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The efficacy and the immunomodulatory effect of rifaximin in prophylaxis of spontaneous bacterial peritonitis in cirrhotic Egyptian patients

LIVER

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Original Article

ABSTRACT

Background/Aims: This study aimed to evaluate the efficacy and the immunomodulatory effect of rifaximin as another promising prophylactic therapy against spontaneous bacterial peritonitis (SBP) in cirrhotics.

Materials and Methods: Seventy cirrhotic patients with ascites were included in the study. Patients were divided into two groups in a randomized single-blind fashion. Group one (n=40) received rifaximin and group two (n=30) received norfloxacin (control group). The treatment duration was 6 months. Serum levels of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) were the primary inflammatory markers of the study to evaluate the effect of the medications used.

Results: Three months after treatment, five cases on norfloxacin therapy showed SBP, whereas all cases on rifaximine therapy were free from SBP. In addition, there was no significant difference between patients on rifaximin and norfloxacin therapy with respect to TNF-α, IL-6, and IL-10 serum levels ($p>0.05$). Furthermore, patients on both rifaximin and norfloxacin therapies showed a statistically significant decrease in TNF-α and IL-6 serum levels compared with their baseline levels ($p=0.000$ and $p=0.000$, respectively). In contrast, serum IL-10 showed a statistically significant increase in both groups in comparison with its baseline level ($p>0.00$). Six-month after treatment, patients on rifaximin therapy showed more effective remission from SBP than those on norfloxacin therapy.

Conclusion: In conclusion, the use of rifaximin not only prevents bacterial translocation but also modulates the immune response of the inflammatory and the anti-inflammatory cytokines in SBP patients. However, the efficacy and the immunomodulatory effect of rifaximin in the prophylaxis of SBP in cirrhotics needs further prospective large-scale, double-blind studies.

Keywords: Rifaximin, norfloxacin, TNF-α, IL-6, IL-10, spontaneous bacterial peritonitis

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is the most frequent and life-threatening infection in patients with liver cirrhosis requiring prompt recognition and treatment (1). SBP is now associated with in-hospital mortality rates ranging from 20% to 40% (2). Furthermore, mortality rates 1 and 2 years after an episode of SBP were reported to be 50%-70% and 70%-75%, respectively (3). However, early diagnosis and prompt treatment with empiric antibiotics decreased SBP mortality rate (3). Bacterial translocation (BT) and migration of viable microorganisms from the intestinal lumen to the mesenteric lymph nodes and other extra-intestinal sites has been postulated as the main mechanism in the pathogenesis of SBP (4-6). In this commentary, selective intestinal decontamination (SID) with poorly absorbable antibiotics decreases intestinal bacterial overgrowth (IBO) and BT in experimental and human cirrhosis, with subsequent prevention of SBP (7,8). The administration of prophylactic antibiotics reduces the risk of recurrent SBP. Norfloxacin (400 mg/day, orally) is the treatment of choice according to the European Association for the Study of the Liver (EASL) guidelines (7).

Rifaximin is a minimally absorbed oral antimicrobial agent that is concentrated in the gastrointestinal tract (9). It has broad-spectrum in vitro activity against gram-positive and gram-negative aerobic and anaerobic en-

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teric bacteria and has a low risk of inducing bacterial resistance (10). The low systemic bioavailability of rifaximin may be more conducive to long-term use than other more bioavailable antibiotics with detrimental side effects (11). Overall, the continuous use of a single antibiotic does not appear to be the optimal solution and efforts should be made to seek alternatives, which could include antibiotic cycling (12).

In this context, the current research aimed to evaluate the efficacy of orally administrated rifaximin as another promising prophylactic therapy against SBP in patients with cirrhosis. In addition, basal concentration of pro-inflammatory and anti-inflammatory cytokines were determined and evaluated before and after treatment.

MATERIALS AND METHODS

In this prospective study, 85 patients with cirrhosis and ascites who had at least one previous episode of SBP were recruited from National liver Institute, Menoufiya University, Shebin El kom, Egypt. Patients were enrolled in a randomized, single blind, and controlled study. Diagnosis of cirrhosis was based on clinical, biochemical, and/or histological criteria. The inclusion criteria were age >20 and <65 years, and participants gave their written informed consent. The protocol was approved by the ethics committee of National liver Institute, Menoufiya University, Shebin El kom, Egypt with Institutional Review Board (IRB) protocol number 0063/2013. The diagnosis of SBP was confirmed if the ascitic fluid polymorphonuclear cell (PMN) count was >250 mm^3 with or without positive culture and by absence of an intra-abdominal source of infection. Ascitic fluid cultures were performed using the conventional culture method and via inoculating 10 ml of fluid in aerobic and anaerobic blood culture bottles at the bedside.

The exclusion criteria were active gastrointestinal bleeding, encephalopathy (>grade 2), hepatocarcinoma, or other malignancies and allergy to medications used.

On admission, there were 15 patients excluded from this study (10 patients had hepatocellular carcinoma, and five patients had severe gastrointestinal bleeding; who subsequently died). Seventy patients were enrolled in the study and divided into two groups: group one (n=40) received a daily oral dose of 800 mg rifaximin (Gastrobiotic 200 mg tablet, Al-Andalous Medical Company, 6-October City, Egypt) for 6 months and group two (n=30) received a daily oral dose of 400 mg norfloxacin (Epinor 400 mg tablet, Egyptian INT. Pharmaceutical Industries CO., 6-October City, Egypt) for 6 months. The etiology of cirrhosis for all patients enrolled in this study was viral hepatitis C. Liver function was evaluated using Child-Pugh classification (14); all patients were classified as Child C.

On admission, physical examination, liver and renal function tests, red and white blood cells count, platelets count, hemoglobin level, prothrombin time, and serum TNF-α, IL-6, and IL-10 concentrations were measured at baseline and 6 months after treatment.

Patients were followed up closely every month with careful assessment to rule out any complications such as fever, abdominal pain, or other symptoms or signs of infection. Study medication was discontinued in the case of recurrent SBP that represents the end point of the trial. The drugs used in the study were withdrawn in patients suffering from other complications such as gastrointestinal bleeding or encephalopathy and then a standard treatment that suits each case was supplied.

Approximately 10 ml of blood was taken from each patient by sterile venipuncture, without frothing and after minimal venous stasis using disposable syringes. Approximately 3 ml of venous blood was delivered in a vacutainer serum separator tube. Immediate centrifugation at 3000 rpm to avoid contamination of the sample with erythrocyte arginase was performed and then serum samples were used for liver and renal function tests (All kits used for biochemical analysis were supplied from Siemens Healthcare Diagnostics Products Gmbh, Germany, Cat. No. OUHP 29). The optical density for all these parameters was measured using Shimadzu UV-PC 1601 spectrophotometer, Japan.

Measurement of liver function parameters

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were measured spectrophotometrically using the kinetic method (15,16), serum bilirubin level (total and direct) was measured spectrophotometrically using the colorimetric (Diazo) method (17), measurement of serum albumin concentration was determined spectrophotometrically using the modified bromocresol green colorimetric method (18), and prothrombin time was determined by the coagulation method (19).

Measurement of renal function parameters

Blood urea nitrogen was determined spectrophotometrically using the enzymatic (fixed rate) UV method with urease and glutamate dehydrogenase (20). Serum creatinine concentration was determined spectrophotometrically using buffered kinetic Jaffé reaction without deproteinization method (21).

Measurement of hematological parameters

Approximately 2 mL of venous blood was delivered in a graduated vacutainer plastic tube containing 3.6 mg of potassium-ethyelenediaminetetraacetic acid (K-EDTA) for complete blood count (CBC), hemoglobin (Hb) (Sysmex® Automated Hematology Analyzer KX-21N, Japan), white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs) (Sysmex Corporation, Kobe 651-0073, Japan).

Cytokine analysis

Approximately 3 mL of venous blood was drawn in EDTA tubes containing the protease inhibitor aprotinin for measurement

of TNF-α, IL-6, and IL-10. These tubes were kept refrigerated before blood sample collection. Serum was separated within 30 min after drawing blood and kept frozen at −70°C for measurement of TNF-α, IL-6, and IL-10. Serum levels of TNF-α, IL-6, and IL-10 were measured by sandwiched ELISA using eBioscience Human TNF-α platinum Elisa BMS223/4/BMS223/4TEN, Human IL-6 platinum ELISA BMS213/2/BMS213/2TEN, and Human IL-10 platinum ELISA BMS215/2/BMS212/2TEN immunoassay, respectively using Biotek Elx 800-UV microtiter plate reader, USA.

Statistical analysis

Sample size was calculated based on the previous study (22) and assuming that prophylaxis with norfloxacin had no infection. Statistical analysis of the data was performed considering an alpha error of 0.05 with a 95% confidence interval. Data were expressed as mean±SD. Continuous data were tested using either chi-square to compare between nominal parameters and t-test as required for quantitative variables. The statistical analysis was performed with IBM©SPSS® Statistics V 20 (SPSS Inc., USA, 2010).

RESULTS

The period of recruitment was from May 2013 to March 2014. At National liver Institute, Menoufiya University, Shebin El kom Egypt, 70 cirrhotic patients were randomized to this study. Demographic data of the participants defined as age, sex, weight, smoking, other systemic disorders such as diabetes and hypertension, Child-Pugh score, model for end-stage liver disease (MELD) score, hepatic encephalopathy, and other concomitant medication used were demonstrated in Table 1.

Table 1. Demographic data of the participants

Parameters (n=40)	Rifaximin (n=25)	Norfloxacin p value	
Age (years)	55.8±4.81	56.5±4.17	0.50
Sex (male)	20 (90%)	16 (88%)	0.829
Weight (kilograms)	80.6±6.26	80.7±7.16	0.740
Smoking (%)	3 (14%)	2 (11%)	0.533
Diabetes (%)	3 (14%)	2 (11%)	0.533
Hypertension (%)	4 (18%)	2 (11%)	0.423
Child-Pugh score	10.25±1.1	10.67±1.8	0.54
MELD score	17.1±4.2	16.25±4.9	0.417
Hepatic encephalopathy	7 (17%)	5 (20%)	0.43
Concomitant medication used during the study (%)			
Spironolactone	38 (95%)	24 (96%)	0.956
Furosemide	36 (90%)	23 (92%)	0.94
Propranolol	5 (12.5%)	3 (12%)	0.988

Plus–minus values are means ±SD. MELD, Model for End-Stage Liver Disease. Differences between groups for each characteristic were tested for significance with Chi square test for nominal variables.

No serious adverse reactions were reported in both groups. There were only minor adverse effects reported with patients on norfloxacin; three patients had nausea (10%) and two patients had headache (6.6%). These adverse effects were symptomatically treated and they disappeared as revealed during patient follow-up. On the other hand, patients on rifaximin did not report any adverse effects.

Liver and renal function tests, complete blood count, and serum cytokine (TNF-α, IL-6, and IL-10) levels at the baseline were presented as mean±SD and showed no significant difference between both groups (p>0.05). Therefore, any changes that occurred after treatments were attributed to the effect of the medication used and hence were not related to individual variations as shown in Table 2. Laboratory characteristics of patients 6 months after treatment presented as mean±SD were demonstrated in Table 3.

Five cases on norfloxacin therapy showed SBP (5/30 or 16.6%) 3 months after initiation of the therapy. On the other hand, all cases on rifaxmine therapy were free from SBP (0/40 or 0%). Organism culture from ascitic fluid for the five infected patients showed infection with gram-positive cocci. All SBP patients were withdrawn from the study and were subsequently treated with third generation cephalosporin that offered an excellent prognosis.

Table 2. Selected laboratory features of patients at baseline

Parameters	Rifaximin	Norfloxacin	p
AST (IU/L)	74.90±14.28	79.80±13.63	0.979
ALT (IU/L)	52.40±14.27	55.90±10.29	0.862
BIL-T (mg/dL)	2.46±0.90	2.36±0.63	0.321
BIL-D (mg/dL)	1.11±0.37	1.10±0.29	0.238
Albumin (g/dL)	2.55±0.37	2.75±0.35	0.810
PT, INR	2.37±0.56	2.33±0.46	0.359
BUN (mg/dL)	57.2±20.83	58.1±20.14	0.885
s.Cr (mg/dL)	1.70±0.25	1.68±0.45	0.226
Hemoglobin (g/dL)	9.11±0.92	9.03±1.16	0.438
RBCs (10^6 /uL)	3.22±0.51	3.39±0.43	0.673
WBCs (10^3 /uL)	8.37±2.70	8.71±2.83	0.863
Platelets (10^3 /uL)	74.96±15.24	76.96±10.45	0.156
TNF-α (pg/mL)	186.67±31.05	182.28±27.48	0.670
IL-6 (pg/mL)	174.98±33.58	170.11±33.49	0.614
IL-10 (pg/mL)	35.05±6.17	39.12±6.44	0.911

Data presented by mean ± SD; AST Aspartate transaminase; ALT Alanine aminotransferase; BIL-T Total bilirubin; BIL-D Direct bilirubin; PT Prothrombin time; INR international normalized ratio; BUN Blood urea nitrogen; s.Cr Serum creatinine; RBCs Red blood cells; WBCs White blood cells; TNF-α Tumor necrosis factor alpha; pg/mL picograms per milliliter.IL-6 interleukin-6; IL-10 interleukin-10.

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Table 3. Table 3. Selected laboratory features of patients six months after treatment

Parameters	Rifaximin	Norfloxacin	p
AST (IU/L)	78.1±10.65	82.50±10.48	0.921
ALT (IU/L)	54.40±11.62	50.40±8.75	0.272
BIL-T (mg/dL)	2.72±0.77	2.60±0.65	0.750
BIL-D (mg/dL)	1.31±0.44	1.13±0.28	0.417
Albumin (g/dL)	2.63±0.30	2.66±0.22	0.558
PT, INR	2.38±0.33	2.43±0.54	0.400
BUN (mg/dL)	55.4±14.52	56.1±9.60	0.133
s.Cr (mg/dL)	1.46±0.24	1.48±0.30	0.543
Hemoglobin (g/dL)	9.01±0.78	8.77±0.64	0.329
RBCs (10^6/uL)	3.39±0.25	3.28±0.37	0.216
WBCs (10^3/uL)	6.90±2.52	7.67±2.03	0.793
Platelets (10^3/uL)	74.43±12.31	75.33±5.86	0.096
TNF-α (pg/mL)	93.29±17.56	90.88±14.03	0.175
IL-6 (pg/mL)	90.45±17.87	83.83±16.12	0.063
IL-10 (pg/mL)	87.02±13.87	90.45±17.87	0.579

Data presented by mean ±SD; AST Aspartate transaminase; ALT Alanine aminotransferase; BIL-T Total bilirubin; BIL-D Direct bilirubin; PT Prothrombin time; INR international normalized ratio; BUN Blood urea nitrogen; s.Cr Serum creatinine; RBCs Red blood cells; WBCs White blood cells; TNF-α Tumor necrosis factor alpha; pg/mL picograms per milliliter.IL-6 interleukin-6; IL-10 interleukin-10.

Six months after treatment, there was no statistically significant difference in the serum cytokine levels (TNF-α, IL-6, and IL-10) between the rifaximin and norfloxacin groups (p>0.05).

For the rifaximin group, TNF-α and IL-6 levels showed a statistically significant decrease compared with their baseline data (p=0.000 and p=0.000, respectively) 6 months after treatment. Furthermore, serum IL-10 level showed a statistically significant increase when compared with its baseline data (p=0.000).

For the norfloxacin group, TNF-α and IL-6 levels showed a statistically significant decrease compared with their baseline data (p=0.000 and p=0.000, respectively) 6 months after treatment. On the other hand, serum IL-10 level showed a statistically significant increase when compared with its baseline data (p=0.000). The changes in TNF-α, IL-6, and IL-10 levels within the treatment groups were demonstrated in Figures 1, 2, and 3, respectively.

Six months after treatment, serum creatinine level in the rifaximin and norfloxacin groups showed an insignificant decrease compared with its baseline data, with a noticeable decline of its levels by approximately 14.09% and 11.46%, respectively. In addition, no statistically significant difference in the serum creatinine level was detected between both the groups under study.

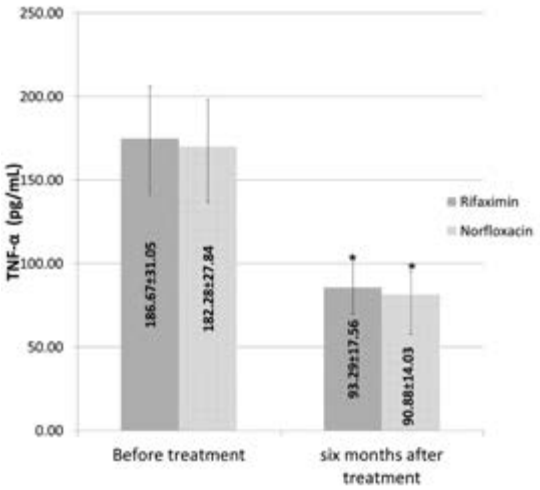

Figure 1. Changes in TNF-α serum level by treatment groups before treatment and six months after treatment. Data presented by mean ±SD. TNF-α level in both groups decrease significantly (p<0.5) six months after treatment in comparison with its baseline level.

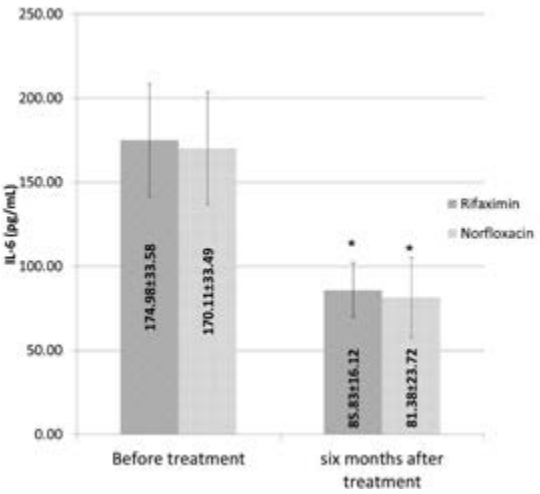

Figure 2. Changes in IL-6 serum level by treatment groups before treatment and six months after treatment. Data presented by mean ±SD. IL-6 level in both groups decrease significantly (p<0.5) six months after treatment in comparison with its baseline level.

DISCUSSION

The prevention of episodes of SBP is an important goal in the treatment of patients with liver disease. The ideal prophylactic agent should be safe, affordable, and effective at decreasing the amounts of these organisms from the gut while preserving the protective anaerobic flora (7). Our study aimed to evaluate the efficacy of rifaximin as a selective intestinal decontaminant on the pro-inflammatory and the anti-inflammatory cytokines in SBP patients and to estimate its role in reducing the risk of breakthrough episodes of SBP in cirrhotics.

IBO and BT increases circulating levels of endotoxin and cytokines such as IL-6 and TNF-α, which have been involved in the hyperdynamic status of cirrhosis (23,24). In addition, IL-10, an

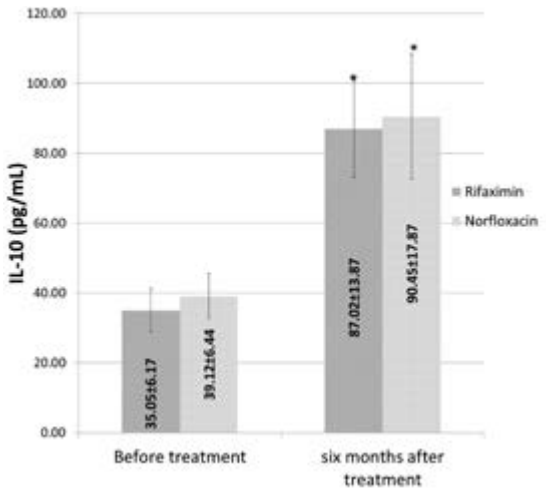

Figure 3. Changes in IL-10 serum level by treatment groups before treatment and six months after treatment. Data presented by mean ±SD. IL-10 level in both groups increase significantly ($p<0.5$) six months after treatment in comparison with its baseline level.

anti-inflammatory cytokine, has been reported to be a useful diagnostic and prognostic marker in patients with community-acquired sepsis (25). In this commentary, serum levels of TNF-α, IL-6, and IL-10 were the primary inflammatory markers of the study to evaluate the effect of the medications used.

The results of our study showed that the use of rifaximin resulted in a reduction of SBP episodes in cirrhotics. Our findings clearly demonstrated that intestinal decontamination with rifaximin resulted in a significant reduction in the mean serum levels of the pro-inflammatory cytokines (TNF-α and IL-6) and a significant increase in the serum level of the anti-inflammatory cytokine (IL-10). Recurrence of SBP episodes in patients on norfloxacin therapy was due to gram-positive organisms that are not sensitive to this therapy (26). Both rifaximin and norfloxacin showed a statistically significant decrease in serum TNF-α and IL-6 levels in comparison with their baseline data. In addition, there was a statistically significant increase in IL-10 serum level in both groups 6 months after treatment. The effect of both rifaximin and norfloxacin on serum TNF-α and IL-6 levels complies with the previous studies which reported that SID as a secondary prophylaxis of SBP not only removes bacterial products but also modulates patients' pro-inflammatory reaction (27,28). The immunomodulatory effect is due to its direct cellular effect on neutrophil response to oxidative stress by reducing secretion of reactive oxygen species and increasing the apoptosis rate (27,28). On the other hand, the increase in the serum level of IL-10 is in accordance with Go´ Mez-Hurtadog et al. (2011) who reported that IL-10 is significantly increased in patients undergoing SID (29).

In addition, it was postulated that IL-10 is responsible for the anti-inflammatory response in patients with cirrhosis using norfloxacin as secondary prophylaxis of SBP (29). The increase in serum IL-10 level and the decrease in both serum IL-6 and TNF-α levels in patients undergoing SID could be explained by the previously reported finding which demonstrated that IL-10 has been found to downregulate a number of different macrophage functions, including cytokine production TNF-α, IL-1, and IL-6 (30). The data obtained from this current study showed that the anti-inflammatory cytokine IL-10 level could be of equal value to the pro-inflammatory cytokines in defining the degree of illness in SBP and could be used as a good diagnostic and prognostic marker for SBP in cirrhotics.

During the current study, rifaximin intake (400 mg/twice daily, orally) was based on the fact that *Escherichia coli* is the most commonly isolated microorganisms from cases of SBP, and it has been shown that uptake of 800 mg rifaximin for 5 days markedly reduces fecal *Escherichia coli* population (31,32).

In the present prospective study, rifaximin was superior to norfloxacin because it is virtually non-absorbable, subsequently minimizing antimicrobial resistance, and maintains the drug safety in all patient populations (9,11). Furthermore, rifaximin has better activity against gram-positive organisms over norfloxacin (10). This is very important, particularly with increasing frequency of gram-positive bacteria in spontaneous bacterial peritonitis (26).

The improvement in survival observed in the current study could be related to the reduction of BT and the subsequent amelioration of hemodynamic alterations, thereby reducing the risk of bleeding, encephalopathy, and infections. It is possible that the shorter follow-up in our study (6 months) may be responsible for the absence of mortality noticed in this study.

According to the data obtained by this study, rifaximin maintained remission from SBP more effectively than norfloxacin. Therefore, when a physician decides to use a poorly absorbable antibiotic for the prophylaxis of an episode of SBP, rifaximin could be preferred. The use of rifaximin as SID not only prevents BT but also modulates the immune response of the inflammatory and the anti-inflammatory cytokines in SBP patients. The anti-inflammatory cytokine IL-10 could be of equal value to the pro-inflammatory cytokines (IL-6 and TNF–α) in defining the degree of illness in SBP. The efficacy and the immunomodulatory effect of rifaximin in the prophylaxis of SBP in cirrhotics needs further prospective large-scale double-blind studies.

A shortcoming of our study could be the small number of patients and the shorter follow-up period. Therefore, this work needs further extension and research on a large scale.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of National liver Institute, Menoufiya University, Shebin El kom, Egypt with Institutional Review Board.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - T.M., G.B., M.A.; Design - T.M., G.B., M.A.; Supervision - T.M., G.B.; Resource - T.M.; Materials - T.M.; Data Collection&/or Processing - M.A.; Analysis&/or Interpretation - M.A.; Literature Search - M.A.; Writing - T.M., M.A.; Critical Reviews - T.M., G.B., M.A.

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