

A Triple Blinded, Randomized, Placebo-Controlled Clinical Trial to Evaluate the Efficacy and Safety of Oral Vancomycin in Primary Sclerosing Cholangitis: a Pilot Study

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ABSTRACT

Background & Aim: Recent studies have suggested the therapeutic effect of antimicrobial agents on primary sclerosing cholangitis (PSC). Therefore, we aimed to evaluate the efficacy of oral vancomycin in patients with PSC.

Method: A triple blinded, randomized, placebo-controlled trial was performed on 29 patients (2015-2016) in the Imam Khomeini Hospital, Tehran, Iran (NCT02605213). Patients were divided into two groups by simple randomization method: placebo 11 (37.9%)/vancomycin 18 (62.1%) and were treated with oral vancomycin (125 mg, four times a day) for 12 weeks. All patients in both groups simultaneously underwent treatment with ursodeoxycholic acid (UDCA, 300 mg, three times a day) before and during the study. Patients' laboratory data and clinical symptoms were recorded at the beginning, first and third month after starting treatment, and the response to treatment was analyzed.

Results: 29 patients with a mean age of 36.27±10.60 years were included in the study. Primary endpoints were accomplished in the vancomycin group showing a significant decline in the mean level of PSC Mayo risk score (decrease rate 3rd month - baseline = -322.03%, p=0.026) during follow up time. Moreover, the analysis of the level of alkaline phosphatase (ALP) in the vancomycin group showed a significant decrease in the third month of treatment as compared to its level in the first month (mean difference 3rd month -1st month = -142.92, Decrease rate= -18.24%, p=0.02). Among secondary endpoints, erythrocyte sedimentation rate (p=0.005), gamma-glutamyl transpeptidase (p=0.02) and patients' symptoms including fatigue, pruritus, diarrhea and anorexia showed a significant decrease in the vancomycin group.

Conclusion: This study demonstrated an acceptable efficacy of vancomycin in the treatment of PSC.

Key words: primary sclerosing cholangitis – oral vancomycin – placebo – Mayo Risk score – liver function tests

Abbreviations: BMI: body mass index ; ESR: Erythrocyte sedimentation rate; FIS-P: Fatigue Impact Scale-Persian; IBD: Inflammatory Bowel Disease; LSD: Least significant difference; PSC: Primary Sclerosing Cholangitis; RCT: Randomized, Controlled Trial; UDCA: Ursodeoxycholic acid; VAS: Visual Analogue Scale.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic, inflammatory, fibro-stenotic, and idiopathic liver disease which is defined by hepatic bile duct dilation and stricture that leads to portal hypertension, cirrhosis, and liver failure with a high morbidity and mortality rate [1-6].

Although the etiology of PSC is still an enigma, studies have

proposed possible hypotheses according to the pathogenesis of the disease, including genetics, overactivation of lymphocytes, excessive immunological responses, bacteremia in the portal vein, and toxicity due to the bile duct salts [7, 8].

In spite of the various clinical trials on different pharmacological agents, up to now there has been no definite therapy for this disease. Liver transplantation has been known as the only effective choice which prolongs survival of the patients, with a recurrence rate of 5-35% in the new grafts [2-4, 9]. Previous studies have discussed the treatment choices including immunosuppressive agents, corticosteroids, and ursodeoxycholic acid (UDCA), with regard to their efficacy in alleviating patients' clinical symptoms. In addition, along with the recently proposed mechanism of PSC regarding

the potential role of gut microbiota and its subsequent immunological reactions due to bacterial remnants, the possible role of antimicrobial agents has manifested itself [2-5, 9-13].

The efficacy of vancomycin has been studied in some case reports, case series, and pilot studies. However, due to the methodology of these studies (mostly case series and case reports) and the small sample size of pilot studies or incidental findings during treatment courses of other clinical conditions, results are variable in the different studies [2-5, 14]. Therefore, we conducted this study to evaluate the efficacy of vancomycin as the recent controversial antibiotic on patients with PSC in a triple blinded, randomized, controlled trial (RCT) with a higher sample size to assess primary end points consisting of the decrease in alkaline phosphatase (ALP) levels and the PSC Mayo risk scores and secondary endpoints including other serum biochemical tests and patient symptoms.

METHOD

Patients

This was a pilot triple blinded RCT that was conducted between 2015 and 2016 on 29 patients in the Imam Khomeini Hospital, Tehran, Iran. Inclusion criteria were: age more than 18 and less than 66 years; diagnosed PSC (chronic liver disease described by advanced course of cholestasis, inflammation with intra- and extra-hepatic bile ducts fibrosis) [1, 9] with cholestasis (reduction in bile flow as a result of impaired secretion by hepatocytes or obstruction of intra- or extra-hepatic bile ducts) [15] for more than 3 months, magnetic resonance cholangiopancreatography (MRCP) and pathological confirmation. While exclusion criteria consisted of symptoms of decompensated cirrhosis including ascites, hepatic encephalopathy, and variceal bleeding, concomitant usage of corticosteroids, immunosuppressive, and other antibiotics within 3 months prior to the study, history of allergy to vancomycin, being considered as on the waiting list for liver transplantation, renal failure with creatinine higher than 1.5 mg/dl, thrombocytopenia with platelet count below 65,000, leukopenia with white blood cell count below 1,500 cells/mm³, different or concomitant etiology of liver disease other than PSC, pregnancy and lactation, drug or alcohol abuse. Informed consent was obtained from all patients after explaining the study and the possible side effects of the drugs. This study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS) and was registered in clinicaltrials.gov with the registration number of NCT02605213.

Randomization, allocation and blinding methods

We used a simple randomization method in order to assign the participants in two treatment groups. Only the principal investigator was aware of the meaning of the codes in order to be able to discontinue the treatment in case of adverse events. The allocation concealment was done by the principal investigator. An independent investigator who was blinded to the treatment group made random allocation cards by using computer-generated random numbers. Both placebo and vancomycin drugs were in the same shape and color and were packed in the same envelope (allocation concealment)

according to the allocation orders. Another investigator who was also blinded was responsible for the patients' enrolments and data collection. Each patient's information was recorded on an envelope and was attached to the patient's file. We used the triple blinding method which meant that patients, investigators who were responsible for the patients' enrolment and the analyzer of the data at the end of the study were unaware of identities to reduce the chance of bias occurrence in the study.

Medication and follow-up

All patients were randomly divided into vancomycin (Vancomycin tablet 125 mg, Jaber Ebne Hayyan Pharmaceutical Company Tehran, Iran, four times a day) and placebo (placebo of vancomycin tablet 125 mg, Jaber Ebne Hayyan Pharmaceutical Company Tehran, Iran, four times a day) groups by a simple randomization method. Treatment duration was of 12 weeks, and the patients were followed-up at week 0, 4 and 12 of treatment: their demographic data, medical and habitual history, laboratory data (including hematological, biochemical and liver function tests) and their symptoms (including fatigue, pruritus, abdominal pain, diarrhea, bloody stool, nausea, vomiting, and anorexia) were assessed. For assessing pruritus a visual analogue scale (VAS) consisting of a 10 cm vertical or horizontal line [16] was used. Fatigue was measured by a valid and reliable Persian version of Fatigue Impact Scale (FIS-P) [17, 18], which consisted of 40 items with score of 160. All patients in both groups simultaneously were under treatment with UDCA (Ursobil, 300 mg, three times a day) before and during the study.

End points

Primary endpoints for this study included a significant decrease in the Mayo PSC risk score and ALP level at week 12. Mayo PSC risk score and ALP were measured at weeks 0, 4 and 12 in each patient. Mayo PSC risk score was calculated as follows: Mayo Risk Score = [0.0295 (age in years) + 0.5373 LN (total bilirubin in mg/dl) - 0.8389 (serum albumin in g/dl) + 0.5380 LN (aspartate aminotransferase in IU/L) + 1.2426 (points for variceal bleeding)]. (LN indicates the natural logarithm).

Secondary end points consisted of a significant decrease in the serum level of erythrocyte sedimentation rate (ESR, mm/hour), alanine aminotransferase (ALT, IU/L), aspartate aminotransferase (AST, IU/L), total and direct bilirubin (mg/dl), white blood cells (WBCs, cells/mm³), platelet (PLT 10³), gamma glutamyl transferase (γ GT, IU/L), and improvement in patients' symptoms (including fatigue, pruritus, abdominal pain, diarrhea, blood in stool, nausea, vomiting, and anorexia) at the end of 12 weeks.

Evaluation of adverse effects

Patients were monitored for any unwanted adverse events associated with drug administration: fever, chills, rash, fatigue, gastroenterological symptoms (abdominal pain, and persistent diarrhea), nephrotoxicity, neutropenia, ototoxicity, thrombocytopenia, antibiotic-resistant infections and neurological symptoms. In case of any adverse events, the intervention was discontinued, the patient was excluded from the study and the treatment files were sent to the principal

investigator to evaluate the patients' condition and evaluate if the adverse events were related to vancomycin treatment or not.

Statistical analysis

All data were analyzed by SPSS software (version 23, IBM Corp). Descriptive statistics were used as the mean ± standard deviation (median) for continuous variables and frequency (percentage) for categorical variables. For comparing different time points in each group of treatment separately, one way repeated measures Anova was done and the Least significant difference (LSD) method was used as a post-hoc test in significant results. For comparing improvement of symptoms in 3 time points in patients of each group separately, the Cochran's Q test was achieved.

Intention to treat analysis was performed for analyzing the results. P-value under 0.05 was considered as statistically significant.

RESULTS

Patient characteristics

Overall 29 patients including 17 (58.6%) male, with a mean age of 36.27±10.60 (19-65) years and a median of 34 years and a mean body mass index (BMI) of 23.94±3.78 (17.04-35.26) kg/m² were considered in the statistical analysis. The PSC duration to the time of study was 4.01±2.20 years and 3.60±3.77 years in the vancomycin and the placebo group, respectively. Twenty-one (75%) patients had concomitant inflammatory bowel disease (IBD). Patients were randomly allocated in the placebo and vancomycin groups (11 and 18, respectively). During the time of the study, one patient in the vancomycin

group due to cholangitis and emergency ERCP and one patient in the placebo group due to pulmonary embolism discontinued the intervention (see study flow diagram, Fig. 1). Table I shows a summary of the patients' demographic data. Patients in both the vancomycin and placebo group did not show any significant difference in the demographic data, which showed a good distribution of the patients in the two groups.

Analysis of treatment efficacy

Mean level and rate of changes of the laboratory results, Mayo PSC risk score and frequency of patient's symptoms during three moments of the study (baseline, first month, third month), are summarized in Table II and III.

Primary endpoints

The primary end point of our study consisted of the Mayo PSC risk scores and ALP levels which were reached at the end of the study. Analysis of the vancomycin and placebo group of treatment showed a significant decrease in the Mayo score in the vancomycin group (P=0.026) at the third month comparing to its level at the baseline: mean difference (3rd month-Baseline) = -0.59, decrease rate= -322.03%, p=0.026, whereas, no significant changes were seen in the placebo group (Figs. 2, 3).

Analysis of the ALP levels in each group separately showed that the ALP level in the vancomycin group significantly declined at the end of month 3 as compared to its level at the month 1 of treatment: mean difference (3rd month-1st first month) = -142.92, decrease rate= -18.24%, p=0.023. It did not show any significant decrease at month 3 or month 1 as compared to its level at baseline: p=0.13, p=0.11 respectively. Also, there was no significant change in the placebo group either (p=0.67) (Fig. 4).

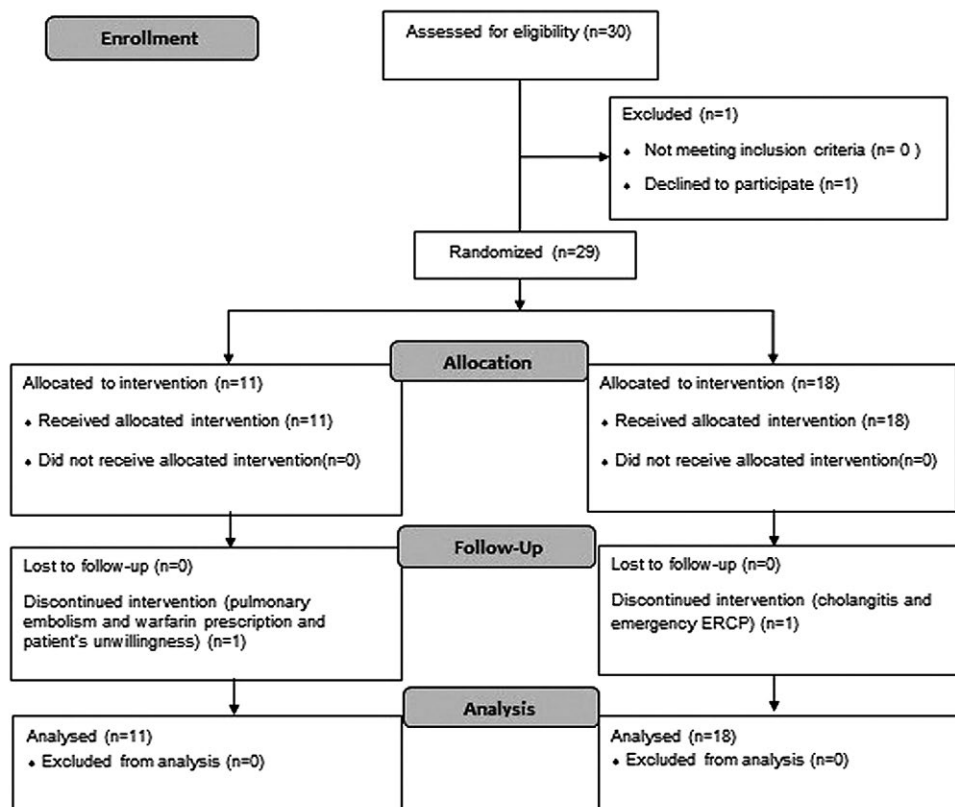


Fig. 1. Study flow diagram.

Table I. Patients' demographics and characteristics stratified by treatment groups.

Variable	Treatment Groups Mean ± SD (Median) Frequency (%)		P-value
	Vancomycin	Placebo	
Age (years)	35.94±9.84 (33)	36.81±12.23 (34)	0.83
Sex (male/female)	M/F: 8 (44.4%) / 10 (55.6%)	M/F: 9 (81.8%)/2 (18.2%)	0.06
Body Mass Index (kg/m ²)	24.19±4.38 (23.99)	23.58±2.87 (23.95)	0.68
Concurrent with Inflammatory Bowel Disease (Yes %)	13 (76.5%)	8 (72.7%)	1
Inflammatory Bowel Disease duration to the time of study (years)	9±6 (7)	11.87±12.77 (8.5)	0.51
Primary Sclerosing Cholangitis duration to the time of study (years)	4.01±2.20 (4)	3.60±3.77 (2)	0.72
Delivery type (Normal vaginal delivery / caesarian section)	NVD/CS: 7 (70%) / 3 (30%)	NVD/CS: 5 (71.4%) / 2 (28.6%)	1
Cigarette smoking	1 (5.6%)	1 (9.1%)	1

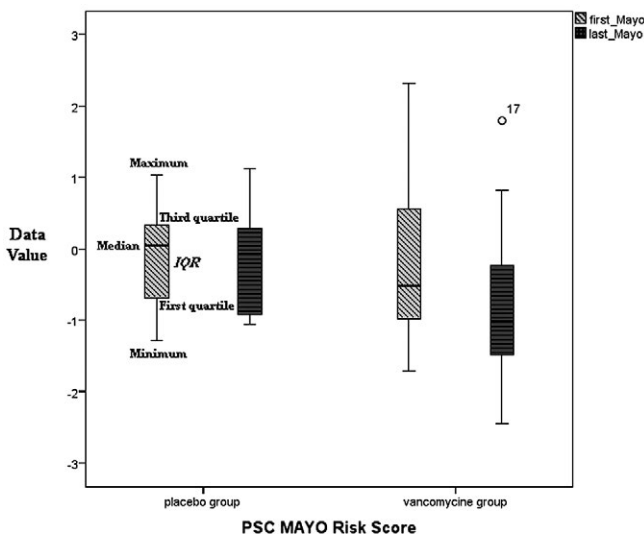


Fig. 2. Primary sclerosing cholangitis (PSC) Mayo Risk score box plot in vancomycin and placebo groups at the start and end of the study.

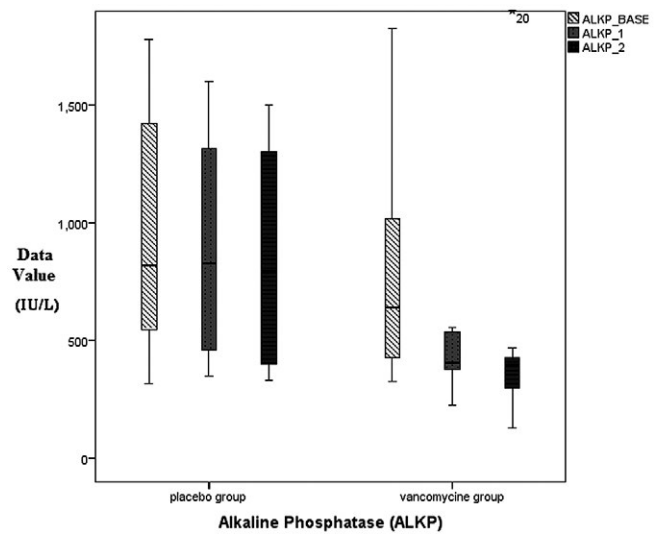


Fig. 4. Serum alkaline phosphatase (ALP) level box plot for the vancomycin and placebo groups during 3 moments of the study (baseline, first month, and third month)

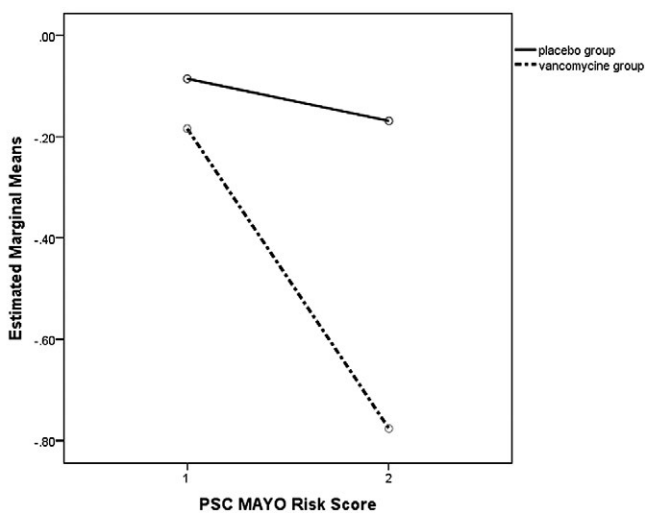


Fig. 3. Mean difference of primary sclerosing cholangitis (PSC) Mayo Risk score in vancomycin and placebo groups during the start and end of the study.

Secondary endpoints

Among secondary endpoints, the ESR level, gamma glutamyl transferase (γGT) level and patients symptoms

showed a significant decrease in the vancomycin group. Separate analysis of the ESR level during the three time points of the study in vancomycin and placebo groups showed a significant decrease in the vancomycin group (F (2,22) =6.83, p=0.005, Partial eta Squared=0.38) (mean difference (3rd month-Baseline) = -15.18, decrease rate= -41.25%, p=0.005). However, there was no significant change in the placebo group (F (2,12) =2.8, p=0.1) (Figs. 5, 7)

Moreover, analysis of γGT level during the three time points of study in the vancomycin and placebo group showed a significant decrease in the vancomycin group in month 3 as compared to its level in month 1 (Fig. 6): mean difference (3rd month-1st first month) = -96.6, Decrease rate= -35.29%, p=0.02.

Patients in the vancomycin group showed significant improvements in their symptoms during follow up time. There was a significant alleviation in patients' fatigue (p=0.002), pruritus (p=0.022), diarrhea (p=0.018), and anorexia (p=0.04). However, except pruritus (p=0.011), there was no significant improvement in the patients of the placebo group (Table III) except in pruritus.

Table II. Mean level and changing rate of serum biochemical tests during the time of study.

Variable Mean ± SD (Median)	Treatment Group	p*	Baseline	1st month	3rd month	p**	Increase / decrease %***	p***	Increase / decrease %****	P****
Mayo Risk score of PSC	Placebo	0.86	-0.11±0.75 (-0.18)	-	-0.16±0.71 (-0.07)	0.33	-45.45	.337	-	-
	Vancomycin		-0.18±1.19 (-0.51)	-	-0.77±1.11 (-1.01)	0.026	-322.03	0.026	-	-
Erythrocyte sedimentation rate (mm/hour)	Placebo	0.59	29.77±30.42 (20)	34.7±34.85 (25)	28.33±28.74 (20)	0.1	-4.83	.845	-	-
	Vancomycin		36.8±30.69 (33)	29.53±25.68 (29)	21.62±22.24 (19)	0.005	-41.25	0.005	-26.78	0.17
Alkaline phosphatase (IU/L)	Placebo	0.59	897.54±481.94 (800)	884.36±458.68 (823)	826.30±429.62 (797)	0.67	-7.93	0.490	-	-
	Vancomycin		1160.05±1570.95 (640)	783.29±1046.33 (414)	640.37±882.15 (395)	0.13	-44.79	0.112	-18.24	0.02
Gamma-glutamyl transpeptidase (IU/L)	Placebo	0.77	455.2±267.86 (444.5)	415.5±230.51 (377.5)	315±130.03 (350)	0.87	-30.7	0.966	-	-
	Vancomycin		414.58±356.66 (350)	223.18±205.24 (153.5)	150±165.18 (86)	0.10	-63.81	0.087	-32.78	0.02
Alanine aminotransferase (IU/L)	Placebo	0.79	75.54±63.74 (45)	70.72±50.64 (50)	81±58.14 (71)	0.45	7.22	0.739	-	-
	Vancomycin		69.38±60.03 (49)	38.88±19.88 (35)	37.25±18.51 (35.50)	0.07	-46.31	0.074	-4.19	0.73
Aspartate aminotransferase (IU/L)	Placebo	0.74	67.45±37.7 (68)	65.18±37.55 (68)	64.1±38.63 (59.5)	0.42	-4.96	0.135	-	-
	Vancomycin		62.17±43.40 (49)	49.41±37.82 (31)	41.43±30.12 (28.5)	0.07	-33.36	0.053	-16.15	0.33
White blood cells (cells/mm ³)	Placebo	0.86	7310±2312.6 (6440)	6709.09±1537.82 (6600)	6830±1595.1 (6500)	0.34	-4.71	0.417	-	-
	Vancomycin		7168.18±1972.8 (7000)	7502.35±2349.0 (7530)	7543.75±2658.81 (7000)	0.89	3.19	0.998	-0.55	0.63
Platelet (PLT x 10 ³)	Placebo	0.81	290.18±142.8 (280)	276.9±119.5 (287)	271.2±118.3 (269)	0.43	-6.54	0.335	-	-
	Vancomycin		277.88±126.3 (260)	297.58±160.42 (241)	271.12±104.08 (227.5)	0.39	-2.43	0.202	-8.89	0.30
Bilirubin conjugated (mg/dl)	Placebo	0.57	1.18±0.98 (1)	1.1±0.77 (1.3)	0.61±0.67 (0.40)	0.64	-48.3	0.637	-	-
	Vancomycin		2.37±4.9 (0.4)	0.89±1.39 (0.20)	0.83±1.75 (0.2)	0.54	-64.97	0.420	-6.74	0.81
Bilirubin total (mg/dl)	Placebo	0.62	2.4±2.29 (1.64)	1.81±1.28 (1.70)	1.49±0.97 (1.20)	0.45	-37.91	0.280	-	-
	Vancomycin		3.49±6.77 (1.1)	2.26±2.70 (1.2)	2.14±3.39 (0.8)	0.28	-38.68	0.410	-5.3	0.35

* P-value of the difference of the Patients' biochemical data at baseline between the two vancomycin and placebo group.
 ** demonstrates the p-value for analyzing the changes of serum biochemical in each group during 3 time points of study with One Way ANOVA analysis for each group.
 ***Increase /Decrease percentage= (Third month Value-Baseline value)/Baseline value) *100, ***p-value = the p-value of difference between Baseline and Third month values.
 ****Increase /Decrease percentage= (Third month Value-First month value)/First month value) *100, ****p-value = the p-value of difference between first month and Third month values.

Analysis of treatment efficacy by considering confounding covariates

By considering the effect of covariates including sex, age, IBD and PSC duration, concomitance with IBD, delivery type, cigarette smoking, in response to treatment, none of the aforementioned covariates yielded any significant change in previously obtained results.

Adverse effects

During the three months of the study there were no adverse effects related to drug administration including fever, chills, rash, fatigue, gastroenterological symptoms (abdominal pain, persistent diarrhea), nephrotoxicity, neutropenia, ototoxicity, thrombocytopenia, antibiotic-resistant infections and neurological symptoms. Overall, two patients discontinued

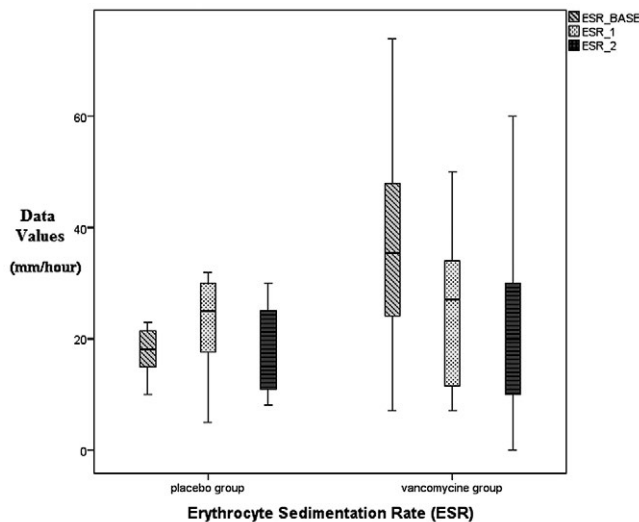


Fig. 5. Serum ESR level box plot for vancomycin and placebo groups during 3 times of study (Baseline, first month, and third month)

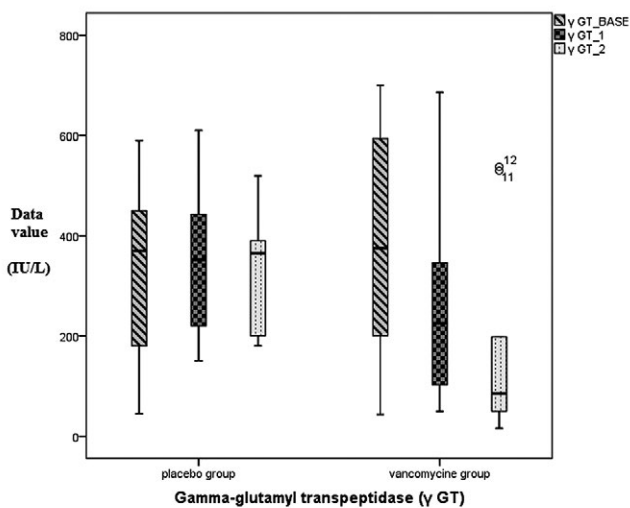


Fig. 6. Serum γ GT level box plot for Vancomycin and Placebo groups during 3 times of study. (Baseline, first month, third month)

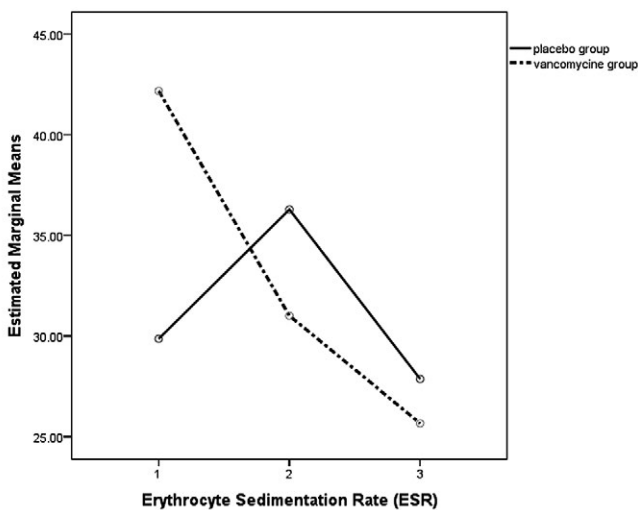


Fig. 7. Mean difference of ESR level in vancomycin and placebo groups during 3 times of study (Baseline, first month, and third month).

the intervention due to pulmonary embolism and cholangitis that were unrelated to drug administration.

DISCUSSION

The pathogenesis of PSC has been presented differently in the literature [11, 19-22]. Infection mechanism is one of the factors that have been discussed in different studies [3]. Various studies mostly including case reports, case series, and pilot studies have discussed the efficacy of antibacterial agents on PSC [2-5, 9]. In this study, we accomplished one of the first triple RCTs on the premier antibacterial agent, vancomycin.

Overall we reached both the primary and secondary endpoint of our study. The PSC Mayo risk score significantly decreased during follow-up. Moreover, the ALP level diminished significantly at the end of month 3 in contrast to its level in month 1. These results were compatible with those of a recent study by Tabibian et al. [2] on 35 PSC patients in four treatment groups treated with vancomycin 125mg, 250mg, metronidazole 250mg, and 500 mg for 12 weeks with primary end point of decreasing ALP levels in week 12, and as a secondary endpoint to decrease serum bilirubin level, Mayo PSC risk score, and pruritus, that significantly reached the primary endpoint of reduction in ALP level. Nevertheless, due to the small sample size in each group, the vancomycin group did not show any significant change in the Mayo score in this study. Moreover, patients of this study who were in the metronidazole group revealed a decrease in the serum bilirubin level, PSC Mayo risk score, and pruritus [2]. Our secondary end point consisted of liver enzyme levels, inflammatory markers, and patients' clinical symptoms. Amongst all, ESR, γ GT, fatigue, pruritus, diarrhea, and anorexia showed a significant decline in patients during the follow-up. The reduction of the ESR level was also reported in a pilot study on 14 children with IBD and PSC that were treated with vancomycin for 43 ± 54 months. This study showed the normalization of ESR and patients' symptoms in all the children. Additionally, drug withdrawal resulted in the recurrence of clinical signs and elevation of liver enzymes in some of the patients, and restarting the drug caused a change in liver enzymes [5]. Furthermore, among the secondary endpoints of our study, total and direct bilirubin, AST and ALT showed a decreasing trend as well. Among the first studies on antibacterial agents, Rankin et al. [23] assessed the efficacy of tetracycline for about 10 months in 5 patients with PSC and IBD, and reported its favorable effect on liver enzymes and patients' symptoms. Furthermore, in a pilot study by Silveira et al. [24] on 16 patients with PSC that underwent minocycline treatment, demonstrated a significant decrease in serum ALP levels. Similarly in our study, patients in the vancomycin group showed a decline in ALP level and a significant decrease at the end of month 3. Cox et al. [3] demonstrated the efficacy of oral vancomycin in the normalization of liver tests and patients' symptoms in 3 children with PSC and IBD. In another study [6], investigation of the effect of UDCA alone and in combination with metronidazole in 80 patients with PSC, showed a significant improvement in liver enzymes, reducing the Mayo risk score, and a decreasing trend in disease progression by endoscopic retrograde

Table III. Analysis of patient's Symptoms

Symptoms	Group	Baseline	First month	Third month	Cochran's Q (df)*	P-value
Fatigue	Vancomycin	11(61.1%)**	6 (33.3%)	2 (11.1%)	12.2 (2)	0.13
	placebo	8 (72.7%)	6 (54.5%)	4 (36.4%)	4 (2)	
Pruritus	Vancomycin	6 (33.3%)	3 (16.7%)	1 (5.6%)	7.6 (2)	0.022
	placebo	7 (63.6%)	4 (36.4%)	1 (9.1%)	9 (2)	0.011
Abdominal pain	Vancomycin	1 (5.6%)	0	0	2 (2)	0.36
	placebo	2 (18.2%)	2 (18.2%)	0	2.66 (2)	0.26
Diarrhea	Vancomycin	4 (22.2%)	0	0	8 (2)	0.018
	placebo	1 (10%)	0	0	2 (2)	0.36
Blood in stool	Vancomycin	1 (5.6%)	0	0	20 (2)	0.36
	placebo	2 (18.2%)	0	0	4 (2)	0.135
Nausea and vomiting	Vancomycin	0	1 (5.6%)	0	2 (2)	0.36
	placebo	0	1 (9.1%)	0	-	-
Anorexia	Vancomycin	5 (27.8%)	1 (5.6%)	1 (5.6%)	6.4 (2)	0.041
	placebo	0	0	1 (9.1%)	2 (2)	0.36

*Cochran's Q test is an extension to the McNemar test for related samples that provides a method for testing for differences between three or more matched sets of frequencies or proportions. Degrees of freedom or „df” is the number of values in the final calculation of a statistic that are free to vary.

** Each number indicates Frequency (percentage %)

cholangiopancreatography (ERCP) in the combination group. However, a longer follow-up with higher doses of drugs has been recommended in this study [6].

In addition, in a case report [25], a coincidental observation during the treatment course of a patient with PSC who had had concomitant bronchitis showed that azithromycin can reduce cholestasis and that its discontinuation led to the recurrence of the patient's symptoms.

Although studies have shown the efficacy of antibiotics in improving patients' clinical symptoms and serum biochemical tests, evidence on the pathophysiology of this outcome is limited. Many assumptions have been proposed, including the presence of bacterial residues in the hepatic tracts (bile, portal tracks, cholangiocytes) in patients with chronic cholestatic liver diseases. This fact is also compatible with animal models that showed inflammation in the hepatobiliary system after induction of bacterial and chemical enterocolitis. Hence, antibacterial agents such as vancomycin can adjust the immunological response due to bacterial triggers, by reducing the synthesis of bacterial biomaterials [2, 11, 22].

Ursodeoxycholic acid, a hydrophilic bile acid, has been recommended at doses between 13 and 15 mg/kg per day in cholestasis induced by PSC. Since our patients had similar weights in both groups with a mean of 70.2 kg and median of 70kg, we used the most feasible dose for each patient (13mg/kg per day, 900 mg per day). We did not use a high dose of UDCA (20-30mg/kg) in order to decrease the adverse events and to assess the efficacy of vancomycin without possible drug interactions of the two drugs.

During the 3 month intervention, no adverse effects related to vancomycin administration were reported in our study. However, Tabibian et al. [2] reported infrequent and mild adverse effects in both high-dose (250 mg orally four times a day) and low-dose (125 mg orally four times a day) vancomycin

groups. As we only used low-dose vancomycin (125 mg orally four times a day) due to safety concerns of the pilot study, none of our patients reported adverse effects or unusual symptoms related to treatment. Among all adverse effects, antibiotic-resistant infections, especially by enterococcus, is one of the main concerns in treating patients with vancomycin. Therefore, finding the best dose and interval between doses that would show its highest effect along with the lowest risk of adverse effects including antibiotic-resistant infections, should be considered [5, 9].

Considering the limitations of our study, we used a simple randomization method in our patients. One limitation of this method is the chance of the unequal number of patients in each treatment group. Since the investigator who was responsible for the patients enrolment was not aware of the allocation orders and the content of each package, at the end of the study we had an unequal number of patients in each treatment group. We assessed the treatment effect by two measures: reduction of laboratory data related to the activity of disease and alleviation of the patients' symptoms during the study. However, there should be larger studies with a longer follow up duration to widely assess the changes in the laboratory data, symptoms, and pathological results by colonoscopy and biopsy and to see changes in the severity of the disease and clinical status of the patients by the discontinuation of vancomycin. Besides, considering this as a pilot study, we had to use a limited sample size due to ethical and economical concerns. Also, we had to use low dose vancomycin to reduce possible adverse events in the patients. Hence, a study with more cases and larger doses should be performed to assess its possible effects and adverse effects in a wider population.

Another limitation of our study was the disproportionate distribution of males and females and of disease severity in our study. In spite of this inequality, the gender of the patients and the severity of disease did not have any influence on the patients' response to treatment in the covariate analysis.

CONCLUSION

The successful treatment of PSC patients with vancomycin strengthen the proposed assumption regarding the infectious mechanism in PSC and its possible role in PSC etiology. However, this study could be the foundation of more population based studies with a higher sample size.

Conflicts of interest: The authors declare no conflict of interest regarding the publication of this paper.

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