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The Effect of Liver Transplantation Surgery on Anaerobic Gut Microbiota Community and Vitamin B12 levels

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ABSTRACT

The Anaerobic Gut Microbiota (AGM) is completely associated with human physiology. Compounds like Vitamins, Short Chain Fatty Acids (SCFAs), bile acids, choline metabolites, indole derivatives, polyamines, lipids, neurotransmitters, and neuroactive compounds were linked by the AGM. This study involved 28 Liver transplant (LT) patients from Gastrointestinal Surgery Center (GEC), Mansoura University. One hundred and twelve stool and blood samples were taken from all LT patients in the first week before and after the surgery. The results showed that the percentage of anaerobic bacteria isolated from patients before and after one week liver transplantation was (67.18% and 32.82%), respectively. On the other hand, the levels of vitamin B12 (VB-12) in the blood decreased simultaneously with the change of AGM in the first week after surgery compared to the first week before surgery, and this affected the levels of VIT-12 in the blood of patients with *p*-value of 0.001.

Key Words: Liver transplantation, Anaerobic Gut Microbiota, Vitamin B12.

1. INTRODUCTION

The tight contact between the liver and the gut is a result of its anatomy. The portal circulation transports nutrients, other signals, and bacteria from the gut along with their byproducts and components to the liver. Then, the gut-liver axis, which is responsible for defense against materials generated from the gut, greatly depends on the liver [1]. The anaerobic gut microbiota (AGM) serves as a bioreactor for independent immune and metabolic processes that might mediate host environment reactions to outside inputs. The AGM functions like an organ, based on its complexity. Because of the great complexity of the AGM components and metabolic processes, the idea of the gut-liver axis must be supplemented with the AGM-liver network [2]. During the metabolism of food and xenobiotics (compounds of non-host origin that enter the gut with the diet or are produced by AGM), the host and its AGM coproduce a wide variety of small molecules, many of which are essential for communication between the host's microbial symbionts and organs. The liver is positioned downstream of the gut and is capable of producing a wide variety of chemicals that are important in controlling the function of distal organs [3]. Numerous biological processes are mediated by the AGM, which produces chemicals such bile acids, short chain fatty acids,

choline metabolites, indole derivatives, vitamins, polyamines, lipids, neurotransmitters, neuroactive substances, and hormones that regulate the hypothalamic-pituitary-adrenal axis [4]. The AGM only has a limited role in human physiology. It plays a crucial role in controlling the process of digestion because commensal bacteria produce, extract, and absorb a wide range of metabolites, such as lipids, vitamins, bile acids, and amino acids. Additionally, AGM can directly stop the colonization of invading bacteria by blocking their growth by stealing resources from other organisms and/or producing antimicrobial chemicals [5]. Studies on germ-free and conventional rodents as well as human volunteers have demonstrated for more than 40 years that the AGM is capable of synthesizing several vitamins, including vitamin K, as well as B group vitamins like biotin, cobalamin, folates, nicotinic acid, panthotenic acid, pyridoxine, riboflavin, and thiamine [6]. While the importance of these vitamins for bacterial metabolism is evident, some of these pathways also appear to have physiological and metabolic implications for mammals. For instance, prothrombin levels are low and hemorrhages occur in germ-free rats raised without a vitamin K dietary supplement, whereas prothrombin levels are normal and clotting activity is normal in conventional rats [7]. Moreover, human participants who had low-vitamin K diets for three to four weeks did not experience vitamin shortage; nevertheless, those who received a broad-spectrum antibiotic to suppress the microbiota experienced a noteworthy reduction in plasma prothrombin levels [8]. Recently, the AGM has using metagenomic sequencing to shed light on the mechanisms involved in vitamin production. investigated the metabolic potential of AGM sequences from two people and discovered that a range of clustered orthologous groups involved in the synthesis of deoxyxylulose-5-phosphate, a precursor to thiamine and pyridoxal, were represented in greater abundance in their microbiomes [9]. have methodically investigated the genomes of 256 common AGM to find evidence of the existence of the biosynthesis pathways for eight B vitamins: pyridoxine, riboflavin, biotin, cobalamin, folate, niacin, pantothenate, and thiamin. Because of this, the authors were able to estimate the percentage of each phylum that might generate a certain vitamin. There were genomes with all eight pathways and genomes without any. Niacin (162 producers) and riboflavin (166 potential producers) were the most frequently synthesized vitamins. A significantly lesser fraction of Firmicutes and Actinobacteria possessed the ability for vitamin B production, whereas nearly all microorganisms from the phyla Bacteroidetes, Fusobacteria, and Proteobacteria possessed the essential pathways for riboflavin and biotin. Compared to 10–50% of the other four phyla, 100% of the Fusobacteria in the instance of VB12 were projected to be producers. In general, the phylum with the highest number of projected manufacturers of B vitamins seemed to be Bacteroidetes. More than 90% of Bacteroidetes were expected to be producers, except VB12. The authors found some interesting pairs of animals whose patterns of vitamin production pathways complemented one another [10]. This suggests that AGM cross-feed each other, supplying vital vitamins for development. This implies that other bacteria that do not make vitamins use a significant amount of the vitamins created by microbes. Their availability to the host is limited by this use. The proportion of each vitamin's daily recommended intake for humans was calculated by the authors using data from the AGM. Out of the eight examined, the AGM were projected to contribute more than 25% of the recommended dietary requirement for four vitamins, excluding bacterial use [10]. Furthermore, research employing diverse human and animal colon preparations has demonstrated that the colonic epithelium may assimilate a variety of B vitamins, such as folate, riboflavin, biotin, niacin, and thiamine, through particular carrier-mediated pathways [11]. A class of water-soluble micronutrients that are mostly obtained from the regular diet make up vitamin B. They mediate several metabolic pathways in humans as cofactors. AGM has the ability to create, ingest, and even compete with the host for vitamin B as a key component of human health. The interaction between the host and AGM may be a significant component influencing VB12 absorption mechanisms. Conversely, deficiencies or overabundance of vitamin B may affect the development of certain bacteria, changing the makeup and functionality of AGM. When combined, vitamin B and AGM may have a systemic positive impact on human health [12]. VB12 is either produced or consumed by the gut bacteria. Furthermore, intestinal flora may have an impact on the intestinal absorption of VB12 [10]. Several bacteria have been reported to be VB12 producers, such as *Lactobacillus reuteri* (*L. reuteri*) and *Enterococcus faecium* [13, 14]. Supplementing with bacteria that produce vitamin B12 is thought to

enhance the gastrointestinal tract's absorption of the vitamin. A hypothesis of this kind has been validated in mice given diets lacking in VB12. The symptoms of VB12 insufficiency were avoided by supplementing with L. reuteri CRL1098—a strain that produces VB12—which suggests that intestinal bacteria may have a therapeutic role in VB12 deficiency [15]. These advantageous benefits, however, might be limited if the bacteria populate the colon, where there aren't enough transporters. It is important to take into account the position of bacterial colonization while creating a probiotic treatment for VB12 insufficiency. Approximately 80% of the gut microbiota are thought to be VB12 users [16]. Therefore, an overabundance of bacteria may cause their host to compete with exogenous VB12, lowering the bioavailability [17]. In small intestinal bacterial overgrowth, consumption of VB12 by the increased anaerobesis was considered a major reason for VB12 deficient symptoms [18]. For VB12 insufficiency, lowering the number of bacteria that consume VB12 is beneficial. For example, a daily probiotic treatment of Lactobacillus showed improvement in bacterial overgrowth and VB12 absorption, indicating that the probiotic treatment may help with VB12 deficit by preventing the expansion of bacteria that consume VB12 [19, 20].

2. MATERIAL AND METHODS

- **Liver transplantation Patients and Sample collection**

The study population included 28 LT patients , In the period from March 2021 to March 2023. All patients were recruited from Gastroenterology Surgical Center (GEC), Mansoura University, Egypt. All individuals provided informed consent and were fully informed about the diagnostic methods involved and the nature of the condition. The study procedure followed the Helsinki Declaration 2013 ethical guidelines and was approved by the research ethics council of Faculty of Medicine, Mansoura University (IRB: MDP.23.08.131.R1). Before taking part in the study, all patients provided written informed consent. The stool and blood samples were collected from the 28 patients at two separate time periods: one week before LT (n=28) (1WBLT), one week after LT (n=28) (1WALT). A total of 112 samples were taken from all patients, divided between stool (n=56) and blood (n=56).

- **Detection of gut microbiota**

Stool samples were collected for detection of AGM, All specimens were transported to the microbiology laboratory in 5 ml sterile Amies transport media [21] at 4^{o}C within 2 hours. Following that cultivated on Columbia chocolate agar base medium [22] and nutritional agar [23]. AnaeroPack (disposable oxygen absorbing and carbon dioxide generating agent) for use in anaerobic jar to support anaerobic bacteria microflora cultivation and inhibit aerobic and pathogenic bacteria. VITEK 2 compact 15 (Biomerieux, France) was used to identify the isolated bacteria visually and biochemically in GEC.

- **Estimation of Vitamin B12 (VB12) Levels in The Blood Samples [24].**

The levels of VB12 in human serum were detected by Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI 800. Competitive immunoluminometric assay: Label ABEI with purified VB12 antigen and FITC with a VB12 binding protein. After thoroughly mixing the sample, calibrator, or control with ABEI label, FITC label, and nano magnetic microbeads coated with sheep anti-FITC, they are incubated at 37°C to form antibody-antigen complexes. The supernatant is then decanted, followed by a one-time cycle of washing. The addition of the starter reagents follows, which starts a flash chemiluminescent reaction. A photomultiplier measures the light signal as RLU in 3 sec, and it is proportional to the amount of VB12 that is contained in the samples. Normal range were: 200-800pg/ml, VB-12 deficiency<200 pg/ml**.**

- **Statistical analysis:**

Every single measurable investigation were performed by Statistical Package for the Social Sciences (SPSS) programming adaptation 15.0 (SPSS Inc., Chicago, IL) and GraphPad Prism bundle; v.8.0

(GraphPad Software, San Diego, CA). Ceaseless factors were communicated as mean±standard deviation [10]. An estimation of $P<0.05$ was considered statistically critical.

3. RESULTS AND DISCUSION

- **Detection of anaerobic gut microbiota in liver transplantation patients**

The objective of this study was to identify the composition of the anaerobic gut microbiota and changes in their composition in patients who had received a liver transplant. A total of 56 fecal specimens obtained from 28 patients with LT at two distinct time points were utilized for the isolation and identification of anaerobic bacteria. The samples were categorized into two distinct groups and organized according to the time period preceding and following the LT surgery. In the present study, we obtained a total of 56 stool samples, anaerobic bacteria were all significantly lower in LT recipients. Were significantly higher isolated bacteria one week before LT (1WBLT) 43 isolates with percentage (67.18%) including, *Bacteroides akkermansia* 6 (%), *Bacteroides stercoris* 4 (%), *Bacteroides melaninogenicus* 2 (%), *Fusobacterium mortiferum* 7 (%), *Clostridium difficile* 5 (%), *Ruminococcaceae* 7 (%), *Peptococcus* 5 (%), *Faecalibacterium prausnitzii* 4 (%), *Bifidobacterium breve* 3 (%). The distribution of the dominating bacterial isolates were changed among the 1 Week After Liver Transplantation (1WALT), Total isolated anaerobic bacteria were 21 (32.82%) including *Bacteroides akkermansia* 4 (%), *Bacteroides stercoris* 2 (%), *Bacteroides melaninogenicus* 1 (%), *Fusobacterium mortiferum* 4 (%), *Clostridium difficile* 2 (%), *Ruminococcaceae* 4 (%), *Peptococcus* 1 (%), *Faecalibacterium prausnitzii* 1 (%), *Bifidobacterium breve* 2 (%). Our results agreed with [25], who reported that while Enterobacteriaceae and *Enterococcus spp*. were significantly higher in LT recipients, anaerobic bacteria, *Bifidobacterium spp*., *Faecalibacterium prausnitzii*, and *Lactobacillus spp*. were all significantly lower. Other bacteria, with the exception of *Enterococcus* species, have demonstrated a propensity to gradually return to normal levels following LT. Additionally, [26], found that in general the majority of infections following LT are primarily caused by bacteria belonging to the Gram-negative spectrum. The most significant Gram-negative germs include *E. coli*, *Klebsiella spp*., and other Enterobacteriaceae, while the most common Gram positive germs are *Enterococcus spp*. and *Staphylococcus spp*. Furthermore, it was demonstrated by [27], that patients experienced dysbiosis of the AGM following surgery. Enterobacteriaceae in particular, which are facultative anaerobe blooms, are linked to inflammatory gut conditions. However, the majority of the colon is anaerobic in a healthy state, and the obligate anaerobes there get their energy from the fermentation of carbohydrates and amino acids. Furthermore, [28] shown that following LT, there is less *Bifidobacterium* and *Lactobacillus* in the stool. Also, [29], reported that the predominant pathogens found in infections related to lung transplantation are particularly *Enterococcus spp*. As well, [30], demonstrated a correlation between post-transplant diarrhea and a decline in diversity in the fecal microbial communities of patients following LT. Additionally, [27] found that the LT recipients had significantly higher Enterobacteriaceae and *Enterococcus spp*. ($P<0.05$) and significantly lower beneficial *Bifidobacterium spp*., *Lactobacillus spp*., and *Faecalibacterum prausnitzii* (all members of the Firmicutes phylum). Furthermore, [25] discovered that all *Eubacteria*, *Bifidobacterium* spp., *Faecalibacterium prausnitzii*, and *Lactobacillus* spp. were significantly reduced in LT recipients. As well, [31] who found an increase in Enterobacteriaceae and Enterococcaceae species, a decrease in *Faecalibacterium prausnitzii* and Bacteroides, and a decrease in the overall variety of gut microbiota following LT.

- **Estimation of Vitamin B12 (VB12) Levels in The Blood Samples.**

The 56 blood samples were classified into two groups: the first group consisted of LT patients through out one week before LT, the second group consisted of LT patients through out one week after LT. The levels of VB12 in the blood samples of LT patients were estimated and the findings are presented in Table (1). In general, the VB12 values decreased in the samples collected one week after surgery compared to the other time periods. The changes in the levels of VB12 was statistically significant ($P=0.001$) in LT. Our findings concurred with those of [32] who found that the mean blood VB12 level

dropped (26%) after LT, from 692±220 pmol/L to 508±248 pmol/L (P=0.002). Furthermore, [13] demonstrated that a number of bacteria, including *L. reuteri* and *Enterococcus faecium*, have been identified as producers of VB12. However, [33] found that increased haptocorrin production was the primary cause of the rise in circulating VB12 levels. Increases in circulating VB12 can also be a sign of several liver diseases, including cirrhosis, hepatocellular carcinoma, acute viral hepatitis, and metastatic liver disease. As well, [34] showed that upon admission, the VB12 levels of patients with acute-on-chronic liver failure were significantly higher than those of healthy controls (P<0.001). A higher 3-month mortality rate and more severe liver disease were linked to elevated VB12 levels. Vitamin B12 levels and the end-stage liver disease score model were found to be independent predictors of mortality (P<0.001) through multivariate analysis. Also, [32] It was discovered that the blood VB12 level in the pre-liver transplant patients had increased highly significantly above the upper limit of normal, and the blood VB12 level in the post-liver transplant patients had significantly decreased, with a P-value of 0.039. Finally, we recommend using molecular techniques in future research to accurately identify and analyze the gut microbiome (e.g., RNA-Seq or qPCR) and confirm a causal relationship between the changes in anaerobic gut microbiota and decreased VB12 levels.

4. CONCLUSION

After liver transplant anaerobic bacteria such as *Bifidobacterium spp*., *Faecalibacterium prausnitzii*, and *Bacteroides akkermansia*. were all significantly lower. Anaerobic Gut microbiota can produce a diverse range of compounds such as VB12 that play a major role in regulating the activity of distal organs. Blood VB12 level has been decreased earlier than other routine liver markers in post-LT when alteration in composition of AGM. The blood VB12 level was normal in the pre-LT patients and a significant decrease in the post-LT patients with a P-value of 0.001. To improve the production of VB12 should promote an improved microbial VB12 production.

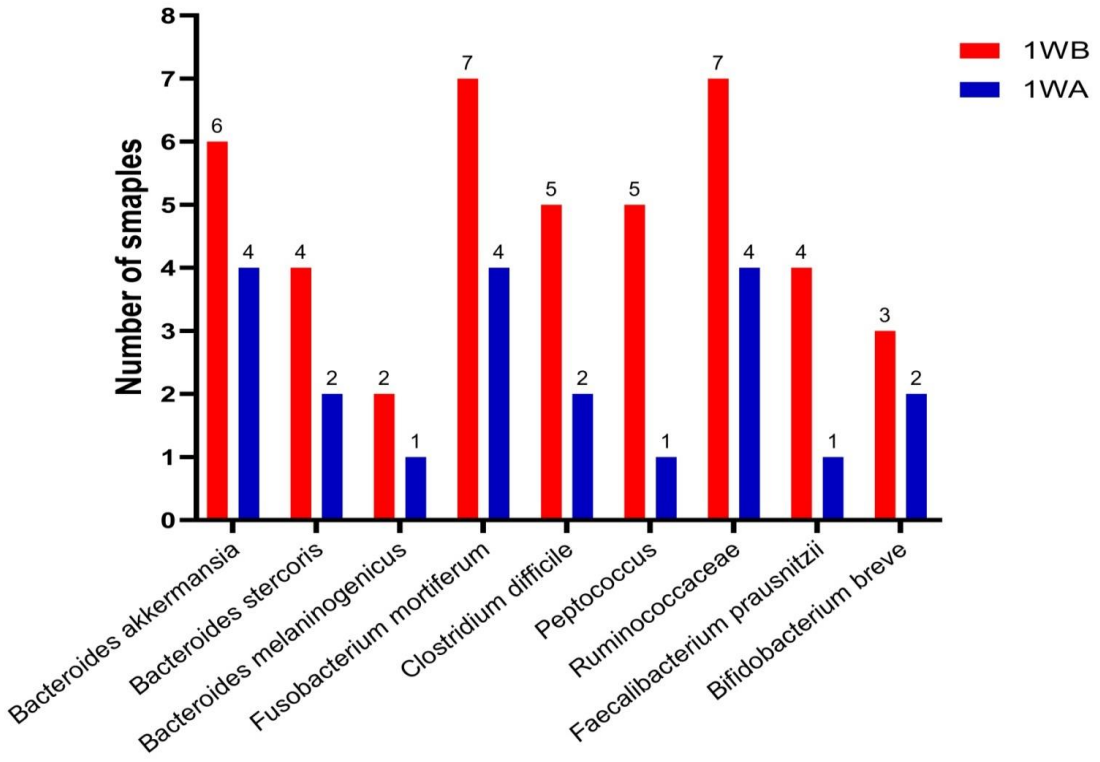

Fig. 1. Isolated anaerobic bacteria from stool cultures of LT patients.

bioMérieux Customer:
System #: 21674
Patient Name: Dr Taher, Almahalla
Isolate: 352022-1 (Approved)
Card Type: ANC Bar Code: 2441816103343917 Testing Instrument: 00001A0FE742 (21674)
Setup Technologist: vitek2c 2(vitek2c2)

Laboratory Report

Printed by: vitek
Patient ID: 352

Bionumber: 0200000000001
Organism Quantity:

Selected Organism: Clostridium difficile

Comments:	

Identification Information	**Card:**	ANC	**Lot Number:**	2441816103	**Expires:**	Nov 16, 2022 12:00 CST
	Status:	Final	**Analysis Time:**	5.80 hours	**Completed:**	May 3, 2020 21:02 CDT
Organism Origin	VITEK 2					
Selected Organism	**Clostridium difficile** Bionumber: 0200000000001 Confidence: Low discrimination					

Analysis Organisms and Tests to Separate:

Low Discrimination Organism

Paraclostridium bifermentans	LECITHIN (100),
Clostridium difficile	LECITHIN (0),
Clostridium group	
Clostridium novyi	LECITHIN (90),
Clostridium innocuum	LECITHIN (10),
Clostridium subterminale	LECITHIN (10),
Hathewaya limosa	LECITHIN (90),
Clostridium sporogenes	LECITHIN (10),

Analysis Messages:

Contraindicating Typical Biopattern(s)

Biochemical Details

4	dGAL	-	5	LeuA	-	6	ELLM	-	7	PheA	-	8	ProA	+	10	PyrA	-
11	dCEL	-	13	TyrA	-	15	APPA	-	18	dGLU	-	20	dMNE	-	22	dMAL	-
28	SAC	-	30	ARB	-	33	NAG	-	34	BGLUi	-	36	URE	-	37	BGURi	-
39	BGALi	-	41	AARA	-	42	AGALi	-	43	BMAN	-	44	ARG	-	45	PVATE	-
51	MTE	-	53	ESC	-	54	BdFUC	-	55	BNAGi	-	56	AMANi	-	57	AIFUC	-
59	PHOS	-	60	IARA	-	61	dRIB2	-	62	OPS	-	63	AARAF	-	64	dXYL	-
	GRAM	+		MORPH	-		AERO	-									

Installed VITEK 2 Systems Version: 9.02
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AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

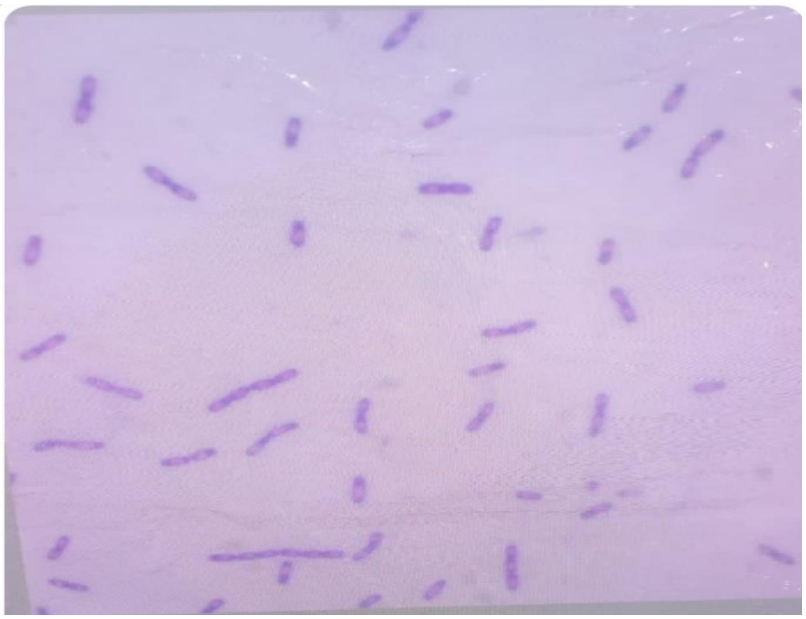

Fig. 2. Identification *Clostridium difficile* by VITEK 2.

Table 1. Estimation of Vitamin B12 (VB12) Levels in The Blood Samples.

	VB12 Levels (pg/ml)	
	1WBLT (n=28)	**1WALT (n=28)**
Mean±SD	513.3±185.75	362.6±105.2
Min-Max	245.4 - 831.2	111.0 – 578.0
***P*-value**	0.001	

1WBLT mean one week before liver transplant. & 1WALT mean one week after liver transplant.

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