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ROLE OF PROBIOTICS IN DECREASING AMMONIA LEVELS. CAN THEY BE USED AS A TREATMENT OF HEPATIC ENCEPHALOPATHY?

[1]Sajjad Hussain Chandio, [2]Tehreem Fatima, [3]Binish Hassan, [4]Saleha Jabeen, [5]Hassan Mumtaz, [6]Muhammad Owais, [7]Ayesha Fatima, [8]Fatima Meer

[1]Post Graduate Resident, Gastroenterology, Holy Family Hospital, Rawalpindi.

[2]House Physician, Holy Family Hospital, Rawalpindi.

[3]Post Graduate Resident, Gastroenterology, Holy Family Hospital, Rawalpindi.

[4]House Physician, Holy Family Hospital, Rawalpindi.

[5]House Physician, Holy Family Hospital, Rawalpindi.

[6]Medical Student, Wah Medical College.

[7]2nd Year Medical Student, Shifa College of Medicine.

[8]House Surgeon, Holy Family Hospital, Rawalpindi.

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***Corresponding Author**
Tehreem Fatima
House Physician Medicine,
Holy Family Hospital,
Rawalpindi.

ABSTRACT

Introduction: Hepatic encephalopathy (HE) is associated with poor prognosis in patients with advanced liver disease. The major pathophysiological mechanism involved in development of HE is raised ammonia levels. Several medications can decrease ammonia levels. Recently, role of probiotics in lowering ammonia levels has been a hot topic of interest. It has already been studies for primary and secondary prophylaxis but use of probiotics for HE treatment has not been studied. Objective of our study was to compare mean change in serum ammonia level with probiotics in the management of hepatic encephalopathy. **Subjects & Methods:** A total of 60 patients fulfilling selection criteria were enrolled in the study. In group-A (study group), patients were given probiotics along with standard treatment. In group-B (reference group), patients were given standard treatment plus placebo. Probiotics were lactobacillus reuteri 100 million cfu OD daily for two weeks. Standard treatment for acute hepatic encephalopathy as per AASLD guidelines was given. Patients were followed up in OPD after 2 weeks. The reduction in serum ammonia level was calculated (as per operational definition). Data were entered and

analyzed by using SPSS v25.0. Data were stratified for age, gender, and grade of hepatic encephalopathy. Post-stratification, an independent sample t-test was applied. A p-value ≤0.05 was taken as significant for all statistical tests in the study. **Results:** A total of 60 patients presenting with hepatic encephalopathy were enrolled in this study. Patients were divided into two groups i.e. Group-A (Probiotics) and Group-B (Placebo). There were 19(63.3%) males and 11(36.7%) females in group-A, while 21(70.0%) were males and 9(30.0%) females in group-B. The age range was from 16 to 75 years with a mean age of 52.1±16.29 years. The mean age of patients in group A was 56.9±14.8 years and in group-B was 45.9±10.4 years. In group-A, mean serum ammonia at baseline was 77.90±10.46 µmol/L and 82.73±8.44 µmol/L in group-B with a p-value 0.054. In group-A, the mean change in serum ammonia was 19.26±11.49 µmol/L and 7.13±6.48 µmol/L in group-B with a p-value 0.000005. **Conclusion:** Probiotics are better as compared to control in reducing serum ammonia levels in hepatic encephalopathy.

KEYWORDS: Hepatic Encephalopathy, Liver Cirrhosis, Serum Ammonia.

INTRODUCTION

Hepatic encephalopathy (HE) is a central nervous system dysfunction caused by liver insufficiency and/or portosystemic shunting. It presents as neuropsychiatric abnormalities, ranging from subclinical alterations to coma.[1] Overt HE is seen in 30% to 45% of cirrhotic patients and in 10% to half of the patients with a transjugular intrahepatic portosystemic shunt.[2] The West Haven classification system has categorized HE in 5 grades: 0-4. Grades are classified according to the state of drowsiness, sleep alteration, cognitive function impairment, etc. Grade 0 is mild and has minimal changes in memory and cognition while grade 4 is severe and is with coma.

The primary pathophysiological mechanism underlying HE involves ammonia which enters the brain.[3] In liver cirrhosis, many factors can elevate ammonia levels. Firstly, there is hepatic functional impairment that leads to inadequate ammonia removal from portal venous circulation. Secondly, there is portal hypertension that leads to the portosystemic shunting of blood, which divert ammonia-containing blood away from the liver to the systemic circulation.[4] Lastly, there is small intestine dysmotility that leads to small intestinal bacterial overgrowth (SIBO). This contributes to increased production of ammonia and hence increased absorption from the gut.[5] Several medications can be given that can decrease ammonia levels in patients with HE; those include LOLA, lactulose, rifixamine, etc.

Recently the use of probiotics is also being considered, to decrease ammonia levels in the blood. Probiotics act by decreasing the urease producing bacteria in the gut. Hence, many study have been done that study the effect of probiotics on ammonia levels but those use probiotics as primary and secondary prophylaxis. Treating patients to prevent the development of the first episode of HE is classified as primary prophylaxis while preventing the recurrence of HE in patients who had a previous episode of HE is classified as secondary prophylaxis.[6], but no study to the best of our knowledge has been done that discuss if they are beneficial for the treatment of HE. Our study aimed to do a Randomized control study and observe if probiotics have any role in the treatment of HE. We hypothesized that probiotics could treat HE by replacing the harmful urease-producing organisms and thereby lowering ammonia levels. Therefore, we used ammonia levels as the endpoint to see if they can be reduced by giving probiotics.

MATERIALS AND METHODS

This is a randomized, prospective study that was conducted at the Department of Gastroenterology, Holy Family Hospital, Rawalpindi, Pakistan from July 25, 2019 to January 25, 2020. The sample size was calculated with the help of WHO calculation, considering the level of significance, power of test, and anticipated population. The calculated sample size was 60.

Patients of both gender with age 16-75 years, presenting with hepatic encephalopathy. It was defined as the presence of HE grades 1-4 on West Heaven criteria. Exclusion criteria included patients with history of, Alcohol use, epilepsy, cardiac diseases, and renal failure (creatinine >1.5mg/dl or on hemodialysis.). Patients with acute confusional state due to drug overdose or those with hypoglycemia were also excluded**.**

After approval from the ethical review committee of the hospital 60 patients who presented in the department of Gastroenterology, Holy Family Hospital, Rawalpindi fulfilling the above criteria were enrolled for the study. The patients were counseled and explained the detail of the study. Written informed consent was taken from patients. Upon presentation, detailed clinical history was taken from each patient. All necessary investigations were done as per standard protocols. The patients were selected through consecutive sampling followed by random allocation in two groups. In group-A (study group), patients were given probiotics along with standard treatment. In group-B (reference group), patients were given standard

treatment plus placebo. Probiotics were lactobacillus reuteri 100 million cfu OD daily for two weeks. Standard treatment for encephalopathy as per AASLD guidelines was given.

All routine and necessary investigations were done. At baseline, a blood sample was obtained through a disposable syringe and sent to assess the serum ammonia level. Reports were assessed and levels were noted. The patients were managed as per standard operating practices. Patients were discharged as per protocols practiced in the department.

Patients were followed up in OPD after 2 weeks. After 2 weeks, the blood sample was again obtained and sent to the laboratory of the hospital for assessment of serum ammonia level. Reports were assessed and the level was noted. The reduction in serum ammonia level was calculated. Mean change in serum ammonia level from baseline to 2 weeks of post-treatment compared between two study groups**.** All this information was recorded on proforma.

DATA ANALYSIS PLAN

Data were entered and analyzed by using SPSS v25.0. Quantitative variables like age, pre and post-treatment serum ammonia levels were presented as Mean±S.D. Qualitative variables like gender and grades of hepatic encephalopathy were presented as frequency and percentage. Both groups were compared for serum ammonia levels using an independent t-test. Data were stratified for age, gender, and grade of hepatic encephalopathy. Post-stratification, an independent sample t-test was applied. A p-value ≤ 0.05 was taken as significant for all statistical tests in the study.

RESULTS

Demographic Characteristics of Enrolled Subjects

A total of sixty (n=60) patients of either gender between age 16-75 years who presented with hepatic encephalopathy were enrolled in this study. Patients were randomly divided into two groups. Initial venous ammonia levels were measured. Group A was administered probiotics along with standard treatment and Group B was taken as a control group and were given only the standard HE treatment. Patients were followed up in OPD after 2 weeks and venous ammonia levels were measured.

Table 1: Demonstrates the characteristics and demographics of patients.

Parameters	Sub-groups	Treatment group (n)	Placebo group (n)
	Number of pts	30	30
Sex	Male	19	21
	Female	11	9
Age groups	16-30 years	2	1
	31-45 years	6	13
	>45 years	22	16
Hepatic Encephalopathy	Grade-I	17	14
	Grade-II	10	12
	Grade-III	2	4
	Grade-IV	1	0

Ammonia levels after Probiotics treatment

Ammonia levels were significantly decreased in probiotic group as compared to control group. , The mean change in serum ammonia in group A (treatment) group was 19.26±11.49 µmol/L and 7.13±6.48 µmol/L in group-B with a p-value 0.000005.

Stratification For effect modifiers

Table 2 shows decrease in ammonia levels with data stratified according to age, gender and grades of HE.

Table 2: Mean change in ammonia levels with respect to gender, age group and grades of HE.

	SUB-GROUPS	TREATMENT (µMOL/L)	PLACEBO (µMOL/L)	P-VALUE
Change In Ammonia Levels (Mean)		19.26	7.13	0.000005
Change In Ammonia Levels With Stratification To Gender	males	20	7	0.00009
	females	17	6	0.021
Change In Ammonia Levels With Stratification To Age Groups	16-30 years	8	4	0.260
	31-45 years	28	9	0.0001
	>45	18	6	0.0003
Change In Ammonia Levels With Stratification To Grade Of Hepatic Encephalopathy	Grade-I	18	9	0.017
	Grade-II	20	5	0.0001
	Grade-III	25	10	0.191
	Grade-IV	23	0	1.000

DISCUSSION

HE is a syndrome observed in patients with cirrhosis or/and portosystemic shunt. It is defined as a spectrum of neuropsychiatric abnormalities and is characterized by intellectual impairment, personality changes, and a depressed level of consciousness.[7] The development

of hepatic encephalopathy is due to accumulation neurotoxic substances such as ammonia in blood, which occurs in the setting of cirrhosis and portal hypertension. The development of hepatic encephalopathy negatively impacts patient prognosis. Common precipitants of HE are renal failure, gastrointestinal bleeding, infections, constipation, dietary protein overload, medication such as benzodiazepines, antidepressants and antipsychotic agents, and diuretics. All these precipitants are involved in increasing blood ammonia levels in one way or the other. Hence, treatment of HE is mainly focused on increasing ammonia clearance or decreasing intestinal ammonia production.

Treatments to Increase Ammonia Clearance include L-ornithine L-aspartate (LOLA) and zinc. LOLA (Hepa-Merz) decrease ammonia levels and utilize it in the conversion of glutamate to glutamine by glutamine synthetase.[8] Zinc improves hyperammonemia by increasing the activity of ornithine transcarbamylase, which is an enzyme in the urea cycle.[9]

Intestinal ammonia production is decreased via protein restriction in diet, use of cathartics**,** and antibiotics. Cathartics include **l**actulose and lactilol. They are non-absorbable disaccharides, which acidify the gut lumen and favor conversion of ammonia (NH3) to ammonium (NH4+) which is not absorbed easily.[10] Antibiotics work by decreasing ammoniagenic bacteria. Antibiotics commonly used are rifixamine, neomycin, metronidazole, paromomycin, oral vancomycin, and oral quinolones.

Another approach to decrease ammonia production is to use probiotics, which tend to decrease urease producing bacteria. In our study, we have done a Randomized Control Study on patients with hepatic encephalopathy. Our study has shown that probiotics can decrease ammonia levels in HE. Treatment with probiotics for 2 weeks with an adjuvant to typical hepatic encephalopathy treatment has shown a remarkable decrease in ammonia levels as compared to placebo. This shows the exceptional effect of probiotics on decreasing ammonia production. In our study, patients taking probiotics have a mean change of 19.26±11.49 µmol/L in serum ammonia levels and those taking placebo have a mean change of 7.13±6.48 µmol/L in serum ammonia levels giving significant results with a p-value 0.000005. It has been reported in another trial that with probiotics, there was a 13.1±3.4 µmol/L decrease in serum ammonia level while with placebo, 2.9±2.2 µmol/L increased in ammonia levels, but here probiotics were used for primary prophylaxis. ($p<0.05$).[11] Another trial also found that with probiotics, there was a 41.25(32.46-50.04) µmol/L decrease in serum ammonia level

while with placebo, 17.31(7.39-42.04) µmol/L mean ammonia level. Here probiotics were used as secondary prophylaxis ($p<0.05$**).**[12]

Our study has shown that probiotics have proved to be very efficient in grade 1 and 2 of hepatic encephalopathy. A decrease of mean 18.24 was seen when probiotics were given to patients with grade 1 encephalopathy as opposed to a change of 8.57 in patients who were given a placebo. Similarly, a change of 19.60 and 4.50 was observed which probiotics and placebo respectively in patients with grade 2 HE. Probiotics use in grade 3 and 4 has also shown a drop in ammonia levels but it is not that significant with respect to the p-value. Similarly, our data has shown that a more meaningful change in ammonia level was seen in patients age 31-45 as compared to age groups 16-30 and >45. In the age group 31-45 mean ammonia change as compared to placebo was 19 µmol/L while in the age group 16-30 and >45 a change of 4 and 12 µmol/L was observed, respectively.

Other similar studies done on the subject have also shown some incredible results with the use of probiotics but in those studies however, probiotics were used for primary[11] or secondary prophylaxis.[12] But our research has studied probiotics for the treatment of HE. Some studies have used MHE (minimal hepatic encephalopathy) as the identifying point to start the treatment with probiotics while others have started probiotics after patients were out of HE. (secondary prophylaxis).

Studies that have been done to assess the prophylactic role of probiotics have lasted longer. For example[13] studying secondary prophylaxis was done for 12 months. On the other hand, research[5] studying primary prophylaxis was done for 3 months however our study was done for 2 weeks, primarily because we were studying the effects of probiotics as a medication for the treatment of hepatic encephalopathy.

In our study, all the patients tolerated the probiotics well without any adverse effects. But a study done on 30 subjects reported self-limited diarrhea was more frequent in LGG patients as compared to placebo.[14] Some other studies have compared lactulose with probiotics for primary and secondary prophylaxis. They have proved lactulose and probiotics to be equally effective, but lactulose can have some adverse effects like diarrhea, abdominal bloating, and distaste, requiring a dose reduction.[15] On the other hand, treatment with probiotics was not associated with any adverse effect.

Whether studies were discussing probiotics as primary or secondary prophylaxis, the endpoint was ammonia levels among others. Ammonia levels have shown a significant decline after treatment with probiotics in all studies. In our study, we used ammonia levels as an endpoint but some studies have used other endpoints like SIBO, and OCTT; increased psychometric hepatic encephalopathy scores; and increased CFF thresholds, compared with baseline.[5]

Strengths of the research include that it's the first of its kind to study the use of probiotics in the treatment of HE. Previous studies typically only investigated probiotics for primary and secondary prophylaxis. It also confirms that probiotics do not have a long onset of action and a considerable response was seen within 2 weeks. This study confirms that probiotics can be used for hepatic encephalopathy treatment.

Our study had some limitations. Firstly, the sample size was very small; the treatment of more patients with hepatic encephalopathy with probiotics will further validate our findings. Secondly, only one kind of probiotic was used: lactobacillus. It is recommended to use clostridium and others for future studies. Also, the use of probiotics, prebiotics, and synbiotics can be used to further explore the best modality for decreasing ammonia levels. Thirdly, the effect of probiotics after 2 weeks was assessed. Long term therapy with probiotics should be done to further understand the benefits and adverse effects. So that a suitable intervention course can be established.

In conclusion, the present study has confirmed that probiotics have a role in decreasing ammonia levels and hence improving HE. It is the first study according to our knowledge that has used probiotics as an adjuvant to HE treatment. Probiotics act at level 1 to decrease the production of ammonia and also tighten the gut junction to prevent escape into circulation and to the brain. They can be used as an adjuvant to normal/ routine treatment of HE and will help the patient in achieving normal functionality soon.

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