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Study gut microbiome 16S rRNA gene versus gut microbiota in HCV patients

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Abstract

Background &Objectives: Still there is percentage of HCV patients not responding to Direct Acting Antiviral Agents (DAAS). Liver can be greatly affected by changes in gut Microbiome, microbiota. The study was done to evaluate the association between gut microbiome versus microbiota in HCV patients and the response to DAAS .**Subjects and Methods:** Thirty HCV patients aged from20-60years old ,both males and femals; group 1 (No=15 of HCV responders and group 2 (No=15 relapser HCV patients) treated by DAAS according to the treatment protocol of the Egyptian National Committee for Control of Viral Hepatitis (NCCVH).Group 3 healthy control subjects (No=15) . Full history taking, clinical examination and all investigations were done plus stool culture by **Vitek 2**and **16srRNA gene** amplification and sequencing were done as per Clinilab Maadi ,Cairo .Statistical analysis were done after tabulation of the results ,$p<0.05$ considered significant. **Results:** 16SrRNA gene microbiome was of statistically significant decrease in (6) micrbiota in non-responders and increased in (2) .Microbiota compared to responder group (with P value: ranged from 0.06 to 0.013) Microbiota which increased in non-responder group than responder group (with P value: ranged from 0.002 to 0.003).The number of microbiota by culture is completely defected and different between the groups compared to 16SrRNA gene for microbiome.. **Conclusion:** Microbiome more accurate and specific than stool culture for detection of microbiota

Keywords: *Microbiota and 16SrRNA gene for Microbiome.*

1-Introduction

Hepatitis C virus (HCV) infection represents an important global cause of chronic hepatitis, cirrhosis and HCC **[1]**. The human gastrointestinal tract contains millions of microorganisms with up to 2000 different species of bacteria **[2]**. The gut flora begin to colonize shortly after birth and plays an important role in keeping the individual healthy by digestion improvement, vitamin production, bile acids generation, and modulation of immune mechanisms of the host **[3]**. Many factors, including diet, drugs, illness, stress, and lifestyle,

affect the structure of community of gut microbiota and microflora, which has a rule in health and diseases **[4]**. In recent years, the relationship between the gut microbiota and the liver has been studied which is described as 'gut-liver axis' **[5].** Many studies documented the involvement of intestinal microflora in alcoholic liver disease (ALD) and non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma (HCC) **[6]**. However, little is known about the relation between hepatic viruses and, especially hepatitis C virus infection and intestinal microflora. especially at Egypt.

2- Subjects and Methods

This is cross sectional study conducted on 30 HCVpatients(responders and relapser) from Benha University Hospitals and 15 healthy subjects as a control group. All patients are adults aging between 20 and 60 years old. Subjects included in this study were classified into the following groups: **Group I**: This group included 15 patients with chronic hepatitis C who achieved SVR.**Group II**: This group included 15 patients with chronic hepatitis C who achieved not SVR. **Group III**: Thi s group included 15 apparently healthy subjects served as control group.

Exclusion criteria: Other all diseases of the liver excuded accordingly by investigations and all percuations for the examination of microbiome.

Inclusion criteria:

1- As per Egyptian National Committee for Control of Viral Hepatitis (NCCVH).

2-Stool culture for Microbita by Vitek 2: VITEK 2 is an automatic system for the identification and susceptibility testing of the most clinically important bacteria.VITEK 2 system can detect more than 90% of gram-positive and gram negative bacilli within 3 hours [7].

1) Sample collection and DNA extraction:-

Stool samples were collected in sterile containers from patients and healthy controls. Stool specimens were processed upon receipt in the microbiology laboratory on the date of collection.

Organism identification:

First day:Routine bacterial stool cultures

Second day:Culture media were examined for bacterial growth.

1- Vitak 2

2) 16S rDNA Identification:

- Isolate genomic DNA from samples:-
- Dilute genomic DNA for PCR:- Vitak 2 for culture stool culture

III. Prepare the PCR reactions

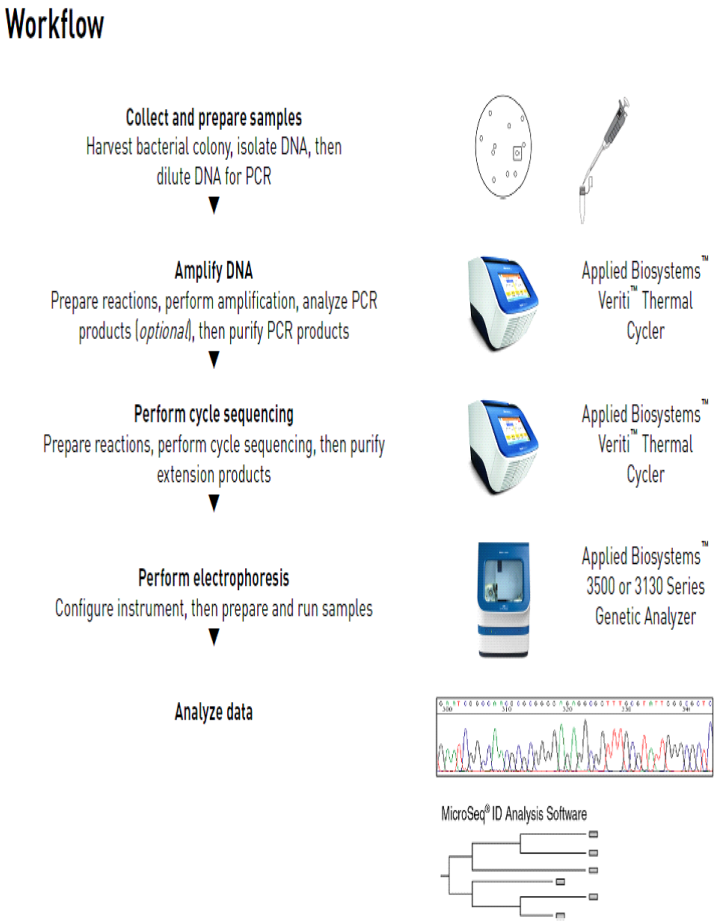
IV. Perform the amplification run:-

VIII. Perform the cycle sequencing run:-

IX. Purify extension products:-

X. Configure the instrument for electrophoresis:-

XI. Prepare samples and perform electrophoresis:-



3- Statistical Analysis

Data management

The clinical data were recorded in a report form. These data were tabulated and analyzed using the computer program SPSS (Statistical Package for Social Science) version 20 to obtain [**8**]

Descriptive data

Descriptive statistics were calculated for the data in the form of:

1) Mean standard deviation (+ SD) Median and inter-quartile range (IQR) for quantitative data.

2) Frequency and distribution for quantitative data.

Analytical statistics

In the statistical comparison between the different groups, the significance difference was tested using one of the following tests:1) Student's t-test and Mann-Whitney test: Used to compare mean of two groups of quantitative data of parametric and non-parametric respectively. 2) ANOVA A test (F value) and Kruskal-Wallis test: Used to compare mean of more than two groups of quantitative data of parametric and non-parametric respectively. 3) Inter-group comparison of categorical data was performed by using Chi square (X2-value) and Fisher's exact test (FET). The statistical analysis was conducted using STATA/SE version 11.2 for Windows (STATA corporation, College Station, Texas).

$$x^2 = \frac{\sum (observed - \exp ected)^2}{Expected} \qquad Expected = \frac{col.total\ x\ row total}{Grand\ total}$$

P value< 0.05 was considered statistically significant (*) while >0.05 statistically insignificant. P value< 0.01 was considered highly significant (**) in all analyses.

4-Results

Table (1) show the Microbiome 16SrRNAgene among the studied groups

Group I : No statistical significant difference between Microbiota in all groups Table (1), Fig. (1).	
1- Escherichia coli	5- Staph aureus
2- Proteus mirablis	6- Bacteroides fragilis
3- Klebsiela pneumonia	7- Clostridium perfringens-3
4- Enterobacter	8- Enterococcus faecium
Group II : Microbiota which decreased in non-responder group than responder group	
1- Lactobacillus brevis strain	P value: 0.013*
2- Pediococcus pentosace	P value: 0.014*
3- Clostridium tetani	P value: 0.006*
4- Shigella flexneri	P value*0.001 > :
5- Shigella dysenteriae	P value: 0.008*
6- Shigella sonnei	P value: 0.026*
Group III : Microbiota which increased in non-responder group than responder group	
1- Pseudomonas aeruginosa	P value: 0.003*
2- Streptococci	P value: 0.002****
Group IV : Microbiota which increased in responder group than non-responder and control groups	
1- Enterobacter hormaechei	P value: 0.013*
2- Enterococcus fecalis	P value: 0.01****
Group V : Microbiota which decreased in responder group than non-responder and control groups	
1- Shigella boydii	P value: 0.001*
2- Enterococcus durans	P value: 0.002****

There is a statistical significant decrease for(6 (microbiome in **non responder** patient **Group II ,However** there was increase for (2 (microbiome in non responders compared to the responders patients **Group III .**In **Group IV and Group V** Responders , there was (2 (microbiota increased and decreased compared to control and non responders,Pvalue ranged from0.013 to 0.002 0.002&to0.001 respectively. In **Group I** No statistical significant between the 3 groups with (8 (microbiota

Table (2) show the microbiota study by Vitek2

Group I: Bacterial species with no statistically significant difference between all studied groups

Bacterial species
Escherichia coli
Enterococcus faecalis
Bacteroides fragilis
Staphylococcus aureus
Clostridium tetani
Pseudomonas aeruginosa

Group II: Increased species in responders than control

Bacterial species	*P* value
Klebsiella	0.04

Group III: Increased species in non responders than responders

Bacterial species	*P* value
Enterobacter	0.05

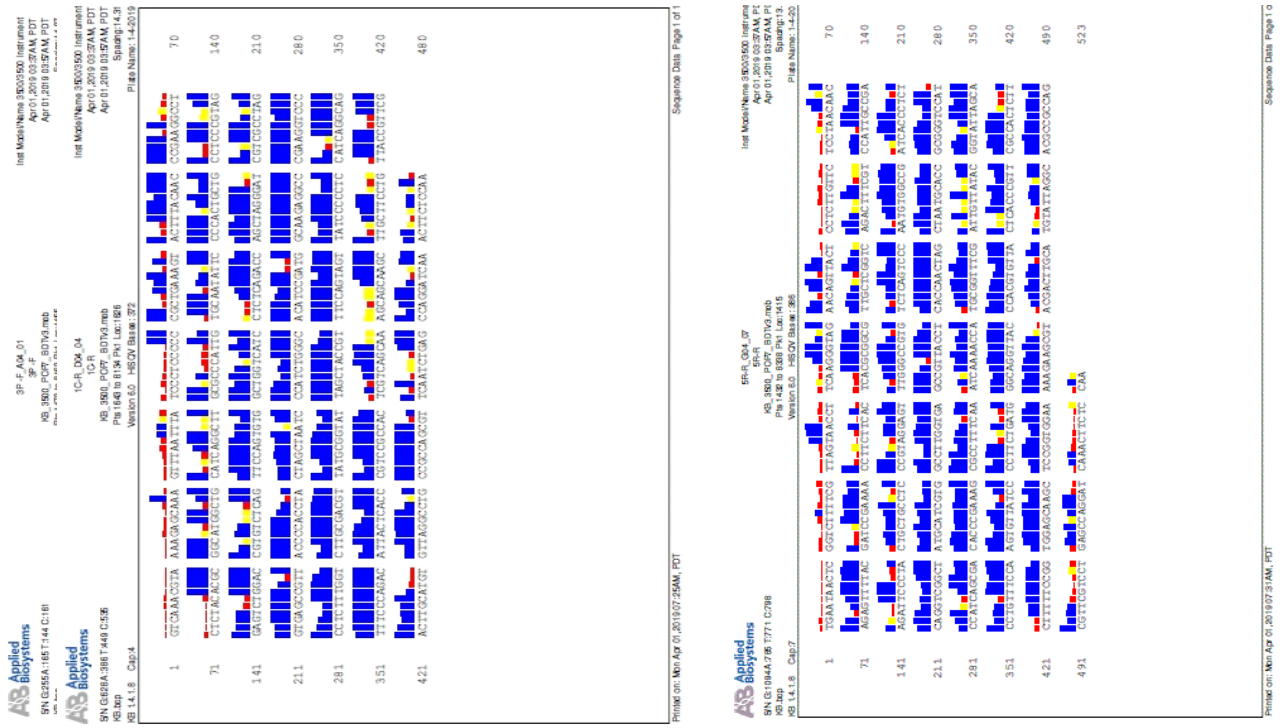
Group IV: Increased species in non responders than control

Bacterial species	*P* value
Enterobacter	<0.001
Proteus mirabilis	0.009
Klebsiella	0.01
Streptococci	0.007

Group V: Decreased species in non responders than control

Bacterial species	*P* value
Clostridium perfringens	0.04

In group 1here was no statistical significant between the 3 groups .Statistical significant for klebsiella speceies increased in group 2 responders than control.while there was 4 microbiota increased in non responders than control.Also there was decreased in Clostridium in non responders ,p value ranged from 0.04 to 0.001.



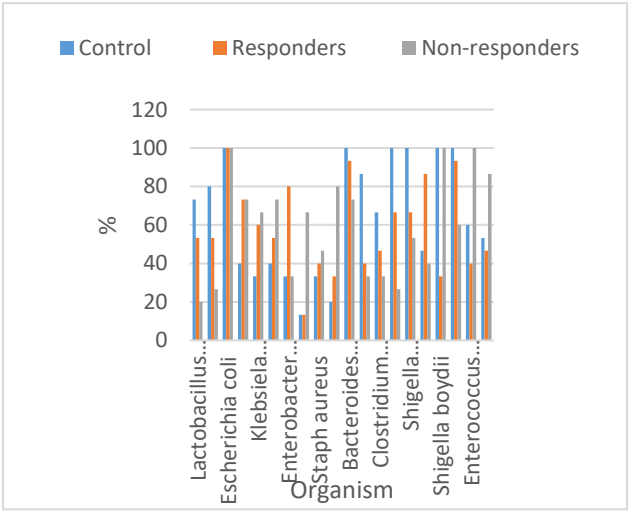


Fig. (1): Results of 16s rRNA gene sequencing in all studied groups.

5- Discussion

Emerging data suggest a strict interaction between the gut microbiota, health, and disease [9]. The gut microbiota of the hepatic patients is affected by diet, nutritional status, alcohol intake, impaired metabolism of bile acids, delayed gastrointestinal motility and use of antibiotics [10]. There are no significant differences between the responders and non-responders groups for all laboratory investigations.

The study revealed that there is no difference between all studied groups as regards Bacteroides fragilis being present in the stool samples of 100% of all subjects. This finding was in agreement with Patricia and colleagues (2019) who reported 100% incidence in both responders and non-responders [10].(table 1,Fig1,2)

In the present study a higher abundance of Enterobacter in HCV-infected patients. This is agree with Bajaj (2016) who showed a peculiar gut microbiota composition in the chronic hepatitis C patients when compared with the healthy subjects including main families of Enterobacteriaceae, Clostridiales, and Lachnospiraceae [8].Concerning Clostridiales, our study showed that Clostridium perfringens were more in healthy subjects .In this regard, Aly and partners (2016) stated that the healthy subjects have shown the abundance of Clostridium genus [11].the difference between the methods of detection of microbiota by 16SrRNA is more accurate and valuable more than stool cuture by Vitek 2 (Table1,2, Fig.1,2) Till now this is the first done about microbiome in HCV patients responders and non responders compared to the control at all there direction for more profile of gut microbiota.

6-Conclusion

Gut microbiota have a crucial role in HCV patients especially the non-responders compared to the control even the responders need to be followed up to adjust the gut flora of them to the normal because this proved to play an important role in micro environmental changes that lead the developments of pathological process to hepatocellular carcinoma (HCC) at the level of Toll receptor 4 of hepatocytes.

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Conflict of interest: The authors declare that they have no conflict of interest.

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