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RESEARCH ARTICLE

Study of the Prevelance of Helicobacter Pylori Infection in Patients with Hepatic Encephalopathy in Medical Intensive Care Unit of Zagazig University Hospitals.

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Abstract

Background: Ammonia is the main culprit and responsible for the development of hepatic encephalopathy (HE) among cirrhotic patients and colonic bacteria are thought to be the main source of ammonia production. Stomach is one of the alternate site for ammonia production in Helicobacter pylori (H. pylori) infected patients.

Aim of the work: (1) To find out the prevalence of helicobacter pylori infection in hepatic encephalopathy who admitted to medical ICU in Zagazig University Hospitals.(2) Study the correlation between the presence of helicobacter pylori and severity of hepatic encephalopathy.

Patients and methods: This cross-sectional study was conducted in Internal Medicine department, in medical intensive care unit of Zagazig University Hospitals from May 2015 to November 2015.

Patients: This study included 450 subjects; divided into three groups; Group 1**:** 300 patients of liver cirrhosis with hepatic encephalopathy; Group 2: 75 patients of liver cirrhosis without hepatic encephalopathy; Group 3: 75 healthy controls without any disease. Cirrhotic patients regardless of its etiology diagnosed by abdominal ultrasonography and laboratory investigations**.** Patients with cirrhosis and hepatic encephalopathy determined by clinical assessment, which included mental status (alertness, mood, worry, and orientation) and complaints of sleep pattern disturbance, i.e., day/ night reversal (insomnia, daytime naps), consciousness.We excluded patients with neuropsychiatric disorders, patients with history of use of psychotropic, antiepileptic drugs or drug abuse, patients with portosystemic shunt operation, patients who had history of gastrointestinal bleeding within the previous 4 weeks, patients who suffer from dehydration or electrolyte imbalance, patients who are their body temperature exceed 37.5 C, patients with other causes of encephalopathy (e.g. metabolic, central, toxic or due to infections), patients who received antibiotics with anti- H. pylori spectrum in the past two weeks and patients with history of H. Pylori eradication treatment within the previous three months.

Methods:All the included patients subjected to full history taking, informed consent was obtained; clinical assessment for hepatic encephalopathy grading and recorded in the pre-designed sheet and laboratory investigations including; Complete blood picture, ESR,Liver function tests including prothrombin time, serum total protein serum albumin, ALT, AST, total and direct bilirubin by automated analyzer Advia 120 Semens,Blood urea, creatinine, blood glucose level and electrolytes,Viral profile (HbsAg and anti-HCV),Fasting venous blood ammonia level,Helicobacter pylori IgG antibodies using ELISA,Helicobacter pylori stool antigen (HpSA),Abdominal ultrasonography,Chest x-ray,Upper gastrointestinal Endoscopy if possible,Child Pugh classification was done on admission by

using parameter of serum bilirubin, serum albumin, prothormbin concentration, hepatic encephalopathy and ascites.

Results: In the current study Mean of age of the 3 groups in our study were (control group, 53.93; cirrhotic group, 53.27 and HE group, 57.22), and there is no statistically significant difference between different groups in age, also it shows the number and percentage of sex in different groups (control; 40 males with percentage of 53.3% and 35 females with percentage of 46.7%), also cirrhotic group showed 35 males with percentage of 46.7% and 40 females with 53.3% percentage. While HE group has 220 males with percentage of 73.3% and 80 females with percentage of 26.7%. Also, there is no statistical significant difference as regard sex between the studied groups. Mean of blood ammonia level in control group is 118.6, in cirrhotic group is 164.6 and in HE group is 283.51 and there is a statistically significant difference between cirrhotic group and HE groupas, also in this study by comparison between H. pylori Ab/Ag positive and negative cases in HE group and mean of blood ammonia level it is found that the mean of ammonia increased significantly among HE patients with H. pylori, antigens, antibiodies positive than negative cases, also by comparison between different grades of hepatic encephalopathy and the mean of blood ammonia level it is found that the LSD test shows that there is significant increase in blood ammonia level in grades II and III in comparison with grade I HE patients.

Conclusion: The frequency of h.pylori Ag&Ab among control group is 46.7%, cirrhotic group Ag is 53.3&Ab is 40% in HE group Ag is 58.3%&Ab is 48.3, also ammonia increased significantly among HE patients with H. pylori; antigens, antibiodies positive than negative cases, also it shows that there is significant increase in blood ammonia level in grades II and III in comparison with grade I HE patients. So, the finding of H. pylori in HE patients may be either co-incidence or co-relation suggesting positive correlation between the presence of helicobacter pylori and severity of hepatic encephalopathy.

Introduction:-

Hepatic encephalopathy (HE)is one of the common causes of death in cirrhotic patients. Although the pathogenesis of HE is not clear, increased serum ammonia is likely an important factor in inducing HE. Clinical manifestations of hepatic encephalopathy include decreased intellectual functions, personality disorders, altered consciousness level, and neuromuscular dysfunction**.** Subclinical hepatic encephalopathy (SHE) has been defined as a condition in which patients with cirrhosis, regardless of its etiology, demonstrates neuropsychological and neuro-physiological defects, yet, having a normal mental and neurological status through global clinical examination (1). Patients exhibit different cognitive deficits (in visual-spatial perception, attention, concentration, constructional ability... etc.), which are not detectable during standard neurological examination, but adversely affect daily functioning. Hepatic encephalopathy has a substantial negative effect on quality of life, even in patients with minimal disease and the treatment of minimal hepatic encephalopathy improves patients' quality of life (2). Moreover, patients have poor navigational skills and an impaired ability to drive and thess impairments place these individuals at an increased risk of road traffic violations and accidents (3).

There are many factors responsible for the development of HE in liver cirrhosis such as: ammonia, production of false neurotransmitters, increase sensitivity of central nervous system neurons to the inhibitory neurotransmitters Gamma Amino Butyric Acid (GABA), increased level of circulating endogenous benzodiazepines, decreased activity of urea-cycle enzymes due to Zinc deficiency, decreased level of myoinositol in brain, deposition of manganese in basal ganglia and swelling of brain astrocytes (4).

Ammonia is a substance most often incriminated in the pathogenesis of encephalopathy; Most of the ammonia is of gut origin where it is produced by the bacterial flora and when stomach infected with Helicobacter pylori (H. pylori)

it is postulated that it is an additional source of ammonia production. H. pylori infection might also stimulate ammonia production in cirrhotic patients. The quantity of ammonia produced by H. pylori to cause HE may depend on; (the number of bacteria and their distribution in the stomach, gastric membrane permeability to ammonia, gastric pH, liver impairment, and hepatic bypass). It has been supposed that H pylori may increase blood ammonia concentration and induce HE when the bacterium is widely distributed in the stomach, and in the presence of severe liver impairment (Child- Pugh class B or C) with abundant hepatic bypass. Some studies showed that hepatic encephalopathy patient with hyperammonaemia that was controlled by eradication of H. pylori (4). Other studies revealed that the prevalence of H. pylori in liver cirrhosis patients was similar to that in controls and no correlation was found between gastric and blood ammonia levels. Thus, H. pylori infection does not seem to play a major role in generation of elevated ammonia associated with hepatic encephalopathy (5).

Patients and Methods:-

This cross-sectional study was conducted in Internal Medicine department, in medical intensive care unit of Zagazig University Hospitals from May 2013 to May 2014. Informed consent and agreement of ethical committee was obtained from all patients and control group.

Patients:-

This study included 450 subjects; divided into three groups; Group 1: 300 patients of liver cirrhosis with hepatic encephalopathy; Group 2: 75 patients of liver cirrhosis without hepatic encephalopathy; Group 3: 75 healthy controls without any disease. Stages of hepatic encephalopathy: Stage I: Mild confusion, agitation, irritability, sleep disturbance & decreased attention, Stage II: Lethargy, disorientation, inappropriate behavior, drowsiness, Stage III: Somnolent but arousable, slurred speech, confused & aggressive, Stage IV: Hepatic coma.. Cirrhotic patients regardless of its etiology diagnosed by abdominal ultrasonography and laboratory investigations. Patients with cirrhosis and hepatic encephalopathy determined by clinical assessment, which included mental status (alertness, mood, worry, and orientation) and complaints of sleep pattern disturbance, i.e., day/ night reversal (insomnia, daytime naps), consciousness.We excluded patients with neuropsychiatric disorders, patients with history of use of psychotropic, antiepileptic drugs or drug abuse, patients with portosystemic shunt operation, patients who had history of gastrointestinal bleeding within the previous 4 weeks, patients who suffer from dehydration or electrolyte imbalance, patients who are their body temperature exceed 37.5 C, patients with other causes of encephalopathy (e.g. metabolic, central, toxic or due to infections), patients who received antibiotics with anti- H. pylori spectrum in the past two weeks and patients with history of H. Pylori eradication treatment within the previous three months.

Methods:-

All the included patients subjected to full history taking, informed consent was obtained; clinical assessment for hepatic encephalopathy grading and recorded in the pre-designed sheet and laboratory investigations including; complete blood picture, ESR by cell counter Sysmex KX 21, liver function tests including prothrombin time, serum total protein serum albumin, ALT, AST, total and direct bilirubin by automated analyzer Advia 120 Semens, blood urea, creatinine, blood glucose level and electrolytes,viral profile (HbsAg and anti-HCV), fasting venous blood ammonia level, helicobacter pylori IgG antibodies using ELISA, helicobacter pylori stool antigen (HpSA), abdominal ultrasonography, chest x-ray, upper gastrointestinal endoscopy if possible, Child Pugh classification was done on admission by using parameter of serum bilirubin, serum albumin, prothormbin concentration, hepatic encephalopathy and ascites.

Blood sampling:-
About 5 ml venous blood sample drawn out from each patient and control subject by vein puncture using sterile disposable syringes. 2 ml EDTA tube for CBC. 1.8 ml on 0.2 ml Na citrate for PT, PTT and INR. Ammonia level was determined in fasting venous blood by kinetic enzymatic method with glutamate dehydrogenase (spectra kit).

Principle:-
α-ketoglutarate reacts with ammonium ions in presence of glutamate dehydrogenase and the coenzyme NADPH to produce L-glutamate and $NADP^+$. The concentration of the $NADP^+$ formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance at 340 nm.

Specimen collection and preservation:-

The samples were collected in EDTA tubes to reduce RBC ammonia production. Collection of blood from stasis free vein of fasting not smoking patients, the tubes were filled completely and were tightly stoppered, transported on ice and ammonia assay was done within 30 minutes of venipuncture, contamination of samples by ammonia from smoking or traffic in laboratory or patient's room,glassware, or water was avoided.

Procedure:-
The reagents were incubated at 37°C for 3 minutes, then the reagents were added in blank, standard and sample as shown.

	Reagent blank	**Standard**	**Specimen**
Reagent (R)	1 ml	1 ml	1 ml
Standard	-	100 µl	-
Specimen	-	-	100 µl

The samples and standard were mixed, the absorbance (A_1) of the standard or specimen or specimen blank was read after 30 seconds and after 2.5 minutes were read again (A_2) on spectrophotometer.

Calculation:-
Automatic calculation of rate of reaction of A_1, A_2 of both standard and specimen using constant factor. Analytical range: 9-1700 µg/dl.

II-Detection of H. pylori IgG antibodies in serum:-

Princple of the test:-
H. pylori IgG antibodies was detected in serum by enzyme immuno-assay. Enzyme linked immunosorbent assays (ELISA) Wampole kit rely on the ability of biological materials, (i.e. antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen-specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of chromogen substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H2SO4, the contents of the wells turn yellow. The color, which is proportional to the concentration of antibody in the serum, can be read on ELISA microwell plate reader (6).

Assay procedure:-
100 µl diluted cutoff calibrator, controls and specimens was pipette to antigen coated microwells, and 100 µl of serum diluent was added to the reagent blank well. 2-A plate sealer was applied and the plate weas swirled gently on a flat surface for 5-10 seconds for mixing &each well was incubated at room temperatue (21°C-25°C) for 20 ± 2 minutes. .3-At the end of the incubation period, the plate sealer was removed. Using a microtiter plate washing device, the liquid was aspirated then dispense. Wash solution was dispensed into the strip and this procedure was repeated on the entire plate two more times. After completing the third wash cycle, all liquid was aspirated out of each strip, then any excess liquid was removed onto a stack of paper towels. 4-100 µl conjugate was added to each

well, including reagent blank well. Bubbles upon addition was avoided. The plate was covered with a plate sealer. 5- Wash step was repeated as described in step 3. 6-100 µl chromogen substrate solution was added to each well, including reagent blank well. 7- The plate was incubated at room temperature (21°C-25°C) for 10 ± 2 minutes. 8- Reaction was stopped by addition of 100 µl of stop solution following the same order of chromogen substrate addition including the reagent blank. The plate was tapped gently along the outsides to mix contents of the well. 9- After 5 minutes, the absorbance of each well was read at 450 nm on ELISA reader instrument (statefax).

Calculation:-

Mean Calibrator O.D. – The mean value for the calibrator from the three calibrator determinations was calculated. Correction Factor – The cutoff calibrator value for each assay is dsetermiend by multiplying the correction factor (printed on calibration vial) by the mean calibrator OD determined in step 1. Immune Status Ratio - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. value by the Cutoff Calibrator Value determined in step 3.

Reference ranges (normal values):

ISR	Results	Interpretation
≤0.90	Negative	No detectable IgG antibody by the ELISA test.
0.91-1.09	Equivocal	Samples that remain equivocal after repeat testing, should be repeat testing, should be retested by an alternate method, e.g., alternate ELISA assay. If the results remain equivocal upon further testing, an additional sample should be taken.
≥1.10	Positive	Indicates presence of IgG

III- Detection of H. pylori Ag in stool using Enzyme Immunoassay (ELISA):-

Principle: Microplates are coated with a cocktail of affinity purified mouse monoclonal antibodies directed to the most specific Helicobacter pylori antigens.In the 1st incubation, the solid phase is treated with the sample, previously extracted from stools, and simultaneously with a mixture of monoclonal antibodies to H. pylori, conjugated with peroxidase (HRP). After washing out all the other components of the sample, in the 2nd incubation the bound enzyme specifically present on the solid phase generates an optical signal that was proportional to the amount of H. pylori antigens present in the sample (7).

Qualitative Assay:-

The samples were prepared according to instruction of the kit for H. pylori extraction. 2- The required number of strips were placed in the plastic holder and wells were carefully identified for standards and samples. First well was left empty for blanking purposes. 3- The following was pipetted 100 µl standard 1 In duplicate, 100 µl standard 2 in duplicate, and then 100 µl samples. 4-100 µl enzymatic conjugate was dispensed in all wells, except for well No. 1, used for blanking operations. 5- Following addition of the conjugate, the microplate was incubated for 120 minutes at +37°C. 6- When the first incubation is over, the microwells were washed according to kit instructions. 7- 200 µl chromogen/substrate was pipetted into all the wells, well No. 1 was included. The microplate was incubated in room temperature for 20 min and was protected from light. 8- 100 µl sulphuric acid was pipetted into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 6. 9- The color intensity of the solution in each well was measured, as a 450 nm filter (reading), blanking the instrument with blank (well No. 1).

Calculation of results:-

The test results are calculated by means of a cut-off value determined from the O 450nm value of the Std. 0 µg/mL (Std. 0) and the OD 450nm of the Std. 0.1 µg/mL (Std. 0.1) with the following formula: Cut-Off = (Std. 0 + Std. 0.1) / 2.

Statistical Methods:-

The collected data were presented, summarized , tabulated, and analyzed by using computerized soft ware statistical package (EPI-info version 6.04 and SPSS version 19). The Chi-square ($\chi 2$) test was used to compare proportion. We consider (+) sign as indication for direct correleation i.e. increase frequency of independent lead to increase frequency of dependent & (-) sign as indication for inverse correleation i.e. increase frequency of independent lead to decrease frequency of dependent, also we consider values near to 1 as strong correleation & values near 0 as weak correleation.. P value < 0.05 was considered statistically significant (S) at 95% confidence interva, P value < 0.005 was considered highly statistically significant (HS), and P value > 0.05 was considered non statistically significant (NS). (Levesque et al., 2010).

Results:-

This study shows that the mean of age of the all groups in our study were (control group, 53.93; cirrhotic group, 53.27 and HE group, 57.22), and there is no statistically significant difference between different groups in age, also this table shows the number and percentage of sex in different groups (control; 40 males with percentage of 53.3% and 35 females with percentage of 46.7%), also cirrhotic group showed 35 males with percentage of 46.7% and 40 females with 53.3% percentage. While HE group has 220 males with percentage of 73.3% and 80 females with percentage of 26.7%. Also, there is no statistical significant difference as regard sex between the studied groups.

Table (1): Comparison between studied groups regarding age and sex.

	Control (n = 75)	Cirrhotic (n = 75)	HE (n = 300)	Test of significance	P
Age					
Mean ± SD	53.93 ± 6.9	53.27 ± 8	57.22 ± 6.78	2.69	0.07
Sex					
Male	40 (53.3%)	35 (46.7%)	220 (73.3%)	4.97	0.08
Female	35 (46.7%)	40 (53.3%)	80 (26.7%)		

Table (2): Comparison between studied groups as regard routine laboratory parameters

	Control Mean ± SD	Cirrhotic Mean ± SD	HE Mean ± SD	ANOVA	p
Hb g/dl	11.92 ± 0.79	11.37 ± 0.67	10.84 ± 0.91*	10.18	0.001
RBCs $\times 10^6$/cmm	4.34 ± 0.62*	3.98 ± 0.55	3.71 ± 0.53	7.9	0.001
WBCs/10^3	5733.66 ± 1099#	5873.55 ± 1664.7	6710 ± 1627	3.41	0.03
Platelet/10^3	206.73 ± 25.2*	139 ± 33.71#	89.98 ± 16.35	184	0.001
Creatinine mg/dl	0.73 ± 0.16	0.99 ±0.39	1.48 ± 0.52*	18.45	0.000
Urea mg/dl	30.9 ± 8.2*	60.2 ± 14.9#	80.07 ± 22.4	38.8	0.000
SGOT U/I	21.87 ± 6.16*	71.87 ± 17.68	75.38 ± 17.6	65.46	0.000
SGPT U/I	21.8 ±6.16*	76.33 ± 15.17#	87.92 ± 22.9	65.54	0.000
Total protein g/dl	6.82 ± 1.68	6.37 ± 0.68	5.73 ± 0.68*	9.5	0.000
Albumin g/dl	3.74 ± 0.26*	3.03 ± 0.35#	2.58 ± 0.45	49.56	0.000
Total bilirubin mg/dl	0.92 ± 0.11*	2.85 ± 0.59#	4.16 ± 2.59	14.22	0.000
Direct bilirubin mg/dl	0.19 ± 0.07*	1.27 ± 0.46	1.77 ± 0.99	7.66	0.000
PT /sec	11.4 ± 1.24*	17.73 ± 1.67#	23.8 ± 6.2	37.22	0.000
PTT /sec	28.8 ± 1.9*	37.33 ± 1.5#	46.6 ± 9.8	31.9	0.000

*This group by post-hoc analysis significantly differs from the other two groups.

#This group by post-hoc analysis significantly differs from HE.

The results of this study in table (2) show that the mean of Hb of 3 groups is 11.92 in control, 11.37 in cirrhotic group and 10.84 in HE group and it shows that there is statistically significant difference between the HE group and the other two groups, the mean of RBCs count in control group is 4.34, in cirrhotic group is 3.88 and in HE group is 3.71 and it shows that there is statistical significant difference in control group from the other two groups, the mean of WBC count in control group is 5733.66, in cirrhotic group is 5873.55 and in HE group is 6710; also it shows that there is statistically significant difference between control group and HE group, the mean of platelet in control group is 206.73 x 10^3, in cirrhotic group is 139 x 10^3 and 89.98 x 10^3 in HE group, also it shows that there is statistically significant difference between control group and the other two groups and there is statistically significant difference between cirrhotic group and HE group.

The mean of cratinine is 0.7 in control group, 0.99 in cirrhotic group and 1.48 in HE group. It shows that there is statistically significant difference between HE group and the other two grops. The mean of urea in control group is 30.9, in cirrhotic group is 60.2 and in HE group is 80.07. Also, there is statistical significant difference between control group and other two groups and also there is statistically significant difference between cirrhotic group and HE group as regard urea.

The mean of SGOT is 21.87 in control group, 71.87 in cirrhotic group, and 15.38 in HE group. Also, there is statistically significant difference between control group and the other two groups.The mean SGPT in control group is 21.8, in cirrhotic group is 20.33 and in HE group is 87.9. There is statistically significant difference between control group and the other two groups and there is statistically significant difference between cirrhotic group and HE group.The mean of total protein in control group is 6.8, in cirrhotic group is 6.37 and in HE group is 5.73. There is statistically significant difference between HE and the other two groups.The mean of albumin is 3.74 in control group, in cirrhotic group is 3.03 and in HE group is 2.58. There is statistically significant difference between control group and the other two groups and there is statistically significant difference between cirrhotic group and HE group.The mean of total bilirubin is 0.92 in control, 2.85 in cirrhotic group and 4.16 in HE group. There is statistically significant difference between control group and the other two groups and there is statistically significant difference between cirrhotic group and HE group.The mean of direct bilirubin in control group is 0.19, in cirrhotic group is 1.27 and in HE group is 1.77. There is statistically significant difference between control group and the other two groups.

The mean of PT in control group is 11.4, in cirrhotic group is 17.73 and in HE group is 23.8; there is statistically significant difference between control and the other two groups, cirrhotic group differs from HE group.The mean of PTT in control group is 28.8, in cirrhotic group is 13.33 and in HE group is 46.6. There is statistically significant difference between control and the other two groups and there is statistically significant difference between cirrhotic group and HE group.

Table (3): Comparison between studied groups as regards the mean of blood ammonia

	Control Mean ± SD	Cirrhotic Mean ± SD	HE Mean ± SD	F	P
Ammonia (mcg/dl)	118.6 ± 59.2	164.41 ± 39.25[#]	283.51 ± 76.28	44.02	0.000

#This group significantly differs from HE only.

Table (4): Frequency of H. pylori antigen and antibody among studied groups

H. pylori	Control		Cirrhosis		HE		X^2	P
	No	%	No	%	No	%		
Antigen								
Negative	40	53.3	35	46.7	125	41.7	0.96	0.7 (NS)
Positive	35	46.7	40	53.3	175	58.3		
Antibodies								
Negative	40	53.3	45	60	155	51.7	0.33	0.84 (NS)
Positive	35	46.7	30	40	145	48.3		

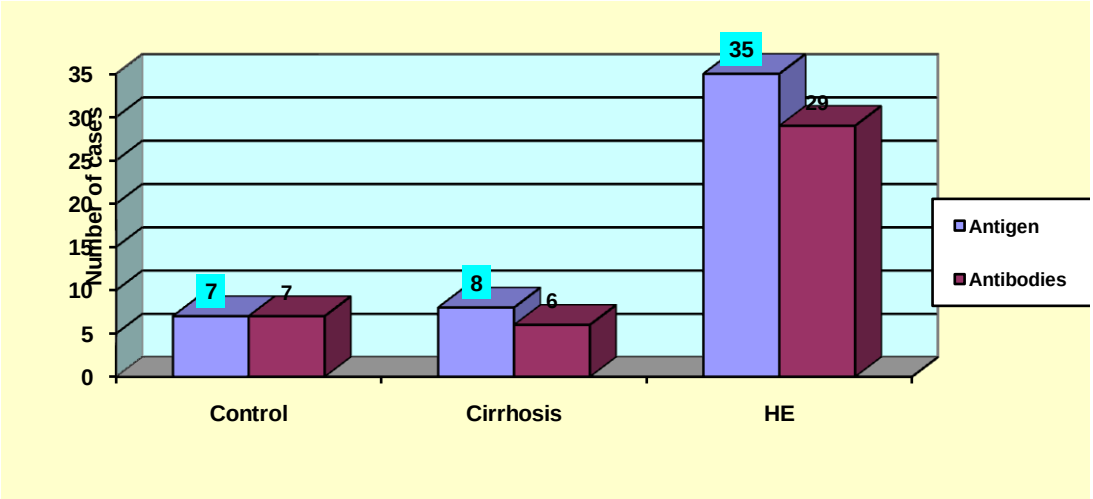

Figure (1): Frequency of H. pylori antigen and antibody among studied groups

This study shows that the mean of blood ammonia level in control group is 118.6, in cirrhotic group is 164.6 and in HE group is 283.51 and there is a statistically significant difference between cirrhotic group and HE group. As regard **f**requency of H. pylori antigen and antibody among studied groups, there is no statistically significant difference between studied groups as regard H. pylori.There is no statistical coefficient correlation between ammonia and variables among studied groups except btween age and blood ammonia level where there is a positive correlation, also between ESR and ammonia where there is opposing correlation.

Table (5): Coefficent correlation between ammonia and different variables in studied groups

Ammonia	**Control**		**Cirrhotics**		**HE**	
	R	**p**	**R**	**p**	**R**	**P**
Age (years)	0.32	0.23	-0.24	0.38	0.38	0.002*
Hb (gm/dl)	-0.44	0.1	-0.02	0.93	-0.01	0.92
RBC $\times 10^6$/cmm	0.41	0.12	0.22	0.41	-0.1	0.96
WBC $\times 10^3$/cmm	-0.35	0.2	0.34	0.21	-0.4	0.28
Platelets10^3/cmm	0.28	0.3	-0.09	0.72	0.14	0.29
Creatinin mg/dl	0.29	0.28	-0.28	0.29	-0.01	0.95
Urea mg/dl	-0.23	0.41	-0.23	0.41	-0.04	0.73
SGOT U/I	0.09	0.74	-0.11	0.7	0.12	0.36
SGPT U/I	0.07	0.81	-0.17	0.53	-0.03	0.82
Total protein g/dl	0.13	0.64	0.07	0.8	-0.03	0.83
Albumin g/dl	0.14	0.61	0.04	0.87	0.05	0.68
Total bilirubin mg/dl	-0.2	0.46	0.25	0.37	0.07	0.58
Direct bilirubin mg/dl	-0.16	0.55	0.15	0.58	-0.03	0.81
PT/ sec	0.42	0.11	-0.09	0.74	-0.12	0.33
PTT/sec	0.08	0.75	-0.26	0.35	0.06	0.63
ESR mm/hour	-0.59	0.02*			-0.01	-0.9

*Significant

Table (6): Comparison between males and females in cirrhotic group as regards the mean of blood ammonia level

	AmmoniaMean ± SD	**T**	**P**
Sex			
Male	170.28 ± 24.6	0.52	0.61
Female	159.27 ± 49.97		

Table (7): Comparison between H. pylori Ab/Ag positive and negative cases in cirrhotic group and mean of blood ammonia level

	Ammonia Mean ± SD	T	P
H. pylori antibodies			
-ve	161.41 ± 48.94	0.35	0.73
+ve	168.9 ± 21		
H. pylori antigen			
-ve	147.23 ± 46.18	1.68	0.11
+ve	179.44 ± 26.46		

There is a non-significnat difference between males and females as regards the mean of blood ammonia level in cirrhotic group patients. There is non-significnat difference between positive and negative H. pylori Ag/Ab cases in cirrhotic group and the mean of blood ammonia level.

The mean of ammonia increased significantly among HE patients with H. pylori, antigens, antibiodies positive than negative cases. The LSD test shows that there is significant increase in blood ammonia level in grades II and III HE patients in comparison with grade I HE patients.

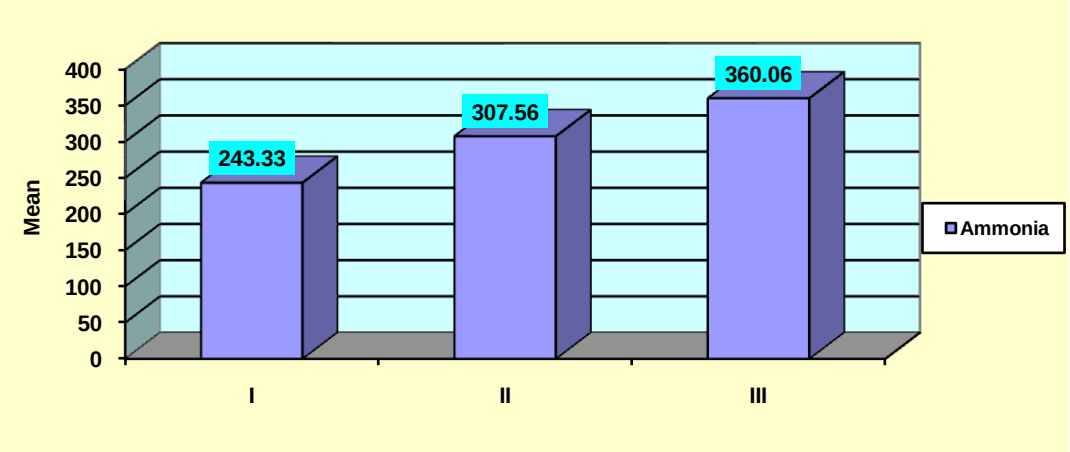

Figure (2) Comparison between different grades of hepatic encephalopathy and the mean of blood ammonia level

Table (8): Comparison between H. pylori Ab/Ag positive and negative cases in HE group and mean of blood ammonia level

	Ammonia Mean ± SD	T	P
H. pylori antigen			
-ve	226.06 ± 50.7	6.37	0.000
+ve	324.55 ± 64.2		
H. pylori antibodies			
-ve	248.81 ± 73.03	4.1	0.000
+ve	320.62 ± 61.53		

Table (9): Comparison between different grades of hepatic encephalopathy and the mean of blood ammonia level

Grade	Ammonia Mean ± SD	T	P
I	243.33 ± 46.22*	12.89	0.000
II	307.56 ± 85.8		
III	360.05 ± 45.32		

Discussion:-

H. pylori, gram-negative, microaerophilic gastric bacteria persistently colonize much of the world's population. Whereas nearly all adults are H. pylori-positive in developing countries, with socioeconomic development, prevalence has decreased substantially (8). H. pylori virulence is affected by the presence of the 35-40-kb cag pathogenicity island that can be detected by identification of the cagA gene or its product (CagA). CagA+ strains are more host-interactive (9). Serologic assays to detect antibodies to the CagA protein enhance overall detection of H. pylori, and specifically detection of the more interactive (CagA+) organisms. Antibodies to CagA persist for at least two decades in the absence of antimicrobial treatments that eliminate H. pylori. H. pylori colonization induces continuous gastric inflammation, which is more pronounced with cagA+ strains, and leads toward diminished gastric acidity.

Hepatic encephalopathy (HE) is a complex neuropsychiatric syndrome characterized by disturbances in consciousness and behavior, personality changes, fluctuating neurologic signs, flapping tremor (asterixis) and distinctive electroencephalographic (EEG) changes, due to hepatocellular dysfunction and porto-systemic shunting. Cirrhosis of liver accounts 50 – 70% cause of HE (10). There are many factors responsible for the development of HE in liver cirrhosis such as; Ammonia, production of false neurotransmitters, increase sensitivity of central nervous system neurons to the inhibitory neurotransmitters Gamma Amino Butyric Acid (GABA), increased level of circulating endogenous benzodiazepines, decreased activity of urea-cycle enzymes due to zinc deficiency, decreased level of myoinositol in brain, deposition of manganese in basal ganglia and swelling of brain astrocytes (4).

Among all above factors ammonia is the main culprit and responsible for the development of HE among cirrhotic patients and colonic bacteria are thought to be the main source of ammonia production. Stomach is one of the alternate site for ammonia production in Helicobacter pylori (H. pylori) infected patients (11). Though in normal circumstances, ammoniawhich produced by H.pylori is not absorbed in circulation except in cases of severe atrophic gastritis, liver and kidney dysfunction. Previous studies have observed that H. pylori eradication may theoretically reduce ammonia concentration in cirrhotic patients (12). Worldwide, data are controversial about the role of H. pylori as an independent risk factor for the development of HE and it is thought that H. pylori eradication therapy has no more role in HE patients (13).
The aim of this study is to find out the prevalence of helicobacter pylori infection in hepatic encephalopathy in our locality and study the correlation between the presence of helicobacter pylori and severity of hepatic encephalopathy.

The present study included 450 subjects; 300 patients of liver cirrhosis with hepatic encephalopathy, 75 patients of liver cirrhosis without hepatic encephalopathy and 75 healthy controls without any disease. Regarding the age, statistical analysis proved proper matching between the three groups, with a mean age ± SD of 53.93 ± 6.9 in control group, 53.27 ± 8 in cirrhotic patients and 57.22 ± 6.78 in hepatic encephalopathy patients ($p = 0.07$). This is in accordance with Correia et al. (2011) who agreed with our results concluded that there were no differences between groups regarding age.

In the present study, there were statistically significant differences between the three groups regarding hemoglobin, red blood cell count, white blood cell count and platelet level. Also, in the present study,cCreatinine&total protien show that there is statistically difference in HE group than the other two groups. urea,SGPT,albumin,total bilirubin,prothrombin time&Ptt show that there is statistical difference in control group from other two groups and cirrhotic group is statistically different form HE group. SGOT&direct bilirubin show that there was statistically difference in control group from the other two groups. These results go hand with hand as reported by Agarwal et al. (2011) (14).

In our study, mean of ammonia in control is 118.6, in cirrhotic group is 164.6 and in HE group is 283.51 and cirrhotic group is statistically different form HE group. There is no statistical coefficient correlation between ammonia and variables among studied groups except in age with ammonia where there is a direct correlation, also between ESR and ammonia where there is opposing correlation. There is no statistical significant association between sex and ammonia in cirrhotic group.Also there is no statistical significant association between ammonia and H. pylori Ag& Ab+ve in cirrhotic patients.,However mean of blood ammonia level significantly increased among HE patients with H.pylori Ag&Ab +ve than –ve.

Many studies have been conducted in contrast with this conclusion that H. pylori does not contribute significantly to the blood ammonia levels and the severity of HE, who determined the contribution of H.pylori to blood ammonia levels in patients of hepatic encephalopathy and correlated various indicators of portal systemic encephalopathy with blood levels of ammonia and H. pylori status. In a prospective study of 47 patients of subclinical hepatic encephalopathy in cirrhosis of liver, aged between 23 and 60 years, 49% showed Helicobacter pylori positivity by rapid urease test (15-18).

The baseline characters of our patients (mean age, serum creatinine, serum albumin, serum bilirubin, prothrombin time) were similar among patients with and without helicobacter infection in all patients. There was no statistically significant difference in blood ammonia levels in either group of patients. Blood ammonia values showed good correlation with the functional state of liver function but they did not show statistically significant difference between two groups of patients in any of Child Pugh classes. It is concluded that Helicobacter pylori does not contribute significantly to blood ammonia levels and the severity of hepatic encephalopathy.

Vasconez et al. (1999) assessed H. pylori status by rapid urease test (19). Kini et al (2001) also reported lower levels of fasting venous blood ammonia in patients with H.pylori infection (29 μ mol/l) than those without H.pylori infection (34 μ mol/l) in cirrhosis of liver disease. They also did not find any significant difference among the two groups. They suggested no role of H.pylori in hepatic encephalopathy (20).

There was a study suggested that H.pylori infection may be risk factor for hepatic encephalopathy only in individuals with advanced cirrhosis, but not in early liver disease (21).

In our study, the H. pylori titre was observed to be rising with the severity of hepatic encephalopathy. This finding correlated with the findings of many studies, where they found H. pylori seropositivity in hepatic encephalopathy-Grade I (77.63%), Grade II (78.13%), Grade III (100.00%), Grade IV (75.00%) (22).

Also many studies agree with our results who found out prevalence of H. pylori in hepatic encephalopathy and determined correlation between presence of H. pylori and severity of hepatic encephalopathy. They stated that prevalence of H. pylori was significantly high in patients of liver disease with hepatic encephalopathy. Prevalence and titre of H. pylori were found significantly increasing with the severity of hepatic encephalopathy, which suggests H. pylori may have a role in the pathogenesis of hepatic encephalopathy (23).

Sethar et al. (2004) study the frequency of H. pylori infection and evaluate its possible role in the pathogenesis of porto-systemic encephalopathy. They documented that frequency of H. pylori antibodies was significantly high in patients of porto-systemic encephalopathy. This may suggest that presence of Helicobacter pylori may have some role or may add to the pathogenesis of encephalopathy in patients with liver disease (24).

A study looked at the relationship of H. pylori infection with Minimal Hepatic Encephalopathy (MHE) and hyperammonemia in patients with liver cirrhosis and the effects of anti-H. pylori treatment in patients with MHE and H. pylori infection. They found that there was a significant association between H. pylori infection and MHE. Anti-H. pylori therapy results in reduction in blood ammonia levels and improvement in MHE (14).

Shaikh et al. (2012) determined the frequency of H. pylori in cases of hepatic encephalopathy with liver cirrhosis and found that before and after 10 day H. pylori eradication therapy, there was no significant improvement in HE grade and other parameters. While the finding of high frequency of H. pylori in HE patients may be either co-incidence or co-relation it needs further vast studies (25).

Conclusion:-

The frequency of h.pylori Ag&Ab among control group is 46.7% ,cirrhotic group Ag is 53.3&Ab is 40% in HEgroup Ag is 58.3%&Ab is48.3 it is also concluded that The mean of ammonia increased significantly among HE patients with H. pylori, antigens, antibiodies positive than negative cases& also by Comparison between different grades of hepatic encephalopathy and the mean of blood ammonia levelit is found that the LSD test shows that there is significant increase in blood ammonia level in grades II and III in comparison with grade I HE patients as in(table 9). So the finding of presince of H. pylori in HE patients may be either co-incidence or co-relation suggesting

positive correlation between the presence of helicobacter pylori and severity of hepatic encephalopathy that needs further studies.

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