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ORIGINAL ARTICLE

STUDY OF BACTERIAL TRANSLOCATION MARKERS IN HEPATITIS C INFECTION PATIENTS AND IN LIVER CIRRHOSIS

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ABSTRACT

Background: Hepatitis C infection is an Increasing problem in Egypt. Bacterial translocation is defined as the migration of live microorganisms or bacterial endotoxins from the intestinal lumen to the mesenteric lymph nodes and extraintestinal sites. **The aim** was to measure bacterial translocation markers namely bacterial DNA and endotoxin in HCV status and cirrhotic patients with correlation of the presence of bacterial markers of translocation and the viral status**. Methods:** This study was conducted on 81 inpatients clinically suffering from liver cirrhosis. Patients were divided into three groups each group included 27 patients. First group patients with hepatitis c virus infection causing liver cirrhosis particulary child A and B groups. The second group included patients with HCV infection who treated with direct antiviral agents (DAA).The last group included patients with compensated liver cirrhosis with negative viral markers. All patients were subjected to laboratory investigations included: Hepatitis markers, CBC, Liver function tests, Kidney function tests. Bacterial DNA and Bacterial Endotoxin **Results:** 67.9% of cases were positive DNA for bacterial translocation. Endotoxin level and DNA results showed significant difference between the three groups as active hepatitis c had highest level of serum endotoxin and all were positive DNA. There was statistically significant difference between DNA positive and DNA negative patients with positive patients had higher median serum endotoxin level 43 Vs 2 in negative patients. **Conclusion:** Bacterial translocation markers (bacterial DNA and endotoxin) are high in cirrhotic and hepatitis c virus infected patients

Key words: bacterial translocation- bacterial DNA- endotoxin- Hepatitis c-cirrhosis.

INTRODUCTION:

Patients with liver cirrhosis and hepatitis C are an increasing problem in Egypt, in terms of social, economic and health care costs. Bacterial infections are frequent in cirrhosis and represent the most common cause of hospitalization. Spontaneous bacterial peritonitis (SBP) and spontaneous blood Stream infections (BSI) represent the most harmful infections in cirrhosis [(1)].

Pathological bacterial translocation (BT) from the gastrointestinal lumen to the mesenteric lymph nodes and into the systemic circulation is a frequent phenomenon, which results in detectable fragments of bacterial DNA(bactDNA) circulating in approximately

30–40% of cirrhotic patients even in the absence of infection [2].

Bacterial translocation is defined as the migration of live microorganisms or bacterial endotoxins (e.g. Bacterial lipopolysaccharide [LPS], peptidoglycan, lipopeptide) from the intestinal lumen to the mesenteric lymph nodes and extraintestinal sites [3].

The most common bacteria involved in bacterial translocation are derived from the family of Enterobacteriaceae (*Escherichia coli* [*E. Coli*], *Klebsiella spp*, etc.), *Enterococci* and *Streptococci spp*., while species of anaerobic microorganisms are rarely responsible for bacterial translocation [4].

It has been shown that bacterial translocation is related to the stage of liver failure and is more prominent in advanced liver disease as estimated by the Child-Pugh score [5]. Moreover, it is more frequent in experimental models with ascites than in those without**.** [6]

One of the countries that was most affected by hepatitis C virus (HCV) is Egypt. According to the data of the Egyptian Health Issues Survey (EIHS), 14.7% of the people aged 15–59 years had an active hepatitis infection in 2009 (7), which decreased to 7% in 2015 and was substantially higher than global levels .To face this challenge, Egypt developed a national strategy for HCV control and established HCV prevention and treatment programs. This strategy covers six main components of prevention and control: surveillance, infection control, improving blood safety, hepatitis B vaccination, health education to providers and communities, and care and treatment.(8)

The prevalence of HCV was 8.7%–40.3% before the national HCV treatment was implemented .Although a lower rate of HCV prevalence (4.6%) was recently reported in a large-scale national study conducted during 2018–2019 (15), a higher rate of HCV prevalence (14.5%–25.9%) was reported by 3 past studies conducted during 2015–2017. The sharp decline in the rate of HCV prevalence by Waked et al. may be explained by the fact that the majority (66.3%) of the included patients in their study were of age <45 years. Indeed, as per some other studies, patients aged >60 years were the most affected by the HCV epidemic in Egypt. (9)

The study **aimed** at Measuring bacterial translocation markers namely bacterial DNA and endotoxin in HCV status and cirrhotic patients with correlation of the presence of bacterial markers of translocation and the viral status**.**

PATIENTS AND METHODS

Study design and settings

Type of study:cross section study.

This study has been conducted in the Internal Medicine Department and the Microbiology and Immunology Department, Faculty of Medicine, Zagazig University during the period from August 2018 to November 2020.

Study participants

This study has been conducted on 81 inpatients clinically suffering from liver cirrhosis in Zagazig University Hospitals. The patient group included 35 females and 46 males and their ages ranged from 20 to 65 years. Patients were divided into three groups each group included 27 patients. First group include patients with hepatitis C virus infection causing liver cirrhosis particularly Child A and B groups. The second group include patient with HCV infection who treated with direct antiviral agents (DAA). The last group include patients with liver cirrhosis Child A and B with negative viral markers.

Inclusion criteria

Age 18-65 years old**.** The patient enrolled in each group were thoroughly selected so that they were matched for age, sex and all other clinical variables to nullify the confounding effect of those variables on bacterial translocation markers and were divided into three groups; HCV (Cirrhotic liver Child A and B) not treated, HCV (Cirrhotic liver Child A and B) treated, and Cirrhotic liver (Child A and B) with negative viral markers.

Exclusion criteria

Child C liver cirrhosis(because complication would affect results), infection (e.g. peritonitis), and hepatocellular carcinoma.

Ethical clearance

Informed consent was obtained from all subjects after taking the approval of the Institutional Review Board, Faculty of Medicine, Zagazig University. The work had been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Process

Full history taking including age, sex, symptoms like fever, abdominal pain and confusion. Presence of hepatic viral infection or not, receiving treatment or not.

Full clinical examination including general examination (pulse, blood pressure, temperature, appearance, pallor and yellowish coloration of sclera in some cases), and abdominal examination (ascites, scratch markers, spider niveai, sharp edge of liver, tenderness, hepatosplenomegally)

Routine investigations including hepatitis markers (HCV Ab ,PCR, and HBs Ag), liver function tests (SGPT, SGOT), kidney function tests, CBC, abdominal ultrasound (search for cirrhosis and ascites), and Child-Pugh classing and scoring were done.

Specific investigations including detection of bacterial lipopolysaccharide (endotoxin) in the serum by Enzyme linked immunosorbent assay (ELISA) and detection of bacterial DNA in blood by polymerase chain reaction (PCR).

Methods:

Specimen collection: Five mL blood was withdrawn from every patient enrolled in the study,2 mL was added to a plain serum separator tube, allowed to clot for 30 minutes, and serum was then separated and kept at -20°C till used for detection of bacterial endotoxin by ELISA via **Human endotoxin ELISA kit (SunRed, China)**.the another 3 mL was added to EDTA tube, mixed well, and kept in the refrigerator at 2-8°C till used for PCR for detection of bacterial DNA via **first DNA extraction** using QIAamp DNA minikit **(Quiagen)**,Lysozyme **(Sigma Aldrich)**.**then DNA amplification via** Taq PCR Master Mix **(Quiagen)** 2x concentrated. It Contains 2.5 U of Taq DNA Polymerase, Qiagen PCR Buffer (with 3 mM MgCl2), and 400 µM of each dNTP. It was stored at - 20°C.

Finally,Detection of the amplified products as follow: Electrophoresis buffer: 50 x Tris acetate EDTA buffer "TAE". It consists of 2 M Tris-HCl, 0.05 M EDTA adjusted to pH 8.0 with glacial acid and made up to 1 liter with distilled water. Dilution of the 50X to give 1X by distilled water was done and the buffer was stored in a capped bottle at room temperature · Agarose **(Boehringer Mannheim, Germany),** Ethidium bromide (10 mg /ml) stored at room temperature in dark bottle , DNA Molecular weight marker **(Quiagen)** ranging in size from (100-1000 bp) andUV transilluminator **(Cole-Parmer, USA).**

Statistical Analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 24. Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies and were compared using Chi square test and fisher exact test when appropriate. Kolmogorov-Smirnov (distribution-type) tests were used to verify assumptions for use in parametric tests. To compare continuous quantitative data of two groups, Mann whitney test (for non-normally distributed data) and independent sample t test (for normally distributed data) were used. The level statistical significance was set at 5% (P<0.05).*P value >0.05 is non-significant (N-S)*

RESULTS

As shown in **table (1)** , the mean age of the studied participants was 51.296±9.83 years ,(56.8%) of them were males. The mean pulse rate was 76.728±6.44 ,the mean systolic blood pressure112.35±11.540 and diastolic blood pressure 72.72±6.89,16% of cases suffer from jaundice while mild ascites was reported in 14.8% of cases. Only (3.7%) of patients were confused. The majority of patients (70.4%) were class a.

The mean values of SGPT and SGOT were 25.619±6.609 and 33.502±7.08 respectively **(Table 2)**.

As shown in **table(3)** , 67.9% of cases were positive DNA for bacterial translocation with mean serum endotoxin 32.291±5.44.

As shown in **table (4)**, there was **statistically** significant difference between the three groups regarding systolic blood pressure with hepatic patients with positive HCV infection had the lowest blood pressure ($p<0.05$) and no statistically significant difference between the three groups regarding Diastolic blood pressure.

As shown in **table(5)** ,there was statistically significant difference between the three groups regarding serum endotoxin level and DNA results for bacterial endotoxin with patients with active hepatitis C had highest level of serum endotoxin and all were positive DNA .

Also in **table (6),**there was statistically significant difference between DNA positive and DNA negative patients with positive patients had lower systolic blood pressure ($p<0.05$). And no statistically significant difference between DNA positive and DNA negative regarding Diastolic blood pressure.

There was statistically significant difference between DNA positive and DNA negative patients regarding SGOT, SGPT**.** There was statistically significant difference between DNA positive and DNA negative patients with positive patients had higher Mean± SD serum endotoxin level 47.14±12.775 Vs 3.25±1.6 in negative patients. **(Table 7)**.

Table (1): Demographic and clinical characteristics of the studied group (n=81)

Variable	Value	
Age (years):		
Mean± SD	51.296±9.831	
Range	20/65	
pulse (beat/min):		
Mean± SD	76.728±6.444	
Range	(65-100)	
Temperature		
Mean± SD	36.812±0.224	
Range	(36.50-37.20)	
Systolic blood pressure		
Mean± SD	112.35±11.540	
Range	(90-140)	
Diastolic blood pressure		
Mean± SD	72.72±6.894	
Range	(60-90)	
Variable	**No**	**%**
Sex:		
Male	46	56.8
Female	35	43.2
Jaundice		
No	68	84.0
Yes	13	16.0
Ascites		
No	68	84.0
Mild	12	14.8
Moderate	1	1.2
Abdominal pain		
No	64	79.0
Yes	17	21.0

Variable	Value	
Confusion		
No	78	96.3
Yes	3	3.7
Child class		
Child a	57	70.4
Child b	24	29.6

Table (3): Bacterial translocation in the studied group (n=81)

Variable	Value	
Serum endotoxin:		
Mean± SD	32.291±45.44	
Range	4.20-154.70	
Variable	**No**	**%**
DNA result:		
Negative	26	32.1
Positive	55	67.9

Table (5): Comparing Bacterial translocation between the studied groups (n=81)

Variable	positive HCV without treatment (n=27)		Patients with treated HCV (n=27)		No previous HCV infection (n=27)		P value
Serum endotoxin:							
Mean± SD	51.140±52.775		37.474±40.702		8.15±1.700		
Median (IQR)	42 (33-45)		30 (22-38)		7 (3.4-8.2)		**0.001***
Variable	**No**	**%**	**No**	**%**	**No**	**%**	
DNA result@							
Negative	0	0	7	26.9	19	73.1	**<0.001****
Positive	27	49.1	20	36.4	8	14.5	

Table (6): Comparing vital signs and clinical characteristics between DNA positive and DNA negative patients for bacterial translocation

Variable	DNA +ve (n=55)	DNA –ve (n=26)	P value
Systolic blood pressure Mean± SD	107.04±11.373	115.81±11.222	**0.022***
Diastolic blood pressure Mean± SD	70.74±6.752	72.44±8.473	0.339

Table (7): Laboratory results between DNA positive and DNA negative patients for bacterial translocation

Variable	DNA +ve (n=55) Mean± SD	DNA –ve (n=26) Mean± SD	P value
SGPT			
Mean± SD	30.381±47.388	15.546±3.813	**<0.001****
Median (IQR)	20 (15.9-27.1)	14.5 (12-18)	
SGOT			
Mean± SD	36.974±29.723	26.157±4.718	0.082
Median (IQR)	29 (22-43.4)	27.5 (22-30)	
Serum endotoxin:			
Mean± SD	47.14±32.775	3.25±1.6	<**0.001****
Median (IQR)	43(12-55)	2(1-3)	

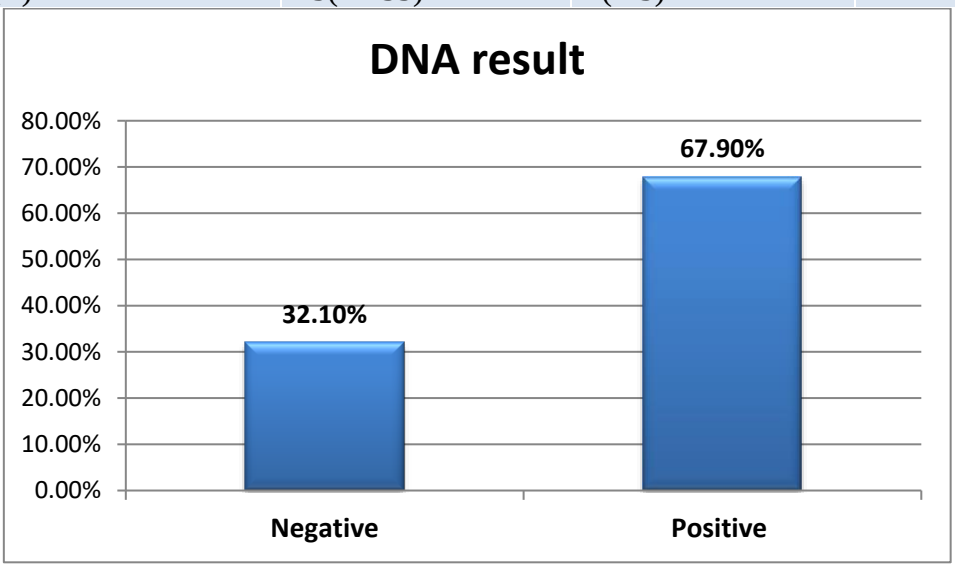

Figure (1): Bacterial translocation in the studied group (n=81) (DNA result)

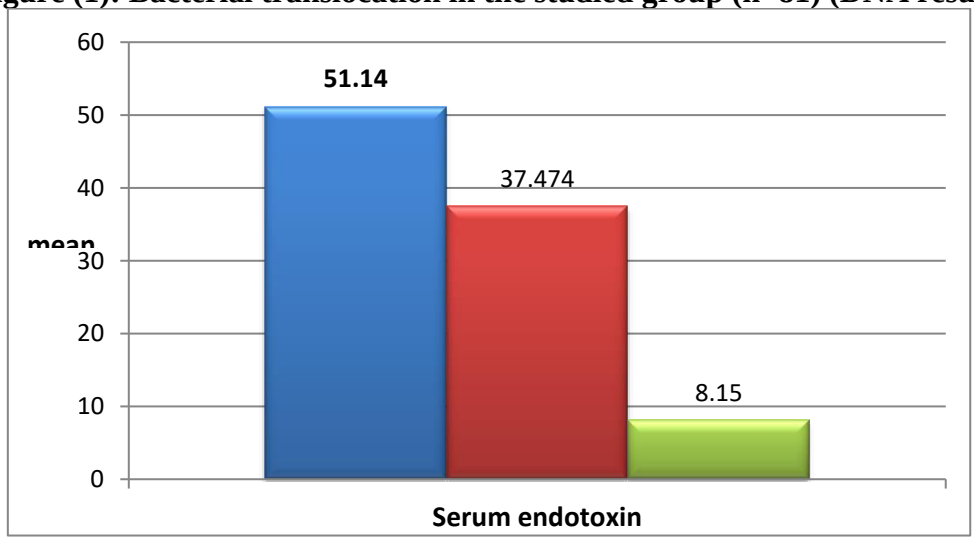

Figure (2): Serum endotoxin between the studied group.

positive hcv without treatment
Patient with treated hcv
No previos hcv infection

DISCUSSION

According to results of current study, Bacterial translocation markers (bacterial DNA and endotoxin) are high in cirrhotic and hepatitis c virus infected patients. Endotoxin level and DNA results showed significant difference between the three groups as hepatitis C without treatment had highest level of serum endotoxin and all were positive DNA. There was statistically significant difference between DNA positive and DNA negative patients with positive patients had higher median serum endotoxin level 43 Vs 2 in negative patients.

In this study the mean age of the studied participants was 51.296 ±9.83 years and (56.8%) of them were males. The mean pulse rate was 76.728±6.44 beat/minute with mean systolic blood pressure112.35±11.540 mmHg and diastolic blood pressure 72.72±6.89 mmHg. Also 16% of cases suffer from jaundice while mild ascites was reported in 14.8% of cases A. The majority of patients (70.4%) were class A (patients with hepatitis C virus infection causing liver cirrhosis).

These results agree with **Cirera et al.** [(5)] where the mean age of patients was 53 years and 56 % of them were males with mean systolic blood pressure 115± 10 mmHg and diastolic blood pressure 75. ±7.5 mmHg and around 40 % of patients were class A.

Our results showed mean values of SGPT and SGOT were 25.619±6.60 and 33.502±7.08 respectively.

This agrees with **Caro et al.** [(10)] study who showed Mean levels of SGPT and SGOT were 28.8±15.5 and 47.1±20.6

We found that 67.9% of cases were positive DNA for bacterial translocation with mean serum endotoxin 32.291±5.44.

Similar finding was previously reported by **Caradonna et al.** [(11)] as DNA positive percentage in HCV patients were 62%.

Mean serum endotoxin level was high also in HCV patients with mean 34.6± 7.3 in a study done at National Taiwan University Hospital by **Nien et al.,** [(12)]**.**

Blood pressure differed significantly between the three groups in systolic blood pressure as the non treated HCV infection group had the lowest blood pressure ($p<0.05$).

This agrees with results of study done by **Marzouk et al.,** [(13)] which was performed in Egypt where SBP were lowest in hepatitis C patients with mean 124.2 mmHg and mean 128.0 mmHg in patients with past hepatitis C infection and with mean 125.2 mmHg in chronic Hepatitis infected patients**.**

In this study there was statistically significant difference between the three groups regarding serum endotoxin level and DNA results for bacterial endotoxin with patients with active hepatitis C had highest level of serum endotoxin and all were positive DNA. In **Bruns et al.** [(14)] study bacterial DNA was detected by multiplex polymerase chain reaction in serum and/or ascitic fluid in 61% of patients with suspected infection at baseline. BactDNA was associated with SBP and bacteremia, acute-on-chronic liver failure**.**

Another study by **Bellot et al.** [(15)] showed that bacterial DNA was positive among 30 % of patients with cirrhosis and ascites and also in **Jun et al**. [(16)] study patients in the Child C group had a high positive rate for bacterial DNA, which was 50.0% compared to 15.6% and 15.4% in the Child A and B groups, respectively.

Regarding systolic blood pressure there was statistically significant difference between DNA positive and DNA negative patients with positive patients had lower systolic blood pressure ($p<0.05$).This correlates with results of **Francés et al.** [(17)] where bacterial DNA positive patients had lower mean arterial pressure (MAP) 80 ±5.32 mmHg while bacterial DNA negative patients had higher pressure with mean MAP 87±7 mmHg also this agrees with **Caro et al.** [(10)] where bacterial DNA positive patients had lower MAP 80.4 (60–100)mmHg while bacterial DNA negative patients had higher pressure with mean MAP 83.3 (64–102) mmHg.

In our study there was statistically significant difference between DNA positive and DNA negative patients with positive patients had higher Mean± SD serum endotoxin level 47.14±12.775 Vs 3.25±1.6 in negative patients.

This agrees with **Caradonna et al.** [(11)] study where 62% of HCV+ patients enrolled in the study, exhibited plasma endotoxins as

bacterial DNA positive patients had mean value 36 compared to bacterial DNA negative patients with mean value 5**.**

Unlike **González-Navajas et al. [18]** study where serum endotoxin and lipopolysaccharide-binding protein were non-significantly higher in patients with bacterial-DNA than in those without bacterial-DNA. Regarding patients with bacterial-DNA from Gram-positive microorganisms (n = 8), these levels were similar to those in patients without bacterial-DNA (n = 16), and significantly lower than in patients with bacterial-DNA from Gram-negative bacteria**.**

In this study we estimate two markers of bacterial translocation rather than one that gave strength to our study.We also compare between three groups ,previous studies compare only cirrhotic patients with healthy indviduals. we discuss hepatitis c status either treated or not.

But we hoped more number of patients in other studies. Also we recommend study the culture of bacteria to help determing type of bacteria and suitable antibiotics.

CONCLUSION

Bacterial translocation markers (bacterial DNA and endotoxin) are high in cirrhotic and hepatitis C virus infected patients.

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Supplementary Tables& Figures

Table (2): Laboratory results of the studied group (n=81)

Variable	Mean± SD	Range
SGPT	25.619±6.609	12-351
SGOT	33.502±7.082	14.60-215

SGPT(serum glutamate pyruvate transaminase)
SGOT(serum glutamic-oxaloacetic transaminase)

Table (4): Comparing vital signs and clinical data between the three studied groups

Variable	positive HCV without treatment (n=27)	Patients with treated HCV (n=27)	No previous HCV infection (n=27)	P value
Systolic blood pressure Mean± SD	107.04±11.373	115.19±10.514	114.81±11.222	**0.012***
Diastolic blood pressure Mean± SD	70.74±6.752	72.96±4.653	74.44±8.473	0.139

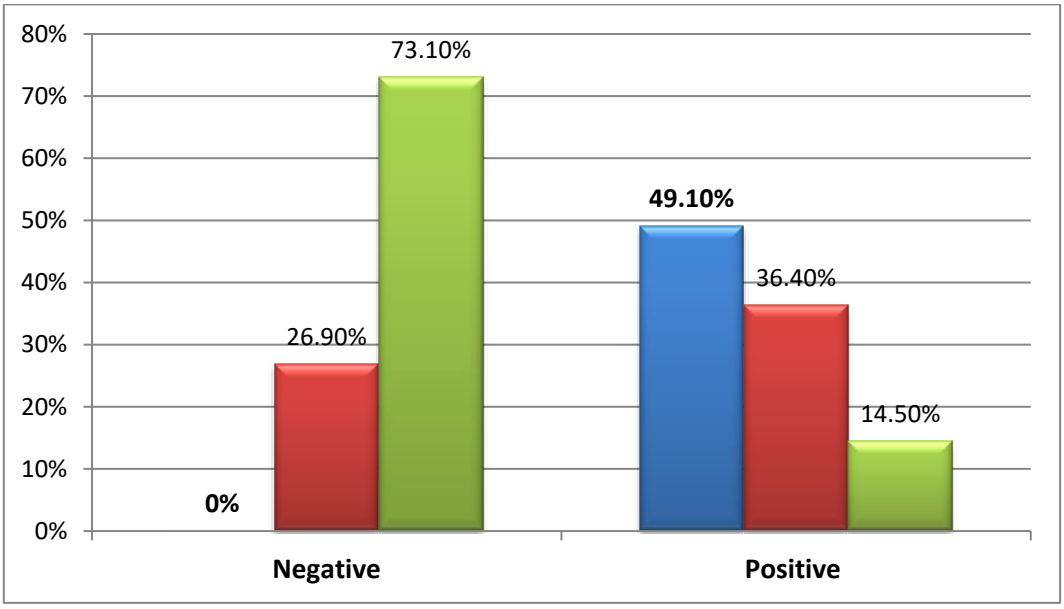

Figure (3): DNA result between the studied group.

positive hcv without treatment

Patient with treated hcv

No previos hcv infection

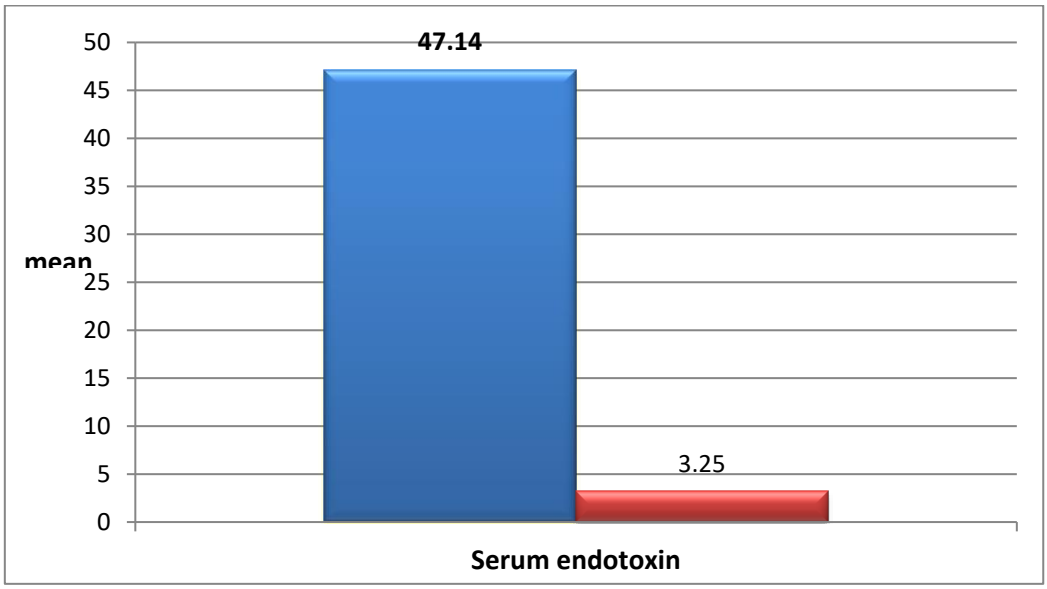

Figure (4): Serum endotoxin between DNA positive and DNA negative.