DonnishJournals

Donnish Journal of Biomedical Research
Vol 3(2) pp. 013-018 August, 2016.
http://www.donnishjournals.org/djbr
ISSN: 2984-8954

Original Research Article

Effect of *Helicobacter pylori* on Treatment of Hepatitis C Virus Egyptian Patients

Naglaa Kamal Madkour[1]*, Azza M.S. Ghanem[2], Sahar Y.H. El-melegy[3], Mohamed O. Abdel-Moneim[3], Soheir S. Abdel-Salam[3], Ghada Hussein[4], Nahla S Kotb[5] and Mohamed A. Attala[6]

[1]Zoology Department, Girls College for Arts, Science and Education, Ain Shams University, Cairo, Egypt.
[2]Immunology Department, Faculty of Medicine, AL-Azhar University, Cairo, Egypt.
[3]Bacteriology Department, Faculty of Science, Benha University, Egypt.
[4]Cilinical Pathology Department, National Institute of Diabetes and Endocrinology, Egypt.
[5]Biochemistry Department, National Organization for Research and Control of Biological product, Egypt.
[6]Tropical Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.

Accepted 24th July, 2016.

Background: **Hepatitis C virus (HCV) is considered the most common etiology of chronic liver disease in Egypt, which may progress to cirrhosis and hepatocellular carcinoma (HCC). Previous studies have documented an association between *Helicobacter pylori (H. pylori)* infection and HCV. *Objective:* This study aimed to investigate the role of *Helicobacter pylori (H. pylori)* seropositivity in the treatment of patients with hepatitis C virus (HCV) infection and the effect of this co-infection on response to interferon-α and ribavirin therapy. *Methods:* The presence of *H. pylori* was tested using a commercially available enzyme immunoassay in serum samples from 49 patients with chronic hepatitis C. Clinical features, HCV markers and response of HCV to interferon-α and ribavirin therapy were compared between *H. pylori* seropositivity and *H. pylori* seronegativity patients. *Results: H.pylori* antigen was detected in 24(49.0%) of 50 HCV patients. There was no association between *H. pylori* seropositivity in the liver and age, gender of patients, liver function tests, AFP levels or viral load. The sustained response rate for HCV clearance following interferon-α and ribavirin treatment did not differ between patients with and without anti-*H. pylori* seropositivity. *Conclusion:* No correlation between coexistent *H.Pylori* infection and clinical course of Hepatitis C Virus and suggest an association between this bacterium and progression of liver fibrosis.**

Keywords: *Helicobacter pylori*, Hepatitis C virus, Interferon, Ribavirin

INTRODUCTION

Hepatitis C virus (HCV) is responsible for the majority of non-A non-B hepatitis with serious complications ranging from chronic inflammatory disease to hepatic cirrhosis and hepatocellular carcinoma (HCC)[3]. The prevalence of HCV infection varies throughout the world, because of high morbidity and mortality from liver disease, Egypt has high prevalence of hepatitis C. Approximately 12% of blood donors are seropositive for HCV antibodies [9].

From one patient to another, the course of HCV-related hepatic disease varies markedly. Several factors including age at exposure, male gender, duration of infection, alcohol intake, viral immune response and steatosis have been shown to be associated with fibrosis progression [4]. However, even in the absence of these factors, disease progression may be observed in some patients, suggesting the role of other factors. Host genetic factors or environmental factors, such as a bacterial co-infection, could be involved [5]. It has been observed that Helicobacter species were associated with the pathogenesis of human enterohepatic diseases [6].

H. pylori is a Gram-negative organism that colonizes mucous layer of the human stomach and known to cause gastrointestinal disorders, including chronic gastritis, peptic ulcers, and gastric adenocarcinoma [2], by PCR, Tolia et al have reported, the presence of genomic sequences of *Helicobacter* spp. in the liver tissue of 40 patients with miscellaneous liver diseases, by sequencing further analysis revealed that most of these species were *H. pylori* [27].

The discovery of the presence of Helicobacter species DNA in liver material from patients with liver disease has led to the challenging hypothesis that these bacteria may play a role in the evolution of hepatic lesions from chronic viral hepatitis to cirrhosis and HCC. Determinants of this evolution are not yet

**Corresponding Author: Naglaa Kamal Madkour. Assistant Prof of Physiology, Zoology Department, Girls College for Arts, Science and Education, Ain Shams University, Egypt.*

fully understood, including those occurring in HCV positive patients [19].

In Egypt the infection with HP is common and acquisition of infection occurs at a very young age [15]. A study performed on Egyptian patients reported that HP antibodies were found in 55.6% of HCV-infected patients vs. 39.4% of the healthy controls. Moreover, the prevalence of HP infection was increased significantly from chronic active hepatitis to cirrhosis [8].

In different parts of the world, the association between HP infection and severity of chronic liver diseases in patients with hepatitis C virus has been documented. But in Egypt, no conclusive data is available till now. These observations prompted us to seek out the possible occurrence and association of HP seropositivity with the pathological stages in liver among CHC Egyptian patients.

This study was conducted to investigate the prevalence of *H.pylori* seropositivity in Egyptian patients with chronic hepatitis C, the influence of *H.pylori* seropositivity on the clinical, virological and histological characteristics of hepatitis C, and the effect of *H.pylori* co-infection on the response of HCV to IFN-α and ribavirin therapy.

PATIENTS AND METHODS

Patients

This prospective study was conducted with 49 participants divided into two groups. The first group composed of 25 patients with HCV only while the second group composed of 24 patients with hepatitis C virus (HCV) and *Helicobacter Pylori (H.Pylori) co-infection.* Diagnosed and treated at Ain Shams University Specialized Hospital, Cairo, between 2012 and 2015. Written consent was obtained from all participants prior to enrollment in the study and all were mentally and physically capable of answering questionnaire.

Inclusion criteria

Adult patients of both sexes (20-54 years old), diagnosis within the previous 6 months, Positive for HCV RNA in serum (by RT-PCR assay), with evidence of chronic hepatitis. Patients were not receiving hepatitis treatment at the time of Sampling. The study excluded patients co-infected with HBV or HIV and patients with clinical or ultrasonography evidence of cirrhosis, all individuals included in this study were subjected to the following:

A. Medical History

Full medical history was taken with special reference to risk factors for liver diseases such as previous HCV exposure in surgical wards, blood transfusions, dental therapy, needle stick injury, history of HCV in the spouse and intravenous injection.

B. Physical Examination

Complete medical examination with particular focus upon the manifestations of hepatitis such as jaundice, hepatomegaly and Abdominal ultrasonography was performed for all patients.

C. Laboratory investigations

Chronic hepatitis C was diagnosed by having either elevated or fluctuating ALT levels for more than 6 months and/or bright liver appearance on abdominal ultrasonography[12]. Patients with other causes of liver disease, including hepatitis B were excluded from the study (standard clinical laboratory methods). Liver function tests, AFP and antischistosomal antibodies were measured using commercially available kits. The HCV viral load was quantified using Real Time PCR technique.

D. Viral Markers

ELISA assays

Sera of all patients and controls were tested for anti-HCV antibodies by ELISA, using third generation kits (DiaSorin, Italy) according to the manufacturer's instructions.

Quantitation of HCV-RNA in serum

HCV-RNA was quantitated in all patients' serum samples using Real Time PCR (RT-PCR) [16] (primers and RT-PCR reagents from Stratagene, Qiagen, USA). Low viremia was defined as viral load lower than 100 $x10^3$IU/L, moderate viremia as viral load 100-1000 × 10^3 IU/L, and high viremia when viral load > 1000 $x10^3$ IU/L [22].

Detection of H. pylori antigen

Samples were tested for *H.pylori* antigen using a commercial test kit, provided by (Immun diagnostic company, Germany) according to the manufacturer's instruction.

Statistical analysis

Mann-Whitney *U* test was used to analyze continuous variables. Chi-square test with Yates correction was used for the analysis of categorical data. Pearson's correlation coefficient was used to evaluate the relationships between the titer of anti-*H.pylori* and fibrosis score. Multivariate analysis was performed using a logistic regression model with a stepwise method. A *P* value of ≤ 0.05 was considered significant. Statistical analyses were performed using Sigma Stat (version 2.03, SPSS Inc., Chicago, IL) and SPSS 6.1J (SPSS Inc., Chicago, IL).

RESULTS

Detection of *H.pylori*

H.pylori antigen was detected in 24 (49%) of 49 patients. Clinical and virological features were compared between patients with and without anti-*H. pylori* in Table 1. It was found that no statistically significant difference between patients with and without *H.pylori* in platelet count (*P*=0.89).Additionally, no significant difference in levels of HCV RNA in serum between patients with and without anti-*H.pylori (P=0.26)*. Fibrosis scores were examined for their correlation with levels of *H.pylori* antigen (Table 2). The titer of *H.pylori* was highly significant correlated with fibrosis score (*P*= 0.001) (Fig. 1).

Seropositivity to *H.Pylori* infection was more frequent in HCV patients with high stage liver fibrosis 14/18(77.7%) than in low stage liver fibrosis 10/31(32.2%) (Table 3). Thus, there was statistically significant (P = 0.003) association between sero-prevalence of *H. Pylori* in the liver and stage of fibrosis (Fig. 2).

Table 1: Lab findings regarding *H.pylori* infection

Variable	*H. pylori* positive (N=24)		*H. pylori* negative (N=25)		P
	Mean	± SD	Mean	± SD	
Age	41.12	8.268	39.16	7.646	0.39
BMI	29.24	9.575	24.72	8.900	0.09
AST	63.8000	20.99802	69.4400	36.45897	0.69 (NS)
ALT	65.5600	27.89576	64.8000	30.02083	0.82 (NS)
T. bilirubin	1.1400	0.51153	1.0760	0.44091	0.88 (NS)
D. bilirubin	0.2560	0.18046	0.2520	0.15578	0.61 (NS)
Albumin	3.90	.31554	3.76	.32772	0.018* (S)
Glucose	98.5200	15.31644	99.9600	19.29050	0.87 (NS)
AFP	12.2400	7.15472	13.8360	16.88113	0.84 (NS)
Hb%	11.8240	1.86107	12.2000	1.80854	0.48 (NS)
PLTs	287.4800	74.55094	294.6800	75.71202	0.89 (NS)
PCR $\times 10^6$	45561.0539968	15733.0674380	6.0104400	17.7013248	0.26 (NS)
TSH	3.8480	.76164	3.5000	.72284	0.27 (NS)
Creatinine	.9800	.14720	.9640	.17531	0.58 (NS)
ALP	115.2800	37.54943	118.9200	57.28796	0.96 (NS)

S: Significant, (*P*<0.05) **NS:** Non-significant, (*P*>0.05) **BMI:** Body Mass Index, **AST:** Aspartate aminotransferase, **ALT:** Alanine aminotransferase, **T. bilirubin:** Total bilirubin, **D. bilirubin:** Direct bilirubin, **AFP:** Alpha-fetoprotein, **Hb:** Hemoglobin, **plt:** platelets, **PCR:** Polymerase chain reaction, **TSH:** thyroid stimulating hormone, **ALP:** Alkaline Phosphatase

Table 2: Fibrosis Score and *H pylori* cases

			H. pylori		
			Negative	Positive	Total
F stage	F1	Count	0	1	1
		% within *H. pylori*	.0%	4.0%	2.0%
	F2	Count	3	4	7
		% within *H. pylori*	12.0%	17.0%	14.0%
	F3	Count	18	5	23
		% within *H. pylori*	72.0%	21.0%	47.0%
	F4	Count	4	13	17
		% within *H. pylori*	16.0%	54.0%	35.0%
	F5	Count	0	1	1
		% within *H. pylori*	.0%	4.0%	2.0%
Total		Count	25	24	49
		% within *H. pylori*	100.0%	100.0%	100.0%

FET= 14.9 P=0.001 (HS)

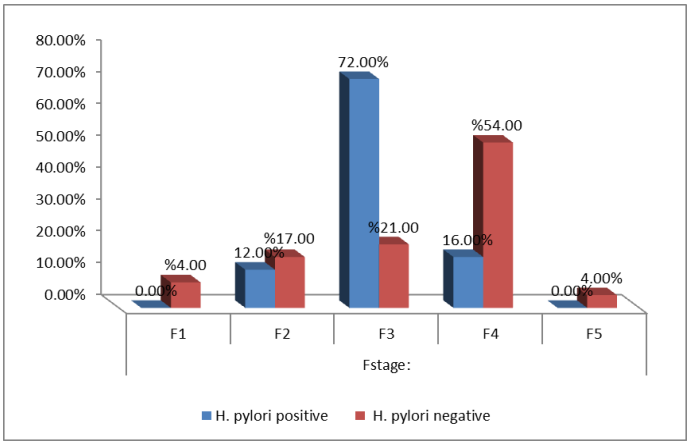

Fig. 1: Comparison of fibrosis score among studied groups

Table 3: Sero-prevalence of *H.Pylori* infection in liver tissues regarding severity of fibrosis

			H. pylori		
			Negative	**Positive**	**Total**
Fibrosis	Low	Count	21	10	31
		% within *H. pylori*	84.0%	42.0%	63.0%
	Advanced	Count	4	14	18
		% within *H. pylori*	16.0%	58.0%	37.0%
Total		Count	25	24	49
		% within *H. pylori*	100.0%	100.0%	100.0%

X^2= 8.68 P=0.003

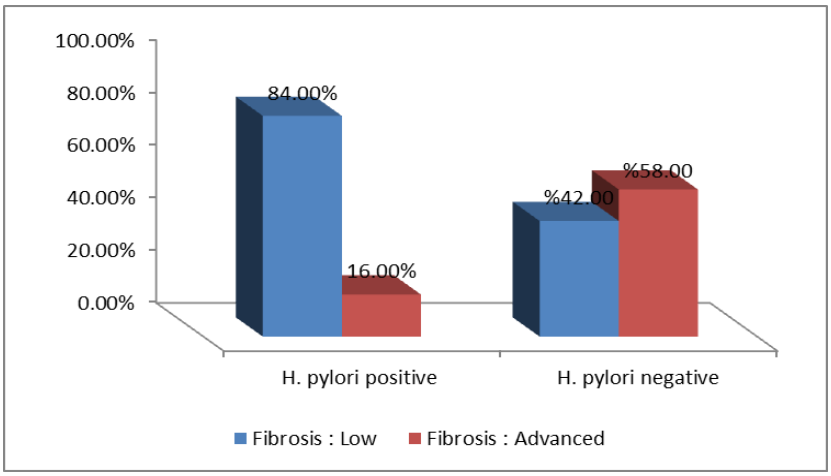

Fig. 2: Sero-prevalence of *H.Pylori* infection in liver tissues according to the severity of fibrosis.

Table 4: Comparing between responders and non-responders regarding lab findings

Variable	**Non-responders (N=18)**		**Responders (N=31)**		**P**
	Mean	± SD	Mean	± SD	
Age	37.9444	6.04369	41.3750	8.67979	0.098(NS)
BMI	24.6667	8.66365	28.2812	9.72272	0.22 (NS)
AST	81.5	39.97385	58.2	17.45360	0.03*(S)
ALT	81.3	t38.66468	56.1	15.56451	0.017*(S)
T. bilirubin	1.1611	.52372	1.0781	.44918	0.87 (NS)
D. bilirubin	.2556	.17896	.2531	.16261	0.88 (NS)
Albumin	3.8444	.40325	3.8281	.28082	0.48 (NS)
Glucose	96.6667	18.68941	100.6875	16.52259	0.15 (NS)
AFP	19.05	18.84448	9.6	5.80422	0.002*(S)
Hb%	11.8111	1.64956	12.1250	1.93441	0.56 (NS)
PLTs	307.1111	72.80101	282.0625	74.98040	0.23 (NS)
PCR ($x10^9$)	6.000	18.46	0.188	0.73751	0.024*(S)
TSH	3.5667	.95054	3.7344	.62970	0.31 (NS)
Creatinine	.9556	.15424	.9812	.16547	0.39 (NS)
ALP	141.9	49.27053	103.1	41.79115	0.009*(S)

S: Significant, (*P*<0.05) **NS:** Non-Significant, (*P*>0.05)

Table 5: Correlation between response to interferon treatment and sero-reactivity of *H. Pylori*

			H. pylori		
			Negative	**Positive**	**Total**
RESPONSE	Non responders	Count	12	6	18
		% within *H. pylori*	48.0%	25.0%	37.0%
	Responders	Count	13	18	31
		% within *H. pylori*	52.0%	75.0%	63.0%
Total		Count	25	24	49
		% within *H. pylori*	100.0%	100.0%	100.0%

X^2=3.12 P=0.077

Effect of H.pylori infection on HCV response to IFN-α and ribavirin therapy:

Of the 49 patients receiving IFN-α and ribavirin therapy, 31 (63%) were responders, There was no significant difference between responders and non-responders according to age, BMI. AST level and ALT level in the responders group was significantly lower than that in the non-responders group (*P*=0.03, 0.017 respectively). There was a significant difference between responders and non-responders group according to AFP level (*P=0.002)* and in contrast, there was no significant difference between responders and non-responders according to PLTs count (*P*=0.23). HCV RNA level in the responders group was significantly lower than that in the non-responders group (*P*=0.024) (Table 4). Sustained HCV response to IFN-α and ribavirin therapy did not differ between patients with and without *H.pylori* infection (Table 5).

DISCUSSION

Since previous studies discovered that Helicobacter species DNA were present in the liver tissue from patients with liver disease, several studies were conducted to investigate the role of these bacteria in the progression of hepatic lesions to cirrhosis and end stage liver failure or HCC [19].

In this study, we found that approximately 49% of our chronic hepatitis C patients were co-infected with *H.pylori*. This *H.pylori antigen* seroprevalence is similar to a report on healthy individuals in Japan [11]. The treatment arm of this study was designed to measure the effect of *Helicobacter pylori* seropositivity in patients with HCV in response to IFN-α and ribavirin therapy.

No evidence was found to suggest that *H.pylori* increases the severity of chronic hepatitis C, since clinical and biochemical evaluations, notably ALT levels, did not differ greatly between patients with HCV infection alone and those co-infected with HCV and *H.pylori (*Table 1). On comparison between studied groups, it was found statistically insignificant. (*P*-value=0.82). This phenomenon comparable to prior reports on single and co-infection of HCV and hepatitis G and SEN viruses [24-31-28-29-30].

Several reports have documented viral interference between hepatitis B virus and HCV [18,21,13]. And that increasing the replication of one agent can enhance replication of the other. Our study showed that no statistically significant difference between negative & positive *H.Pylori* group as regarding the viral load of HCV RNA as appear in Table 1, (*P*-value = 0.26) and in contrast, this result doesn't agree with data obtained in Umemura and co-worker [26] who mentioned that the HCV RNA titer in patients with HCV and *H.pylori* co-infection was significantly lower than in patients with HCV infection alone (*P*=0.013), suggesting that *H.pylori* infection might interfere with HCV replication. At present, we can only describe the observation of possibility of viral-bacterium interference and cannot provide a sound scientific basis for its occurrence. Additional studies, for instance, HCV replicon system analysis *in vitro* would be required to validate this result [25].

Over the past few years, *Helicobacter* species have been found to be present in the liver of HCV-negative patients, and have been associated with hepatocellular carcinoma (HCC) development in the non-cirrhotic liver [7,1,17]. Rocha *et al*. have reported that virtually all patients with HCC are *Helicobacter* species positive in their HCC, and 61-68% of those with cirrhosis are *Helicobacter* positive in liver tissue, compared with 4.5% and 3.2% of hepatitis patients and controls, respectively [20]. This suggests that the presence of *Helicobacter* species DNA sequences in the liver may be a co-risk factor in the progression of chronic HCV liver disease.

In the present study, the titer of *H.pylori* was highly significant correlated with fibrosis score (*P*= 0.001) (Table 2). This finding is in agreement with Umemura and co-workers[25], who demonstrated a strong correlation between the titer of anti-*H.pylori* and the degree of fibrosis (*P*=0.0083; r=0.33). However, since no patients with HCC were enrolled, we were unable to assess whether the level or positivity of *H.pylori* was associated with the development of HCC. Since there are no reports that clarify the clinical significance of *H.pylori* level in patients with liver disease, this significant finding should be expanded in larger populations that contain patients with chronic hepatitis and cirrhosis.

Recently, *H. pylori* has been suspected to be involved in various autoimmune disorders, including idiopathic thrombocytopenia [10, 14]. Several studies have also reported that the eradication of *H. pylori* is often accompanied by a significant increase in platelet count in patients with idiopathic thrombocytopenia [14, 23]. Although this clinical observation suggests the involvement of *H. pylori*, little is known about the pathogenesis of *H.pylori*-associated idiopathic thrombocytopenia. We found that serum platelet count had no

significant difference between patients with HCV/*H.pylori* co-infection compared to the HCV group (*P*-value=0.89) (Table 1).

Our results raise the possibility that *H.Pylori* infections were more frequent in HCV patients with high stage liver fibrosis (14/18, 77.7%) than in low stage liver fibrosis (10/31, 32.3%). Thus, there was statistically significant (*P* = 0.003) association between sero-prevalence of *H. Pylori* in the liver and stage of fibrosis (Table 3). This finding is in agreement with Umemura and co-worker [25], who reported that there was a strong correlation between the titer of anti-*H. Pylori* antibody and the degree of fibrosis (P=0.0083; R=0.33).

Since the treatment arm of the study was initially designed to measure HCV response only, eradication of *H.pylori* had not been performed. Eradication of *H.pylori* in patients with chronic hepatitis C prior to IFN and ribavirin therapy is currently being planned in a forthcoming study.

We found that the rate of HCV response to IFN-α and ribavirin therapy did not differ between patients with and without *H.pylori.* On comparison between studied groups, it was found no statistically significant difference, (*P*-value=0.077) (Table 5). This finding is in agreement with Umemura and co-worker [25], who reported that there was no significant difference in the sustained HCV treatment response between those who had HCV infection alone and those with HCV and *H.pylori* co-infection (*P*-value=0.89) .

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

REFERENCES

1. Avenaud P, Marais A, Monteiro L, et al, 2000: Detection of Helicobacter species in the liver of patients with and without primary liver carcinoma. Cancer; 89:1431-9.
2. Blaser MJ, 1998: Helicobacter pylori and gastric diseases.BMJ.; 316(7143):1507-1510.
3. Butel JS, 2007: Virology. In: Brooks GF, Carroll KC, Butel JS, Mores SA, editors. Medical microbiology. New York: Mc Graw Hill; p. 367–620.
4. Caste´ra L, Hezode C, Roudot-Thoraval F, Bastie A, Zafrani ES, Pawlotsky JM, et al, 2003: Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. Gut; 52:288–92.
5. Caste´ra L, Pedeboscq A, Rocha M, Le Bail B, Asencio C, de Ledinghen V, et al, 2006: Relationship between the severity of hepatitis C virus-related liver disease and the presence of Helicobacter species in the liver: a prospective study. World J Gastroenterol; 12(45):7278–84.
6. de Magalhaes Queiroz DM, Santos A, 2001: Isolation of a Helicobacter strain from the human liver. Gastroenterology; 121:1023–4.
7. Dore MP, Realdi G, Mura D, Graham DY and Sepulveda AR, 2002: Helicobacter infection in patients with HCV-related chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Dig Dis Sci; 47:1638-43.
8. El-Masry S, El-Shahat M, Badra G, Aboel-Nour MF, Lotfy M, 2010: Helicobacter pylori and Hepatitis C virus co-infection in Egyptian patients. J Global Infect Dis; 2:4–9.
9. El-Zanaty F, Way A, 2008: Egypt demographic and health survey. [accessed 06.01.11].
10. Franchini M, Veneri D, 2004: Helicobacter pylori infection and immune thrombocytopenic purpura: an update. Helicobacter; 9: 342–346.
11. Fujisawa T, Kumagai T, Akamatsu T, Kiyosawa K and Matsunaga Y, 1999: Changes in sero epidemiological pattern of Helicobacter pylori and hepatitis A virus over the last20 years in Japan. Am J Gastroenterol; 94:2094-9.
12. Hamilton SR, Aaltonen LA, 2000: World health organization classification of tumors. Pathology and genetics of tumors of digestive system. Lyon IARC Press, 157-202.
13. Jardi R, Rodriguez F, Buti M, et al, 2001: Role of hepatitis B, C, and D viruses in dual and triple infection: influence of viral genotypes and hepatitis B precore and basal core promoter mutations on viral replicative interference. Hepatology; 34:404-10.
14. Michel M, Cooper N, Jean C, Frissora C, Bussel JB, 2004: Does Helicobater pylori initiate or perpetuate immune thrombocytopenic purpura? Blood; 103: 890–896.
15. Mohammad MA, Hussein L, Coward A, Jackson SJ, 2008: Prevalence of Helicobacter pylori infection among Egyptian children: impact of social background and effect on growth. Public Health Nutr; 11(3):230–6.
16. Moucari R, Ripault M-P, Martinot-Peignoux M, Voitot H, Cardoso A-C, Stern C, Boyer N, Maylin S, Nicolas Chanoine M-H, Vidaud M, Valla D, Bedrossa P, Marcellin P, 2009: Insulin resistance and geographical origin: major predictors of liver fibrosis and response to peginterferon and ribavarinin HCV-4. Gut, 58:1662-1669.
17. Nilsson HO, Mulchandani R, Tranberg KG, Stenram U and WadstromT, 2001:Helicobacter species identified in liver from patients with cholangio carcinoma and hepatocellular carcinoma. Gastroenterology; 120:323-4
18. Pontisso P, Ruvoletto MG, Fattovich G, et al, 1993: Clinical and virological profiles in patients with multiple hepatitis virus infections. Gastroenterology; 105:1529-33.
19. Rocha M, Avenaud P, Menard A, Le Bail B, Balabaud C, Bioulac-Sage P, et al, 2005: Association of Helicobacter species with hepatitis C cirrhosis with or without hepatocellular carcinoma. Gut; 54:396–401.
20. Rocha M, Avenaud P, Menard A, et al, 2005: Association of Helicobacter species with hepatitis C cirrhosis with or without hepatocellular carcinoma. Gut; 54:396-401
21. Sagnelli E, Coppola N, Scolastico C, et al, 2000: Virologic and clinical expressions of reciprocal inhibitory effect of hepatitis B, C, and delta viruses in patients with chronic hepatitis. Hepatology; 32:1106-10.
22. Shaker O, Ahmed A, Doss W, Abdel-Hamid M, 2010: MxA expression as marker for assessing the therapeutic response in HCV genotype 4 Egyptian patients. J Viral Hepat, 17:794-799.
23. Stasi R, Rossi Z, Stipa E, Amadori S, Newland AC, Provan D, 2005: Helicobacter pylori eradication in the management of patients with idiopathic thrombocytopenic purpura. Am J Med; 118: 414–419
24. Tanaka E, Alter HJ, Nakatsuji Y, et al, 1996: Effect of hepatitis G virus infection on chronic hepatitis C. Ann Intern Med;125:740-3.
25. Tanaka E, Ohue C, Aoyagi K, et al, 2000: Evaluation of a new enzyme immunoassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating that of genomic amplification of HCV RNA. Hepatology; 32:388-93.
26. T. Umemura, H. Muto, E. Tanaka, et al, 2007: Anti-Helicobacter pylori seropositivity: influence on severity and treatment response in patients with chronic hepatitis C. Journal of Viral Hepatitis: 14, 48–54.
27. Tolia V, Nilsson HO, Boyer K, Wuerth A, Al-SoudWA, Rabah R, Wadstrom T, 2004: Detection of Helicobacter ganmani-like 16S rDNA in pediatric liver tissue. Helicobacter; 9(5):460-468.
28. Umemura T, Alter HJ, Tanaka E, et al, 2001: Association between SEN virus infection and hepatitis C in Japan. J Infect Dis; 184:1246-51.
29. Umemura T, Alter HJ, Tanaka E, et al, 2002: SEN virus: response to interferon alfa and influence on the severity and treatment response of coexistent hepatitis C. Hepatology;35:953-9.
30. Umemura T, Tanaka E, Ostapowicz G, et al, 2003: Investigation of SEN virus infection in patients with cryptogenic acute liver failure, hepatitis-associated aplastic anemia, oracute and chronic non-A-E hepatitis. J Infect Dis; 188:1545-52.
31. Umemura T, Yeo AE, Sottini A, et al, 2001: SEN virus infection and its relationship to transfusion-associated hepatitis. Hepatology; 33:1303-11.