Does Nifuroxazide Enhance the Effect of Metronidazole Oral Administration in the Treatment of *Clostridium difficile* Infection?

ABSTRACT

Aims: To evaluate the efficacy of metronidazole monotherapy and modified therapy with metronidazole + nifuroxazidefor the for treatment of a mild form of *Clostridium difficile* infection (CDI).
Study design:A prospective, randomized, controlled clinical trial.
Place and Duration of Study: University of Applied Sciences Tuzla in the period from June 2018 to June 2019.
Methodology: Sixty patients were included in the study, divided into two groups. One group received standard therapy (metronidazole) for the treatment of a mild form of CDI, while the other group was treated with modified therapy (metronidazole + nifuroxazide). Subjects with a developed clinical picture and a positive toxin test for *Clostridium difficile* were surveyed on the day of admission, then on the 4^{th}, 10^{th}, 14^{th}, and 30^{th} days from the start of therapy. The goal of the research was to determine the impact of the modified therapy protocol on the number of stools and the presence of pain compared to standard therapy.
Results: The modified therapy with metronidazole + nifuroxazide showed better pharmacological efficacy in the treatment of CDI compared to the standard therapy with metronidazole alone. The group of subjects who were treated with modified therapy reported a significantly lower number of stools (*P*=.001) and the absence of pain at the first and second check-ups.
Conclusion: Nifuroxazide and metronidazole represent a combination of drugs that reduce the number of stools in the shortest possible time and result in the absence of abdominal pain in patients diagnosed with a mild form of CDI.

Keywords: Clostridium difficile infection, nifuroxazide, modified therapeutic protocol

1. INTRODUCTION

Infections caused by the bacterium *Clostridium difficile* (CDI) occur due to changes in the flora of the digestive tract, usually after therapy with antibiotics and other drugs. Most of the available antibiotics can trigger CDI, but the highest probability for the development of the disease is after the use of third-generation cephalosporins, penicillin antibiotics, namely ampicillin and amoxicillin, and clindamycin. The cause of CDI can be the use of other drugs, such as proton pump inhibitors, but also other risk factors, for example, hospitalization of the patient and older age [1].

The infection is manifested by the appearance of watery stools with a mixture of blood and mucus. In addition to symptoms from the digestive tract, patients experience: an elevated body temperature of 38—38.5°C, leukocytosis, abdominal pain, malaise, hypoalbuminemia, intestinal bleeding and dehydration [2,3]. A significant symptom of infection is diarrhea that begins 5—10 days after the start of antimicrobial therapy.

Not long after the identification of *Clostridium difficile*, therapy with a positive pharmacological effect was introduced. Antibiotics, metronidazole, and vancomycin, appeared as a very effective therapy. Metronidazole is more often used in the treatment of mild to moderately severe forms of infection, in a dose of 500 mg three times a day for 10—14 days.

As a new patent of the researcher Maurice Claude Ernest Carron, the drug nifuroxazide was mentioned for the first time in 1966, when the first generic drug was made in France. Since then, it has been used in the treatment of many bacterial infections. Nifuroxazide is a derivative of 5-nitrofuran, which belongs to the group of antidiarrheal and intestinal anti-inflammatory and anti-infective drugs. The mechanism of action of nifuroxazide has not been fully elucidated, but it is assumed that the free nitro (-NO_2) group in its structure is responsible for its antimicrobial effect. The nitro group has a pronounced electro-attractive power, which is an important prerequisite for antibacterial activity [4]. After penetrating the bacterial cell, it interacts with bacterial enzymes, impairs the activity of specific dehydrogenases, and by inhibiting protein synthesis in the bacterial cell, inhibits their growth. What makes it unique among nitrofurane derivatives is its local effect on pathogens in the intestines, where it appears as a practically insoluble substance at the target site, and absorption into the systemic circulation is excluded. Numerous studies have proven that more than 99% of the amount of nifuroxazide remains in the intestine five hours after administration, and a maximum of 0.005% of the drug is absorbed intestinally. Given that it is absorbed in an insignificant amount; it is impossible to detect it in human urine after oral administration [5]. Thanks to this, systemic side effects rarely occur during its use [6].

Research shows that the use of nifuroxazide does not change the fecal flora of a healthy person even with a dosage of 1200 mg per day. There are no recorded conditions in which the use of nifuroxazide is not recommended because it does not interact with other drugs [7].
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This research aimed to examine the effectiveness of a modified therapeutic protocol which includes a combination of the drugs nifuroxazide (4 x 200 mg) + metronidazole (3 x 500 mg) compared to metronidazole monotherapy in the treatment of a mild form of CDI. The research confirms the positive pharmacological effect of nifuroxazide on the number of daily bowel movements of the patient and the presence or absence of pain. The vision of the research was to prove that nifuroxazide affects the reduction of the number of stools in the shortest possible period, as well as that it affects the cessation of pain in the patient, in a shorter time than under the influence of standard therapy, which includes only metronidazole. Also, the goal of the research was to evaluate the adverse events due to the application of the mentioned modified therapy, during the treatment of a mild form of CDI.

2. METHODOLOGY

STUDY AREA

The research was conducted at the Department of Gastroenterology and Hepatology of the Clinic for Internal Diseases of the University Clinical Center Tuzla, as well as at the Infectious Diseases Clinic of the University Clinical Center Tuzla in the period from June 2018 to June 2019.

STUDY DESIGN

Sixty patients with CDI were included in the study, divided into two groups of 30 participants each. One group was treated with metronidazole and the other group with a combination of metronidazole + nifuroxazide.

INCLUSION AND EXCLUSION CRITERIA

The patients included in the study first gave their consent for participation in writing, after being informed about the details, type, possible side effects, duration, risks, complaints, procedures, and confidentiality of research data. The inclusion criteria were age older than 18 years and a positive CDI toxin test result. Exclusion criteria were: age less than 18 years and comorbidities: heart failure classified according to NYHA, chronic obstructive disease of severe form, renal insufficiency grades III and IV, pregnancy and a stroke within a month before the start of therapy.

2.1 Data collection

Patients with a confirmed toxin test for CDI were monitored for 30 days. On the day of admission, they filled out a questionnaire and were randomized into two equal groups, with 30 subjects in each. One group was treated with standard therapy- metronidazole 500 mg 3 times a day (group M), and the other group was treated with modified therapy, namely the combination of the same metronidazole dose + nifuroxazide 200 mg 4 times a day for 10—14 days (group N). All subjects were treated with a diet regimen. The number of stools per day and stomach pain was recorded in the patient's questionnaire during the first control in the morning of the fourth day from the start of therapy, and on other controls 10^{th}, 14^{th}, and 30^{th} day from the start of therapy. To acess abdominal pain we used Pain Scale Chart 1 to 10 levels. The patients did not have access to the questionnaires, which were filled out and administered by the responsible medical staff. During the research, potential adverse drug reactions were carefully monitored.

2.2 Statistical analysis

The testing methods used were the chi-square test, Krippendorf alpha (K-alpha), and the Kruskal-Wallis agreement test. All tests were performed at the 5% significance level. The chi-square test was used for data of nominal type, age, gender structure, and the number of stools. To analyze the differences between ratings in individual phases of patient follow-up, the Kruskall-Wallis test was used as a non-parametric alternative to the analysis of variance for the level of the entire sample, as well as the Mann - Whitney U test (Wilcoxon T-test), for testing differences between two groups of data.

3. RESULTS

The average age of all respondents was 55.7 ± 12.44 years (mean ± standard deviation), 58.3% (n=35) were women and 41.7% (n=25) were men. In the corresponding groups, the mean age of the M group was 51.63 ± 9.01 years with 63.3% (n=19) females and 36.7% (n=11) males and there was no statistical difference in the age between the groups. In the N group, there were 53.3% women (n=16) and 46.7% men (n=14) with a mean age of 59.76 ± 14.11 years. When processing the data, we divided the patients into seven age groups (Figure 1).

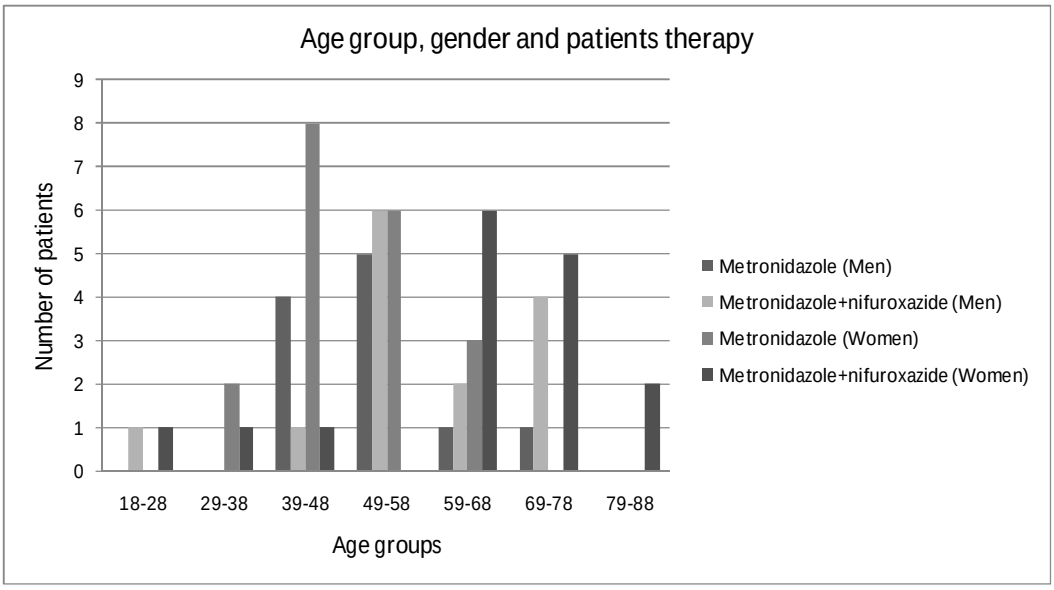

Figure 1. Age structure, gender and therapy by the groups of patients included in the study

Out of 60 patients, 11 patients (18%) had already established therapy for hypertension, other cardiovascular or respiratory diseases (Figure 2).

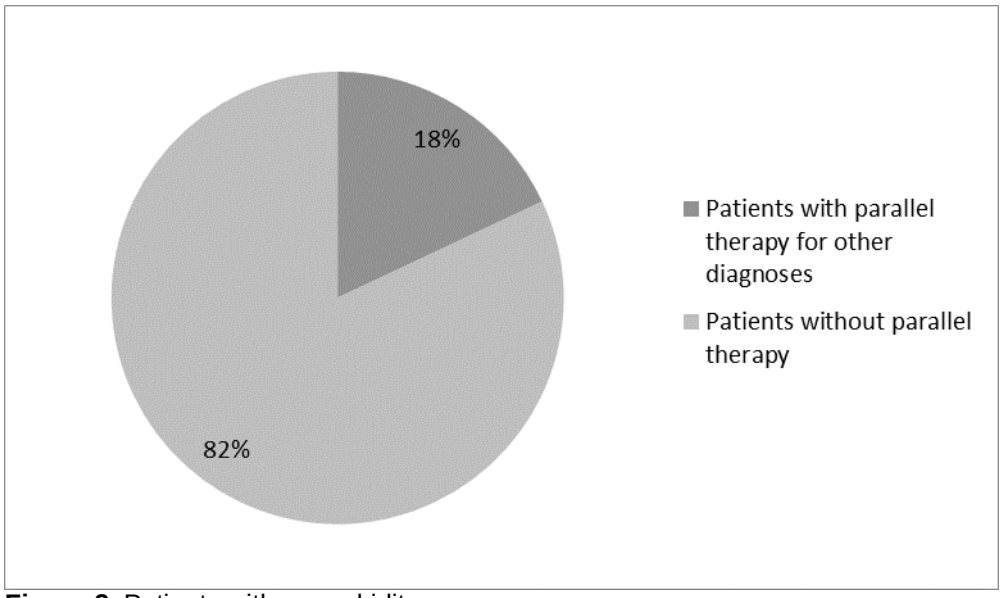

Figure 2. Patients with comorbidity

3.1 Number of stools

There was a significant difference in the effects of combined therapy compared to metronidazole monotherapy. The difference was detected in terms of the arrangement and number of stools. In combination therapy, the number of stools decreased during therapy, with a dynamic that has a stable downward trend, which is not the case with the use of

monotherapy. The first indicator of the difference is the average number of stools according to the controls, given in Table 1.

Table 1. Mean number of stools, a comparative review of metronidazole versus metronidazole + nifuroxazide groups

Group (n=60)	Baseline	4th	10th	14th	30th
Metronidazole (n=30) (SD)	7.83 (0.75)	7.67 (0.61)	7.17 (0.83)	5.03 (1.52)	2.70 (1.06)
Metronidazole +nifuroxazide (n=30) (SD)	5.27 (2.21)	3.50 (1.78)	2.53 (1.25)	1.73 (1.14)	1.50 (0.57)

Table 1 shows a significantly lower number of stools in the combined therapy. The nifuroxazide group (group N) reported fewer stools after days 4 and 14 than the metronidazole-only group (*P*=.001). The reduction in the number of stools per day after 30 days showed no statistical difference.

Another indicator is the comparative diagram of the incidence of the number of stools. The chi-square test (*P*<.001) shows a statistically significant difference between the two treatments. This difference between the two groups of patients is evident from Figure 3. mean values for the group that used combined therapy. The nifuroxazide group (group N) reported fewer stools after days 4 and 14 than the metronidazole-only group (*P*=.001). The reduction in the number of stools per day after 30 days showed no statistical difference.

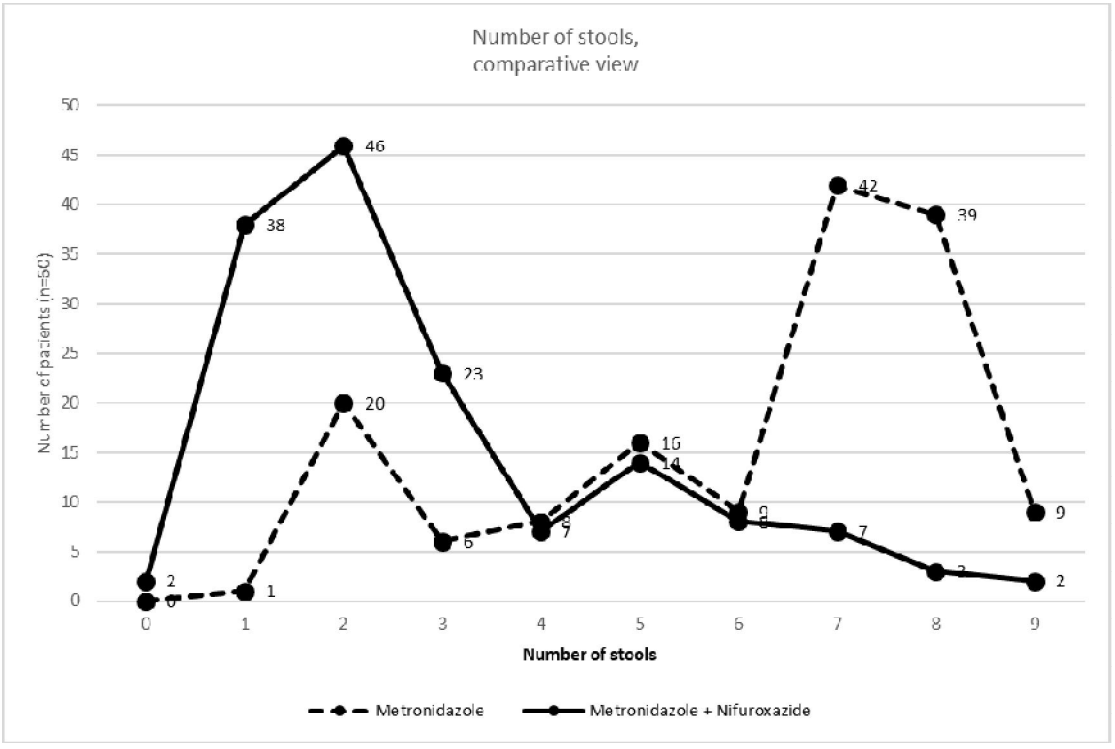

Figure 3. Diagram of the comparative review of the incidence of the number of stools

3.2 Pain

The analysis shows that there was a significant difference in the effects of the combined therapy, metronidazole + nifuroxazide, compared to the monotherapy with metronidazole. The difference was detected in terms of the distribution of the incidence of pain scores in controls. At the same time, the effect of the combination of drugs is such that the pain disappears soon after the application of the combined therapy, which is not the case if only metronidazole is used. The following table shows a comparative overview of the incidence of scores 1 (presence of pain) and 2 (absence of pain) by group.

Table 2. Comparative overview of the incidence of scores 1 (presence of pain) and 2 (absence of pain) by group.

Group (n=60)	Score 1	Score 2
Metronidazole	110	40
Metronidazole +nifuroxazide	48	102

The results show that themetronidazole + nifuroxazide group recorded lower pain levels than the metronidazole group (Figures 4 and 5).

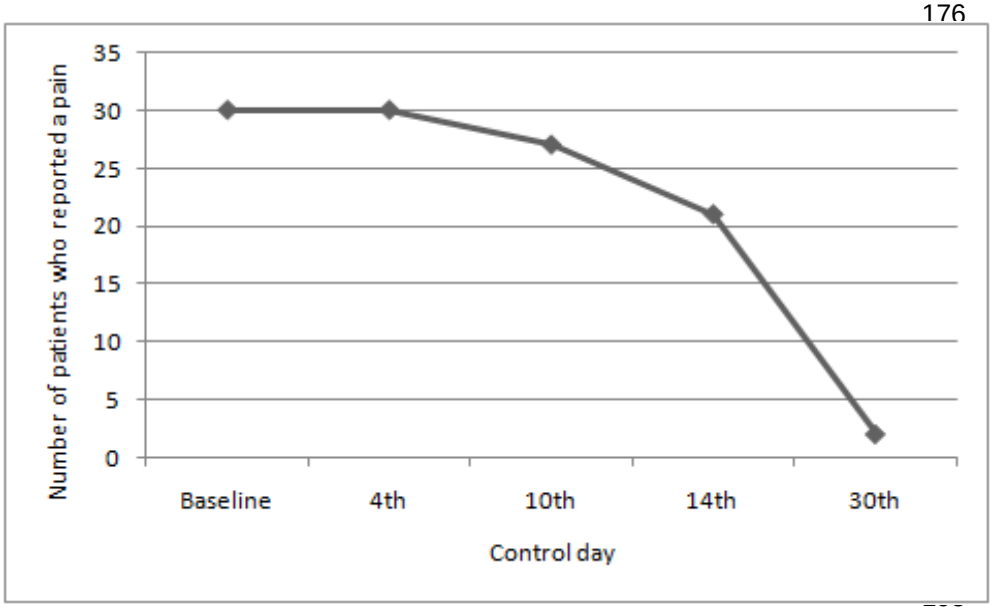

Figure 4. Diagram of pain, incidence of patients with pain by stages of the trial, treated with metronidazole monotherapy

The figure 4 shows a large sample of patients (n=21) in the group treated with monotherapy, who had pain until the 14th day from enrollment, while the group treated with combined therapy has a downward trend of the pain presence, already after the first control (Figure 5).

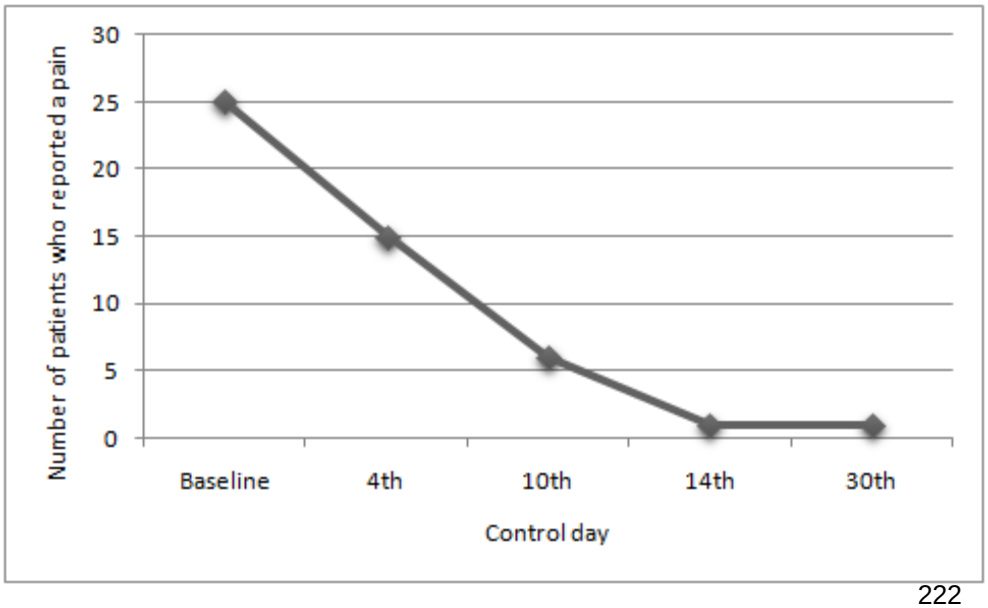

Figure 5. Diagram of pain, incidence of patients with pain by stages of the study, treated with metronidazole + nifuroxazide combined therapy

4. DISCUSSION

From the total sample, the largest number of respondents (n=17) was in the age group of 49 to 58 years, followed by the age group of 39 to 48 years (n=14). The analysis of the age structure by group gave the information that the largest number of respondents (n=23) of group M belong to the age range from 39 to 58 years, while the same number of respondents of group N belong to the age category between 49 and 78 years. According to the results of the Daniel and Rapose research from 2015 conducted in North America, the average age of the participants was about 60 years old in the total examined sample with 87% suffering from a mild form of CDI [8]. Data published in BMC Infect Dis. from 2016, show an average age greater than 62 years in hospitalized patients with mild CDI, during 2016 [9].

In the analyzed group of participants, there were 41.7% male patients and 58.3% female patients. The chi-square test showed that there was no statistically significant difference in the distribution of patients based on gender and age groups at the significance threshold of *P*=.087. Data from the Australian group of authors, conducted in the state of Victoria, show a greater representation of female respondents with 61% of the total number of respondents. Analyzing the results of this research, we can state that the characteristics of the examined group according to age and gender correspond to the characteristics of the respondents in other international studies [10].

Out of the 60 examined patients, 11 (18%) simultaneously received another, permanent therapy for one of the cardiovascular diseases, namely hypertension as the most common diagnosis, and diseases of the respiratory organs. Given that potential side effects were monitored throughout the entire study, none were recorded, which tells us that nifuroxazide therapy, as a modified therapy, guarantees safety as a parallel therapy.

The representation of patients with parallel therapies in research involving patients with CDI is discussed in a prospective study from 2013 at Cleveland Medical Center, conducted by Venkata et al. They show the involvement of 7% of patients with diabetes mellitus, 4% with liver diseases and 2% of patients with kidney diseases [11].

As the number of stools was analyzed for each patient on the day of admission, and at each of the four controls, we can state that the average number of stools at the enrolment of participants who received metronidazole was 7.83 with the final outcome of the number of stools being 2.70. The results show an uneven distribution of the number of stools in the controls. At the first and second control, the number of stools remains above 7, and a significant drop occurs only at the fourth control. On the other hand, when it comes to the number of stools in patients who received combined therapy (metronidazole + nifuroxazide), the average number of stools, taking into account admission and four controls, was 2.91. The average number of stools at the enrolment for this group of subjects was 5.27 with the final outcome of the average number of stools being 1.5. The results show a tendency for the maximum number of stools per day to decrease after the controls, with a convincing drop even at the first control (3.5). The third and fourth control almost do not differ, so this leads to the conclusion that combined therapy has the greatest impact on the number of stools in the period up to the third control. The Kruskal-Wallis test (P=.009) shows that there is a statistically significant difference in the incidence of the number of stools during all controls, so taking into account the results of the Mann-Whitney test, the results can be interpreted in such a way that this combination of drugs reduces the number of stools per day significantly and in a short period.

From the diagram of the comparative examination of the number of stools incidence, it is noticeable that in all controls there were the most subjects with 7 or 8 stools when it comes to the group of participans who were treated only with metronidazole, while the largest number of participants, with only two stools per day, was recorded in the group of participans treated with nifuroxazide. The Kruskal-Wallis test shows that there is no statistically significant difference regarding the incidence of the number of stools at the sample level.

The decrease in the number of daily stools with the use of nifuroxazide is shown by the results of research from 2016. This study described the greater effectiveness of nifuroxazide in the treatment of intestinal infections, compared to probiotics. According to the aforementioned research, already 72 hours after the start of therapy, the average number of stools for the group treated with nifuroxazide was drastically reduced compared to the group treated with probiotics [12].

The pain parameter, in the group of respondents who were treated with metronidazole, shows the prevalence of grade 1 (presence of pain) in about 73% of cases, which shows that the consumption of the drug in most of the sample did not lead to the disappearance of pain. On the other hand, the condition of patients treated with a combination of metronidazole and nifuroxazide in terms of pain is reflected in the rapid growth of incidence 2 (absence of pain), which indicates the effective action of the combined therapy that relieves pain in the short term. If we make a comparative analysis based on the mentioned data, we can conclude that there is a significant difference in terms of the effects of metronidazole in combination with nifuroxazide.

A more detailed presentation of these data is presented through diagrams, by groups and test phases. Namely, in all participants of the group receiving monotherapy, the pain persists until the first control, which is the fourth day of treatment, while it decreases very slightly until the second or third control, when as many as 21 patients with pain were recorded. An absolutely rapid and proportional decrease is shown by the curve that indicates patients who

feel pain and were treated with the combination of metronidazole + nifuroxazide. The curve shows a tendency to eliminate pain and already by the second control the pain has disappeared in 80% of patients. These individual analyzes imply the conclusion that the effect of the combination of drugs metronidazole + nifuroxazide is such that the pain disappears soon after the therapy, which is not the case if metronidazole is used as monotherapy.

According to the results of a study by Layth et al. from 2016, published in GastroenterolHepatol, metronidazole monotherapy was recommended only in the mildest cases of CDI, and the exclusive effect of the drug on achieving negativity in the CDI toxin test was verified, without a significant effect on pain reduction, in the period treatment of 14 days [13]. The results of this research fully agree with our analyses, when it comes to the treatment of CDI with metronidazole monotherapy.

5. CONCLUSION

The finding of this study support metronidazole + nifuroxazide combination therapy over metronidazole monotherapy for the treatment of CDI. Frequency of important clinical symptoms of CDI such the number of daily stools reduced within a shorter period. Besides, the modified therapy was more active in pain relief among the CDI patients compared to the metronidazole therapy alone.
During the research no side effects of nifuroxazide, as part of the modified therapy, were recorded, and we can state that it has a very good safety profile, and certainly represents an efficient protocol in the treatment of mild CDI.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

J. Rahimić and E. Alibegović were in charge for Project planning and implementing and conceived the original Project idea. A. Kurtćehajić collected and sorted questionnaries from patients. A. Kurtćehajić and D. Alibegovic worked out majority of technical details, calculations and statistical analysis. L.Lekić collected some datas for Project and supervised the Project. E. Dautović gave critical overview of the manuscript. J.Rahimić wrote the manuscript withsupport from E. Alibegovic, L.Lekić, E. Dautović. All authors discussed the results and approved the final version of the manuscript.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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