ORIGINAL ARTICLE

Analysis of Gastric Juice in Acid Peptic Diseases

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Abstract:

Background: Peptic ulcer disease is an imbalance between offensive and defensive gastric factors. A bacterium called *Helicobacter pylori* has been considered a major causative agent for gastric and duodenal ulcers. This is a major cause of mortality in developing countries. *Aims and Objectives:* The aim of this study was to assess the biochemical parameters in gastric juice of acid peptic disease patients (Study Group) and normal healthy individuals (Control Group) in humans. *Material and Methods:* A total of 70 patients suffering peptic ulcer disease with *H. pylori* infection and 15 non-infected individuals were chosen as control group. *Results:* We observed an increased significant level ($p<0.05$) in pH, β-glucuronidase activity, malondialdehyde (MDA) and superoxide dismutase (SOD) as well as catalase(CAT) levels in gastric juice and decrease significant level ($p<0.05$) in pepsin activity, urea, mucin and nitric oxide level. *Conclusion:* The present study showed variations in levels of MDA, β-glucuronidase, SOD, CAT activities, pepsin, mucin urea and nitric oxide. Therefore these parameters can be used as additional parameters for diagnosis and prognosis of acid peptic diseases. These can be used by clinicians to adopt treatment strategies in betterment of acid peptic disease patients.

Keywords: Catalase, β-glucuronidase, *Helicobacter pylori*, Pepsin, Mucin, Urea, Nitric oxide, Malondialdehyde, Superoxide dismutase.

Introduction:

Peptic ulcer disease is a group of disorders characterized by the presence of ulcers in any portion of gastrointestinal tract (GIT) exposed to acid in sufficient concentration and duration. An ulcer is a crater like lesion in a membrane; ulcers that develop in areas of the GIT exposed to acidic gastric juice are called peptic ulcers [1]. It is one of the most common gastrointestinal disorders, which causes a high rate of morbidity. It comprises both gastric and duodenal ulcers [2].

Peptic disease is a worldwide problem among all age groups and both gender [3]. Peptic ulcer diseases are still an important cause of morbidity and mortality in developing countries though its prevalence has shown decrease in developed countries. Almost 5-15 % of adult population of world is suffering from peptic ulcer disease [4]. According to the latest WHO data published in April 2011 peptic ulcer disease, deaths in India reached 1, 08,392 or 1.20% of total deaths. The age adjusted death rate is 12.37 per 100,000 of population and ranks India 5th in the world [5].

Helicobacter pylori (*H. pylori*) and Non Steroidal Anti-Inflammatory Drugs (NSAID) disrupt the normal mucosal defense and repair, making the mucosa more susceptible to acid. *H. pylori* infection is present in 80% to 90% of patients with duodenal ulcers and 70% to 90% of patients with gastric ulcers. If *H. pylori* are eradicated, only 10% to 20% of patients have recurrence of peptic ulcer disease, compared with that of 70% recurrence in patients treated with acid suppression alone [6].

Peptic ulcer disease is an imbalance of aggressive

gastric luminal factors like acid and pepsin and defensive mucosal barrier function may be environmental and host factors, contribute to ulcer formation by increasing gastric acid secretion or weakening the mucosal barrier. Elaborately, peptic ulcer disease is characterized by the imbalance between gastric offensive factors like acid, pepsin secretion, lipid per-oxidation, nitric oxide and defensive mucosal factors like mucin secretion, mucosal cell shedding, glycoprotein, proliferation & antioxidant enzymes like catalase, superoxide dismutase & glutathione levels. Peptic ulcers include both gastric and duodenal ulcers. A bacterium called *Helicobacter pylori* has been considered a major causative agent for gastric and duodenal ulcers [1].

Material and Methods:

The present study was carried out in the Department of Biochemistry and General Surgery Department, Mahatma Gandhi Mission's Medical College, Kamothe, Navi Mumbai. Patients attending OPD and admitted in General Surgery ward with diagnosis of acid peptic disease at MGM, Navi Mumbai Maharashtra, during the period of February 2012 to March 2013 were enrolled for the present study. Total 85 subjects comprising of males and females of aged between 20-60 years were selected from General Surgery Department and their gastric juice was collected using endoscopy for the further analysis. The informed written consent concern forms were collected from patients & healthy control subjects. The present study was approved by Institutional Ethics Committee. The present study includes total 85 subjects out of which 70 were acid peptic diseases patients with *H. pylori* infection and 15 were healthy control subjects. Among control group 09(60%) were male and 06(40%) were females. In study group 45(64%) were male and 25(36 %) females.

Sample collection:

10 ml of gastric juice was collected from both normal healthy subjects and acid peptic diseases patients by endoscopy. Endoscopy was performed after an overnight fast, 8–12 hrs after the last dose of medication. At each endoscopy, immediately upon the endoscope entering the stomach, gastric juice was aspirated for fasting gastric juice. Samples of gastric juice were collected and centrifuged at 3000 rpm for 10 minutes and was used for estimation of pepsin, mucin, nitric oxide ($NO^{\cdot}$) and MDA, SOD, urea, catalase and β-Glucuronidase activity in gastric juice.

Biochemical Analysis:

Following biochemical parameter estimated by spectrophotometrically, pH was measured by pH-meter. Principle of pepsin in gastric juice was estimated by Joan Fourie and R. S. Arnot method: The acidified human haemoglobin was used as substrate for pepsin activity. Trichloroacetic acid soluble products resulting from the proteolytic action of pepsin (including tyrosine and phenylalanine) were measured at 280 nm in comparison with the effect of a standard crystalline pepsin [7]. Principle of mucin in gastric juice was estimated by Narman Siplet, S.A. Kmaroy and Harry shay Method: The hydrolysis of alkaline or neutral mucus with hydrochloric acid gives rise to large amounts of furfural, which interfered with the development of color with naphthoresorcinol. Furfural and glucuronic acid produce entirely different colors, as may be seen from the absorption spectra. Maximum absorption in the visible spectrum in the case of furfural takes place in the range of light transmitted by Filter 400 and in the case of glucuronic acid in the band transmitted by Filter

565 [8]. Principle of urea in gastric juice was estimated by Diacetyl Monoxime (DAM) method: under acidic condition diacetyl monoxime is heated it gets decompose into hydroxylamine and diacetyl. The diacetyl is then condensed with diazine, which is unstable and reacts with thiosemicarbazide and ferric ions (Fe++) to form pink colour complex. Thus thiosemicarbazide and ferric ions acts as a colour intensitifier and gives stability of colour obtained. The intense pink colour formed is measured at 540nm/ yellow green filter. The intensity of the color complex is directly proportion to the amount of urea present [9]. Principle of β-glucuronidase activity in gastric juice was estimated by Gobor Szasz method: The rate of hydrolysis of phenolphthalein glucuronide serves to assay the activity of β-glucuronidase at alkaline pH. The absorbance was read at 546 nm against water blank [10]. Principle of malondialdehyde (MDA) in gastric juice was estimated by K Satoh's method: In an acidic medium lipoproteins are precipitated and MDA elaborated as a result of lipid peroxidation reacts with thiobarbituric acid (TBA) to form pink coloured complex of MDA-TBA adduct. The colour is proportional to the MDA concentration of the sample which is compared with 1,1,3,3-ethoxypropane as primary standard treated similarly. The colour intensity is measured spectrophotometrically at 535 nm [11]. Principle of nitric oxide in gastric juice was estimated by Najawa K.C. and Nabil W. method: Nitrate the stable product of nitric oxide is reduced to nitrite by cadmium reduction method after deproteinisation of sample. The nitrite produced is determined by diazotization of sulphanilamide and coupling to naphthylene ethylene diamine. The color complex produced is measured at 540 nm [12]. Principle of superoxide dismutase (SOD) in gastric juice was estimated by Marklund and Marklund method: Superoxide anion is involved in the auto- oxidation of pyrogallol at alkaline pH. SOD dismutates superoxide radicals and thus inhibits the auto-oxidation of pyrogallol which can be observed by decrease in absorbance when measured by spectrophotometer at 420nm [13]. Principle of catalase in gastric juice was estimated by Sinha K method: Dichromate in acetic acid is reduced to chromic acetate when heated in presence of H_2O_2 with formation of per chromic acid as an unstable intermediate. The chromic acetate thus produced is measured spectrophotometrically at 620nm. The catalase preparation is allowed to split H_2O_2 for different period of time. The reaction is stopped at particular time by the addition of dichromate/ acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate spectrophotometrically at 620 nm after heating the reaction mixture [14]. Confirmation tests for *H. pylori*, acid peptic ulcer patients with symptoms of *H. pylori* infection and confirmed by Card test (serum antibodies test) and Rapid urease test carried out in Gastroenterology Department.

Statistical analysis:

Statistical analysis of the data was carried out with SPSS, version 16; Data was reported as mean ± SD. The comparisons between two groups were tested by unpaired t-test. A 95% confidence interval was used. P values less than 0.05 were considered statistically significant. Correlation between two continuous outcomes was evaluated using Pearson correlation coefficients.

Results:

Table 1: Mean ± SD of pH, Pepsin, Mucin, Urea and β-Glucuronidase of Control and Study Groups

Parameter	Control Group (mean ± SD) N=15	Study Group (mean ± SD) N=70
pH	02.54 ± 0.78	04.67 ± 00.99*
Pepsin (mg/dl)	77.59 ± 7.99	59.72 ± 14.98*
Mucin (mg/dl)	01.36 ± 0.17	00.41 ± 00.14*
Urea (mg/dl)	70.82 ± 4.20	37.78 ± 03.47*
ß-glucuronidase (mU)	00.66 ± 0.03	01.00 ± 00.22*

**p<0.05 (Statistically Significant)*

Table 2: Mean ± SD of MDA, $NO^{\cdot}$ Levels, SOD and CAT activities of Control and Study Groups

Parameter	Control Group (mean ± SD)	Study Group (mean ± SD)
MDA (ηmole/ml)	000.54 ± 00.34	003.02 ± 00.84***
$NO^{\cdot}$ (µmole/l)	124.25 ± 15.44	102.97 ± 19.72***
SOD (U/ml)	010.15 ± 05.33	028.34 ± 11.67***
CAT (U/mg of protein/ml)	002.44 ± 00.14	003.54 ± 00.77 ***

**** P<0.001 (highly statistically significant), Malondialdehyde-MDA, Nitric Oxide-$NO^{\cdot}$, Superoxide Dismutase-SOD, Catalase-CAT*

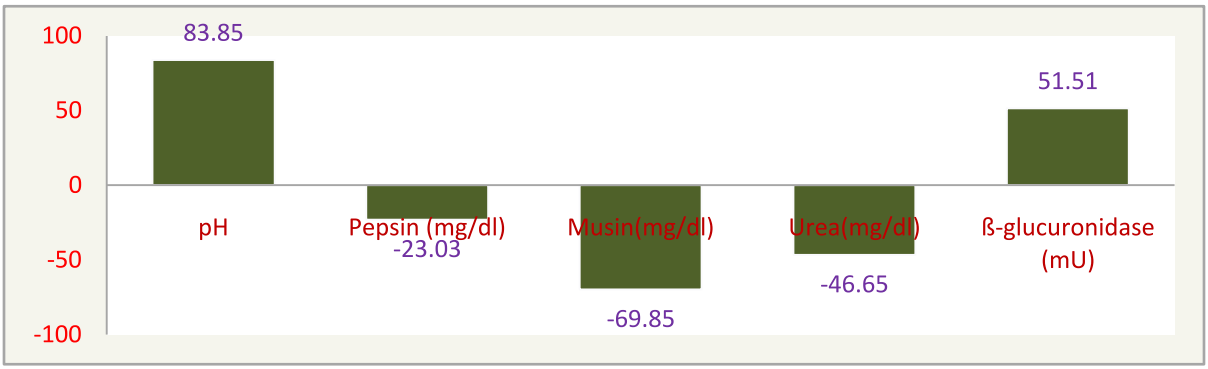

Fig. 1 : Percentage (%) change of Gastric juice pH, Pepsin, Mucin, Urea and β-Glucuronidase of Study Group with Respect to Control Group

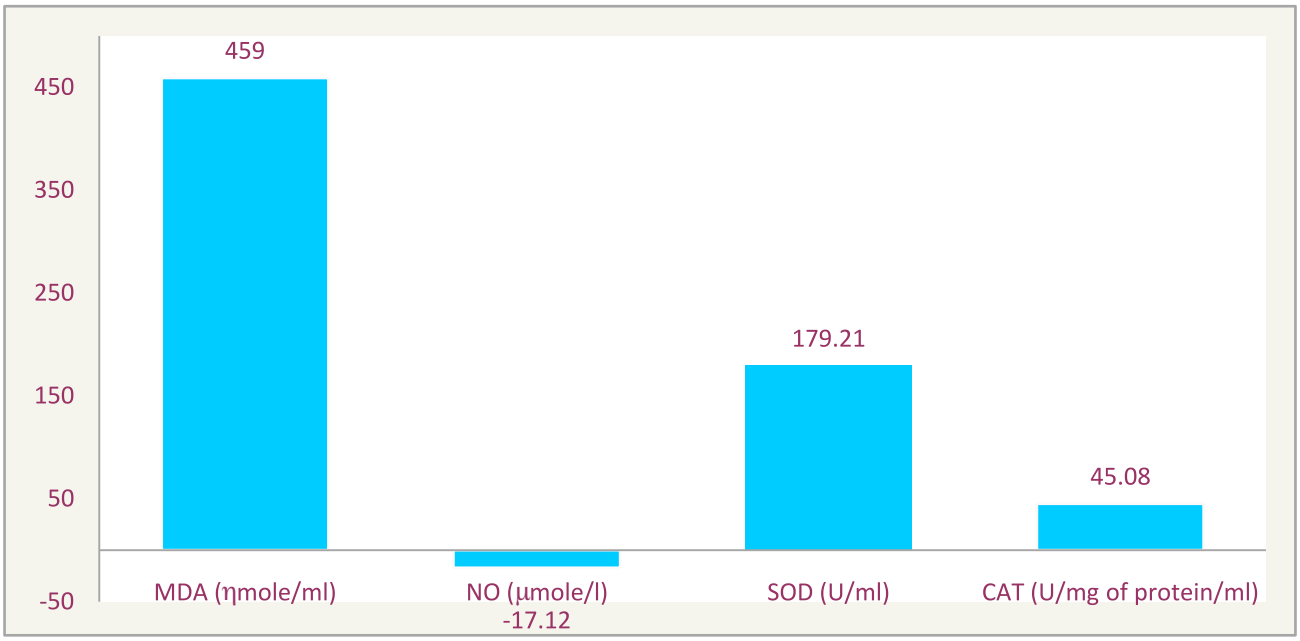

Fig. 2 : Percentage (%) Change of Gastric Juice MDA, NO, SOD and CAT levels of Study Group with Respect to Control Group

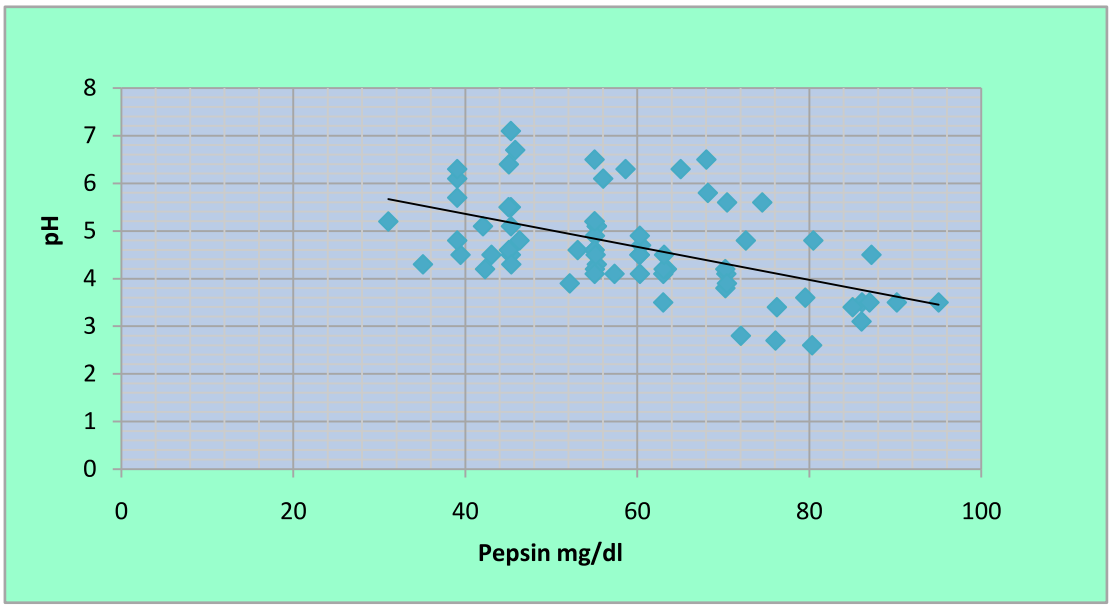

Fig. 3 : Graph-3 show negative correlation between Pepsin and pH in Acid Peptic Diseases (APD), r = - 0.51

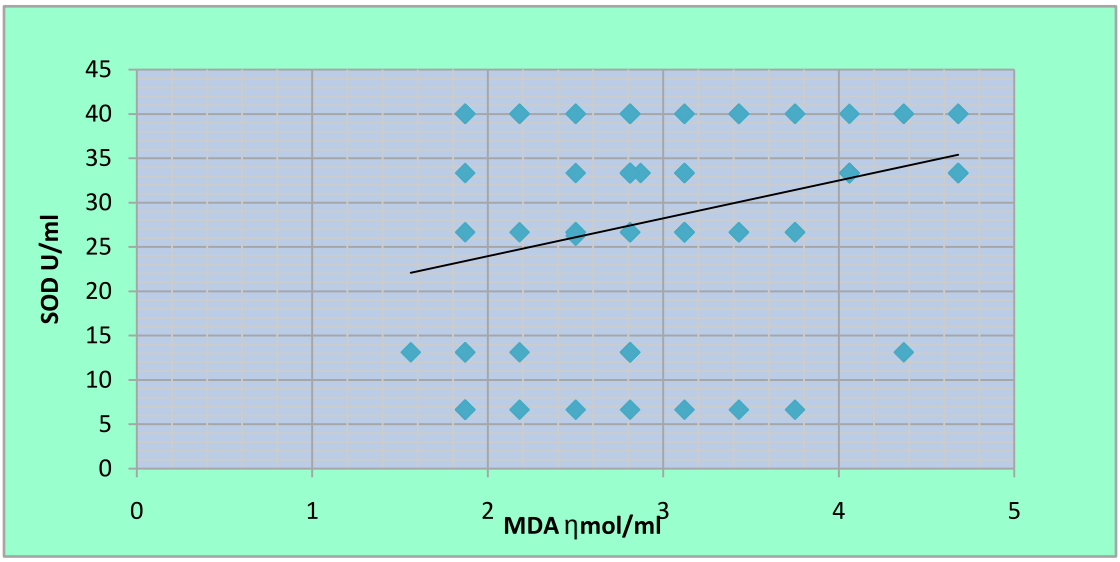

Fig. 4 : Show positive correlation between MDA and SOD in Acid Peptic Diseases (APD), r = 0.30

Discussion:

Peptic Ulcer Disease (PUD) is one of the most common gastrointestinal disorders, which causes a high rate of morbidity. It comprises both gastric and duodenal ulcers. These are benign defects in the gastrointestinal mucosa that extends beyond the muscular is mucosa, and are perpetuated by acid peptic activity [15]. *H. pylori* infection plays a crucial role in the pathogenesis of peptic ulcer disease. More than 95% of patients suffering from the duodenal ulcers and about 70-80% of patients with gastric ulcers are *H. pylori* positive [16].

Alteration in the biochemical parameters and oxidative stress markers in acid peptic diseases were rarely studied and which is not getting attention so far. In the present study, determination of gastric juice pH, pepsin, mucin, urea and β-glucuronidase enzymes activity as well as oxidative stress markers (MDA, $NO^{\cdot}$, SOD and CAT) in acid peptic ulcer patients with symptoms of *H. pylori* infection and confirmed by Card test (serum antibodies test) and Rapid urease test carried out in Gastroenterology Department. Correlation between these biochemical parameters and oxidative stress markers were also determined.

In present study, we observed that the percentage change for gastric juice pH levels (83.85%) which is more than in acid peptic diseases patients as compared to control. (Table 1) This may be due to H. pylori having high urease activity that hydrolyze urea to ammonia and bicarbonate and thus increased gastric pH. It is positively correlated with β-glucuronidase, MDA, SOD, CAT and negatively corrected with pepsin, mucin, urea, nitric oxide. Our results are concurrent with findings of OJ Lee *et al* [17], studies 143 patients with dyspepsia and reported that gastric juice pH and ammonia concentration were higher in 94 *H. pylori* infected patient (3.16 vs.1.55, p=0.0001; 5.58 ± 2.69 vs. 2.00 ± 1.49 μmol/L, p= 0.0001). Among 28 patient who received eradication therapy, 19 (67%) were successful, and their gastric pH levels and ammonia concentration were significantly lower than those in the eradication failure group (1.60 vs. 2.33; p= 0.007; 1.77 ± 1.28 vs. 4.02 ± 1.20 μmol/L; p= 0.0001),

and suggest that intragastric ammonia produced by *H. pylori* may have a partial role in an increased gastric juice pH, T Furuta *et al* [18] and D Stevanovic *et al* [19] also observed similar findings .

We observed that the percentage change in pepsin activity (-23.03%) of gastric juice in acid peptic disease patients was found to be significantly decreased ($p<0.05$) as compared to control group. (Table 1) Activity of pepsin decreased due to *H. pylori* infection, *H. pylori* produces urease enzyme which is capable of breaking urea into bicarbonate and ammonia thereby neutralizing the acidic pH and decreasing the activity of pepsin. It is negatively correlated with pH, SOD, MDA, CAT, β-glucuronidase and positively correlated with mucin, urea, nitric oxide. Our results are concurrent with findings of JL Newton *et al* [20], reported that mean pepsin activity was significantly lower in those with *H. pylori* compared to those without *H. pylori* infected subjects ($P<0.001$). SM Park *et al* [21] and M Feldman *et al* [22] also reported similar findings.

The percentage change mucin levels of (-69.85%) gastric juice which was found decreased in acid peptic disease patients ($p<0.05$) as compared to control. (Table 1) Low levels of mucin found in acid peptic disease patients indicate that *H. pylori* might be involved in decreasing total mucin synthesis. The gastrointestinal mucosa contain glycosylated mucin in high concentration, *H. pylori* binds to glycosylated mucin and causes destruction of this mucin layer thereby leading to low mucin levels. It is negatively correlated with MDA, SOD, CAT, β-glucuronidase, pH and positively correlated with pepsin, urea, nitric oxide. Our results of mucin are concurrent with the findings of JC Byrd *et al* [23], determined the effect of *H. pylori* on mucin synthesis in cultured gastric epithelial cells, they found that *H. pylori* inhibits total mucin synthesis *in vitro* and decreases the expression of MUC5AC and MUC1. A decrease in gastric mucin synthesis in vivo may disrupt the protective surface mucin layer. I. Radziejewska *et al* [24], studied 14 gastric juice samples of duodenal ulcer patients infected with *H. pylori*; assessed before and at the end of eradication. They reported that the bacterium affects the soluble form of MUC 1 mucin. RQ Wang and DC Fang [25], reported that *H. pylori* infection can alter the expression of some mucin genes in pericancerous mucosa and cancerous tissues of gastric carcinoma, then destroy the gastric mucosa barrier. S Tanaka *et al* [26], found that the amount of intracellular mucin in antrum epithelial cells of *H. pylori* positive patients was significantly lower than that of *H. pylori* negative patients ($p<0.01$).They suggested that *H. pylori* infection decreases gastric mucin synthesis via inhibition of UDP-galactosyltransferase.

We observed that the percentage change in urea level (-46.65%) of gastric juice in acid peptic disease patients which was less ($p<0.05$) as compared to control. (Table 1) It indicated that patients infected by *H. pylori* infection, produces urease enzyme. This urease enzyme converts urea to ammonia that makes gastric juice alkaline. Gastric juice urea levels were negatively correlated with pH, β-glucuronidase, MDA, SOD, CAT and positively correlated with pepsin, mucin, nitric oxide. Similar finding are also reported by K Blusiewicz *et al* [27], studied 30 patients end-stage renal disease and 31 patients with dyspeptic symptoms with *H. pylori* infection and found that *H. pylori* infection caused a significant decrease in urea concentration.

There was an inverse correlation between urea and ammonia concentration in gastric juice in both groups. Similar results have been reported by BJ Marshall *et al* [28] and AA Nijevitch *et al* [29].

In present study, we observed that the percentage change for β-glucuronidase activity (51.51%) in acid peptic diseases patients were found to be increased ($p<0.05$) as compared to control (Table 1). This increased β-glucuronidase activity may be due to tissue destruction in acid peptic diseases. The increased of gastric juice β-glucuronidase activity in acid peptic diseases patients positively correlated with MDA, SOD, pH, CAT and negatively correlated with pepsin, mucin, urea, nitric oxide. Our results of β-glucuronidase are concurrent with findings of RI Russell and C Watts [30], found that gastric juice activity of β-glucuronidase in all group higher as compared to normal. K Rogers *et al* [31], reported that measurement of gastric-juice enzymes is useful in the diagnosis of gastric cancer and may be beneficial in the identification of high-risk groups and MM Khanolkar [32] estimated the serum β-glucuronidase activity in gastrointestinal carcinoma and they found that increased β-glucuronidase activity as compared to control.

We observed that the percentage change in MDA levels of (459%) gastric juice in acid peptic diseases patients were found to be increased ($p< 0.001$) as compared to control. (Table 2) Increased lipid peroxidation may due to *H. pylori* infection induced oxidative stress which leads to damage of gastric mucosa. Elevated gastric juice MDA levels were positively correlated with pH, SOD, CAT, β-glucuronidase and negatively correlated with pepsin, urea, mucin, $NO^{\cdot}$ Similar results have been reported by Santra *et al* [33], found that gastric mucosal MDA levels were higher in duodenal ulcer patients with or without *H. pylori* infection as compared to control subjects ($p<0.01$) while serum MDA levels in duodenal ulcer patients with *H. pylori* infection were also significantly higher than in patients without *H. pylori* infection. They reported that increased MDA level due to the infection of *H. pylori* was associated with generation of reactive oxygen molecules, which leads to oxidative stress in the gastric mucosa. Similar study was carried out by R Tandon *et al* [34], Galaktinova *et al* [35], S Demir *et al* [36], ZA Inas *et al* [37]. they reported that mean serum MDA levels in gastric ulcer, duodenal ulcer and gastric carcinoma patients were found to be significantly higher compared to the control ($p<0.001$).

Our results demonstrated that the percentage change in gastric juice nitric oxide levels (-17.12%) were significantly decreased in acid peptic diseases as compared to control subjects ($p<0.001$), (Table 2) indicating that *H. pylori* may reduce the levels of $NO^{\cdot}$ in gastric juice to escape from host immunity response and to colonize successfully in human stomach mucosa. Secondly, the $NO^{\cdot}$ as an oxygen free radical can react with other oxygen intermediates, reaction with a superoxide anion-radical, also generated in abundance during *H. pylori* colonization. Due to increased level of superoxide anion-radical production, $NO^{\cdot}$ will be inactivated. As a result of this reaction a reactive anion of peroxynitrite acid (ONOO-) is formed. This anion joins protein sulphydrylic groups (SH) and oxidises aromatic group nitrogen, causing lipid peroxidation. It is positively correlated with pepsin, mucin, urea but negatively correlated with pH, SOD, CAT, β-glucuronidase, MDA. Similar findings were reported by M Ansari1 *et al* [38], found that, significant reduction in the mean levels of nitric oxide in the gastric juice of the patients with *H. pylori* infection as compared to non infected individual ($p <0.0001$). *H. pylori* may reduce the levels of NO in gastric juice to escape from host immunity response and to colonize successfully in human stomach mucosa and Chojnacki J *et al*

[39], show that the concentration of nitric oxide metabolites was higher in asymptomatic infections ($p< 0.05$). After eradication of *H. pylori* the concentration of nitric oxide metabolites in the non ulcer dyspepsia group increased ($p< 0.05$), whereas in asymptomatic infection group, it did not undergo significant changes.

In present study, we observed that the percentage change for gastric juice SOD(179.21%) and CAT(45.08%) activity in acid peptic diseases patients were found to be increased as compared to control($p<0.001$). (Table 2) elevated activities of these enzymes in gastric juice of *H. pylori* infected patients could be due to excess production of ROS by *H. pylori* which are in turn scavenged by increased levels of enzymatic antioxidants i.e. SOD and CAT. Secondly there might be release of intracellular enzyme (SOD and CAT) from damaged mucosal cells. It is positively correlated with pH, β-glucuronidase, MDA, CAT and negatively correlated with pepsin, mucin, urea, nitric oxide. Our results of SOD are concurrent with findings of M Ansari1 *et al* [38], showed that activity of SOD in gastric juice was significantly increased in *H. pylori* infected patients as compared to control ($P < 0.05$). they observed negative correlation with between nitric oxide level and SOD activity in gastric juice ($r = -0.35$, $P = 0.023$). Dolatkhah *et al* [40], studied 43 smoker patients with active peptic ulcer and 43 nonsmokers without peptic ulcer, 43 smokers without peptic ulcer and 43 non-smokers with active peptic ulcer as control group were selected. They showed that in smokers with active peptic ulcer, superoxide dismutase activity were significantly high rather than control group. Smoking gives rise to the increase in some free radicals in different body tissues that lead to severe oxidative pressure on tissues. Ray *et al* [41], reported that the activity of SOD and CAT activity in gastric ulcer rat was significantly elevated as compared to control ($p<0.05$). Kumar *et al* [42], Patra *et al* [43] and Raji *et al* [44] also observed similar finding in rat.

In present study MDA, β-glucuronidase, pH level and SOD and CAT activities were significantly increased whereas pepsin, mucin urea and nitric oxide were significantly decreased in acid peptic disease with *H. pylori* infection. MDA, β-glucuronidase and pH level were negatively correlated with pepsin, mucin urea and nitric oxide.

Conclusion:

Present study showed that acid peptic disease with *H. pylori* infection produces urease enzyme which is capable of breaking urea into bicarbonate and ammonia thereby neutralizing the acidic pH and decreasing the activity of pepsin. *H. pylori* infection is also involved in decreasing total mucin synthesis. The gastrointestinal mucosa contain glycosylated mucin in high concentration, *H. pylori* binds to glycosylated mucin and causes destruction of this mucin layer thereby leading to low mucin levels. *H. pylori* infection induced oxidative stress which leads to damage of gastric mucosa by lipid peroxidation that's increases β-glucuronidase activity may be due to tissue destruction in acid peptic diseases in *H pylori* infection. Nitric oxide level decreased due to *H. pylori* infection generates large amounts of superoxide radicals, that can rapidly react with $NO^{\cdot}$ and generate peroxynitrite leads to damage of gastric mucosa. SOD and CAT activity increased enzyme expression caused by ROS derived from activated macrophages or release of the enzyme from the damaged mucosal cell.

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