treatment of patients with full-thickness tears. After 1 year of follow-up, patients in the surgery group showed better results than the physiotherapy group; however, they reported that 82% of patients in the second group also showed acceptable improvement. In the literature, physical therapy applications are more effective on pain and functional status than placebo in the treatment of rotator cuff syndrome[8].

There are few studies in the literature regarding the use of PEMF therapy in rotator cuff syndrome. Hypotheses on this

Table 1. Demographic data of the groups.

	Group 1 (PEMF)	Group 2 (Sham)	p
Average age	65.6±6.6	64.6±11.5	0.727
Gender (females/males)	13/7	14/6	0.736
Impaired shoulder (right/left)	10/10	7/13	0.337
Impaired shoulder (dominant/nondominant)	11/9	7/13	0.204

PEMF: pulsed electromagnetic field.

Table 2. In-group evaluation results of the scales.

		Pretreatment Median (min–max)	Posttreatment Median (min–max)	*z*	p
NRS	PEMF	4.5 (0–8)	2 (0–5)	-3.49	<0.001
	Sham	4.5 (0–8)	0.5 (0–4)	-3.53	<0.001
UCLA	PEMF	14.5 (11–31)	25 (21–35)	-3.828	<0.001
	Sham	15 (10–29)	24.5 (14–31)	-3.632	<0.001
SPADI	PEMF	70 (13.07–90.7)	50.75 (0–80)	-3.823	<0.001
	Sham	69.2 (26.15–86.1)	46.9 (16.9–62)	-3.921	<0.001

NRS: Numerical Rating Scale; UCLA: University of California–Los Angeles Shoulder Scale; SPADI: Shoulder Pain and Disability Index; PEMF: pulsed electromagnetic field.

Table 3. Comparison of changes in groups before and after treatment.

	Group 1 (PEMF) Median (min–max)	Group 2 (Sham) Median (min–max)	*z*	p
NRS	2 (0–5)	2.5 (0–8)	-1.38	0.165
UCLA	10 (0–23)	9 (0–14)	-1.471	0.141
SPADI	21.5 (0–68)	21.53 (6–46.9)	-0.203	0.839

NRS: Numerical Rating Scale; UCLA: University of California–Los Angeles Shoulder Scale; SPADI: Shoulder Pain and Disability Index; PEMF: pulsed electromagnetic field.

subject are based on the effect of the magnetic field to promote soft tissue healing. Tucker et al.[9] showed earlier improvement of the rotator cuff tendons and an increase in bone quality histopathologically after the application of PEMF therapy to the shoulder joint in rats. In a study conducted with cell cultures, it was shown that growth factors and the expression of cytokines increased in tendon cells with a low-frequency pulsed magnetic field, and it was concluded that PEMF stimulates tendon cell proliferation and has no toxic effect[10].

We found that the oldest clinical study regarding the use of PEMF on the shoulder was published by Binder et al.[11] in 1984. They reported that PEMF therapy is beneficial in severe and persistent chronic rotator cuff lesions.

Aktaş et al.[12] examined the effectiveness of PEMF therapy in two groups of patients with 46 subacromial impingement syndromes in a double-blind randomized controlled study. For the first group, PEMF therapy has been applied for 15 sessions, and Sham–PEMF therapy has been applied to the second group for the same period. At the end of 3 weeks, there was a significant improvement in the Visual Analog Scale (VAS) scores, Constant score, and Shoulder Disability Questionnaire scores of the two groups compared with the baseline, but they did not find any difference between the two groups.

Freitas et al.[13], in a study investigating the effectiveness of PEMF in patients with impingement syndrome, have applied PEMF therapy to the treatment group and Sham–PEMF therapy to the control group. Then, they included the patients in both groups on a 6-week home exercise program. From the patients who were followed up for 3 months after the treatment, the group receiving active PEMF therapy showed more functional improvement and fewer pain symptoms at all stages than the initial value. The authors concluded that PEMF therapy had positive effects on functional improvement, muscle strength, and pain relief in patients with subacromial impingement syndrome.

Finding the results of these studies in opposition to each other may be due to the difference in the methods used. Both studies were conducted in a double-blind randomized prospective and placebo-controlled process. The most important methodological difference is in the intensity of the applied electromagnetic field (EMF). DeFreitas et al. stated that the optimal dosimeter was not developed in the treatment with EMFs and remained true to the parameters (i.e., 200 G and 50 Hz) previously determined by the device manufacturer. Although we also adopted this method, we found that the default dose of our device for shoulder pathologies was significantly lower than that of DeFreitas (i.e., 25 G and 50 Hz). Aktaş et al. did not give a reason in their study, but they used a magnetic field close to the dose we used (i.e., 30 G and 25 Hz). However, it is possible to assume that they use the default settings on the device when determining the dose.

The main factor that distinguishes our study from previous studies is that the use of MRI for the detection of rotator cuff tear and the comparison of PEMF therapy with Sham. We found a significant improvement in the NRS, SPADI, and UCLA Shoulder Scale scores in both groups compared with baseline values. The score changes in the NRS and SPADI scores of both groups were above the minimum clinically significant difference value and were statistically significant, but there was no significant difference between the groups. Significant improvements were observed in the total scores of the UCLA Shoulder Scale questionnaires of both treatment and control groups and all subsection scores (i.e., pain, function, active forward flexion, flexion muscle strength, and patient satisfaction) at the end of treatment compared with baseline. However, there was no statistically significant difference in the UCLA Shoulder Score change in the between-group evaluation.

The main limitation of this study was low sample size and single-center design. Other limitations of this study were that the long-term effects of PEMF and the effect of PEMF without conventional therapy were also not investigated.

CONCLUSIONS

Our study showed that standard physical therapy in rotator cuff tears had a positive effect on the symptomatic and functional status in the early period. However, the combination of PEMF therapy with other physical therapy agents was found to be similarly effective with the placebo group, and adding it to our routine treatment program did not provide additional benefit. PEMF is supported as an inexpensive, reliable, and alternative therapy method. More studies are needed to establish the standard protocols in PEMF applications.

ETHICS STATEMENT

All participants provided written informed consent forms before enrolling in this study. This study was carried out in compliance with the principles of the Declaration of Helsinki. The study protocol was approved by Bolu Abant Izzet Baysal University Clinical Research Ethics Committee (No. 2018/04).

AUTHORS' CONTRIBUTIONS

MÖ: Conceptualization, data curation, formal analysis, investigation, and writing – original draft. **MFY:** Conceptualization, supervision, and writing – review and editing. **EY:** Conceptualization, supervision, and writing – review and editing.

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Salivary glands of fetuses are adversely affected by artificial food colorings in rats

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SUMMARY

OBJECTIVE: Artificial food colorings, as types of food additives, are widely used at present in daily life. We aimed to investigate the effects of exposure to artificial food colorings during the intrauterine period on the salivary glands in adulthood.

METHODS: A total of 30 Wistar albino female pregnant rats were included in this study. The treatment group was given a mixture of nine artificial food colorings at no observed adverse-effect-level doses. Neither mothers nor offspring were fed with food colorings after delivery. When the offspring became adults, they were sacrificed, and the possible histopathological and immunohistochemical effects of artificial food colorings on the salivary glands were investigated. In these glands, anti-matrix metallopeptidase 2 (anti-MMP2), anti-MMP9, anti-tissue inhibitor of metalloproteinases (TIMP) metallopeptidase inhibitor 1 (anti-TIMP1), anti-TIMP2, and anti-TIMP3 were examined.

RESULTS: The expressions of anti-MMP2, anti-TIMP1, anti-TIMP2, and anti-TIMP3 parameters were found to be higher in treatment groups ($p<0.05$).

CONCLUSION: It was suggested that intrauterine exposure of synthetic food colorings may lead to deterioration of the tissue structure of the salivary glands in adulthood, thereby increasing susceptibility to chronic illnesses including malignancy and chronic inflammation. Therefore, pregnant women should give importance to their nutrition in terms of foods containing synthetic colorings.

KEYWORDS: Food coloring agents. Saliva. Rats. Toxicity. Inflammation.

INTRODUCTION

Food additives are used to fix, maintain, and improve biological and nutritional values during the processing, preparation, manufacturing, packaging, and storage stages of foods. These additives are also defined as mixtures of substances or substances of natural or artificial origin that are purposefully used to prevent undesirable changes that may occur in the food and improve the quality and shelf life of the product[1,2].

Most of the food additives like drugs are substances that can be metabolized by the body, and toxic effects may occur when they are used at higher doses than the approved ones[3]. A toxicological test is carried out mainly on experimental animals to determine the safety of a chemical compound that is present in foodstuffs for any reason and that is determined to be present in the final product. The overall objective of toxicological tests on experimental animals is to determine the no-observed-adverse-effect level (NOAEL) value[1,2]. Then, the acceptable daily intake (ADI) levels are determined based on the NOAEL values[4,5].

Chronic and neuropsychological illnesses, such as cancer, diabetes, hypertension, autism, and hyperactivity, are influenced

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by the genetic and environmental factors. One of the environmental factors worsening these illnesses may be food additives. Synthetic food colorings are used in many foods[1,2].

Due to the widespread use of colorings, high exposure is inevitable. Parallel to the rapid climb of the number of consumers and unconscious consumption of higher quantities than recommended, several adverse effects may occur[6-9].

This study aimed to find a possible link between the maternal exposure to synthetic food colorings and the structural deterioration of the salivary glands in adulthood.

METHODS

Animal experiments

All materials and tests carried out in this study were approved by the SDU Animal Experiments Local Ethics Committee (dated April 2, 2015, Decision n°: 08) and used in accordance with the rules of the ethics committee. In this study, a total of 30 samples were initially used to provide genetic diversity from female Wistar albino rats.

Throughout the experiment, the rats were fed with water and feed (Standard Rat Fodder), and they were kept alive under standard light (12 h light/12 h dark) and heat (23°C) conditions. The rats were randomly divided into two groups of 15 each, namely, the "control" and "food coloring" (treatment) groups. For the groups, drinking water was given from 1 week before the experimental animals became pregnant and continued until birth.

At the end of the first week, male rats were left for 7 days to provide mating in each cage. Food coloring and control group rats were conceived this way. The day the male rats were placed in the cages was accepted as the first day of the pregnancy of the female rats. Thirteen rats from the food coloring group and 12 rats from the control group gave birth. After the birth, the pups were kept with the mother for 1 month during the breastfeeding period, and then 48 rats were taken from the food coloring and control group rats. The results were as follows: control female (n=12), treatment female (n=12), control male (n=12), and treatment male (n=12), each group consisting of 12 rats. When the rats were 3 months old, they were sacrificed under anesthesia, and their salivary glands were removed for pathological examination.

Synthetic food colors

Based on the NOAEL values, the maximum dose that each rat could receive per day was calculated. The NOAEL values are 100 times the ADI values[1,2]. The content of the coloring mixture that was used and the NOAEL values were as follows: erythrosine 10 mg/kg/day, ponso 4R 70 mg/kg/day, allura red AC 700 mg/kg/day, sunset yellow FCF 250 mg/kg/day, tartrazine 750 mg/kg/day, amarant 15 mg/kg/day, brilliant blue FCF 600 mg/kg/day, azorubine and carmoisine 400 mg/kg/day, and indigotin 500 mg/kg/day[1,2]. The coloring mixture was given by oral gavage once daily in the order of 1 mL/100 g weight of rats.

Histopathological and immunohistochemical studies

An immunohistochemical analysis was performed with a Ventana BenchMark ULTRA automatic coloring device using the Ventana ultraView universal DAB detection kit. All antibodies (Abcam, Cambridge, MA, USA) were scored semiquantitatively as follows: score 0: no coloring, score 1: focal-weak coloring, and score 2: extensive coloring.

Statistical analysis

The statistical analysis was carried out using Mann–Whitney *U*-test for two independent ordinal data and Kruskal–Wallis *H* test for more than two groups[10,11]. Statistical significance was defined as $p<0.05$.

RESULTS

In this study, the results of the immunohistochemical analyses regarding the effects of food colorings on the salivary gland structure are shown in Table 1.

In these glands, anti–matrix metallopeptidase 2 (anti-MMP2), anti-MMP9, anti–tissue inhibitor of metalloproteinases (TIMP) metallopeptidase inhibitor 1 (anti-TIMP1), anti-TIMP2, and anti-TIMP3 were examined. The expressions of all parameters, except anti-MMP9, were found to be higher in treatment groups ($p<0.05$).

Immunohistochemistry imaging

In our study, anti-MMP2, anti-TIMP1, anti-TIMP2, and anti-TIMP3 expressions were found to be high. Images for the significant results are shown in Figure 1. Accordingly, the control and treatment groups were statistically significantly different in terms of immunohistochemistry imaging scores in MMP2 parotid duct (Figure 1.1 and 1.2), TIMP1 parotid stroma (Figure 1.3 and 1.4), TIMP1 submandibular (Figure 1.5 and 1.6), TIMP2 sublingual duct (Figure 1.7 and 1.8), TIMP2 submandibular serous (Figure 1.9 and 1.10), TIMP3 parotid serous (Figure 1.11 and 1.12), and TIMP3 submandibular stroma (Figure 1.13 and 1.14).

DISCUSSION

All food additives are considered safe when the codec is properly consumed. Considering the results of such studies, the use of some additives is terminated, while the safe limits of some additives are reduced. For example, Red2G, a colorant, has been identified as

aniline, a carcinogenic substance, and prohibited in our bodies[1,2]. European Food Safety Authority has extensively investigated concerns about synthetic food colors, and investigations have been conducted on ADI (E123), brown HT (E155), sunset yellow FCF (E110), quinoline yellow (E104), and Ponso (ponceau) 4R[1,2].

Synthetic food colorings may have negative effects on the cellular level. For example, mitochondrial respiration may be suppressed by 16–100%. However, it is not known how these effects are reflected in the clinic[12]. In some studies, relationships between salivary gland pathologies and nutrition and the application of certain chemicals have been identified[13-15]. For example, administration of chronic isoproterenol leads to hypertrophy and hyperplasia in the salivary glands[13]. In the atrophic parotid glands that are induced by the liquid diet, asper cell apoptosis increases, while proliferative activity decreases[14]. Deferoxamine was shown to be effective in correcting the damage caused by radiation in the salivary glands[15]. It is important to reveal the negative effects of synthetic colors to which people are continuously being exposed at this point.

The aim of this study was to investigate the effects of nine food colors on many tissues. The tissues were analyzed, and this

Table 1. The p-values of immunohistochemical analyses determined using Kruskal–Wallis *H* tests at α value of 0.05.

Name of the group	Name	Comparison	Mean rank	p
Anti-MMP2	Parotid gland duct	Control male	7.50	0.044
		Treatment female	13.50	
Anti-TIMP1	Parotid gland stroma	Control male	11.06	<0.001
		Treatment male	27.50	
	Submandibular gland serous gland	Control male	11.61	<0.001
		Treatment male	28.50	
		Control female	15.20	0.016
		Treatment male	22.80	
	Submandibular gland mucous gland	Control female	9.50	<0.001
		Treatment male	27.50	
		Control female	9.50	<0.001
		Treatment female	27.50	
	Submandibular gland stroma gland	Control Male	12.50	0.048
		Treatment female	24.50	
		Control male	12.50	0.002
		Treatment male	28.50	
		Control female	16.50	0.048
		Treatment male	28.50	
Anti-TIMP2	Submandibular gland serous gland	Control female	12.70	0.039
		Treatment male	24.86	
	Sublingual gland duct	Control female	11.11	0.033
		Treatment female	21.58	
Anti-TIMP3	Parotid gland serous gland	Control female	12.50	0.048
		Control male	24.50	
		Control female	12.50	0.012
		Treatment male	26.50	
	Submandibular gland stroma	Control female	15.30	0.035
		Treatment male	27.00	

MMP2: matrix metallopeptidase 2; TIMP: tissue inhibitor of metalloproteinases; TIMP1: TIMP metallopeptidase inhibitor 1; TIMP2: TIMP metallopeptidase inhibitor 2; TIMP3: TIMP metallopeptidase inhibitor 3.

study was published progressively as a financial support was found. Maternal exposure to artificial food colors and additives (AFCAs) apparently affects the expression of cytochrome P450 family 1 subfamily A polypeptide 1 (CYP1A1), glutathione-*S*-transferase (GST), and vascular endothelial growth factor (VEGF) in the skin[2]. When the laryngeal tissue was investigated, significant decreases in goblet cell count and cilia loss were observed with AFCAs in maternally exposed rats (p<0.05). The other study demonstrated that maternal exposure of AFCAs plays a role in the mucosal defense system and possibly in carcinogenesis[1]. In this part of the study, the salivary tissues were investigated.

In this study, anti-MMP2, anti-MMP9, anti-TIMP1, anti-TIMP2, and anti-TIMP3 were examined. These parameters provide information about salivary gland morphogenesis. MMP9 and MMP2 are homologous enzymes containing the extracellular matrix and zinc in the active site, which can break down the basement membrane components[16]. MMPs are thought to play a role in the inflammatory cascade[17]. Tissue inhibitors of metalloproteinases (i.e., TIMP1, TIMP2, and TIMP3) are thought to inhibit the action of collagen-reducing enzymes and play an important role in the accumulation of increased extracellular matrix underlying fibrosis[18]. In our study, anti-MMP2, anti-TIMP1, anti-TIMP2, and anti-TIMP3 expressions were found to be high. These results suggest that the intrauterine exposure to synthetic food colorings may lead to deterioration of the tissue structure of the salivary glands in adulthood, thereby increasing the susceptibility to chronic illnesses including malignancy and chronic inflammation.

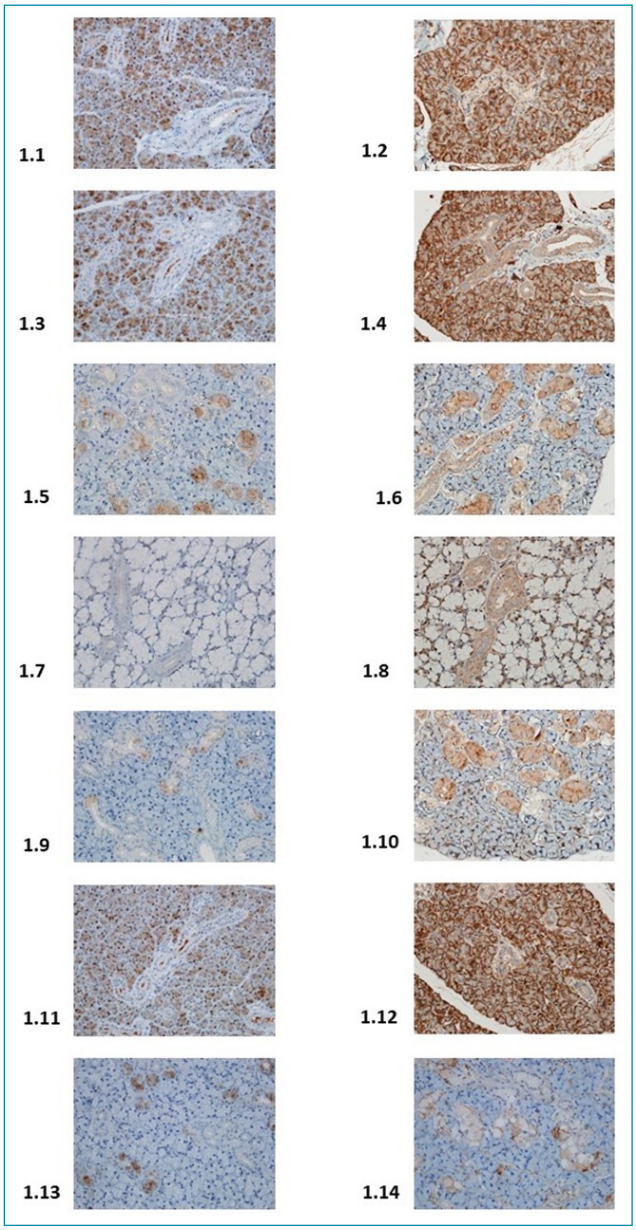

Figure 1. Immunohistochemical imaging.

The effects of synthetic food colorings, which are types of food additives, may be due to their own effects, or they may be caused by the synergistic effect resulting from the interaction with other substances in the organs[12]. As a matter of fact, it is known that some additives have a synergistic effect. For example, in a study conducted on mice and the synergistic effects of synthetic food colorings on nerve development, both tartrate and Brilliant Blue FCF showed no adverse effects when administered alone but suppressed nerve growth in the central nervous system when given in combination and at high doses[19]. In another *in vitro* nerve cell study, Brilliant Blue FCF, made by using quinolone yellow aspartame, increased the harmful effects of L-glutamic acid[20].

In a study that investigated the synergistic effects of food colorants in rat liver cell cultures, it was shown that additives may increase the harmful effects of these carcinogens if they are exposed to a carcinogenic agent[21,22].

Consequently, anti-MMP2, anti-TIMP1, anti-TIMP2, and anti-TIMP3 expressions were found to be higher in treatment groups in our study. These results suggest that the intrauterine exposure to synthetic food colors may lead to deterioration of the tissue structure of the salivary glands in adulthood, thereby increasing the susceptibility to chronic illnesses including malignancy and chronic inflammation.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, and writing the original draft, review, and editing.

DISCLOSURE

Preliminary data of this study was presented in summary at the International Congress of Molecular Medicine in 2017.

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ORIGINAL ARTICLE
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Abdominal drain amylase on the first day after pancreatectomy: a predictive factor for pancreatic fistula

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SUMMARY

OBJECTIVE: To analyze abdominal drain on the first postoperative day and evaluate its predictive nature for the diagnosis of Pancreatic Fistula exclusion, seeking to establish a cutoff point from which lower values demonstrate safety in excluding the possibility of this complication.

METHODS: From August 2017 to June 2020, data from 48 patients undergoing pancreatic resection were collected and analyzed from a prospective cohort. The patients were divided into two groups, one group consisting of patients who did not develop PF (Group A), and the other composed of patients who developed PF (Group B). The receiver operation characteristic curve was constructed, and cutoff points were evaluated by calculating sensitivity and specificity.

RESULTS: Group A brought 30 patients together (62.5%) and Group B brought 18 patients together (37.5%). The 444 U/L value was the most satisfactory cutoff point for the receiver operation characteristic curve (CI 0.690–0.941), with a sensitivity of 94.4% and a specificity of 60%, thus being able to select 18 of 30 patients who did not succumb to PF.

CONCLUSIONS: Abdominal drain on the first postoperative day can be used as a predictive factor in the diagnosis of PF exclusion (CI 0.690–0.941), with the value of 444 U/L being the best performance cutoff point.

KEYWORDS: Pancreatectomy. Pancreatic neoplasms. Amylases. Drainage. Pancreatic fistula.

INTRODUCTION

Pancreatic resection surgeries, such as pancreatoduodenectomy (PD) and distal pancreatectomy (DP) are the most common modalities in treating pancreatic neoplasms. Despite the improvement in the operative mortality rate for these surgeries in the last three decades, morbidity still remains high, with the pancreatic fistula (PF) being the main complication, as well as the most feared[1-4].

To establish a definition for this complication, the International Pancreatic Fistula Study Group (ISGPF-2016)[5] reviewed the literature and established that the content of the abdominal drain with an amylase dose greater than three times the serum limit, associated with a worsening of the patient's clinical condition from the third postoperative day defines the diagnosis of PF with clinical repercussion (PF-CR).

The considerable incidence of PF-CR has prompted other studies to find clinical and laboratory criteria predictive of which patients would evolve or would not with this complication. In this scenario the amylase from the drain on the first postoperative day (AD1PO) stands out, in which research has

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been carried out to investigate whether this parameter can be used as a tool in the diagnostic exclusion of this complication, and thus asses the removal of the abdominal drain in the first postoperative[6-12] days of. This practice, which has already been performed in some institutions, is associated with lower rates of intra-abdominal and pulmonary complications, length of hospital stay, and lower hospital costs[13,14].

The data have shown that lower values in AD1PO are good indicators of exclusion in the diagnosis of PF, but the cutoff points vary widely between studies, thus lacking external validity[6-11,15,16]. As such, this study analyzed the AD1PO in patients who underwent pancreatic resection surgery at Hospital São Paulo (São Paulo, Brazil).

Objective

To analyze the AD1PO and evaluate its predictive nature in diagnosing exclusion of PF-CR, and to establish a cutoff point in which lower values (below) demonstrate safety to exclude diagnosis of this complication.

METHODS

The study was submitted and approved by the Research Ethics Committee with the Plataforma Brasil (CAAE) 94208718.7.0000.5505 and the Research Ethics Committee of UNIFESP 2.823.557.

A prospective cohort of patients with pancreatic neoplasms was instituted who underwent pancreatoduodenectomy, distal pancreatectomy, and pancreatic enucleation by the Group of Biliary Tract and Pancreas of the discipline of Surgical Gastroenterology of the Department of Surgery at UNIFESP at Hospital São Paulo from August 2017 to June 2020. Data were recorded on an Excel spreadsheet.

The measurement of amylase from the abdominal drain was performed on the first, third, and fifth postoperative days, with a diagnosis of PF-CR defined according to criteria of the ISGPF-2016[5]. Epidemiological variables, postoperative evolution data, histopathological diagnosis, readmission, and mortality rates were also analyzed.

Patients were divided into two groups for statistical analysis of clinical and surgical variables: one group of patients developed PF-CR and the other group of patients did not deal with this complication. Statistical analysis was performed by Stata version 15.1, College Station, TX, USA. The categorical variables were measured in absolute and relative frequencies, quantitative variables in median and interquartile ranges (IQT), and mean and standard deviation as appropriate. To verify the association between groups in categorical variables, Fisher´s exact test or the Chi-square test were used; for quantitative variables, the Student´s T-test or the Mann-Whitney non-parametric test were used.

The Receiver Operating Characteristic Curve (ROC) was set up to assess the predictive component of AD1PO in the diagnosis of PF exclusion. From this curve, the cutoff points were evaluated by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and likelihood ratio. The chosen cutoff point was the one with the highest sensitivity associated with the best specificity.

RESULTS

Within the period described, 48 patients underwent pancreatic resection surgery, and were divided into two groups, according to the presence or absence of PF-CR. Group A was comprised of 30 patients (62.5%), 26 of whom did not develop PF (86.7%), and four had a biochemical leak, without clinical repercussion (13.3%). Group B was comprised of 18 patients (37.5%), 11 of whom progressed to grade B PF (61.1%), while seven progressed to grade C PF (38.9%).

Patient characteristics are described in Table 1. Pancreatic adenocarcinoma was the main histological type of neoplasm in this sample. When analyzing groups A and B, it was observed that pancreatic adenocarcinoma occurred with lower PF-CR rates (22.2%), compared to other histological types (77.8%).

For postoperative variables, with the exception of the number of deaths, other variables showed a statistically significant difference between groups ($p< 0.05$). In group B, 16 patients developed some postoperative complications (88.9%); in group A, only six patients progressed poorly (20%). Among the most frequent complications, abdominal collection was present in 15 patients in group B (83.3%), and only one patient in group A (3,3%). Days of hospitalization and days with the abdominal drain were higher in group B (30.5 and 33 days, respectively) vs. group A (11 and 10 days, respectively). The rate of readmission was also higher in group B, with 27.8% of patients being readmitted.

Table 1 shows the distribution of patients among the surgical modalities. Comparing surgical procedures and PF classification in patients who developed this complication, group B saw a higher frequency of grade B PF in patients who underwent DP (87.5%) compared to the group who underwent PD (44.4%); however, this association did not present a statistically significant value, despite being borderline ($p=0.06$).

The AD1PO ROC curve, represented in Figure 1, showed an area under the curve equal to 0.815, with a confidence interval (CI) of 0.690–0.941. The curve did not cross the null line and the CI was not lower than half (0.5), demonstrating that the AD1PO presents good performance as a diagnostic test for

Table 1. General Population Characteristics.

	Group A		Group B		p
	N	%	N	%	
	30	62.50	18	37.50	
Age	55.4±13.9		56.1±16.6		0.88[I]
Sex					0.94[II]
Male	13	43.3	8	44.4	
Female	17	56.7	10	55.6	
Histopathological diagnosis					0.09[II]
Pancreatic adenocarcinoma	14	46.7	4	22.2	
Others	16	53.3	14	77.8	
Complications	6	20.0	16	88.9	<0.01[II]
Abdominal collection	1	3.3	15	83.3	<0.01[II]
Deaths	1	3.3	4	22.2	0.06[III]
Readmission	1	3.3	5	27.8	<0.05[III]
Number of days with drain	10 (8–13)		33 (21–40)		<0.01[IV]
Number of days hospitalized	11 (9–13)		30.5 (17–41)		<0.01[IV]
Surgical procedure					0.44[III]
Pancreatoduodenectomy	20	66.7	9	50.0	
Distal Pancreatectomy	8	26.7	8	44.4	
Pancreatic Enucleation	2	6.7	1	5.6	

I: Student's T-test; II: χ^2 test; III: Fisher's exact test; IV: Mann-Whitney test.

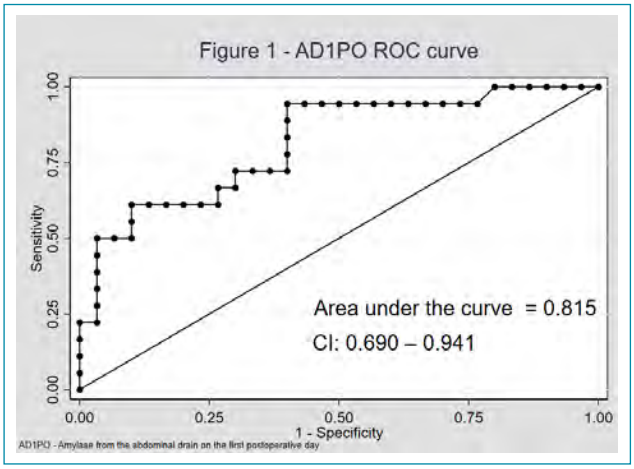

Figure 1. ROC curve of the amylase from the drain on the first postoperative day.

PF-CR. In Table 2, cutoff points were listed and evaluated to analyze exclusion capacity in the diagnosis of PF-CR. The cutoff point of 47 U/L showed higher sensitivity (100%) and higher NPV (100%) in this study; that is, none of the six patients below the cut had to deal with PF, being able to select 20% of patients in group A. The cutoff point of 444 U/L showed a sensitivity and NPV of about 94.4% and 94.7%, respectively, with high clinical applicability, as 18 of 30 patients without PF were below the cutoff value, which addressed about 60% of patients in group A.

DISCUSSION

In pancreatic resection surgeries, placement of drains is a widely used measure, in contrast to other intra-abdominal surgeries (cholecystectomy, colectomy, hepatectomy, and splenectomy, for example)[17] for which this routine is not always indicated.

The Verona group[13] chose 114 patients and divided them into two groups, which had early (3rd postoperative day) versus late removal of the drain (5th day or more). The result was that in the early group, we observed reduced rates of PF, intra-abdominal, and pulmonary complications, length of hospital stay, and lower hospital costs. However, the cutoff point of 5,000 U/L in the AD1PO includes patients with a high probability of developing a PF, as demonstrated in the study by Linneman et al.[18] with 1,402 included patients, demonstrating a 7% incidence

Tabela 2. Amylase from the drain on the first postoperative day cutoff points.

Cutoff points	S (%)	E (%)	PPV (%)	NPV (%)	A (%)	PLR	Patients below the cutoff with PF
47	100.0	20.0	42.8	100.0	50.0	1.25	0
444	94.4	60.0	58.6	94.7	72.9	2.36	1
634	77.8	60.00	53.8	81.8	66.7	1.94	4

S: sensitivity; E: specificity; PPV: positivie predictive value; NPV: negative predictive value; A: accuracy; PLR: positive likelihood ratio

of PF in the group with AD1PO <5,000 U/L. Due to this fact, safer cutoff points have excluded the diagnosis of PF-CR.

Several studies evaluated AD1PO as a predictive factor in the diagnosis of the exclusion of PF-CR[6-12] to select patients at a low risk of developing this complication, and to remove abdominal drains in the first postoperative days[13-14]. Studies show favorable results and demonstrate that it can be used as a predictive factor in this situation; however, the cutoff points between studies have been quite variable.

The ROC curve of this study demonstrated that AD1PO can be used as a predictive factor for PF-CR. From this graph, cutoff points were analyzed, with the best performance value being 444 U/L, selecting 60% of 30 patients who did not have PF-CR; however, this cutoff point missed one patient who was forced to deal with PF-CR, with sensitivity and NPV not reaching 100%, as shown in Table 2. It should be noted that this patient, even with a low AD1PO, maintained the hypothesis of PF: other clinical criteria, including the patient´s general condition, level of consciousness, and the issue of the drained secretion were also altered, factors which are considered in assessing the prognosis of PF associated with an abdominal infectious focus.

The cutoff point of this study was lower than that by Fong et al.[11]. In their initial cohort of 126 patients who underwent pancreatoduodenectomy, a cutoff point of 612 U/L was estimated, with a sensitivity of 93% and a specificity of 79%. After this analysis, a validation cohort was assessed with 369 patients, divided into two groups, the first composed of patients with AD1PO below 600 U/L, and the other exceeding or equaling that value. In the first group of 62.1% patients, only two developed into PF-CR, demonstrating that this cutoff point was satisfactory for the sample.

There are some systematic reviews evaluating the studies of AD1PO and their predictive character in the diagnosis of PF and PF-CR[15,18]. All came to the conclusion that, despite evidence showing that AD1PO has a high predictive capacity for PF-CR, there are several cutoff points that can vary between institutions; that is, the external validity of these values is the main limitation of the studies. In the meta-analysis by Giglio et al.[15], they analyzed cutoff intervals to guarantee safety and rule out a diagnosis of PF-CR, which would select a number of patients with a low risk of developing the complication. Performing this analysis, the value of 350 U/L showed a sensitivity of 91% (CI 76–97%) and a specificity of 84% (CI 59–95%), a value close to that of 444 U/L.

An important limitation of this study was the joint analysis of three surgical modalities: PD, DP, and pancreatic enucleation (EN). Due to the low number of PD and DP, there was not a high incidence of PF in relation to the others with any statistical significance, nor an analysis of AD1PO between the two groups was performed due to the reduced number of patients.

The decision for permanence or removal of the drain varies according to protocols of each hospital; however, data such as the behavior of the amylase drain for several days, appearance of the drained effluent content, and the clinical condition of the patient are criteria when deciding to keep or remove the drain. Using AD1PO as the only decisive criterion to decide on its removal can lead to errors in a minority of cases, as observed in this study. Thus, combining clinical criteria with a low dosage of AD1PO can be a viable alternative in maintaining or removing the abdominal drain in the first postoperative days.

CONCLUSION

AD1PO can be used as a predictive factor in the diagnostic exclusion of PF-CR, because its ROC curve did not exceed the nullity line (CI0.690 – 0.941), with the value of 444 U/L being the best performance point, since it was able to detect about 60% of patients who did not develop PF, in addition to presenting high sensitivity and NPV.

AUTHORS' CONTRIBUTIONS

PHBR: Conceptualization, Data Curation, Writing – Original Draft. **AS:** Data Curation Formal Analysis. **EJL:** Conceptualization, Methodology, Writing – Review & Editing. **AG:** Conceptualization, Methodology, Writing – Review & Editing. **GJLF:** Conceptualization, Methodology, Writing – Review & Editing. **FAT:** Conceptualization, Methodology, Writing – Review & Editing.

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Is there a relation between computed tomography findings and electrocardiography findings in COVID-19?

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SUMMARY

OBJECTIVE: COVID-19 can cause lung damage and may present with pneumonia in patients. In the present study, the correlation between the severity of pneumonia and electrocardiography parameters of COVID-19 were examined.

METHODS: A total of 93 COVID-19 patients and a control group consisting of 62 volunteers were studied. Computed thorax tomography evaluation was performed; each lung was divided into three zones. For each affected zone, scores were given. The main computed thorax tomography patterns were described in line with the terms defined by the Fleischner Society and peer reviewed literature on viral pneumonia. We compared Computed thorax tomography of patients with corrected QT (QTc) and P wave dispersion (Pd) time.

RESULTS: There is a significant difference between the patient and control groups in terms of QTc values (413.5±28.8 msec vs. 395.6±16.7 msec p<0.001). Likewise, the Pd value of the patient group is statistically significantly higher than that of the control group (50.0±9.6 ms computed thorax tomography ec vs. 41.3±5.8 msec p<0.001). In the patient group, a reverse correlation was detected between computed thorax tomography score and Pd value according to partial correlation coefficient analysis (correlation coefficient: -0.232, p=0.027). In the patient group, the correlation between computed thorax tomography score and QTc value was similarly determined according to partial correlation coefficient analysis (Correlation coefficient:0.224, p=0.017).

CONCLUSIONS: COVID-19 prolongs QTc and P wave dispersion values; and as the severity of pneumonia increases, QTc value increases. However, whereas the severity of pneumonia increases, P wave dispersion value decreases.

KEYWORDS: Coronavirus infections. Electrocardiography. Tomography, X-Ray Computed.

INTRODUCTION

The COVID-19 disease first appeared in Wuhan, province in China, in December 2019. It quickly spread to other regions of China and other countries, and caused a pandemic[1]. COVID-19 has the feature to affect many organs, especially the lungs, but lung involvement and pneumonia are among the most important mortalities[2]. Reverse transcription polymerase chain reaction and computed tomography (CT) are generally used in diagnosis[3]. In this manner, pneumonia can be interpreted with CT. CT is frequently used in treatment follow-up and prognosis[4]. Aside from the lungs, the heart is also one of the affected organs, presenting with symptoms such as arrhythmia, heart muscle damage, and heart failure[5]. In a study, the QT duration in electrocardiography (ECG) of COVID-19 patients

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was shown to be longer than that of populations without the disease[6]. Furthermore, drugs used in COVID-19 treatment prolong QT duration[7]. There is no study examining the correlation between COVID-19 pneumonia and QT interval. In addition, diseases affecting the lungs are known to have an effect on the atrium and thus the P wave in ECG[8,9]. It is possible that COVID-19 causes pneumonia, which increases the volume load in the atrium and affects the P wave. The condition of the atrium can be estimated by the dispersion of the p wave (Pd) in ECG[8,9]. In this study, the correlation between the lung involvement severity in COVID-19 patients and Pd and QT was examined.

METHODS

A total of 93 COVID-19 patients and a control group consisting of 62 volunteers who applied in our hospital from April to July 2020 were enrolled. The study was approved by the Local Medical Ethics Committee of Van Yuzuncu Yil University. Positive Polymerase chain reaction (PCR) test for COVID-19 was identified as the inclusion criteria.

Patients with pregnancy, chronic kidney failure, chronic liver failure, malignancy, coronary artery disease, heart failure, and heart valve disease were excluded from the study. Moreover, patients with a known rhythm disorder (atrial fibrillation, subraventricular tachycardia, Wolf Parkinson White, ventricular rhythm disorders, etc.) or a pathological (pathological Q, T negativity, ST changes etc.) ECG findings were excluded.

12-lead ECG records (Nihon Kohden brand) were received after 30 minutes of rest, at room temperature (20–24°C). All ECGs (filter range 0.5 Hz to 150 Hz, AC filter 60 Hz, 25 mm/s, 10 mm/mV) were analyzed by two independent clinicians, who were blinded to the study design and clinical data. All ECG records were scanned, the data was transferred to a personal computer to reduce possible errors, and Adobe Photoshop software was used for 400% magnification. The participants having U wave in their ECGs were not included in the study. For each lead, three values were calculated averagely. The duration from the beginning of the P wave to the end were measured in all leads. Pd value was calculated by subtracting the Pmin width from the Pmax width QT interval, measured from the beginning of the QRS complex to the end of the T wave; corrected QT interval was calculated by using Bazett formula ($QTc=QT\sqrt{(R\text{-}R\ interval)}$).

Non-contrast, 3 mm slice thickness CT findings of the patients with COVID-19 pneumonia pre-diagnosis and positive PCR test were evaluated.

The CT images were taken using a 16-slice multidetector CT (MDCT) device (Somatom Emotion 16-slice; CT2012E- Siemens AG, Berlin and Munich, Germany). CT evaluation was done by two independent radiologists. Briefly, each lung was divided into three zones: upper (above the carina), middle, and lower (below the inferior pulmonary vein) zones. For each affected zone, scores were given. The main CT patterns were described in line with the terms defined by the Fleischner Society and peer-reviewed literature on viral pneumonia[10,11].

Typical CT findings are *ground-glass opacification (GGO)*, air bronchogram, traction bronchiectasis, subpleural atelectasis, and peribroncovascular thickening; atypical findings are mediastinal lymph node, pleural effusion, tree-in bud, pneumothorax and cavitation.

As to the findings, 3 points for ground-glass opacity, 2 for peripheral-subpleural involvement, 2 for consolidation, and 2 for air bronchogram. Besides that, centrilobuler nodular opacity, subpleural atelectasis, halo-reverse halo, air buble sign were evaluated, and 1 point was given for each finding.

Pleural-pericardial effusion and mediastinal lymph node are atypical findings with no score.

The impact rate is multiplied by 1 if the lung involvement rate is 0–25%, by 2 if ti is 26–50%, by 3 if it is 51–75%, and by 4 if it is more than 76%. (Figure 1).

For example, the patient has involvement in the upper right zone, it is 1 point For ground-glass opacity; 3 points are given; 40% of all zones is affected; (1+3) χ^2=8 points were calculated (Figure 1).

CT and ECG examinations of the participants were performed before treatment.

Echocardiography examination was conducted in all patients. The Echocardiography examination was performed at least 15 min after the rest using a Vivid E9 (Vivid 9 Pro, General Electric Medical Systems, Milwaukee, Wisconsin, USA) device and an X5-1 transthoracic probe in the left lateral position (two-dimensional, M-mode, color Doppler echocardiography)

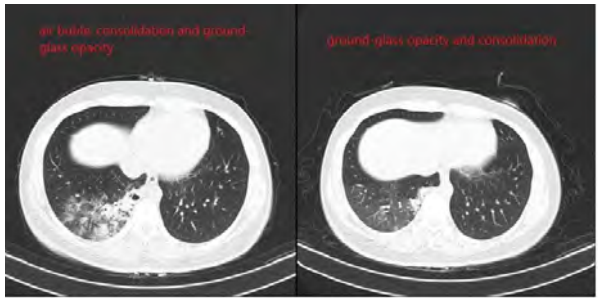

Figure 1. Examples of ground glass opacity, air bubble and consolidation.

using parasternal and apical windows. All the Echocardiography examinations were performed in accordance with the American Society of Echocardiography (ASE) guidelines and the European Standard Echocardiography Guidelines[12].

Statistical analysis

Whereas descriptive statistics for continuous variables are expressed as average and standard deviation, it is expressed as numbers and percentages for categorical variables. Student t test was used in comparison of groups according to continuous variables. The group and categorical variables were compared using χ^2 test. Partial correlation coefficient was calculated considering Pd and age in determining the correlation between QTc and CT Score values, likewise between Pd and CT score values. In calculations, 5% was considered statistically significant, and all statistical analyses were performed using SPSS (ver. 22.0) statistical package programme.

RESULTS

The clinical and demographic characteristics of patients and control group are shown in Table 1. There is a statistically significant difference in QTc values between patient and control groups (413.5±28.8 msec vs. 395.6±16.7 msec p<0.001). Similarly, the Pd value of the patient group is statistically significantly higher than the control group's Pd value (50.0±9.6 msec vs. 41.3±5.8 msec p<0.001). Between the patient and the control group, age, gender, Hypertension (HT), Diabetes Mellitus (DM), and Ejection Fraction (EF) parameters are not different significantly.

In the patient group, a reverse correlation was detected between CT score and Pd value according to Partial correlation coefficient analysis (Correlation coefficient: -0.232, p=0.027). Pd value of the patients with higher scores were calculated lower. The correlation between CT score and QTc value was similarly determined according to Partial correlation coefficient analysis in the patient group (Correlation coefficient: 0.224, p=0.017). Patients with high scores also had a high QTc value (Figure 2). No statistically significant correlation was detected between CT score and gender, HT, DM, EF in the patient group.

In addition to this, the correlation between the categories of QTc, Pd and score variables is shown visually in Figure 2. 30–44 msec category of Pd, 6–56 categories of the score value and 397–431 mseccategories of QTc are indicated in Figure 2, in the positive region according to the first dimension. It is expected

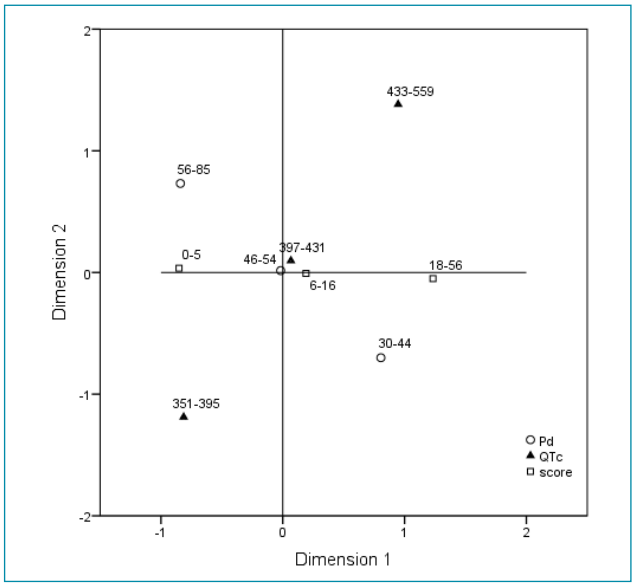

Figure 2. Configuration of the P wave dispersion, QTC and score on two dimensional map, categorical principal component analysis.

Table 1. Descriptive statistics and comparison results for clinical and demographic characteristics.

	Paient (n=93)		Control (n=62)		p-value
	Female n (%)	Male n (%)	Female n (%)	Male n (%)	
Sex	34 (36.6)	59 (63.4)	23 (37.1)	39 (62.9)	0.946
HT	10 (10.8)		6 (9.7)		0.830
DM	7 (7.5)		5 (8.1)		0.903
	Mean±SD		Mean±SD		
Age	43.4±17.2		45.6±14.3		0.408
QTc msec	413.5±28.8		395.6±16.7		0.001
Pd msec	50.0±9.6		41.3±5.8		0.001
EF	59.8±4.5		60.3±4.0		0.476
LA	3.81±0.34		3.82±0.33		0.885

HT: hypertension; DM: diabetes mellitus; QTc: corrected QT; SD: standard deviation; Pd: p wave dispersion; EF: ejection fraction; LA: left atrium.

that the score values should be between 6–56 in individuals with a QTc of 397–431 msec and a Pd in the range of 30–44 msec. Similarly, in Figure 2, in the negative region according to the first dimension, 56–85 msec of Pd, 351–395 msec of QTc and 0–5 categories of score value are shown and according to this, the score value is expected to be in the range of 0–5 in patients with a QTc value of 351–395 msec and a Pd value of 55–86 msec.

Moreover, there is no statistically significant relation between the right atrium and Pd (p=0.572). Similiarly, we did not find a significant relation between troponin value, Pd and QTc (p=0.125, p=0.181, respectively)

DISCUSSION

Prolonged QTc and Pd values in COVID-19 patients were detected. A positive correlation between the degree (severity) of the lung involvement and the QTc value was also found, whereas the reverse correlation between the degree (severity) of the lung involvement and the Pd value was determined. These findings may be an important guide for approach and treatment in COVD-19.

COVID-19 was shown to cause cardiac arrhythmia[13]. Additionally, studies show that it causes ventricular arrhythmia and prolongs the QTc duration, being the predictor of ventricular arrhythmia[6,14]. There are also case reports about the correlation between COVID-19 and atrial arrhythmias[15]. Pd is used as an indicator for atrial arrhythmias (especially atrial fibrillation)[16]. In the present study, we found higher Pd and QTc values in the patient group. These values were calculated before treatment; in the given circumstances, COVID-19 prolonged the duration of Pd and QTc, regardless of the treatment. The fact that Pd is an indicator for atrial fibrillation (AF) and the high Pd value in COVID-19 shows COVID-19 may be a predictor for the development of AF.

Furthermore, autonomic system, endothelial dysfunction, and inflammatory response have an impact on p wave duration and dispersion[17,18]. The fact that autonomic imbalance affects intra-atrial and interatrial conduction times may be prone to atrial arrhythmias[19]. The virus SARS-CoV-2, the virus that causes COVID-19, had neurotropic feature and involvement of the brainstem cardiorespiratory nuclei[20]. CT has an important role in guiding the diagnosis and treatment of COVID-19[21]. Various scores were used to determine the severity of COVID-19 pneumonia with lung findings in CT; severity of the disease tried to be determined[22]. There is no broad-scale research conducted between CT findings of lung involvement and ECG parameters. In the present study, patients with high lung involvement severity had high QTc values. The fact that lung involvement also affects the conduction system of heart and impairs the ventricular repolarization phase are the indicator for increasing potential of ventricular arrhythmias (ventricular tachycardia and ventricular fibrillation).

Among the causes of cardiac arrhythmias, hormonal disorders, electrolyte imbalances, myocardial cell damage, adrenergic stress, and inflammation are shown. COVID-19 can also cause these pathologies[23]. Even though studies examining COVID-19 in terms of ventricular arrhythmias are available in the literature, there are not enough studies on atrial arrhythmias. In the present study, the correlation between Pd value and pneumonia severity as well as Pd value impaired due to COVID-19 were shown. The reverse change in the lung involvement of COVID-19 and Pd value shows that the mechanism of the disease affecting atrial conduction and causing pneumonia is different. The high Pd value of patients without severe pneumonia means that they are more prone to atrial arrhythmias, such as atrial fibrillation[24]. This situation indicates that patients tend to suffer with thromboembolic events in the future[25].

Our study is not sufficient to answer the question why Pd is less affected in severe pneumonia. Many factors may be discussed and large scaled studies are needed in this field.

Limitations

Among the limitations of the present study, the relative small sample size is one of them. The hormonal conditions of patients affect the ECG parameters, and no evaluation of hormones was performed herein, which is another limitation. The effect of hormone values on the ECG parameters is known, and the fact that no evaluation of hormones was made is one of the limitations of present study. Since the pathophysiology of COVID-19 is not clear, trying to explain the mechanism affecting ECG parameters is not enough, another limitation.

CONCLUSIONS

As a result, COVID-19 prolongs the QTc and Pd values. Whereas the severity of pneumonia increases in COVID-19, QTc is prolonged. Pd value, which is another ECG finding, decreases as the severity of pneumonia increases.

AUTHORS' CONTRIBUTIONS

FÖ: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing. **MT:** Conceptualization, Data Curation, Writing – Review & Editing. **CG:** Conceptualization, Data Curation,Writing – Review & Editing. **ST:** Conceptualization, Formal Analysis. **MD:** Data Curation, Formal Analysis, Writing – Original Draft. **RÇ:** Data Curation, Formal Analysis, Writing – Review & Editing, **NB:** Data Curation, Writing – Original Draft, Writing – Review & Editing.

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Effectiveness of early therapeutic intervention in phases one and two after COVID-19 infection: systematic review

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SUMMARY

OBJECTIVES: Although research in relation to new vaccines for the coronavirus, SARS-CoV-2 (COVID-19), is ongoing, it has been reported that medical teams are also considering the use of antiviral drugs in patients in order to verify their effectiveness when infection signs and symptoms present, mainly in stages one and two of the disease.

METHODS: For the selection of studies, the combination based on the Medical Subject Heading Terms (MeSH) was used, and the databases Medline (Pubmed), LILACS, SciELO, SCOPUS, Web of Science, and BIREME were searched. The search period for articles consisted of manuscripts published between January 2010 and July 2020 without language and localization restrictions.

RESULTS: Initially, 20 articles were selected and then reduced to 19 after exclusion based on repetititve articles. Titles and abstracts were analyzed, and 14 articles were excluded because they did not meet the inclusion criteria and did not answer the guiding question. Studies show that patients receiving certain medications in the initial stages (one and two) indicate a reversal of complications during hospitalization or often do not require hospitalization in addition to being discharged in a shorter period of time.

CONCLUSION: Studies have reported that effective drugs for treating COVID-19 exist. In addition, this study emphasizes the importance of performing therapeutic interventions in the initial stages of infection aimed at reversing the disease and minimizing public health costs.

KEYWORDS: Coronavirus Infection. Therapy. Drug therapy. Systematic review.

INTRODUCTION

The new coronavirus, SARS-CoV-2 (COVID-19) was initially identified in December 2019 in Wuhan, China. The epidemic's epicenter quickly moved to the European continent and, in March 2020, it was classified as a pandemic by the World Health Organization (WHO), making it a worldwide public health concern[1,2]. The disease has reached more than 12,102,328 million cases and 551,046 thousand deaths worldwide, in addition to more than 5 million confirmed cases worldwide[3].

Disease symptoms usually begin with fever associated with dry cough and fatigue and, after a week, can cause breathing difficulties with many patients needing hospitalization and hospital treatment due to the evolution of the disease. The time from the onset of symptoms to the need for a patient to be taken to the intensive care unit (ICU) occurs on an average of 10 days after infection[4]. So far, the treatment of individuals infected with COVID-19 with moderate/severe symptoms that give an indication of hospitalization consists of offering respiratory support, in addition to other measures, until the immune

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system is able to fight the virus[4].COVID-19 can be divided into three phases with different manifestations, treatment, and prognosis. The antirheumatic drug with antiviral and antibiotic activities seems to be capable of playing an important role in the treatment of COVID-19 to avoid complications that require invasive ventilation[5].

COVID-19 can be differentiated in three progressive stages[6-8]. The first phase is a viral phase normally characterized by fever, fatigue, dry cough, anosmia, altered taste, nasal congestion, and diarrhea[7]. The second phase is a pulmonary phase characterized by dyspnea and hypoxia. The third phase is characterized by respiratory distress that can develop into a clinical manifestation of vasculitis with embolism, septic shock, and metabolic acidosis[9]. These symptoms correspond to those reported by the patients and the health team but are not exclusive of other observations of symptoms.

Although much research has been done in relation to new vaccines, it is reported that medical teams are also trying to reconcile the use of antiviral drugs in order to verify their effectiveness in the presence of infective disease symptoms and to adapt to their local conditions and populations, seeking to avoid the collapse of hospitals in both the public and private healthcare sectors[4]. In this sense, the main and guiding objective of the present research was to verify the scientific evidence on the therapeutic intervention in phases one and two after infection by COVID-19 in order to answer the research question: How effective are the intervention phases one and two after COVID-19 infection?

METHODS

Protocol and registration

The systematic review was conducted according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA)[10]. Searches for scientific articles were conducted by two independent researchers using the electronic databases MEDLINE (PubMed) (https://www.ncbi.nlm.nih.gov/pubmed/), LILACS (http://lilacs.bvsalud.org/), SciELO (http://www.scielo.br/), BIREME (https://bvsalud.org/), SCOPUS, and WEB OF SCIENCE from January 2010 to July 2020. The research was structured and organized in the PICOS format, which represents an acronym for target population, intervention, comparison, outcomes, and study (Table 1).

Research strategy

The descriptors were selected from the Health Sciences Descriptors (DeCS) and Medical Subject Heading Terms (MeSH) dictionaries, given their wide use by the scientific community for indexing articles in the PubMed database. The following keywords and Boolean operators were proposed for the searches: ([coronavirus] and [covid-19] and [phases] and [treatment] and [disease progression]). The articles were identified through electronic search and then organized and reviewed independently for duplication by the two authors. Subsequently, the titles of the articles were also analyzed independently. Articles that did not fulfill any inclusion criteria were excluded. In the sequence, the abstracts of the articles selected in the second step were examined. Articles that did not contain characteristics of the question to be answered were excluded at this stage.

Eligibility criteria

The designs of epidemiological studies with possibilities for admission to this research were descriptive, cross-sectional, cohort, and case study. Publications were included without language and localization restrictions. The article search period ranged from January 2010 to July 2020. Studies published in the form of letters to the editor, guidelines, literature and systematic reviews, meta-analyses, and abstracts were excluded. Table 2 represents the inclusion and exclusion criteria used in this research.

Risk of bias

The quality of the methods used in the included studies was independently assessed by the reviewers according to the PRISMA recommendation[10]. The assessment prioritized clear description of the information. At this point, the review was carried out blindly, masking the names of the authors and magazines, and avoiding any potential bias and conflict of interests.

Exclusion criteria

Studies published in the form of letters to the editor, guidelines, literature, narrative, and systematic reviews, meta-analyses

Table 1. Description of the pico components.

Acronym	Definition
P	Patients
I	COVID-19
C	Therapy
O	Intervention
S	Descriptive study Cross-sectional study Observational study

Source: developed by the authors.

Table 2. Summary of the inclusion and exclusion criteria.

Inclusion criteria	
Design	Case reports Case-control studies Controlled clinical trials Cohort studies Screening studies Observational studies
Localization	No restriction
Language	No restriction
Exclusion criteria	
Design	Letters to the editor Guidelines Literature reviews Systematic reviews Meta-analyses
Studies	Conducted with animals Unclear, inadequate, or poorly described studies
Form of publication	Only abstract

Source: developed by the authors.

and abstracts were excluded. Studies with unclear descriptions, or unavailable in their entirety were also excluded (Table 2).

DATA ANALYSIS

The extraction of data for the eligibility process of the studies was performed using a specific form for systematic review prepared by two researchers in Excel®, in which the extracted data were initially added by one of the researchers and then checked by the other. They were initially selected according to the title, after which abstracts were then analyzed and only those that were potentially eligible were selected. Based on their abstracts, articles were selected for full reading and those that met all predetermined criteria were included. In case of disagreement between evaluators, a third evaluator made the decision on the eligibility of the study in question.

Selection of studies

Initially, the eligibility reviewers were trained to perform the systematic review by other reviewers. After calibration and clarification of doubts, the titles and abstracts were independently examined by the two eligibility reviewers. Those who presented a title within the scope but with an unavailable abstract were also obtained and analyzed in full. Subsequently, the eligible studies were obtained in full text and evaluated. In specific cases, when the study with potential for eligibility presented incomplete data, the authors could be contacted by e-mail for further information; however, this communication was not necessary for any of the articles. In case of disagreement between the reviewers, a third party was involved for the final decision.

Data collection

After screening, the texts of the selected articles were reviewed and extracted in a standardized manner by two authors under supervision by identifying the year of publication, place of research, language of publication, type of study, sample, method, results, and conclusion of the study.

Clinical outcome

The clinical result of interest consisted of verifying the scientific evidence about the therapeutic intervention in phases one and two after infection of COVID-19. Those who did not use this approach were not used for the literature review sample.

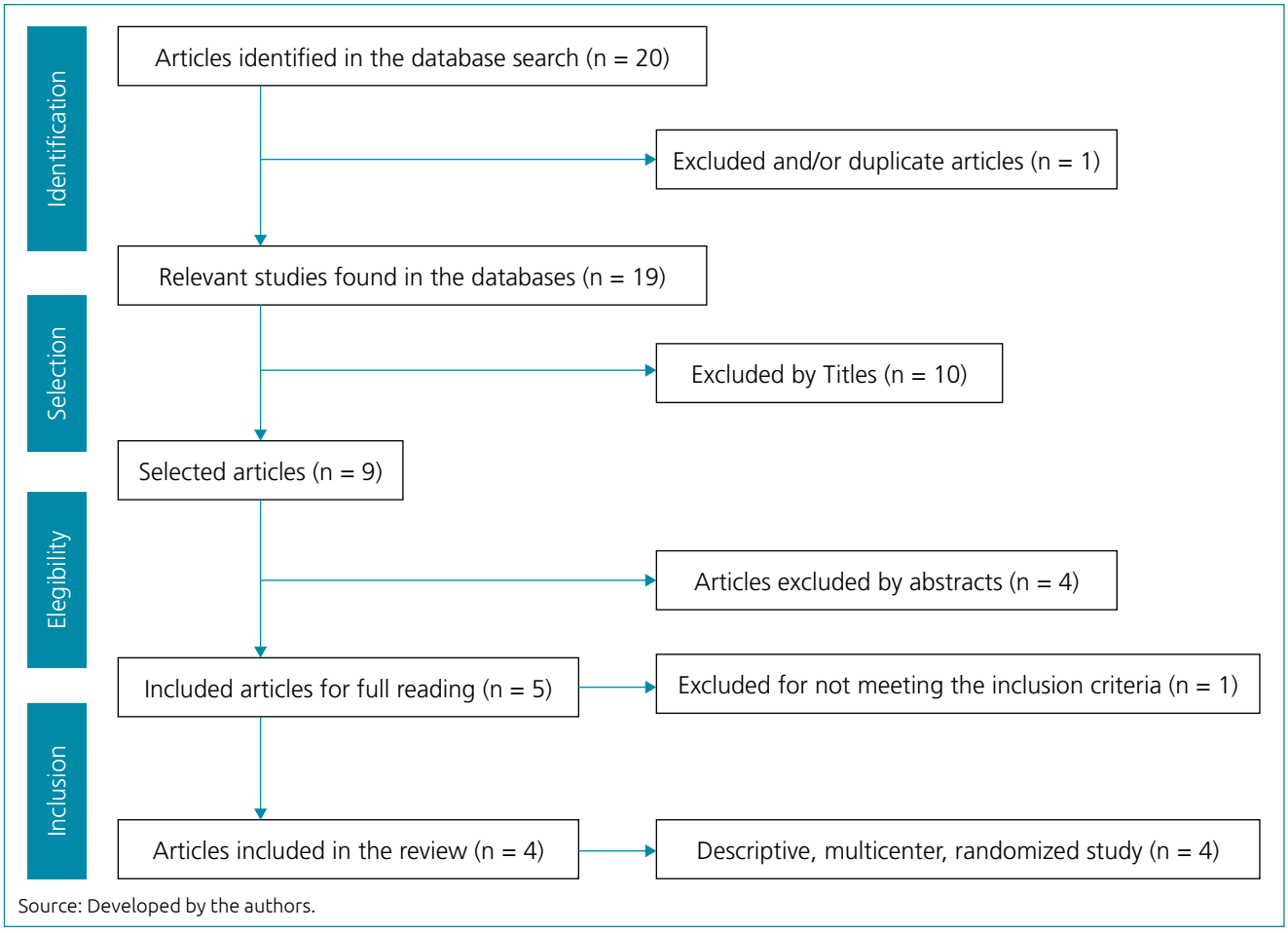
RESULTS

Initially, 20 articles were selected and then reduced to 19 after exclusion by repetition. Titles and abstracts were analyzed and 14 papers were excluded for not meeting the inclusion criteria that characterized the guiding question, so 4 articles were admitted for the final analysis[1-14]. The studies selected for the research were of the randomized controlled, multicenter, and descriptive type, as they answered the guiding question and were eligible according to the PRISMA[10] criteria used for the development of this research (Figure 1).

From the chosen descriptors, the databases of the scientific bases were consulted and the results available in Table 3 were obtained.

Regarding the description of the results of the articles eligible in this study, the information is verified in detail in Table 4. The information of the study samples, selected intervention protocols, associated comorbidities, therapeutic management, symptoms present at the beginning of treatment, medication use, recovery time, and age of individuals are highlighted in this table.

As for the sample of studies included in this research, the number of studies corresponded to n=1,010 individuals. The individuals undergoing antiviral therapy using remdesivir were 41, oseltamivir 599, and individuals with no therapy formed the rest of the patient sample. In the study by Wang et al.[13], the authors aimed to evaluate the efficacy and safety of using the drug remdesivir in adults who had COVID-19 symptoms for 12 days or less. Patients received intravenous remdesivir (200 mg on day 1 followed by 100 mg on days 2



Source: Developed by the authors.

Figure 1. Flowchart of the search process.

Table 3. Classification of references obtained from the PubMed, SciELO, LILACS, Web of Science, and Scopus databases.

Keywords	Number of articles	References excluded	Reason	Selected	Database
(coronavirus) and (covid-19) and (phases) and (treatment) and (disease progression)	0	0	0	–	SciELO
(coronavirus) and (covid-19) and (phases) and (treatment) and (disease progression)	0	0	0	–	LILACS
(coronavirus) and (covid-19) and (phases) and (treatment) and (disease progression)	12	10	Excluded by title (9), addressing another topic (1);	2	BIREME
(coronavirus) and (covid-19) and (phases) and (treatment) and (disease progression)	0	0	0	–	Scopus
(coronavirus) and (covid-19) and (phases) and (treatment) and (disease progression)	0	0	0	–	Web of Science
(coronavirus) and (covid-19) and (phases) and (treatment) and (disease progression)	8	6	Duplicates (1); Excluded by title (5)	2	PubMed
Total	20	16		4	BIREME PubMed

Source: Developed by the authors.

Table 4. Summary of the included articles.

Author/ Year	Objective of the Study	Study Design Sample	Description/ Intervention Protocol	Results/ Outcomes	Conclusion
Beigel et al.[11], 2020	To evaluate the clinical effectiveness and safety of using remdesivir in adults hospitalized with COVID-19	RCT Multicenter	The individuals in the study sample came from different countries and were hospitalized with or without supplemental O_2, non-invasive MV, and invasive MV. TG: n=538 Intravenous remdesivir: 200 mg on day 1 and 100 mg from day 2 to day 10, or until hospital discharge. PG: n=521 Received a placebo infusion on the same days as the treatment group.	Mean age TG=58.6 years/PG=59.4 years Mean days of symptoms before randomization TG=9/PG=9 Associated Comorbidities (%): GT=SAH (49.3), Obesity (37.7), DM2 (30.6) PG=SAH (49.9), Obesity (36.2), DM2 (28.7) Therapeutic Management Score *Hospitalization without the need for supplemental O_2 (%) TG=12.4/PG=11.5 *Hospitalization with O_2(%) TG=41/PG=38.1 *Hospitalization+Non-invasive ventilation (%) TG=18.1/PG=19 *Hospitalization+invasive ventilation (%) TG=23.1/PG=28.2 Mean Recovery Time TG=11 days/PG=15 days *TG randomized within 10 days of symptom onset; had a recovery rate of 1.28. *TG randomized after 10 days of symptoms; had a recovery rate of 1.38.	Using remdesivir for 10 days in the TG had better results than the placebo when treating hospitalized patients with COVID-19. The results were better in the TG patients who were given remdesivir while receiving supplemental O_2. The findings of the study highlight the need for identifying cases in the initial stages of hospital treatment for COVID-19 and begin the antiviral treatment before the disease progresses and requires mechanical ventilation.
Goldman et al.[12], 2020	To assess the effectiveness and safety of remdesivir treatment for 5 or 10 days in patients with severe COVID-19 disease	RCT	The individuals in the study sample came from different countries and had access to treatment in the clinic. Individuals already using MV for randomization and concomitant drug treatment were excluded. TG5: n=200 Intravenous remdesivir for five days: 200 mg	Mean age: TG5=61 years TG10=62 years Mean days of symptoms before randomization: TG5=8/TG10=9 Associated Comorbidities (%): TG5=SAH (50), Dyslipidemia (20), DM2 (24) TG10=SAH (50), Dyslipidemia (25), DM2 (22)	In this study, no significant difference in effectiveness was found between using remdesivir for 5 or 10 days.

Continue...

Table 4. Continuation.

Author/ Year	Objective of the Study	Study Design Sample	Description/ Intervention Protocol	Results/ Outcomes	Conclusion
			on day 1 and 100 mg from day 2 to day 4. TG10: n=117 Intravenous remdesivir for ten days: 200 mg on day 1 and 100 mg from day 2 to day 9.	Therapeutic Management Score (%) *Not hospitalized TG5=60/TG10=52 *Hospitalization without the need for supplemental O_2 TG5=6/TG10=7 *Hospitalization with O_2 TG5=10/TG10=7 *Hospitalization+Non-invasive ventilation TG5=4/TG10=5 *Hospitalization+invasive ventilation TG5=8/TG10=17 *Death TG5=16/TG10=21 Recovery Time Day 5 TG5=16/TG10=14 Day 7 TG5=36/TG10=26 Day 11 TG5=58/TG10=49 Day 14 TG5=64/TG10=54	
Wang et al.[13], 2020	To report the results found after using remdesivir in adult patients with severe symptoms of COVID-19 infection	RCT Multicenter	The individuals in the study sample came from hospital health services in Wuhan, China. They were admitted in a serious condition due to COVID-19 infection and were 12 days or less from the onset of symptoms. TG: n=155 Intravenous remdesivir: 200 mg on day 1 and 100 mg from day 2 to day 10. PG: n=78 Received a placebo infusion on the same days as the treatment group.	Mean age TG=66 years/PG=64 years Associated Comorbidities (%): TG=SAH (46), CAD (9), DM2 (25) PG=SAH (38), CAD (3), DM2 (21) Therapeutic Management Score *Duration of invasive MV (days) TG=7 days/PG=15.5 days *Duration of O_2 support (days) TG=19 days/PG=21 days *Hospitalization days TG=25/PG=24 *Hospitalization+invasive ventilation (%) TG=23.1/PG=28.2	Using intravenous remdesivir did not significantly improve the time of clinical recovery, mortality, or time to eliminate the virus in patients with severe COVID-19 when compared with the placebo. The study is limited because the lack of hospital beds during the pandemic hindered patients in earlier stages of the symptoms from being admitted.

Continue...

Table 4. Continuation.

Author/ Year	Objective of the Study	Study Design Sample	Description/ Intervention Protocol	Results/ Outcomes	Conclusion
				*Death (%) Day 7 TG=6/PG=5 Day 14 TG=10/PG=9 Day 28 TG=15/PG=13 Recovery Time TG=21 days/PG=23 days Clinical Improvement Rates (%) Day 7 TG=3/PG=3 Day 14 TG=27/PG=23 Day 28 TG=65/PG=58	
Huang et al.[14], 2020	To present the epidemiological, clinical, laboratory, radiological, treatment, and clinical results of patients with COVID-19	Cross-sectional	41 hospitalized patients were admitted to the study because they were diagnosed with COVID-19. The members were managed with doses of antibiotics (orally and intravenously) and oseltamivir (75 mg orally, twice a day).	Age (%) 20 (49) of infected patients were between 25 and 49 years old, and 14 (34) were between 50 and 64 years old. Mean age The mean age of patients was 49 years Symptoms at the beginning of the disease Fever (40), cough (3), and myalgia or fatigue (1). Medicines All patients were administered empirical antibiotic treatment and 38 (93%) patients received antiviral therapy (oseltamivir). Hospital discharge (%) On January 22, 2020, 28 (68) of the 41 patients were discharged and six (15) died. The discharge was based on subsided fever for at least 10 days, evidenced radiographic chest improvement, and viral release in upper respiratory tract samples.	

RCT: randomized clinical trial; USA: United States of America; n: sample number; TG: treatment group; PG: placebo group; SAH: systemic arterial hypertension; DM2: Diabetes Mellitus type 2; COVID-19: coronavirus disease 2019; O2: oxygen; MV: mechanical ventilation; CAD: coronary artery disease.

to 10 in single daily infusions) or the same volume of placebo infusions for a total of 10 days.

The second study included[12] involved hospitalized patients aged at least 12 years old and had confirmed COVID-19 infections. For this study, patients were allocated to 55 hospitals from March 6 to 26th, 2020. Patients were randomly assigned and received intravenous treatment with remdesivir for 5 or 10 days. All patients received 200 mg remdesivir on the first day, followed by 100 mg remdesivir once daily on subsequent days.

In another study[11], 1,063 patients were randomly divided into two groups: (1) remdesivir or (2) placebo). Remdesivir was administered intravenously as a dose of 200 mg on day 1 followed by a maintenance dose of 100 mg administered daily on subsequent days until hospital discharge or until the patient's death. A corresponding placebo was administered in the same way as that of the active drug. In this study, the 236b patients were divided into a remdesivir (n=158) or placebo (n=78) group. Patients had a mean age of 65 years and the majority were male, 89 (56 %) in the remdesivir group and 51 (65%) in the placebo group. The authors observed that patients who received remdesivir had a shorter clinical improvement time compared to those who received placebo (18 *versus* 23 days)[11].

For patients assigned to the remdesivir group, the duration of invasive mechanical ventilation (IMV) was shorter compared to the placebo group, suggesting that the use of the drug was more efficient in the remdesivir group. Adverse events were reported in 102 (66%) of 155 patients in the remdesivir group and in 50 (64%) of 78 subjects in the control group. The most common adverse events in the remdesivir group were hypoalbuminemia, hypokalemia, anemia, and thrombocytopenia; and in the placebo group, constipation and an increase in blood lipids were noted[13].

In the second study[12], 200 patients were assigned to receive remdesivir for 5 days and 197 individuals for 10 days. As a result, patients in the 10-day group showed significantly less effective clinical behavior compared to the 5-day group. In total, 65% of patients who received remdesivir treatment for 5 days showed significant clinical improvement compared to 54% of patients who received treatment for 10 days. The median length of hospitalization among discharged patients was seven days for the 5-day group and eight days for the 10-day group (60 and 52%, respectively), and mortality was numerically lower in the 5-day group compared to the 10-day group (8 *versus* 11%). The mean recovery time was 10 days among patients in the 5-day group and 11 days among patients in the 10-day group[12].

In the third study[11], of the 1,063 patients who were evaluated, 541 were assigned to the remdesivir group and 522 to the placebo group. The authors reported that patients in the remdesivir group had a shorter recovery time than patients in the placebo group. Serious adverse events occurred in 114 patients (21.1%) in the remdesivir group and 141 patients (27.0%) in the placebo group. Twenty-eight serious adverse events involving respiratory failure in the remdesivir group (5.2% of patients) and 42 in the placebo group (8.0% of patients) occurred. Acute respiratory failure, hypotension, viral pneumonia, and acute kidney injury were somewhat more common among patients in the placebo group. Grade 3 or 4 adverse events occurred in 156 patients (28.8%) in the remdesivir group and in the placebo group, 172 (33.0%) experienced adverse events (Grade 3 or 4).

The most common adverse events in the remdesivir group were anemia or decreased hemoglobin (43 events [7.9%] compared with 47 [9.0%] in the placebo group), acute kidney injury, decreased estimated glomerular filtration rate, creatinine clearance and/or increased blood creatinine (40 events [7.4%] compared with 38 [7.3%]), pyrexia (27 events [5.0%] compared to 17 [3.3%]), hyperglycemia or increased blood glucose level (22 events [4.1%] compared with 17 [3.3%]), and increased levels of aminotransferases, including alanine aminotransferase, aspartate aminotransferase, or both (22 events [4.1%], compared with 31 [5.9%])[11].

In the study by Huang et al.[14], doses of antibiotics (orally and intravenously) and oseltamivir (orally, 75 mg twice daily) were administered to 41 participating patients. Of these, 20 (49%) infected patients were between 25 and 49 years old, and 14 (34%) were between 50 and 64 years old. The mean age of patients was 49 years, and 13 (32%) were admitted to the ICU, provided they needed a high-flow nasal cannula or higher-level oxygen support measures to correct hypoxemia. The most common symptoms upon disease presentation were fever (n=40 [98%]), cough (n=31 [76%]), and myalgia or fatigue (n=18 [44%]). More than half of the patients (n=22 [55%]) developed dyspnea. All patients were administered empirical antibiotic treatment and n=38 (93%) patients received antiviral therapy (oseltamivir). On January 22nd, 2020, 28 (68%) of the 41 patients were discharged and six (15%) died. The condition for discharge was based on the reduction of fever for at least 10 days with concurrent improvement in radiographic evidence of the chest and viral release in upper respiratory tract samples[14].

DISCUSSION

This research aimed to verify the importance of therapeutic intervention in the initial stages after a COVID-19 infection in order to prevent the evolution of symptoms. It was found in the studies in which drugs used in the COVID-19 population were analyzed that the drugs were effective for the time of clinical improvement[13], led to shorter times to discharge

from the hospital, and caused a decrease in the use of invasive mechanical ventilation (IMV)[11-13]. However, research has also reported adverse reaction to medications[12,13] and more than 5 days of drug administration led to less effective drug efficiency[12]. It is important to highlight that the placebo groups also had health-related adverse effects. From this analysis, the importance of developing research with a larger population that seeks to verify scientific evidence is highlighted, as well as elucidating the effectiveness of drugs with early intervention in patients diagnosed with COVID-19. To date, no therapy has proven effective to the point of being advised for patients with COVID-19, but it is noteworthy that several ongoing randomized controlled trials will provide more meaningful information regarding the effectiveness of drugs used to treat it.

A study carried out by Grein et al.[15] demonstrated that hospitalized COVID-19 patients who were in serious condition and treated with compassionate remdesivir presented clinical improvement in 36 of the 53 patients (68%). These data corroborate the findings of the present research[11-13]. However, in one of the studies[13], the authors reported that remdesivir did not result in significant reductions in SARS-CoV-2 RNA loads in patients with severe disease stage or in detectability in upper respiratory tract samples, despite having observed strong antiviral effects in preclinical models of coronavirus infection. However, the pharmacokinetics of remdesivir in the respiratory tract cells of critically ill patients is unknown[16].

Regarding mortality, lower rates were observed in patients in the 5-day group who received the remdesivir dose compared to the 10-day group (8 *versus* 11%)[12]. In a recent, controlled, and randomized study in which drugs for patients hospitalized by COVID-19 were used, the mortality at 28 days was 22%[17].

In addition, remdesivir-related adverse events, such as hypoalbuminemia, hypokalemia, anemia, and thrombocytopenia, were reported in two studies[11,13]. However, in a previous study[18], patients significantly improved, and no adverse effects were observed. The current dose recommendation for remdesivir in COVID-19 is 200 mg (loading dose) on the first day followed by 100 mg on subsequent days[19]. In the studies presented in this analysis[11-14], both used the dosage according to this indication.

With regard to other administered drugs, one study[14] used antibiotics and oseltamivir in its patient sample. Although COVID-19 is a viral disease, initial hospital data shows that patients are being treated with antibiotics to cure or protect against secondary infections during respiratory illness or hospitalization. Two studies[20,21] reported improvement in the clinical picture with the use of antibiotic therapy and another associated medications without the evolution of the picture for bacterial infection. However, as reported by two other studies[22,23], patients' clinical conditions were not improved with the early initiation of antibiotic therapy.

Oseltamivir has no documented *in vitro* activity against SARS-CoV-2. However, its use is indicated in patients with severe acute respiratory syndrome or flu-like syndrome[24]. To assess the outcome of *in vitro* evidence suggesting that zinc may be effective against COVID-19, a study compared patients who received hydroxychloroquine and azithromycin in combination with zinc in patients who received hydroxychloroquine or azithromycin. As a result, the authors emphasize that the addition of zinc did not affect the length of hospital stay, ventilation duration, or the length of stay in the ICU[25]. Another study, with the aim of evaluating the role of hydroxychloroquine for the treatment of patients diagnosed with COVID-19, compared 821 participants who started treatment with hydroxychloroquine or placebo. No significant differences in the incidence of new cases between the two groups were noted, and side effects were greater in the hydroxychloroquine group (40.1%) compared to the other group (16.8%). No serious adverse effects were reported[26].

However, a study by Gautret et al.[27], which involved treatment with hydroxychloroquine combined with azithromycin, showed a negative viral titer in all patients who used this treatment combination. So far, the effectiveness and safety of this intervention for patients with SARS-CoV-2 infection are considered inconclusive. Caution is recommended with respect to the use of this association.

In Brazil, some medical teams use a combination of hydroxychloroquine and azithromycin in the population, and are currently achieving positive levels of effectiveness with this intervention, significantly reducing hospitalizations and virus-associated effects, thus demonstrating treatment effectiveness[28-30].

CONCLUSION

Patients submitted to treatment with certain antiviral drugs were less likely to have complications during the hospital stay, in addition to having less symptom evolution and a sooner hospital discharge. However, adverse events may occur in individuals submitted to such medication. Considering the studies included in this research, the importance of therapeutic interventions in the initial phases of the infection is evident, aiming to minimize viral activity with a reduced length of hospital stay and a direct impact on health costs, as the number of patients in ICU beds decreased significantly.

It should be noted that there is a considerable range of antiviral drugs being studied, and the specific indication of any given medicine must be adjusted – regarding its dosages

as well. The combination of some of these medications has proven effective and must be studied in a broader population while highlighting that the existing studies have already demonstrated important therapeutic possibilities to treat COVID-19.

AUTHORS' CONTRIBUTION

LFG: Methodology, Formal Analysis. **GSC:** Investigation. **KMP:** Data Curation, Resources. **AIG:** Validation, Visualization. **PH:** Conceptualization, Supervision.

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REVIEW ARTICLE
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Predictors associated with sickle cell nephropathy: a systematic review

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SUMMARY

INTRODUCTION: Sickle cell anemia affects more than 30 million people worldwide. Chronic kidney disease develops in 40% of individuals. The death rate of patients with sickle nephropathy is still high, with little known predictors related to its development. To answer the question "What predictors are associated with the onset of chronic kidney disease in patients with sickle cell anemia?", this article seeks to contribute to a better understanding of sickle nephropathy, making possible a new look at the sickle cell anemia and its kidney complications.

METHODS: A systematic review was developed, using the PRISMA recommendation, for cohort studies on predictors related to the outcome of sickle nephropathy in patients with sickle cell anemia.

RESULTS: Initially 321 studies were identified in Pubmed, of which six were selected to compose this systematic review. Lower hemoglobin levels, increased ages and albuminuria were the most pointed predictors associated with chronic kidney disease.

CONCLUSION: The main predictors associated with the development of chronic kidney disease in individuals with sickle cell anemia were lower hemoglobin levels, increased ages, and albuminuria. New studies evaluating predictors for the development of chronic kidney disease in sickle cell anemia are needed to better understand its installation and prevent its progression.

KEYWORDS: Acute kidney injury. Renal insufficiency, chronic. Anemia, sickle cell.

INTRODUCTION

Sickle cell anemia (SCA) was presented by Linus Pauling in 1949 as an autosomal recessive disease in which multiple organs are affected[1] and ever since it has become a prevalent genetic disease in the world: more than 30 million people are affected worldwide[2]. A meta-analysis published in 2018 estimated the prevalence of patients with SCA to be 111.91 for every 100,000 live births[3]. In Brazil, between 25,000 and 50,000 cases of SCA are estimated, according to the Ministry of Health[4]. Chronic kidney disease (CKD) develops in 40% of individuals, and 15–30% die due to kidney complications[5]. Among individuals who reach the fourth decade of life, half have end-stage kidney damage[6].

In the last decades, the average life expectancy of patients with SCA has increased thanks to better management of the numerous complications of the disease, including sickle nephropathy (SN)[7]. However, the death rate of patients with SN is still high, with little known predictors related to its development[8]. To answer the question "What predictors are associated with the onset of CKD in patients with SCA?", this article seeks to contribute towards a better understanding of SN, making possible a new look at the SCA and its kidney complications, which will contribute towards early detection, better management and, consequently, an improvement in the prognosis of the disease.

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METHODS

A systematic review was developed, using the PRISMA[9] recommendation, for cohort studies on predictors related to the outcome of SN in patients with SCA.

Through 4 strategies with the keywords "sickle cell anemia", "renal insufficiencies" and "chronic renal insufficiencies", as well as their synonyms, with the interlocutors AND or OR, through PubMed database (http://www.pubmed.gov.), original full text articles were searched, as the example: {("renal insufficiency, chronic" [MeSH Terms] OR ("renal" [All Fields] AND "insufficiency" [All Fields] AND "chronic" [All Fields]) OR "chronic renal insufficiency" [All Fields] OR ("chronic" [All Fields] AND " renal "[All Fields] AND" insufficiencies "[All Fields]) OR" chronic renal insufficiencies "[All Fields]) AND (" sickle cell anemia "[All Fields] OR" anemia, sickle cell "[MeSH Terms] OR ("anemia" [All Fields] AND "sickle" [All Fields] AND "cell" [All Fields]) OR "sickle cell anemia" [All Fields] OR ("sickle" [All Fields] AND "cell" [All Fields] AND "anemia" [All Fields])) AND ("2009/10/28" [PDat]: "2019/10/25" [PDat] AND "humans" [MeSH Terms]).

Two researchers (LVSM and SMSR) independently and blindly analyzed the titles and abstracts of the articles found. The divergences were analyzed by a third researcher (LRS). After that, a full-text reading was done applying the inclusion criteria: articles published in the period between 10/27/2009 and 10/25/2019; cohort original articles; studies in which measures of creatinine clearance have been obtained by previously validated means; studies carried out in humans. The exclusion criteria were: articles which only address patients with sickle cell trait/other hemoglobinopathies and those that do not address predictors in patients with kidney injury.

For each full text selected, the CASP[10] questionnaire was applied to assess the methodological quality and possible bias. A manual search was performed on the references of the selected articles, to select an article that had not been included in our search key. Then, the predictors cited by the articles that had a p-value less than 0.05 were extracted and arranged in a table according to the highest frequency.

RESULTS

Initially, 321 studies were identified in PubMed, of which six were selected to compose this systematic review, as shown in Figure 1.

Of the six selected studies, five were developed in the United States[11-15] and one in Brazil[16]. Table 1 summarizes the main

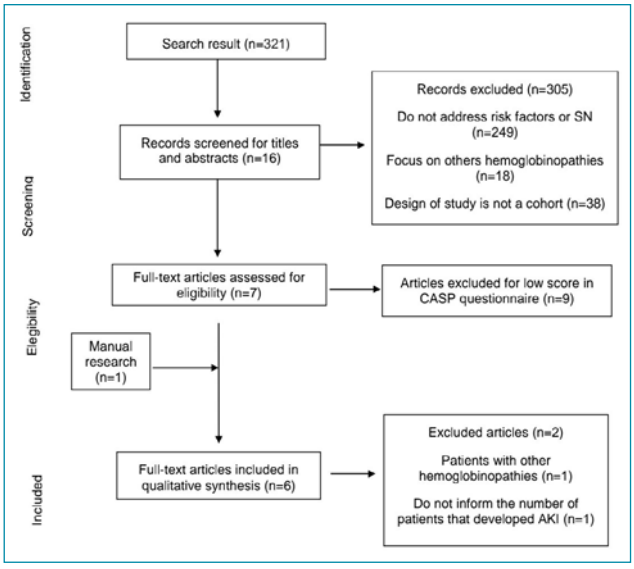

Figure 1. Flow of phases of the research.

Table 1. Results of analyzed articles.

Article	n	Age (Years/mean)	Risk factors (p<0.05)
Lebensburger, 2011[13]	144	–	Lower Hb levels, lower HbF levels and incresead LDH.
Aygun, 2011[11]	260	≤30	Increased cystatin C levels, higher systolic blood pressure, decreased leukocytes count and decreased absolute neutrophil counts.
McPherson Yee, 2011[15]	261	2–21	Increased age, lower Hb levels.
Silva Junior, 201216	98	19–67	Lower Hb levels, lower hematocrit levels, lower platelet levels, leukocyturia, proteinuria and increased age.
Saraf, 201414	795	24–46	Higher mean arterial pressure, lower Hb levels, macroalbuminuria, haemoglobinuria, increased age, AST, incresead LDH.
Gosmanova, 201412	98	21–42	Increased age, higher systolic and diastolic blood pressure, albuminuria.

CKD: chronic kidney disease; AKI: acute kidney injury; Hb: hemoglobin; HbF: fetal hemoblogin; CRP: C-reactive protein; AST: aspartate transaminase; LDH: lactate dehydrogenase; NGAL: urine neutrophil gelatinase-associated lipocalin; BMI: body mass index.

characteristics of the six studies and the respective predictors associated with CKD in patients with SCA.

Lower levels of hemoglobin, albuminuria and increased age were the main predictors shown in the studies. Other important predictors presented were increased LDH (lactate dehydrogenase), higher blood pressure, lower levels of fetal hemoglobin, among others listed in Table 2.

DISCUSSION

Lower levels of hemoglobin were suggested as important predictors for CKD. However, CKD caused by other diseases can also lead to anemia, which raises the question: is CKD linked to anemia or is anemia a predictor of SN? Naik et al. described the pathophysiology of SN, correlating with anemia. The red blood cells of patients with AF when in vessels with lower oxygen tension or lower pH polymerize, taking the form of a sickle, occluding small diameter vessels and leading to ischemic lesions in multiple organs, including the kidney. This also leads to glomerular microinfarctions and apoptosis of epithelial cells, which can decrease the glomerular filtration rate[17].

Patients that developed CKD had greater ages when compared to patients without kidney injury. These data are consistent with a 4 decades study developed with 1056 patients, which demonstrated that renal injury tends to occur later, around the third to fourth decades of life[18].

Albuminuria and proteinuria were also prevalent predictors. The literature points to other studies that demonstrate albuminuria as a predictor linked to the pathogenesis of podocyte loss and disruption of the glomerular filtration barrier[19]. Podocytes are the epithelial cells that line the glomerular capillaries through their foot processes and are responsible for the composition of the glomerular filtration membrane, together with the porous endothelial cells and the glomerular basement membrane. They serve as a barrier by holding proteins through size and load-dependent blocking. As podocytes have their own metabolism, ischemic lesions, toxins, complement deposition, among other situations, lead to the production of reactive oxygen species that induce apoptosis and podocyte processes effacement, through the destruction of the actin cytoskeleton, changes in the negative podocyte surface and failure of calcium metabolism. Vasooclusive episodes occur in SCA patient's vessels, culminating with ischemic lesions to the kidneys. All of these result in the failure of the filtration barrier and trigger proteinuria[20,21].

Arterial hypertension was positively correlated with the development of CKD in individuals with SCA, through higher systolic and diastolic blood pressure seen in CKD patients. Corroborating these findings, a cohort of 158 patients with SCA showed a 50% increase in creatinine levels in patients with systolic blood pressure levels above 140 mmHg or diastolic blood pressure above 90 mmHg[22].

Urinary and serum markers such as lactic dehydrogenase, AST, cystatin C, leukocyturia, and hemoglobinuria were related to the development of renal injury, which suggests that hemolysis and inflammation are present in the pathophysiology of the nephropathy. Fetal hemoglobin was shown as a protective factor as it prevents red blood cells from acquiring the sickle shape[17].

A small sample study demonstrated a decrease in lactic dehydrogenase in patients with SCA who were treated with hydroxyurea. Also, the same study reported lower reticulocyte counts and higher levels of fetal hemoglobin, findings that suggest a reduction in hemolysis in patients receiving hydroxyurea and also show that hemolysis is an important feature in patients with SCA. A larger cohort also demonstrated that lactic dehydrogenase is associated with higher mortality in individuals with SCA, reaffirming that hemolysis is a predictor of poor prognosis in these patients[23,24].

Higher levels of AST were pointed in the studies of this review as predictors of SCA, but other articles in the literature have correlated higher levels of AST, as well as bilirubin and alanine aminotransferase, with another organ failure in SCA: the sickle cell hepatopathy, as demonstrated in the cohort

Table 2. Risk factors associated with SN (n=7).

Risk factors	CKD
Lower Hb levels	4
Increased age	4
Albuminuria	3
Increased LDH	2
Higher systolic blood pressure	2
Higher diastolic blood pressure	1
Lower HbF levels	1
Lower Hematocrit levels	1
Decreased leukocytes count	1
Decreased absolute neutrophil count	1
Lower platelet levels	1
Haemoglobinuria	1
Leukocyturia	1
AST	1
Higher MAP	1
Increased cystatin C levels	1

SN: sickle nephropathy; Hb: hemoglobin; HbF: fetal hemoglobin; CRP: C-reactive protein; LDH: lactate dehydrogenase; MAP: mean arterial pressure; NA: not assessed.

developed with more than 1100 subjects. On the other hand, this study does not present any parameter related to kidney function, which provides the missing information of whether AST is related or not with SN[25]. However, Madu et al. correlated AST levels and proteinuria, without statistical significance, which also indicates a poor correlation between this predictor and kidney injury[26].

A study published in 1985 with patients with renal dysfunction caused by multiple diseases, associated decreased glomerular filtration rate with increased levels of cystatin C[27]. Our study also demonstrated an association between this marker and reduced renal function in patients with SCA, but recent studies have conflicted with these findings. Unal et al. for example, showed no difference in cystatin C levels between SCA patients and healthy controls[28]. On the other hand, a Greece study developed with 87 patients found an inverse correlation between glomerular filtration rate and cystatin C levels, corroborating with the previous findings of the 1985 study[29]. All this controversy raises the need for further research on this marker.

Regarding urinary markers, leukocyturia and hemoglobinuria did not correlate with proteinuria in a cross-sectional study conducted in Africa, which differs from our findings[30]. However, other reports have shown that hemoglobinuria is related to SN as a sign of papillary necrosis, congestion of the renal vasculature, and even renal medullary carcinoma[31-33].

Our study has some limitations. Few cohort studies evaluating the development of kidney injury in patients with SCA have been found in the databases, which demonstrates the need for further studies on this topic. In addition, there is high heterogeneity among the articles, with several measures of creatinine clearance and different criteria of albuminuria.

CONCLUSION

In conclusion, the main predictors associated with the development of CKD in individuals with SCA were lower hemoglobin levels, albuminuria, and increased age. New studies evaluating the pathophysiology of kidney injury in SCA are needed to better understand its installation and prevent its progression.

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AUTHORS' CONTRIBUTIONS

LM: Conceptualization, Data Curation, Methodology. **SR:** Conceptualization, Data Curation, Methodology, Writing – Original Draft. **LS:** Writing – Original Draft, Writing – Review & Editing. **DBM:** Methodology, Writing – Original Draft, Writing – Review & Editing.

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Guillain-Barré syndrome associated with SARS-CoV-2 infection: a scoping review

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SUMMARY

BACKGROUND: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections can affect the nervous system, triggering problems such as the Guillain-Barre Syndrome (GBS), an association that can bring complications to the patient.

OBJECTIVE: This scoping review aimed to clarify the clinical features and analyze patients with GBS associated with SARS-CoV-2 infection, looking at morbidity, mortality, and neurological outcomes.

SEARCH STRATEGY: The search was conducted through Medline, Web of Science, Embase, CINAHAL, Latin-American and Caribbean Literature in Health Sciences (LILACS), clinicaltrials.gov, SCOPUS, and the Cochrane Central Register of Controlled Trials.

SELECTION CRITERIA: Observational studies, published after 2019, describe patients with GBS associated with SARS-CoV-2 infection. There were no language restrictions while selecting the studies.

DATA COLLECTION AND ANALYSIS: Three authors, Kleyton Santos de Medeiros, Luíza Thomé de Araújo Macêdo, and Wederson Farias de Souza, independently screened the search results using titles and abstracts. Duplicate studies were excluded. The same authors then went through the entire text to determine whether the studies met the inclusion criteria. Discrepancies were resolved by other reviewers, Ana Paula Ferreira Costa, Ayane Cristine Sarmento, and Ana Katherine Gonçalves. Finally, the selection of the studies was summarized in a PRISMA flow diagram.

MAIN RESULTS: Main manifestations were fever, coughing, dyspnea, sore throat, ageusia, anosmia, and respiratory failure, in addition to paresthesia of the upper and lower limbs, tetraparesis, facial diplegia, areflexia, asthenia, mastoid pain, acute ataxia, fatigue, numbness, swallowing disorder, and moderate low back pain.

CONCLUSION: Coronavirus disease 2019 (COVID-19) can trigger the GBS, despite the few studies on this topic. Patients had clinical manifestations of COVID-19 infection and neurological manifestations characterizing GBS.

KEYWORDS: Coronavirus infections. COVID-19. Guillain-Barre syndrome.

INTRODUCTION

In December 2019, an outbreak of SARS-CoV-2, the virus that causes COVID-19 was detected in Wuhan City, Hubei Province of China. COVID-19 primarily affects the respiratory tract and the lungs and the appearance of symptoms depends on the age and the patient's underlying medical illness as well as on the condition of the immune system[1,2].

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Infected individuals usually have simple respiratory symptoms, fever, dry cough, and tiredness, which can progress to pneumonia and dyspnea[3]. The reported neurological manifestations and complications of COVID-19 include anosmia, headaches, dizziness, delirium, stroke, epilepsy, encephalitis, encephalopathy, myalgias, and Guillain-Barré syndrome (GBS)[1,2,4].

GBS is an acute immune-mediated disease of the peripheral nerves and nerve roots (polyradiculoneuropathy) usually preceded by various infections[2]. Classical clinical manifestations include paresthesia, progressive, ascending, and symmetrical flaccid limbs paralysis, muscle weakness, and areflexia. It may also present an infection of the gastrointestinal or respiratory tract before neurological symptoms[1].

The aims of this scoping review was to clarify the clinical features of patients with GBS associated with SARS-CoV-2 infection, their morbidity and mortality, as well as this important neurological manifestation caused by COVID-19.

METHODS

The Scoping Review was carried out following the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) checklist[5].

Protocol and registration

The review was not registered in the International Prospective Register of Systematic Reviews (PROSPERO), and corresponding authors were not contacted due to time constraints. Ethical approval was not required for this review.

Eligibility criteria

This scoping review included the following studies: observational studies (case report, case series, case-control, and cohort) describing patients with GBS associated with SARS-CoV-2 infection; and studies published after 2019, as the first case of COVID-19 was registered in Wuhan, China, in December 2019[6]. There were no language restrictions while selecting studies.

Information Sources

Medline, Web of Science, Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHAL), Latin American and Caribbean Literature in Health Sciences (LILACS), clinicaltrials.gov, Scopus, and the Cochrane Central Register of Controlled Trials were used to search for articles published between December 2019 and April 2020. We selected the publications starting from December 2019 because the first case of COVID-19 was registered in Wuhan, China, in December 2019[6].

Search

The medical subject headings (MESH) terms were (COVID-19 OR severe acute respiratory syndrome coronavirus 2 OR SARS-CoV-2) AND (Guillain Barre Syndrome OR Guillain-Barré Syndrome OR Landry-Guillain-Barre Syndrome OR Acute Autoimmune Neuropathy). Eligible studies were also selected from the reference lists of the retrieved articles. The research included articles published until June 26th.

Selection of sources of evidence

Three authors, KSM, LTAM, and WFS, independently screened the search results using the titles and abstracts. Duplicate studies were excluded. The same authors then went through the full text to determine whether the studies met the inclusion criteria. Discrepancies were resolved by others reviewers, APFC, ACAS, and AKG. The selection of the studies was summarized in a PRISMA flow diagram (Figure 1).

Data items and Synthesis of results

Various characteristics of the eligible studies were extracted, including the first authors' last names, year of publication, location of the study (country), study design, primary objective, level of evidence, number of patients, gender, mean age of patients, comorbidities, clinical manifestations, muscle strength assessment, patient outcome, chest imaging, laboratory tests, tests diagnosis, and treatment. Standardized data extraction forms were specifically created in Excel for this review, and the results were entered into a database. All data entries were double-checked. Subsequently, the qualitative synthesis was summarized.

Critical appraisal of individual sources of evidence

The quality of the included studies was assessed using the New JBI Levels of Evidence developed by the Joanna Briggs Institute Levels of Evidence and Grades of Recommendation Working Party of October 2013[7]. Then, a Checklist for Case Series[8] and a Checklist for case reports were used[9].

RESULTS

Selection of sources of evidence

The database search identified 196 articles. Excluding duplicates, a total of thirty-eight articles; one hundred and fifty-eight were considered eligible. However, forty-seven were excluded because titles and abstracts were considered

irrelevant to the topic or published before 2019. Subsequently, one hundred and eleven full-text articles were identified and assessed for eligibility. However, eighty-two publications were excluded because the data was insufficient to be extracted or calculated. Thus, twenty-nine articles were analyzed. The PRISMA-ScR flowchart for selecting the available studies is shown in Figure 1.

Characteristics of sources of evidence

The articles were carried out in different places, being Iran[1], Italy[10-15], China[16], the United States[17-20], France[21-24], Spain[25-30], Canada[31], Switzerland[32,33], Austria[34], Holland[35], Turkey[36], and Germany[37]. Twenty-seven articles were in English and three in Spanish, published in 2020 and presented in the data extraction Table 1.

Critical appraisal within sources of evidence

Twenty-six articles were case reports (level of evidence 4.d) and three case series (level of evidence 4.c). Therefore, it was observed that the studies included in this review have low levels of evidence, according to the New Levels of Evidence from JBI[7]. This can be explained due to the recent appearance of the disease.

Despite this, all studies were well designed and well evaluated by the JBI Critical Appraisal Checklist for Case Series[8] and

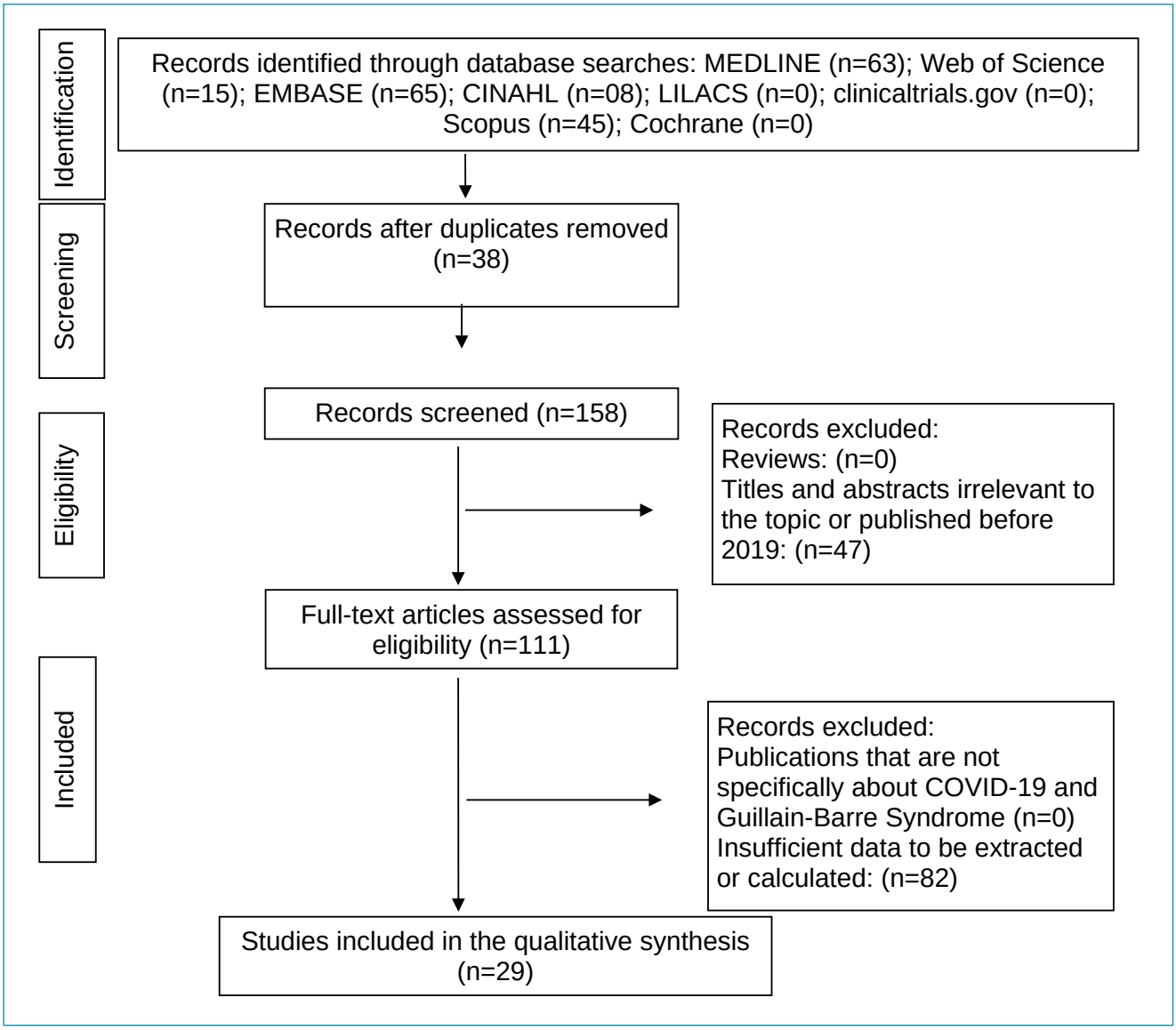

Figure 1. Flow diagram of the search for eligible studies COVID-19 and Guillain-Barre Syndrome: CENTRAL. Cochrane Central Register of Controlled Trials.

Table 1. Description of the characteristics of included studies.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Sedaghat et al.[1]	Male	65	Diabetes Mellitus 2	Presented neurological manifestations such as acute progressive weakness of the distal lower extremities, progressing from the distal to the proximal limbs and, shortly afterward, he presented quadriplegia and facial paresis bilaterally.	On physical examination, the patient had normal vital symptoms and was conscious.	CT showed diffused consolidations and ground-glass opacities in both lungs, and bilateral pleural effusion.	Hydroxychloroquine, LPV/RTV, and Azithro- mycin. And 0.40 g/kg/ day IVIg; and metformin 2 diabetes mellitus.	RT- PCR; chest CT and EMG.
Toscano et al.[10]	P1 – Female	77	NA	Paresthesia in the lower limbs and hands. Flaccid areflexic tetraplegia evolving to facial weakness, upper-limb paresthesia (36 h), and respiratory failure (day 6)	Lymphocytopenia, Raised CRP, LDH, and ketonuria.	CT scan of the thorax revealed interstitial bilateral pneumonia.	IVIg treatment.	RT-PCR and EMG.
	P2 – Male	23	NA	Upper and lower facial weakness, which became bilateral and complete within 2 days, accompanied by mastoid pain, loss of taste, and lower limb paresthesia.	Lymphocytopenia, raised ferritine, CRP, LDH, and AST.	Normal thorax imaging.	Amoxicillin for five days and IVIg.	RT-PCR, EMG, and brain MRI.
	P3 – Male	55	NA	Flaccid tetraparesis and facial weakness evolving to areflexia (day 2) and respiratory failure (day 5).	Lymphocytopenia, raised CRP, LDH, AST, GGT, and ketonuria.	A CT scan of the thorax revealed multiple bilateral, ground-glass opacities compatible with interstitial pneumonia.	Azithromycin and received 2 cycles of IVIg.	RT-PCR and EMG.
	P4 – Male	76	NA	Lumbar pain and lower limb weakness and, on the 4th day after admission, muscle weakness rapidly evolved to a flaccid areflexic tetraparesis.	Lymphocytopenia, raised CRP, ketonuria. IVIg treatment resulted in motor improvement, more evident in upper limbs, but still unable to stand.	Normal thorax imaging.	IVIg treatment.	RT-PCR
	P5 – Male	61	NA	Complained of asthenia, loss of taste and smell, for one week.	Lymphocytopenia, raised CRP, LDH, and AST. Developed respiratory failure with neuromuscular features (hypercapnia, paradox respiration, acidosis) and was referred to the ICU, were he received mechanical ventilation through tracheostomy. The patient developed acinetobacter pneumonia.	Thorax X-ray and CT showed interstitial pneumonia, without parenchymal opacities nor alveolar damage.	Received IVIg and plasma exchange; had bacterial pneumonia during IVIg treatment, which delayed plasma exchange.	RT-PCR and EMG.

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Padroni et al.[11]	Female	70	NA	Complaining of asthenia, hands and feet paresthesia, and gait difficulties progressing within 1 day. On March 4th she had developed fever (BT P= 38.5°C) and dry cough.	Arterial blood gas analysis showed pO_2=76 mmHg with normal p/f ratio (=363). The patient was intubated and mechanical ventilation was applied, because of respiratory failure due to the worsening of muscle weakness.	A chest high-resolution computed tomography revealed some small "ground glass" areas in both lungs.	IVIg 400mg/die for 5 days was started.	RT-PCR and the neurological examination disclosed moderate.
Alberti et al.[12]	Male	71	Hypertension, abdominal aortic aneurysm, and lung cancer treated with surgery only in 2017 with negative oncological follow-up; no previous neurologic history was reported.	Paresthesia at limb extremities, followed by distal weakness rapidly evolving to a severe, flaccid tetraparesis over the previous 3 days. Neurologic examination showed symmetric limb weakness, symmetric and extensive stocking-and-glove hypesthesia at the 4 limbs (more pronounced at lower limbs), absent deep tendon reflexes, and normal plantar response. Moderate low back pain were present.	He showed hemodynamic disturbances with severe drug-resistant hypertension. Arterial blood gases indicated severe hypoxia (paO_2 65 mm Hg without supplemental oxygen). Unresponsive to continuous positive airway pressure ventilation and prone positioning. The patient died a few hours later because of progressive respiratory failure.	Brain CT scan was normal, whereas chest CT scan showed multiple bilateral ground glass opacities and consolidations, typical of COVID-19 pneumonia.	High-dose IV immunoglobulins (0.4 g/kg/d for 5 days) were started few hours after admission, together with high-flow 60%–80% oxygen via nonrebreather mask, antiviral therapy (LPV + RTV), and hydroxychloroquine	RT- PCR; chest CT and EMG.
Assini et al.[13]	P1 – Male	55	NA	Severe respiratory syndrome preceded by anosmia and ageusia, fever, and cough; acute onset of bilateral eyelid ptosis, dysphagia, and dysphonia.	Neurological examination showed bilateral masseter weakness, tongue protrusion deficit due to bilateral paralysis of the hypoglossal nerve, and hyporeflexia of upper and lower limbs, without muscle weakness. The patient was moved to ICU for invasive ventilation. Lymphocytopenia.	NA	Hydroxychloroquine, Arbidol, RTV and LPV; IVIg.	RT- PCR; EMG.
	P2 – Male	60	NA	Fever and cough; weakness in lower limbs with distal distribution and foot drop on the right side.	Simultaneously, massive disorders of the vegetative nervous system, consisting of gastroplegia, paralytic ileus, and loss of blood pressure control occurred. Neurological examination showed distal weakness at four limbs, with foot drop. Tracheostomy and assisted ventilation. Blood tests showed lymphocytopenia, increased LDH and GGT, and leukocytosis.	NA	Hydroxychloroquine, antiretroviral therapy, and tocilizumab. IVIg therapy.	RT-PCR and thoracic CT scan.

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Ottaviani et al.[14]	Female	66	NA	History of increasing difficulty walking and acute fatigue; she had mild fever and cough 10 days earlier. She also manifested a transient pruriginous dorsal rash, in addition to mild hypertension treated with beta-blockers. On evaluation, she was paraparetic with a rapidly progressive symmetric weakness in the lower limbs, leading to falls and paraplegia. Progressively developed proximal weakness in all limbs, dysesthesia, and unilateral facial palsy.	Maintaining reasonable respiratory function with supplemental oxygen. Moreover, gas exchanges worsened with a sudden desaturation, requiring intubation and ICU admission, where she was treated for multi-organ failure along with a leg deep vein thrombosis and a superimposed bacterial infection (*ab ingestis* pneumonia).	Lung CT scan showed bilateral ground glass opacities.	IVIg; antiretroviral drugs (LPV and RTV) and hydroxychloroquine.	RT-PCR and the neurological examination disclosed moderate (Medical Research Council grade 4/5).
Riva et al.[15]	Male	60	NA	Three-day history of progressive limb weakness and distal paresthesia at four-limbs. His past medical history was unremarkable. Twenty days before, he had developed fever (37.7–38.5 °C), headache, and myalgia followed by anosmia and ageusia.	Cell blood count, CRP, creatine phosphokinase, arterial blood gases, renal and hepatic function tests were normal. Anti-ganglioside antibodies tested negative.	Chest CT scan showed bilateral ground-glass opacities, consistent with COVID-19 pneumonia.	IVIg;	Antibodies for SARS-CoV-2 IgM/IgG and the neurological examination disclosed moderate.
Zhao et al.[16]	Female	61	NA	Presented with acute weakness in both legs and severe fatigue. Neurological examination disclosed symmetric weakness.	Her clinical condition improved gradually and her lymphocyte and thrombocyte counts normalized on day 20. At discharge on day 30, she had normal muscle strength in both arms and legs and return of tendon reflexes in both legs and feet.	Chest CT showed ground-glass opacities in both lungs.	IVIg; infection isolation room and received supportive care and antiviral drugs of arbidol, LPV, and RTV.	RT-PCR
Virani et al.[17]	Male	54	NA	Complaints of numbness and weakness of his lower extremities of 2-day duration. The weakness progressed. The patient complained of difficulty breathing and weakness was noted to ascend up to his nipples.	He was electively placed on mechanical ventilator support for concerns of impending respiratory failure. His clinical course showed improvement in his respiratory status with liberation from mechanical ventilation on day 4 of IVIg therapy. Neurologically, his upper extremity weakness resolved after completion of the course of IVIg. Lower extremity weakness persisted.	MRI of thoracic and lumbar spine that did not reveal any abnormal spinal pathology. This imaging, however, did reveal incidental findings of bilateral basilar opacities in the lungs.	Oral amoxicillin and steroids. 400mg/kg of IVIg therapy for a planned 5-day course. Hydroxychloroquine 400 mg for the first two doses with subsequent 200 mg dose twice a day for an additional eight doses.	RT-PCR and MRI.

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Rana et al.[18]	Male	54	Hypertension, hyperlipidemia, restless leg syndrome, and chronic back pain.	Ascending limb weakness and numbness that followed symptoms of a respiratory infection. Two weeks before presentation, he initially developed rhinorrhea, odynophagia, fevers, chills, and night sweats; he developed watery diarrhea; Over the next few days, he noted worsening paresthesias of his distal extremities bilaterally. His symptoms progressed to weakness of all limbs and difficulty voiding urine, developed progressive shortness of breath requiring intubation. Quadriparesis and areflexia with mute plantar responses.	He was extubated on hospital day 4. On hospital day 7, he was discharged to an inpatient rehabilitation facility. While in the inpatient rehabilitation, he was noted to have resting tachycardia and persistent difficulty urinating, which eventually required an indwelling catheter. He reported burning dysesthesias in his distal extremities and trunk, and complained of diplopia, which was worse on rightward.	Chest X-ray was negative other than an incidental finding of bibasilar lung infiltrates *versus* atelectasis. MRI of the thoracic and lumbar spine was reported to show no evidence of myelopathy or radiculopathy.	Amoxicillin; metronidazole. Hydroxychloroquine and azithromycin; IVIg.	RT-PCR; the neurological examination disclosed moderate (Medical Research Council grade 4/5) and EMG.
Su et al.[19]	Male	72	Coronary artery disease, hypertension, and alcohol abuse	Symmetric aresthesias and ascending appendicular weakness. Seven days earlier he had mild diarrhea, anorexia, and chills, without fever or respiratory symptoms. This condition resolved in 5 days. Weakness began 6 days after diarrhea, and the patient presented 1 day after neurological symptom onset. On admission, he was afebrile with normal vital signs. Mental status and CN were normal.	On day 3, the patient developed respiratory distress with a negative inspiratory force of –20 cm H_2O and vital capacity of 1.350 mL. He was transferred to the ICU and intubated. He remained afebrile and followed commands. Oxygen saturation was normal on ventilator settings positive end-expiratory pressure 5 cm H_2O and fraction of inspired oxygen 30%. Chest X-ray was stable. Sputum culture grew Stenotrophomonas maltophilia.	Chest X-ray showed mild bibasilar atelectasis *vs.* patchy consolidations. Computed tomography of the head was normal. Incompatible implant precluded MRI. On day 10, his oropharyngeal secretions increased, and chest X-ray showed new right lower lobe consolidation.	IVIg	RT-PCR and the neurological examination disclosed moderate (Medical Research Council grade 4/5).
Lantos et al.[20]	Male	36	NA	Presenting with left eye drooping, blurry vision, and reduced sensation and paresthesia in both legs for 2 days. He was in his usual state of health until 4 days before presentation, when he developed viral symptoms in a COVID-19-endemic region, reporting subjective fevers, chills, and myalgia.	Physical examination was notable for a partial left third nerve palsy and decreased sensation below the knees to all modalities. Nonetheless, the patient's hospital course was characterized by progressive ophthalmoparesis (including initial left CN III and eventual bilateral CN VI palsies), ataxia, and hyporeflexia, and the clinical picture was thought to be consistent with MFS from COVID-19 infection.	Brain MRI: prominent enhancement with gadolinium, and T2 hyperintense signal of the left CN III. No other CN demonstrated abnormal signal. No cerebellar lesions were seen to explain the patient's ataxia.	IVIg; hydroxychloroquine.	RT-PCR and RMI.

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Camdessanche et al.[21]	Male	64	NA	The patient fell and hurt his left shoulder leading to a tear of the rotator cuff. Eleven days after the symptom onset, the patient complained of paresthesia in feet and hands. In three days, he installed a flaccid severe tetraparesia. The patient complained of swallowing disturbance with a risk of suffocation.	Clinical presentation was moderate with high grade fever for three days requiring oxygen 2–3 L/ min through nasal cannula for five days. The patient was admitted in ICU and mechanically ventilated because of respiratory insufficiency.	Thoracic CT scan showed only 10–25% of ground glass opacities.	Paracetamol, preventing thromboembolism by low molecular weight heparin and LPV/RTV 400/100 mg twice a day for ten days. IVIg (0.4g/ kg per day during 5 days).	EMG
Arnaud et al.[22]	Male	64	Diabetes mellitus type 2	Cough, dyspnea, diarrhea, and fever. Fast progressive lower-limb weakness; The neurological examination showed generalized areflexia, severe flaccid paraparesis, mainly affecting proximal muscles, and a decreased proprioceptive length-dependent sensitivity involving the four limbs. We also found hypoesthesia to light touch and pinprick in lower extremities rather.	Respiratory rate was 30 breaths/min and oxygen saturation was 93% on ambient air. Lung auscultation revealed diffuse crackles.	A chest CT showed bilateral, diffuse and subpleural ground-glass opacities with a crazy-paving appearance, and a band of air space consolidation.	Cefotaxime, Azithromycin; IVIg and Hydroxychloroquine.	RT-PCR and EMG.
Bigaut et al.[23]	P1 – Male	43	NA	Presented with cough, asthenia, and myalgia in legs, followed by acute anosmia and ageusia with diarrhea the next day. Symptoms resolved spontaneously after 2 weeks. Twenty-one days after the beginning of respiratory symptoms, he presented with a rapidly progressive manner paraesthesia, hypoesthesia, and distal weakness in the lower limbs. In the following 2 days, these symptoms extended to the midthigh and tip of the fingers associated with ataxia, and he was hospitalized at day 4 due to a right peripheral facial palsy.	His BT was 36.9°C and oxygen saturation was 99%.	CT of the chest showed ground-glass opacities; MRI at day 7 showed multiple cranial neuritis (in nerves III, V, VI, VII, and VIII), radiculitis, and plexitis on both the brachial and lumbar plexus.	IVIg.	RT-PCR and the neurological examination disclosed moderate (Medical Research Council grade 4/5).
	P2 – Female	70	Obesity	Anosmia and ageusia, followed by diarrhea for 2 days. She complained of mild asthenia and myalgia without fever. Seven days later, she presented with acute proximal tetraparesis and distal forelimb, perioral dyspnea, and loss of ambulation.	Rapidly transferred to an ICU for noninvasive ventilation for acute respiratory failure with hypercapnia. She was discharged from the ICU 9 days later, without requiring invasive mechanical ventilation. Her clinical condition improved slowly with physiotherapy, needing a transfer in a rehabilitation center.	CT of the chest showed moderate ground-glass opacities in both lungs.	IVIg.	RT-PCR and the neurological examination disclosed moderate (Medical Research Council grade 4/5).

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
El Otmani et al.[24]	Female	70	Rheumatoid arthritis	Presented with a rapidly, bilateral weakness and tingling sensation in all four extremities resulting in a total functional disability within 48 hours. The patient denied any sphincter disturbances, dyspnea or swallowing difficulties. Neurological examination showed quadriplegia, hypotonia, areflexia, and bilateral positive Lase`gue sign. CN were intact. Three days prior to the ongoing symptoms' onset, the patient presented an episode of dry cough without dyspnea or fever, spontaneously resolving within 48 hours.	Temperature, lung, and cardiac auscultation were also normal.	Chest CT (day 10) revealed ground-glass opacities in the left lung.	IVIg (2 g/kg for 5 days) and a combination of Hydroxychloroquine (600 mg per day) and Azithromycine.	RT-PCR
Juliao Caamaño et al.[25]	Male	61	NA	Fever and coughing without dyspnea on day 1 of the illness; right peripheral facial nerve palsy.	NA	Brain CT and MRI were performed without any acute pathological findings.	Hydroxychloroquine and LPV/RTV; oral prednisone.	X-ray and RT-PCR.
Galán et al.[26]	Male	43	NA	Consultation for symmetric and global weakness of the 4 extremities of progressive intensity with impossibility for walking, as well as alteration in the sensitivity of the 4 members at the distal level. Three days before, there was a self-limited diarrhea episode, followed by symptoms of infection of the upper respiratory tract, bilateral facial paresis, and dysphagia.	NA	In the X-ray of thorax there are alterations suggestive of Early pneumonia by COVID-19.	IVIg; sulfate hydroxychloroquine, antiretro virals (LPV and RTV), antibiotic (amoxicillin), corticosteroids and oxygen therapy low flow.	RT-PCR; EMG and the neurological examination disclosed moderate (Medical Research Council grade 4/5).
Marta-Enguita et al.[27]	Female	76	NA	Evolution of low back pain radiating to the posterior aspect of both legs and progressive tetraparesis with paresthesias of distal onset. The pain was bilateral, with right predominance and greater night intensity. He associated progressive weakness predominantly proximal in the lower extremities, and 2 days before our evaluation, he presented weakness in the upper extremities, with functional limitation. Eight days before the onset of the symptoms, he had started with a cough and fever without dyspnea, with 72 hours of evolution; He associated global areflexia and hypoesthesia in both legs.	The patient was admitted and at 4 h presented dysphagia for liquids and progressively for solids, with a nasal voice and difficulty swallowing her own saliva, with progressive onset of a picture of ventilatory failure. She presents progressive deterioration, requiring oxygen therapy (FiO_2 60%), with maintained $SatO_2$ levels of around 91%, which do not show a problem of alveolar capillary junction or gas exchange. Finally, she dies at 12 h.	Normal cranial CT and cervical spine were performed, showing degenerative signs at the level of the vertebral bodies, without invasion of the spinal canal. On chest CT, a pattern compatible with the level of pulmonary impairment due to SARS-CoV-2 infection was observed.	NSAID, pyrazolones, and transdermal morphics. amoxicillin-clavulanic acid, and azithromycin.	RT-PCR

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Molina et al.[28]	Female	55	Dyslipemia and active smoking.	Fever, unproductive cough and dyspnea after 15 days of evolution. In the past 24 hours, she reported paresthesias in the hands and feet, as well as weakness in the lower extremities. Severe low back pain radiating to both legs with progressive weakness in the 4 extremities associated with dysphagia. At 48 hours, the patient presented worsening of neurological symptoms, with areflexic tetraparesis. Along with this, liquid dysphagia, bilateral facial diplegia, weakness in closing the eyelids, lingual and perioral paresthesias. No meningeal signs.	At initial examination, the patient is conscious and oriented. Blood pressure 155/102 mmHg, heart rate 103 beats per minute, temperature 36.6 ºC, oxygen saturation 93% basal ($SatO_2$). Eupneic with 20 breaths per minute. Bibasal crackles on pulmonary auscultation. Strength and sensitivity preserved in the 4 limbs. Rest of physical examination without significant changes. Adequate ventilatory mechanics and SatO2 without the need for respiratory support. In this context, it was decided to transfer to the ICU.	Chest radiography revealed consolidation in the left lower lobe; Using MRI, a slight leptomeningeal improvement is observed in the brain stem and cervical cord.	hydroxychloroquine, ceftriaxone and azithromycin; IVIg.	RT-PCR and the neurological examination disclosed moderate (Medical Research Council grade 4/5).
Sancho-Saldaña et al.[29]	Female	56	NA	Recent unsteadiness and paraesthesia in both hands. Fifteen days earlier, she had reported fever, dry cough, and shortness of breath that was controlled with symptomatic treatment. she developed lumbar pain and progressive proximal lower limb weakness, bilateral facial nerve palsy, oropharyngeal weakness, and severe proximal tetraparesis with cervical flexion.	She was transferred to the ICU for 5 days due to the risk of respiratory insufficiency and began rehabilitation, not needing mechanical ventilation. She started recovering by day 7 after the onset of weakness.	Her chest X-ray showed a lobar consolidation.	hydroxychloroquine and azithromycin; IVIg.	RT-PCR and the neurological examination disclosed moderate (Medical Research Council grade 4/5).
Reyes-Bueno et al.[30]	Female	51	NA	Diarrhea, odynophagia, and cough. The condition lasted approximately 10 days, after which she kept feeling discomfort in the throat. She did not refer ageusia or anosmia.	From March 30th, she started having intense root-type pain in all four limbs, especially in the legs as well as dorsal and lumbar back pain. On April 4th she started with weakness in her lower limbs, which progressed to the point of preventing her from walking in a few days, associated with double binocular vision. The neurological exploration showed paresis of the left external rectus muscle with horizontal diplopia when looking to the left, discrete predominantly inferior bilateral facial paresis, symmetrical paraparesis with 3+/5 weakness in psoas, hamstrings, gluteus, and quadriceps, 3/5 in gastrocnemius, 2/5 in posterior tibial and peroneal; and global areflexia. She also presented symptoms of autonomic dysfunction such as dry mouth, diarrhea, and unstable blood pressure.	NA	IVIg.	RT-PCR and ELISA technique; the neurological examination disclosed moderate (Medical Research Council grade 4/5).

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Chan et al.[31]	Male	58	NA	Presented with acute-onset bilateral facial weakness, dysarthria, and paresthesia in his feet. He denied any other neurological symptoms, including anosmia and ageusia. He denied fever, fatigue, cough, shortness of breath, or any other symptoms on review of systems. Neurological examination demonstrated complete facial diplegia and areflexia in the lower extremities. he had slight movements of his facial muscles and the distal paresthesias of his lower extremities were unchanged.	Temperature of 36.6°C, maximum heart rate of 140 beats/minute, maximum blood pressure of 187/103 mmHg, maximum respiratory rate of 34 breaths/ minute, and an oxygen saturation of 96% on room air, with resolution of tachycardia, hypertension, and tachypnea within 12 hours. Auscultation of the lungs revealed diffuse crackles bilaterally.	Chest x-ray demonstrated diffuse heterogeneous infiltration in both lungs. CT and CTA of the head and neck did not demonstrate any intracranial or vascular abnormalities but demonstrated ground-glass opacities in both lung apices.	Empiric ceftriaxone and azithromycin; IVIg.	RT-PCR and EMG;
Coen et al.[32]	Male	70	NA	Paraparesis, distal allodynia, difficulties in voiding and constipation. Ten days before he developed myalgia, fatigue, and a dry cough.	Physical examination revealed fine crackles in the left base, bilateral lower limb flaccid paresis, absent deep tendon reflexes of the upper and lower limb and idiomuscular response to percussion of the muscle tibialis anterior, indifferent plantar reflexes. There was no sensory deficit.FilmArray Meningitis/ Encephalitis (ME) Panel testing (BioFire Diagnostics, Salt Lake City, UT) and SARS-CoV-2 RT-PCR were negative. showed decreased persistence or absent F-waves in tested nerves.	Chest X-ray was normal. Contrast-enhanced MRI excluded myelopathy. Nerve conduction study showed sensorimotor demyelinating polyneuropathy with "sural sparing pattern"; F wave study showed decreased persistence or absent F-waves in tested nerves.	IVIg.	RT-PCR and ELISA technique.

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Lascano et al.[33]	P1 – Female	52	NA	Dry cough, fever, odynophagia, arthralgia, diarrhoea. Back pain, limb weakness, ataxia, distal paresthesia, dysgeusia, cacosmia. Developed respiratory failure, dysautonomia, and tetraplegia with areflexia.	Improvement of tetraparesis. Able to stand up with assistance. GBS disability clinical score 4/6. Spinal cord: no nerve root gadolinium enhancement.	NA	IVIg	RT-PCR and Antibodies for SARS-CoV-2 IgM/IgG.
	P2 – Female	63	Diabetes mellitus type 2	Dry cough, shivering, odynophagia, breathing difficulties, chest pain. Lower limb pain, mild weakness and normal deep tendon reflexes. Developed tetraparesis, distal paresthesia and areflexia.	Dismissal with full motor recovery. Persistence of lower limb areflexia and distal paresthesia. GBS disability clinical score 1/6.	NA	IVIg	RT-PCR
	P3 – Female	61	NA	Productive cough, fever, myalgia, vasovagal syncope, diarrhoea, nausea and vomiting. Lower limb weakness and distal paresthesia, dizziness, dysphagia, dysautonomia, areflexia. Presented worsening of bulbar symptoms and bilateral facial palsy.	Improvement of tetraparesis and ability to walk with assistance. Persistence of neuropathic pain and distal paresthesia. GBS disability clinical score 3/6. Spinal cord: lumbosacral nerve root enhancement. Normal brain imaging.	NA	IVIg	RT-PCR
Helbok et al.[34]	Male	68	NA	Cough, headache, fatigue, myalgia and fever up to 39°C followed by anosmia and ageusia. but still complained of severe fatigue and developed symmetric distal tingling in both feet followed by ascending dysesthesias up to the knees and proximal weakness.	His respiratory condition worsened, and the patient required oxygen supplementation (3L/min) followed by pressure support non-invasive ventilation after 36 h. The next day he presented inability to walk. On examination, the patient was alert and fully oriented, afebrile with normal vital signs (oxygen saturation 98% on room air, blood pressure 143/90mmHg, heat rate 85 bpm). Due to muscle weakness accompanied by respiratory failure the patient underwent elective intubation in a fully conscious state.	Chest Computed tomography was performed and revealed residual ground-glass opacities in both lower lungs	IVIg and plasma exchange	RT-PCR and Antibodies for SARS-CoV-2 IgM/IgG; the neurological examination disclosed moderate (Medical Research Council grade 4/5).

Continue...

Table 1. Continuation.

Author	Gender	Age	Comorbidities	Clinical manifestations	Patient outcome	Chest imaging	Treatment	Diagnosis
Kilinc et al.[35]	Male	50	NA	Four days of progressive bilateral facial weakness, paresthesia of distal extremities and an unsteady gait. Four weeks earlier he had experienced an episode of dry cough lasting several days without fever or other symptoms of infection. Neurologic examination showed facial diplegia, normal eye movements, mild symmetric proximal muscle weakness and impaired propriocepsis in the legs. Patient had an ataxic gait and tendon reflexes were absent.	Routine blood examination showed no abnormalities. Routine analysis of CSF showed a normal cell count and total protein level.			RT-PCR and Antibodies for SARS-CoV-2 IgM/IgG; EMG.
Oguz-Akarsu et al.[36]	Female	53	NA	History of dysarthria associated with progressive weakness and numbness of the lower extremities. She had a mild fever (37.5°C) but no cough, dyspnea, anosmia or ageusia.	NA	Focal intensities suspicious for COVID-19 pneumonia were incidentally identified in peripheral areas of lungs on STIR sequence of the brachial plexus MRI; Chest computed tomography showed bilateral peripheral ground-glass opacities and consolidations on both lungs.	Plasma exchange; hydroxychloroquine and azithromycin.	RT-PCR
Scheidl et al.[37]	Female	54	NA	Areflexia, numbness, and tingling of all extremities were also found, with initial maintained gain ability. She did not experience fever, respiratory or gastrointestinal symptoms, but reported about a transient loss of smell and taste 2 weeks before the GBS symptoms occurred.	The first electrophysiological evaluation (at admission) showed significantly prolonged distal motor latencies and temporal dispersion of the CMAP of the common peroneal nerve bilaterally.	MRI of the cervical spine and the chest x-ray examination did not show pathological findings. Electrophysiological studies were performed using a Nicolet Viking EMG device.	IVIg	RT-PCR and EMG.

LPV: lopinavir; RTV: ritonavir; RT-PCR: real-time reverse transcriptase; CT: chest tomography; EMG: electromyography; NA: Not Applicable; CRP: C-reactive protein; LDH: lactate dehydrogenase; IVIg: intravenous immune globulin; AST: aspartate aminotransferase; MRI: magnetic resonance imaging; GGT: gamaglutamiltranspeptidase; ICU: intensive care unit; BT: body temperature; CN: cranial nerve; MFS: Miller Fisher Syndrome; NSAID: non-steroidal anti-inflammatory drugs; ELISA: enzyme-linked immunosorbent assay; CTA: CT angiography; GBS: Guillain-Barre Syndrome; CSF: cerebrospinal fluid; STIR: Short-Tau Inversion Recovery; CMAP: compound muscle action potentials.

Case Reports[9], that is, they achieved a high score and, thusly, were included in the review.

Synthesis of results

Clinical manifestations

Main clinical manifestations were fever, coughing, dyspnea, sore throat, ageusia, anosmia, respiratory failure, and diarrhea, as shown in Figure 2.

Toscano et al.[10] describing three patients [P1, P3, and P5] who received mechanical ventilation and two who were admitted to the Intensive Care Unit (ICU) [P3 and P5]. The condition of P5 deteriorates during hospitalization, presentation of hypercapnia, paradoxical breathing, and acidosis, leading to admission to the ICU, where mechanical ventilation by tracheostomy and pneumonia by acinetobacter is allowed.

Alberti et al.[12] describing a patient with hemodynamic disorders with severe drug-resistant hypertension and arterial blood gases indicate severe hypoxia.

Assini et al.[13] described a patient who needs tracheostomy and assisted ventilation [P2].

Ottaviani et al.[14] described a patient who was treated for organ failure, in addition to deep venous thrombosis of the legs and overlapping bacterial infection (pneumonia *ab ingestis*).

Rana et al.[18] described a patient who developed persistent difficulty in urinating, or who ended up requiring a permanent catheter.

Su et al.[19] described a patient who had a sputum culture Stenotrophomonas maltophilia, an organism associated with pneumonia associated with mechanical ventilation.

However, Chan et al.[31] described an asymptomatic patient. In addition, other patients require ventilatory support[11-14,16,17,19,21,23,27,29,34], five need intubation[11,14,18,19,34], and eight were admitted to the ICU[11,13,14,17,19,21,23,29]. However, two[12,27] of the twenty-nine patients died during treatment from progressive respiratory failure.

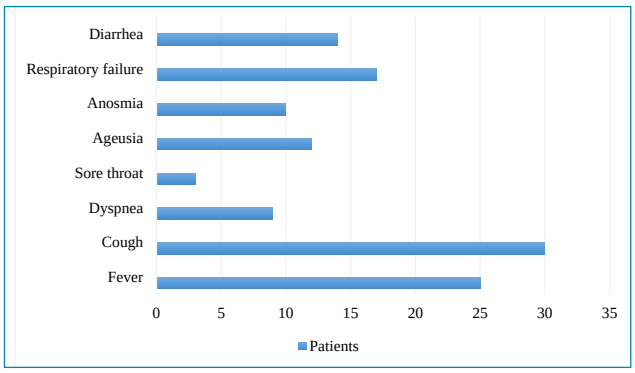

Figure 2. Prevalence of symptoms.

Diagnosis

The main methods for diagnosing SARS-Cov-2 infection (COVID-19) were nasopharyngeal swabs for polymerase chain reaction with real-time reverse transcriptase (RT-PCR), enzyme-linked immunosorbent assay (ELISA) technique, chest radiography, chest tomography (CT), and clinical examination[1,10-37].

Sixteen studies used CT and RT-PCR in the chest[1,10-14,16,17,21-25,27,36,37]; six studies used chest radiography and RT-PCR[17-19,26,28,29]; five studies used only RT-PCR[20,31-33,37]; two studies used the ELISA and CT technique[15,34]; and two studies used only the ELISA technique[30,35].

Electromyography and clinical methods were used for the diagnosis of GBS [1,10-37], with strong muscle evaluation using the Medical Research Council (MRC)[1,10,12,14-16,18,19,21,23,26,29,34,36].

Treatment

The main treatment methods mentioned were empirical antibiotics[1,10,16-18,21,22,24,26-29,31,36]; Hydroxychloroquine[1,12-14,16,18,20,22-26,28,29,36]; antivirals (lopinavir and ritonavir)[1,10,12-14,21,25,26]; room isolation[10,16]; and plasma exchange[17,34,36]. Thirty-six patients were treated with intravenous immunoglobulin (IVIg)[1,10-24,26,28-35,37].

Neurological outcome

The main neurological manifestations were: weakness in the lower extremities[1,10,12,14-18,22-24,29,30,33,35]; paresthesia of the upper and lower limbs[11,12,15,17-19,21,23,24,28,29,31,33-35,37]; tetraparesis[1,12,17,21,23,27-29,33]; facial diplegia[1,14,17,23,25,26,28-31,33,35]; areflexia[10,17,18,22,24,27,30,31,33]; asthenia[11,17,23]; mastoid pain and sensitive ataxia[17]; fatigue[10,14,32,34]; numbness[16,18,36,37]; swallowing disorders[21,26-28,33]; low back pain[12,27-29]; difficulty or loss in walking[14,23,26,30,35]; myalgia[15,20,23,30,32-34]; odynophagia[18,30,33]; hypoesthesia[22,23,27]; paraparesis[22,30,32]; dysarthria[31,36]; hyporeflexia[13,20]; bilateral eyelid ptosis[13]; progressive ophthalmoparesis[20]; desesthesia[14,34]; dysgeusia, cacosmia, disautonomy, arthralgia, and tetraplegia[33].

Patient outcomes

Main patient outcomes were:

- Only nine studies revealed comorbidities, type 2 diabetes mellitus[1,22,33]; hypertension, abdominal aortic aneurysm and lung cancer[12]; obesity[23]; dyslipidemia and active smoking[28]; rheumatoid arthritis[24]; hypertension, hyperlipidemia, restless legs syndrome, and back pain[18]; and coronary artery disease, hypertension, and alcohol[19];
- Patients with lymphopenia[10,13,17,28,33,36];
- Images showing multiple opacities in the ground glass[1,10-12,14-17,19-24,26,28,29,31,34,36] or inflammation in the lungs and a small amount of pleural effusion[1,17];

- Muscle strength testing showed failure in four limbs using a MRC scale[1,10,12,14-16,18,19,21,26,29,34,36];
- Hospitalizations in ICU[11,13,14,17,19,21,29] and patients with advanced support for mechanical ventilation of the airways[11-14,16,19,21,23,27,29,34];
- Patients undergoing physical therapy for rehabilitation[16-18,23];
- Lung auscultation revealed diffuse rales[22];
- Cases presenting variant forms of GBS, such as acute sensory-motor neuropathy, acute axonal neuropathy, and Miller-Fisher syndrome[1,13,25,30].

DISCUSSION

Until now, little is known about the neurological manifestations from COVID-19 and its direct relationship with GBS. The first case where neurological characteristics were observed standing out from the COVID-19 clinical symptoms was recently described; main symptoms included acute weakness in the legs and severe fatigue, with rapid progression[10]. For this reason, there are concerns that this virus is a possible trigger for GBS.

Sedaghat & Karimi[1], in one case report, described GBS for the first time in a patient infected with COVID-19. The patient reported acute progressive symmetric ascending quadriparesis. Two weeks before hospitalization, the patient suffered from cough, fever, and RT-PCR was reported positive for COVID-19 infection. The electrodiagnostic test showed that the patient had an Acute Motor-Sensory Axonal Neuropathy (AMSAN) variant of GBS.

In the study carried out by Toscano et al.[10], five patients with GBS after the onset of Covid-19 were examined. The first symptoms were lower-limb weakness and paresthesia in four patients and facial diplegia, followed by ataxia and paresthesia in one patient. In summary, flaccid tetraparesis or tetraplegia evolved from 36 hours to 4 days in four patients; three received mechanical ventilation. The interval between the onset of symptoms of Covid-19 and the first symptoms of GBS ranged from 5 to 10 days. This interval is similar that seen with GBS that occurs during or after other infections. As in previous studies, the authors point out that a possible relationship between these two diseases is the fact that COVID-19 through stimulation of inflammatory cells produces various inflammatory cytokines, and as a result, creates immune-mediated processes. As the GBS is an immune-mediated disorder, molecular mimicry as a mechanism of autoimmune disorder plays a vital role in its creation.

Zhao et al.[16] reported a woman who presented with acute weakness in both legs and severe fatigue, progressing within one day. Neurological examination disclosed symmetric weakness and areflexia in both legs and feet. Three days after admission, her symptoms progressed. Oropharyngeal swabs were positive for SARS-CoV-2 with RT-PCR assay. Considering the temporal association, it was speculated that the SARS-CoV-2 infection might have been responsible for the development of GBS.

Virani et al.[17], in their study, described a case where the patient with COVID-19 presented neurological symptoms, including numbness and weakness of the extremities; consequently, there was a decrease in tendon reflexes with rapid progression. The mechanism proposed for this association is an autoimmune reaction where antibodies to surface glycoproteins are developed in the offending pathogen that also corresponds to similar protein structures of peripheral nerve components (molecular mimicry), leading to neurologic involvement.

Camdessanche et al.[21], in their study, also reported on one patient without medical history who was admitted after he fell and hurt the left shoulder, leading to a tear of the rotator cuff. He had a fever and cough for two days. SARS-CoV-2 RT-PCR with nasopharyngeal swab was performed and proved to be positive. Eleven days after symptom onset, the patient complained of paresthesia in both feet and hands. In three days, he demonstrated severe flaccid tetraparesis. The patient complained of swallowing disturbance with a risk of suffocation as liquids took the wrong path. The patient was admitted to ICU and mechanically ventilated due to respiratory insufficiency.

Padroni et al.[11] described a case of GBS following a clinically resolved paucisymptomatic COVID-19. The patient complained of asthenia, hands, and feet paresthesia, and gait difficulties, progressing within one day. Symptoms of COVID-19 were resolved in a few days. Neurological examination disclosed moderate symmetric distal upper and lower limb weakness, loss of deep tendon reflexes, preserved light touch, and pinpricking sensation.

Assini et al.[13] described two cases of GBS and COVID-19. In one of them, the patient needed invasive ventilation in the ICU and had an acute onset of bilateral eyelid ptosis, dysphonia, and dysphalgia 20 days after admission. Furthermore, through neurological examination, he demonstrated a deficit in the protrusion of the tongue due to bilateral paralysis of the hypoglossal nerve and hyporeflexia of the upper and lower limbs, along with bilateral masseter weakness.

Putting together all of these findings, the causal association between GBS and COVID-19 remains speculative but very probable. Neurologists and other clinicians should be aware of the essential early recognition and treatment of the potential neuromuscular and autonomic worsening leading to cardio-respiratory failure in patients with GBS and mild or controlled pulmonary COVID-19. More in-depth research should be

carried out about this association, so that there is an established protocol of suitable diagnosis and treatment, in order to avoid high degrees of debilitation caused by GBS.

Limitations

The main limitation of this review was the lack of studies with a larger number of patients.

CONCLUSION

In conclusion, through well-designed primary studies, it is evident that COVID-19 can trigger GBS, as patients had clinical manifestations of COVID-19 infection and neurological manifestations characterizing GBS. Although the small number of patients limited our estimates, we believe that the results listed here are important for a better diagnosis and treatment of patients with neurological symptoms concomitant with respiratory symptoms

AUTHORS' CONTRIBUTIONS

KSM: Conceptualization, Data Curation, Formal Analysis, Supervision, Writing – Original Draft, Writing – Review & Editing. **LTAM:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft. **ACS:** Conceptualization, Formal Analysis, Writing – Original Draft. **WFS:** Data Curation, Formal Analysis. **AKG:** Supervision, Writing – Review & Editing. **APF:** Writing – Review & Editing.

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REVIEW ARTICLE
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Nuances between sedentary behavior and physical inactivity: cardiometabolic effects and cardiovascular risk

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SUMMARY

OBJECTIVE: The aim of this study was to highlight the differences between the cardiometabolic effects and the cardiovascular risk of physical inactivity and sedentary behavior.

METHODS: A narrative bibliographic review was conducted. In the research, national and international articles were selected from the PubMed, SciELO, and LILACS databases using the descriptors "sedentary lifestyle, cardiovascular risk, physical inactivity, sedentary behavior, and cardiovascular risks."

DISCUSSION: Both physical inactivity and sedentary behavior are related to metabolic and organic changes, promoting a chronic proinflammatory state, cardiac remodeling, increased body adiposity, and skeletal muscle dysfunction. It is possibly stated that both of them result in a higher risk of developing chronic diseases, resulting in higher global and cardiovascular morbidity and mortality, with nuances in their intrinsic effects.

CONCLUSIONS: It is inferred that both physical inactivity and sedentary behavior are cardiovascular risk factors that can be modified with the correct clinical approach. It is necessary to differentiate physically inactive individuals from those with a high number of sedentary behaviors. These concepts need better clinical applicability to improve the prevention of primary and secondary cardiovascular risks.

KEYWORDS: Sedentary behavior. Cardiovascular diseases. Metabolism. Risk factors. Lifestyle.

INTRODUCTION

One out of four adults currently does not meet the physical activity recommendations established by the World Health Organization (WHO). In Brazil, about 47% of the population shows insufficient levels of physical activity. This pandemic of physical inactivity imposes a high cost in terms of health assistance and loss of labor productivity[1,2].

Physical inactivity is defined as insufficient levels of practice of physical activity, as recommended by the WHO for each age range[3], and is considered the fourth largest cause of death on a global scale[4].

Physical inactivity is also a significant risk factor for cardiovascular diseases (CVD), which represent about 30% of deaths in Brazil in the last decades, ranking as the leading cause of mortality in both low- and high-income countries[5]. In this study, a new strategy was proposed to reduce health risks by combating physical inactivity and minimizing the time spent in sedentary behaviors (Table 1)[6,7].

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Contrary to our understanding, sedentary behavior is not a synonym for physical inactivity but rather defined as any behavior in which the corresponding energy expenditure is ≤1.5 metabolic equivalents (*metabolic equivalent of task* [MET]) in a sitting, reclining, or lying position while at rest (Figure 1A)[8]. The MET is a unit that corresponds to the energy required by an individual to keep at rest, representing an oxygen uptake of ~3.5 mL/kg/min. Through the MET unit, it is possible to classify the physical activity based on light, moderate, or vigorous intensity (Figure 1B)[9].

Sedentary behavior is also an important factor related to cardiovascular and metabolic morbidity and mortality. It is associated with a higher prevalence of overweight, obesity, and the risk of developing type 2 diabetes mellitus (DM), although it may have its deleterious effects mitigated or even eliminated in highly active individuals[10,11].

It is relevant to assess the metabolic effects and the cardiovascular risk associated with sedentary behavior and physical inactivity given the high prevalence of cardiometabolic diseases and several deleterious health effects related to these conditions, in particular, changes in cellular metabolism with the increase in peripheral insulin resistance, changes in lipid metabolism, body fat accumulation, musculoskeletal dysfunction, systemic pro-inflammatory state, and cardiac remodeling (Table 2).

Furthermore, it is essential to understand the difference between sedentary behavior and physical inactivity, which are often used as synonyms but with distinct intrinsic risk factors[12]. Therefore, an individual can be physically active while spending most of the time in sedentary behaviors, such as watching television or using the computer (Figure 2)[13]. Thus, this study aimed to evidence the differences between the cardiometabolic effects and the cardiovascular risk of physical inactivity and sedentary behavior.

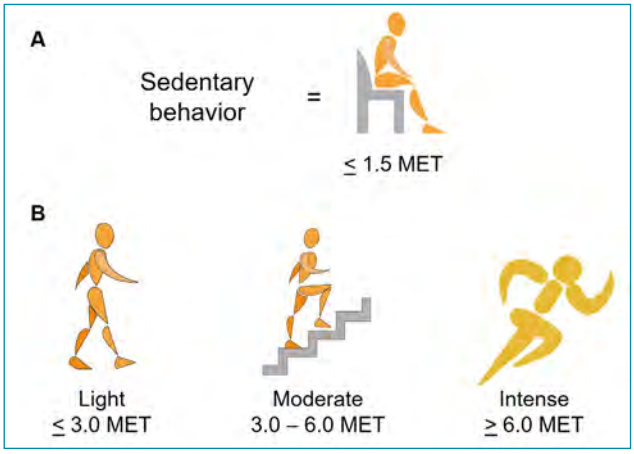

Figure 1. Scheme depicting the classification of sedentary behavior and the types of physical activity that result in different categories of metabolic equivalents (MET).

Table 1. Physical activity recommendations and sedentary behavior of different age groups, according to the World Health Organization[7].

Group	Sedentary behavior	Physical activity
Children and adolescents (5–17 years)	Children and adolescents should limit the amount of time spent in sedentary behaviors, particularly the amount of recreational screen time.	Minimum of 60 min/day of moderate-to-vigorous intensity physical activity, preferably aerobic.
Adults (18–64 years)	Limit the amount of time spent in sedentary behaviors. Adults who spend a significant time in sedentary behaviors should aim to achieve or exceed the upper levels of recommended moderate-to-vigorous intensity physical activity.	At least 150–300 min/week of moderate-intensity aerobic physical activity, or at least from 75 to 150 min of vigorous-intensity aerobic physical activity, or an equivalent combination of both types. Adults should do muscle-strengthening activities of moderate or greater intensity which involve all major muscle groups on ³2 days/week.
Elderly adults (≥65 years)	Limit the amount of time spent in sedentary behaviors. Elderly adults who spend a significant time in sedentary behaviors should aim to achieve or exceed the upper levels of recommended moderate-to-vigorous intensity physical activity.	Elderly adults should do at least 150–300 min of moderate-intensity aerobic physical activity throughout the week, or do at least from 75 to 150 min of vigorous-intensity aerobic physical activity, or the equivalent combination of both types. Elderly adults should also do muscle-strengthening activities of moderate or greater intensity which involve all major muscle groups on ³2 days/week. Elderly adults should also do multicomponent physical activity, with emphasis on functional balance and strength training, with moderate or greater intensity on ³3 days/week.

Table 2. Data of the main studies included in this review.

Study	Objectives	Methods	Main conclusions
Guthold et al., 2018 Switzerland[2]	To describe the levels of insufficient physical activity across countries, and to estimate regional and global trends.	Data from a population-based study reporting the prevalence of insufficient physical activity.	The prevalence of physical inactivity in 2016 was two times higher in high-income countries and increased with time (from 2001 to 2016).
Leão et al., 2020 Brazil[3]	To describe the sedentary behavior in elderly people residing in the rural area.	Data from eight aspects were used to evaluate the sedentary behavior of elderly people residing in the rural area.	The mean sedentary behavior was lower compared with the literature.
Rissardi et al., 2018 Brazil[5]	To evaluate the prevalence of physical activity in the adult population and its effects on blood pressure, glycemia, and lipid profile.	Cross-sectional, population-based study with stratified simple random sampling performed with 1,717 adults of different age ranges.	High prevalence of physical inactivity and correlation with cardiovascular risk factors, especially arterial pressure and glycemic and lipid profiles.
Costa et al., 2017 Brazil[14]	To evaluate the practice of physical activity, sedentary behavior, and the association with cardiovascular risk.	Cross-sectional study with 576 adolescents using socioeconomic, demographic, lifestyle, and clinical variables.	Abdominal adiposity and male sex represent important cardiovascular risk factors in adolescents.
Alvarez et al., 2019 Chile[15]	To investigate whether arterial pressure and other cardiometabolic risk factors differ across physical activity levels in school students of different ethnicities.	Cross-sectional study with 540 school students from 6 to 13 years of age divided into two groups, according to their ethnicity and physical activity level.	It was observed that, compared with physically active individuals, the physically inactive individuals showed higher levels of arterial pressure, abdominal circumference, and body mass index.
Díaz-Martínez et al., 2018 Chile[16]	To investigate the association of physical activity with obesity, metabolic markers, diabetes mellitus, hypertension, and metabolic syndrome in Chilean adults.	Through the 2009/2010 National Health Survey, 5,157 participants were evaluated regarding their body mass index, waist circumference, metabolic biomarkers, and physical activity levels.	Failure to comply with physical activity recommendations is associated with obesity, diabetes, hypertension, and metabolic syndrome, which are important cardiovascular risk factors.
Phillips et al., 2017 Ireland[17]	To determine the relationship between the intensity and duration of physical activity with a range of inflammatory markers.	Intensity and duration of physical activity were measured in 396 participants for 7 consecutive days.	Replacing sedentary behavior with moderate to vigorous-intensity physical activity is associated with the improvement of the inflammatory profile.
Giurgiu et al., 2019 Germany[18]	To investigate the dynamic relationships between sedentary behavior and mood dimensions in everyday life.	Ambulatory assessment study on the sedentary behavior and everyday life mood level of 92 college students for 5 days.	Sedentary participants showed worse satisfaction and energy levels. Sedentary behavior was considered a general health risk factor for affecting somatic and mental health.

Continue...

Table 2. Continuation.

Study	Objectives	Methods	Main conclusions
Park et al., 2018 South Korea[19]	To evaluate the association between sedentary time and cardiovascular risk factors among Korean adults.	Cross-sectional study analyzing the sedentary time and cardiovascular risk factors among 3,301 adults.	Prolonged sedentary time was significantly associated with increased diastolic arterial pressure and low HDL cholesterol levels. The associations were independent of general or abdominal obesity and moderate to vigorous-intensity physical activities.
Crichton and Alkerwi, 2015 Luxembourg[20]	To assess the relationship of physical activity intensity with HDL and LDL levels, total cholesterol, and triglycerides.	Participation of 1,331 adults subjected to cardiovascular health assessment and analysis of the level of physical activity.	Spending less time with sedentary behaviors and engaging in medium levels of intense physical activity may be associated with a more favorable blood lipid profile, particularly with regard to HDL and triglyceride levels.
Qi et al., 2015 United States[21]	To evaluate the associations between sedentary time and cardiometabolic biomarkers.	Analysis of the associations between sedentary time and a range of cardiometabolic biomarkers in 12,083 adults.	Prolonged sedentary time was associated with decreased HDL levels and increased triglycerides, glycemia, fasting insulin, and insulin resistance. Even in physically active individuals, sedentary time was negatively associated with several cardiometabolic biomarkers.
Matta et al., 2016 Lebanon[22]	To evaluate whether physical inactivity is an independent predictor of diastolic dysfunction.	Evaluation of the level of physical activity and the presence of diastolic dysfunction in 1,356 outpatients.	Physically inactive patients with increased left ventricular mass index had 2- to 3-fold increased odds of having diastolic dysfunction.
Andersen et al., 2015 Sweden[23]	To investigate the relationships between skeletal muscle morphology and the risk of cardiovascular events.	Population-based cohort study with 466 men with 71 years of age and no cardiovascular diseases.	Higher skeletal muscle proportion of type-I fibers was associated with lower risk of cardiovascular events and a higher proportion of type-IIx fibers was associated with higher risk of cardiovascular events in physically active men.
Cavedon et al., 2020 Italy[24]	To investigate the role of physical inactivity on the bone mineral density and body composition of oldest–old women.	The bone mineral density and the mass of fat-free soft tissue were measured in 11 oldest–old wheelchair-bound women, 11 oldest–old mobile women, and 11 young healthy women all matched for weight and height.	Alterations in bone and body composition parameters are exacerbated in the physically inactive oldest–old women. These negative effects of physical inactivity are not limited to locomotor limbs, and a systemic decline of bone and muscle parameters is likely associated with physical inactivity.

HDL: high-density lipoprotein; LDL: low-density lipoprotein.

METABOLIC AND ORGANIC EFFECTS

Both physical inactivity and the time of sedentary activities are among the leading modifiable cardiovascular risk factors and have contributed to the burdening and development of chronic non-communicable diseases (CNCD), with small nuances in their intrinsic effects[13,25].

Several deleterious health impacts related to physical inactivity have been described by associating it with the marked increase of cardiovascular morbidity and mortality and the increase of risk factors, such as dyslipidemia, insulin resistance, obesity, and systemic arterial hypertension (SAH)[14,26].

A Chilean study performed by Alvarez et al. (2019) with children and adolescents evidenced that physical inactivity was associated with the increase of cardiometabolic risk factors. Physically inactive individuals showed increased arterial pressure and body mass index compared with physically active individuals[15]. In contrast, in another Chilean study performed with adults, physical inactivity was a significant cardiovascular risk factor related to obesity, DM, SAH, and metabolic syndrome[16].

Physical inactivity is considered one of the leading causes involved in the development of chronic diseases and is associated with higher cardiovascular risk, decreased life expectancy, increased mortality, and acceleration of biological aging[27].

Prolonged sedentary behavior was also associated with cardiovascular risk factors, such as insulin resistance, dyslipidemia, increased arterial pressure and body mass index, and decreased cardiorespiratory fitness. This type of behavior may damage vascular function due to blood flow reduction, increased production of reactive oxygen species, and the presence of a proinflammatory state that generates endothelial dysfunction and increases cardiovascular risk[25].

Recent studies with children and adolescents have highlighted the association between prolonged sedentary activities and increased cardiovascular risk. Sedentary behavior was responsible for compromising the metabolic profile in this population as it may result in the development of CVD[28]. Higher obesity rates at adult age result from sedentary behavior during childhood and adolescence since these behaviors persist until adulthood[29]. Furthermore, this population is connected to the digital world increasingly earlier and for a prolonged time, offering risks to mental health. The dependency generated by these means can unleash anxiety, depression, and behavioral changes[30].

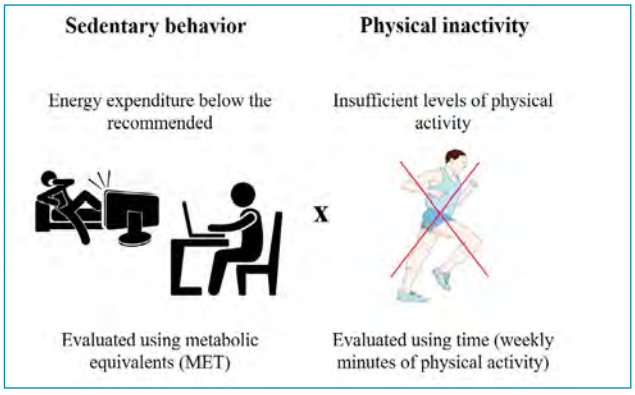

Figure 2. Representative model of the differences in definition between sedentary behavior and physical inactivity.

Sedentary individuals who spent excessive sitting time along with lower levels of physical activity showed a worse metabolic profile for cardiovascular risk and a range of inflammatory biomarkers[17]. Among the health effects caused by the accumulation of sedentary behaviors is the increase of inflammatory mediators, weight gain, damaged lipid metabolism, insulin resistance, decreased muscle mass, nitric oxide, and sleep quality[31]. Prolonged sedentary behavior may also be associated with a high risk of insomnia and other sleep disorders[32].

Sedentary behavior affects both somatic and mental health, thus being considered a general health risk factor. Negative results regarding mood were proportional to the sedentary behavior levels adopted in everyday life[18].

It is noted that physical inactivity is associated with CVD risk factors, such as dyslipidemia, SAH, obesity, insulin resistance, and DM. Sedentary behavior is also related to the development of CVD, compromised vascular function, proinflammatory state, decreased muscle mass, and sleep disorders. Therefore, it is inferred that both physical inactivity and sedentary behavior play a key role in increasing the cardiovascular risk.

BODY ADIPOSITY

Physical inactivity resulted in abdominal and visceral fat gain and a higher risk of type 2 DM regardless of age, sex, ethnicity, or body mass index. The two major risk factors associated with type 2 DM were obesity and physical inactivity. The prevalence of DM was higher in obese, overweight, and physically inactive individuals. Furthermore, physical inactivity was related to an increased risk for each of these diseases[10].

A clinical study with nonobese adults verified that only one day of physical inactivity, long sitting hours, and minimum walking time decreased insulin sensitivity even under reduced caloric intake[33].

Appetite control occurs through a complex interaction between human physiology and behavior. Limited physical activity levels seem to interact with body fat, deregulating the appetite and acting as an excessive intake source. Hormonal responses to changes in energy consumption and structured exercises have been verified, although few studies have investigated their response to the increase of the time spent in sedentary behaviors[33].

Sedentary behavior resulted in abdominal and visceral fat gain[19]. One hour per day of sedentary behavior increased the risk of overweight and resulted in a higher risk of developing abdominal fat. The increase in visceral and intermuscular fat possibly stimulates the release of proinflammatory cytokines and decrease of anti-inflammatory markers from adipose tissue, with a catabolic effect on muscle tissue[6].

This process occurs due to immobilization, which is considered a stressor mechanism, resulting in decreased glucose use by the muscles, increased insulin resistance, and less energy use by inactive muscles. This energy, which is redirected to the liver, increases the production of lipids, which are preferably stored in the adipose tissue in the central region of the abdomen. These adipocytes become metabolically active when filled with fat, thus producing inflammatory molecules while simultaneously reducing the secretion of the anti-inflammatory adiponectin[34].

Sedentary behavior was also associated with the reduction of high-density lipoprotein (HDL) cholesterol[20,21]. Among adults aged from 30 to 50 years, sedentary time was associated with reduced HDL cholesterol levels but not with other lipid profile markers or arterial pressure. Sedentary behavior suppressed the activities of the lipoprotein lipase enzyme, and this suppression was associated with decreased triglyceride uptake in the plasma and reduced plasma HDL levels[19]. However, the long-term interventions are necessary to change the lipid levels[25].

The increase in caloric intake, usually associated with sedentary behavior, was another factor associated with health damages due to fat accumulation in the liver and adipocytes. This fat accumulation results in difficulties in performing aerobic activities, reduced maximum oxygen consumption, and increased death risk by other causes. Furthermore, decreased cardiorespiratory fitness may result from the increased time exposed to sedentary behaviors[34].

Therefore, it may be inferred that both sedentary behavior and physical inactivity are harmful to lipid metabolism; in the long term, their consequences may lead to the marked accumulation of visceral and central abdominal fat, constituting a risk factor for several CNCD.

PROINFLAMMATORY STATE

The relevance of interventions to combat physical inactivity relies on the fact that this is a modifiable risk factor, besides being one of the main factors responsible for obesity[35].

Obesity is related to chronic inflammation and insulin resistance, which may be the link between obesity, DM, and CVD. Adipose tissue excess was related to the increased production of proinflammatory and atherogenic cytokines, such as IL-6, TNFα, and MCP-1, and the reduction of anti-inflammatory adipokines, such as adiponectin[36].

Physical inactivity and adipose tissue excess are inflammation triggers that may be associated with circulating humoral factors with deleterious effects on the heart and multiple other organs. Low-grade chronic inflammation can contribute to the pathogenesis of diseases directly related to cardiovascular risk[36,37].

The C-reactive protein, an inflammatory marker widely used to predict cardiovascular risk, shows an inverse relationship with physical activity levels. However, it is unknown if the reduction in C-reactive protein levels is the direct effect of physical activity or the consequence of unintentional weight loss due to exercise[17].

Sedentary behaviors such as watching television or using the computer are strongly related to the risk of developing dyslipidemia, obesity, type-2 DM, SAH, metabolic syndrome, and CVD. The effects of long periods of sedentary behavior on physically active individuals seem to be characterized by metabolic changes commonly observed in diabetogenic and atherosclerotic profiles. Harmful changes in the serum levels of insulin and glucose and in the systolic and diastolic pressures have been experimentally demonstrated after long and uninterrupted sitting periods[25]. Although the correlation between the time spent in sedentary behaviors and the increase of cardiovascular and metabolic risk is well documented, the precise mechanisms by which this occurs have not been elucidated[38].

CARDIAC REMODELING

Although the relationship between physical inactivity and the cardiovascular system is well discussed, its cardiometabolic effects on cardiovascular health are complex and not fully elucidated. Strong CVD predictors, such as the stiffening of large arteries and vascular endothelial dysfunction, have been documented among physically inactive men and women. However, most of the data on the vascular consequences of this inactivity are based on extreme models, such as bed rest or the immobilization of a limb[39].

Imbalances between the production and destruction of reactive oxygen species by antioxidant systems associated with physical inactivity promote the decoupling of endothelial nitric oxide synthase, resulting in reduced bioavailability of nitric oxide and increased superoxide production. The prolonged disturbance of endothelial function associated with the reduction in vascular compliance by physical inactivity is particularly harmful to cardiovascular health. High loads in the left ventricle may lead to the stiffening of the muscle, ventricular remodeling, and increased risk of cardiac insufficiency[13].

Physically inactive patients with normal systolic function of the left ventricle (LV) often present reduced functional capacity, similar to those with diastolic dysfunction. Physical inactivity was associated with 70% increased odds of developing diastolic dysfunction of the left ventricle, compromising its relaxation capacity[22]. Patients who reached a higher increase in physical conditioning and reduction in abdominal fat showed a trend toward improvement in the diastolic function of the LV[40]. However, the relationship between physical activity and diastolic function keeps controversial, with studies that show a limited effect of physical activity on cardiac remodeling related to age, diastolic function, and training performance[41].

Aging is known to be the most powerful predictor of diastolic dysfunction of the LV, with a threefold increase in odds for every 10 years of aging. Remarkably, in addition to elderly patients, the predicted probability of developing diastolic dysfunction was higher among physically inactive patients of all age ranges in a population study. Thus, it may be inferred that since this lifestyle is modifiable, diastolic dysfunction may also be reversible (Figure 3)[22].

In the study by Park et al. (2018), sedentary time was associated with increased diastolic arterial pressure but not with increased systolic pressure. The biological mechanisms to explain this association remain unclear, although there is a possibility that these behaviors may affect arterial pressure in different ways. Systolic arterial pressure can be less affected by changes in peripheral vascular resistance than diastolic arterial pressure[19].

The harmful effect of physical inactivity on cardiac remodeling is notorious compared with sedentary behavior, with irreversible consequences for the cardiomyocytes, such as cardiac insufficiency, especially diastolic insufficiency. The effects of sedentary behavior on cardiac remodeling are still not well established.

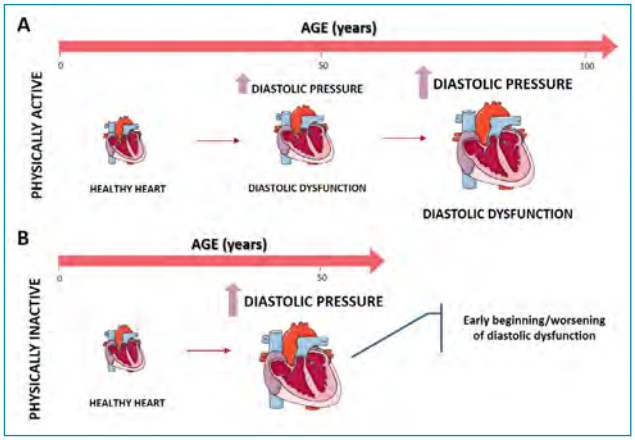

Figure 3. Comparison of the effects of physical inactivity throughout time on cardiac remodeling in physically active individuals (A) and in physically inactive individuals (B).

MUSCULOSKELETAL DYSFUNCTION

The value of skeletal muscle mass is relevant to identify individuals with higher cardiometabolic risk. It was evidenced that people with low skeletal muscle mass had a higher risk of developing CVD than people with normal muscle mass[42]. Physical inactivity may increase myostatin, inhibiting skeletal muscle myogenesis. However, the levels of this cytokine decrease with exercise, inducing a beneficial adaptative response through the growth of muscle fibers[43,44].

The study conducted by Andersen et al. (2015) with elderly men analyzed the type of skeletal muscle fiber that may influence in increasing the risk of CVD. Each type of fiber has different metabolic and anti-inflammatory properties. It was observed that there is an association between a higher proportion of type-IIx fibers (fast-twitch and glycolytic action) and a higher risk of cardiovascular events. Type-I fibers (slow-twitch and oxidative action) were related to a lower risk. However, these associations were mostly seen in physically active participants, and the mechanisms involved remain unknown[23].

The study performed by Cavedon et al. (2020) with elderly women evaluated the repercussions of physical inactivity on the bone mineral density and body composition of these participants. Besides aging, physical inactivity was a significant factor that implies musculoskeletal damage[24].

The decrease in physical inactivity through intense periodic training promotes the maintenance of the muscle cell environment through the synthesis of cytoprotective markers and the increased degradation of damaged proteins. This creation of a resistant cell environment to the stress induced by exercise becomes beneficial to post-training adaptation, and cardiovascular health seems to benefit from these physiological adaptations[45].

Skeletal muscle inactivity may lead to mitochondrial dysfunction due to signaling changes and the increased release of reactive oxygen species, changes that may result in muscle atrophy[46]. Prolonged sedentary behavior may cause early muscle fatigue and decreased muscle and bone mass, besides promoting the decrease of nitric oxide, which may result in poor vascularization and oxygenation for the musculoskeletal system[31].

It is verified that physical inactivity has negative effects on bone mineral density and body composition. Sedentary behavior is responsible for harmful changes in the musculoskeletal system. Furthermore, skeletal muscle dysfunction implies increased cardiovascular risk when related to low muscle and bone mass, early muscle fatigue, and poor vascularization and oxygenation.

CONCLUSIONS

It is inferred that physical inactivity and sedentary behavior are cardiovascular risk factors that can be modified with a certain degree of practicality, faced with the proper clinical approach.

It is necessary not only to characterize the individual as sedentary but also to differentiate physically inactive individuals from those with a high number of sedentary behaviors, even if presenting regular practice of physical activity. These are well-defined concepts in the current literature that require better clinical applicability to improve the prevention of primary and secondary cardiovascular risks.

AUTHORS' CONTRIBUTION

EASM: Conceptualization, data curation, methodology, visualization, and writing (original draft). **LESF:** Conceptualization, data curation, methodology, visualization, and writing (original draft). **RJFC:** Conceptualization, data curation, methodology, visualization, and writing (original draft). **CALBF:** Supervision, validation, visualization, and writing (review and editing). **MRL:** Formal analysis, project administration, supervision, validation, visualization, and writing (review and editing). **RHAB:** Conceptualization, investigation, project administration, supervision, validation, visualization, and writing (review and editing).

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