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Peptic Ulcer Disease and *Helicobacter Pylori* Infection: Does Serum Zinc Level Play a Role?

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A B S T R A C T

The pathogenesis of peptic ulcer is multifactorial and arises from an imbalance between protective and aggressive factors. This work aimed to assess the possible protective role of serum zinc in patients with peptic ulcer disease and declare any correlation between serum zinc level and *H. pylori* infection. Fifty consecutive patients with symptoms suggestive of peptic ulcer disease were included. All underwent complete clinical evaluation, laboratory investigations, upper gastrointestinal endoscopy and rapid urease test. Measurement of serum zinc level was done for all the included patients in addition to twenty five age and sex matched healthy control subjects. The mean serum zinc level of the studied patients was highly significantly decreased in comparison to healthy controls (P= 0.002). Moreover, a significant stepwise decrease in serum zinc level was observed with increased severity of gastric (P<0.01) and duodenal mucosal injury (P>0.05). At serum zinc cut-off level of 87 µmol/L, patients had gastric and duodenal endoscopic lesions with sensitivity, specificity, positive predictive value and negative predictive value of 58%, 92%, 93.5% and 52.3%; respectively. On the other hand, no significant difference was detected in serum zinc levels between *H. pylori* positive and negative patients (P>0.05). Zinc may play a protective role against gastric and duodenal mucosal membrane injury. Serum zinc cut-off level of 87 µmol/L could predict positive gastric and duodenal endoscopic findings.

Introduction

The pathogenesis of peptic ulcer is multifactorial and arises from an imbalance between protective and aggressive factors (Feinstein *et al*., 2010). *Helicobacter pylori (H. pylori)* infection, alcohol, bile salts, acid and pepsin can alter the mucosal defense by allowing back diffusion of hydrogen ions and subsequent epithelial cell injury. The defensive mechanisms include tight intercellular junctions, mucus, mucosal blood flow, cellular restitution and epithelial renewal (Suerbaum *et al*., 2002).

A strong link has been established between *H. pylori* and a diverse spectrum of gastrointestinal diseases, gastric adenocarcinoma, MALT lymphoma and gastric non Hodgkin lymphoma (Noto *et al*., 2012).

The gastric mucosa may be impaired in conditions of zinc deficiency. Moreover, clinical studies have shown the anti-ulcer action of zinc in humans. Previous studies revealed that zinc acts as the essential element in the physiology of the digestive system, accelerating the process of wound healing of various types of tissues including gastric ulcer (Watanabe *et al*., 1995). Zinc deficiency may affect the structure of intercellular junctional complexes of gastro-intestinal epithelial cells destroying membrane barrier function and integrity. This leads to increase in neutrophil accumulation and starts a positive regulation of chemokines that play an important role in neutrophil migration and inflammatory development (Christudoss *et al*., 2010).

This work aimed at assessing the possible protective role of serum zinc in patients with peptic ulcer disease and also to declare any correlation between serum zinc level and *H. pylori* infection.

Patients and Methods

This prospective study was conducted on fifty consecutive patients with symptoms suggestive of peptic ulcer disease like epigastric pain, dyspepsia, heartburn who were candidates for upper gastrointestinal endoscopy. They were presented to Tropical Medicine and Internal Medicine Departments at Ain Shams University Hospital, during the period from October 2013 to August 2015.

Patients who received zinc supplementation, proton pump inhibitors (PPI), non-steroidal anti-inflammatory drugs (NSAIDs), those presented with hematemesis or melena, as well as those with chronic liver disease, renal failure, malabsorption or previous gastric surgery were excluded.

Informed written consent was obtained from each patient prior to inclusion. The study protocol was approved by the Research Ethics Committee of Faculty of Medicine, Ain Shams University according to the ethical guidelines of the 1975 Declaration of Helsinki.

All of the included patients underwent:

(1) A complete clinical evaluation;

(2) Routine laboratory investigations:

CBC and PT, INR ; in addition to liver profile (serum ALT & AST) and kidney function test (serum urea & creatinine) that were measured on Synchron CX-9 Pro auto analyzer (Beckman Instruments Inc.; Scientific Instruments Division, Fullerton, CA 92634, 3100, USA).

(3)Measurement of serum zinc level:

Serum zinc was assayed for all the included patients in addition to twenty five age and sex matched healthy control subjects using Colourimetric method. Kits were supplied from Qumica Clinica Aplicada S.A, Spain.

Principle: In an alkaline solution, the zinc ions of the sample produce a red coloured complex with 2-(5-bromo-2-pyridylazo)-5-(N-n-propyl-N-3-sulfopropylamino)-phenol. The colour intensity is directly proportional to the zinc ions' concentration present in the sample. Normal range of serum zinc level is 84-159 µmol/L.

(4)Upper gastrointestinal endoscopy: Thorough examination of the esophagus, stomach and duodenum was done to all patients using Olympus CV-150 or Pentax EPM-3500.

Gastroesophageal reflux disease was graded by using the Los Angeles classification into: *Grade A:* one (or more) mucosal break no longer than 5 mm, that does not extend between the tops of two mucosal folds (a mucosal break being defined as an area of slough or erythema with discrete demarcation from the adjacent mucosa), *Grade B:* one (or more) mucosal break more than 5 mm long that does not extend between the tops of two mucosal folds, *Grade C:* one (or more) mucosal break that is continuous between the tops of two or more mucosal folds which involves less than 75% of the circumference and
Grade D: one (or more) mucosal break which involves at least 75% of the esophageal circumference (Lundell *et al*., 1999).

Gastritis was classified according to its site into antral, body, fundus, or pangastritis. Ulcers were classified as gastric or duodenal according to their site (Todd *et al*., 2004). Biopsies were taken from gastric and duodenal lesions.

(5) Rapid urease test:

The test was performed at the time of gastroscopy. A biopsy of mucosa was taken from the antrum of the stomach, and is placed into a medium containing urea and an indicator such as phenol red. The urease produced by *H. pylori* hydrolyzes urea to ammonia, which raises the pH of the medium, and changes the color of the specimen from yellow (negative) to red (positive) (Versalovic, 2003).

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. Qualitative data were presented as number and percentage while quantitative data were presented as mean, standard deviation and range. Comparison between two groups with qualitative data was done using Chi-square test and/or Fisher exact test. Comparison between two independent groups with quantitative data was done using Independent t-test, while comparison between more than two independent groups with quantitative data was done using One Way ANOVA test.

Receiver operating characteristic (ROC) curve was used to assess the best cut off point with sensitivity and specificity.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the P-value was considered as: $P > 0.05$: Non significant, $P < 0.05$: Significant and $P < 0.01$: Highly significant.

Results and Discussion

This prospective study included 50 consecutive patients presented with symptoms suggestive of peptic ulcer disease. Their mean age was 40.98±12.58 years and included 24 males (48%) and 26 females (52%). The clinical presentations of studied patients were epigastric pain (100%), heart burn (56%), regurgitation (22%), vomiting (22%) and dyspepsia (16%).

The mean serum zinc level of studied patients was 87.18±17.99µmol/L which was highly significantly decreased in comparison to healthy controls (99.64 ± 11.63 µmol/L) ($P= 0.002$).

Table.1 shows that the most common upper GIT endoscopic findings in our studied patients were diffuse gastritis (44%), GERD grade A (38%), duodenitis (26%) and antral gastritis (22%).

A highly significant difference was observed in serum zinc levels among different gastric endoscopic findings (P<0.01) as the lowest serum zinc level was detected in patients with gastric ulcer compared to diffuse gastritis. In addition, there was a significant difference in serum zinc levels among duodenal endoscopic findings; the lowest serum zinc level was detected in duodenal ulcer compared to duodenitis (P<0.05). However, there was no significant difference was observed in serum zinc levels among different esophageal endoscopic findings (P>0.05).

Figure.1 shows Receiver Operating Characteristic (ROC) curve for serum zinc at cut-off level of 87µmol/L. At this cut-off level; patients had positive gastric and duodenal endoscopic findings with sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 58%, 92%, 93.5% and 52.3% respectively [Area under the Curve (AUC) = 0.729].

In the current study, *H. pylori* infection was detected by rapid urease test in 34/50 patients (68%).There was no significant difference of serum zinc levels between positive versus negative *H.pylori* infection (P>0.05, Table 2).In addition, the presence of *H.pylori* infection didn't show a significant difference in relation to positive endoscopic findings (P>0.05, Table 3).

Table.1 Comparative analysis of serum zinc level among different endoscopic findings in studied patients

Endoscopic findings		No. (%)	Serum zinc (µmol/L)	One Way ANOVA		Sig.
			Mean ± SD	F	P- value	
Esophagus (n=50)	Normal	23 (46%)	89.26 ± 14.08	2.448	0.076*	NS
	GERD† grade A	19 (38%)	81.32 ± 18.89			
	Incompetent cardia	6 (12%)	101.33 ± 23.29			
	Barrett's esophagus	2 (4%)	76.50 ± 13.44			
Stomach (n=50)	Normal	10 (20%)	104.70 ± 16.78	6.221	0.000**	**HS**
	Antral gastritis	11 (22%)	90.18 ± 11.37			
	Diffuse gastritis	22 (44%)	80.91 ± 14.58			
	Gastric ulcer	3 (6%)	64.00 ± 2.65			
	Hiatus hernia	4 (8%)	87.00 ± 25.18			
Duodenum (n=50)	Normal	35 (70%)	91.29 ± 17.97	3.702	0.032***	**S**
	Duodenitis	13 (26%)	79.00 ± 15.03			
	Duodenal ulcer	2 (4%)	68.50 ± 2.12			

†GERD: Gastro Esophageal Reflux Disease.
*NS: No significant difference, **HS: highly significant difference, ***S: significant difference.

Table.2 Comparative statistics of serum zinc levels between *H. pylori* positive versus negative infections

H. pylori	Serum zinc level (µmol/L)		Independent t-test		Sig.
	Mean	SD	t	P-value	
Negative (n=16)	92.19	17.38	1.038	0.303*	NS
Positive (n= 34)	88.00	16.03			

*NS: No significant difference

Table.3 Comparison between *H.pylori* Positive and Negative Patients Regarding Endoscopic Findings

Endoscopic findings		*H. pylori* Positive		*H. pylori* Negative		Chi-square test		Sig*
		No.	%	No.	%	X^2	P-value	
Esophagus	Normal	18	52.9%	5	31.2%	1.280	0.257	NS
	GERD† grade A	12	35.3%	7	43.8%	0.068	0.793	NS
	Incompetent cardia	2	5.9%	4	25.0%	2.173	0.141	NS
	Barrett's esophagus	2	5.9%	0	0.0%	0.046	0.828	NS
Stomach	Normal	6	17.6%	4	25.0%	0.052	0.820	NS
	Antral gastritis	7	20.6%	4	25.0%	0.001	0.988	NS
	Diffuse gastritis	17	50.0%	5	31.2%	0.885	0.347	NS
	Gastric ulcer	3	8.8%	0	0.0%	0.345	0.557	NS
	Hiatus hernia	1	2.9%	3	18.8%	1.859	0.173	NS
Duodenum	Normal	22	64.7%	13	81.2%	2.682	0.102	NS
	Duodenitis	10	29.4%	3	18.8%	0.208	0.648	NS
	Duodenal ulcer	2	5.9%	0	0.0%	0.018	0.894	NS

†GERD: Gastro Esophageal Reflux Disease,
*NS: No significant difference.

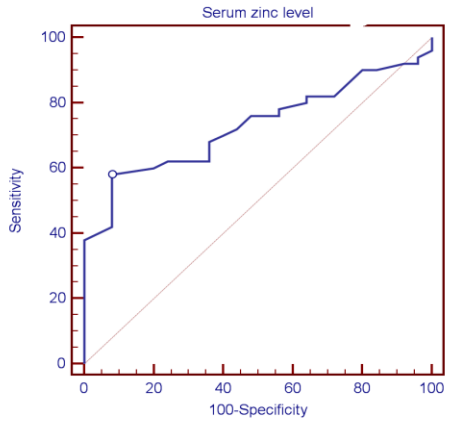

Figure.1 Receiver Operating Characteristic (ROC) curve for serum zinc at cut-off level of 87 µmol/L. At this cut-off level; patients had positive gastric and duodenal endoscopic findings with sensitivity 58% and specificity 92%. [Area under the Curve (AUC) = 0.729].

Zinc contributes a role in the host defense mechanism by maintaining the structure and function of the membrane barrier that is especially important in the gastro-intestinal tract, which is continuously exposed to plenty of pathogens and noxious agents (Finamore *et al*., 2008).

In the current study, we aimed to assess the possible protective role of serum zinc in patients with peptic ulcer disease and also to declare any correlation between serum zinc level and *H. pylori* infection.

All included patients in the current study (100%) presented by epigastric pain and

56% by heart burn. Less common clinical presentations were regurgitation, vomiting and dyspepsia.

The present study revealed that the most common upper GIT endoscopic findings were diffuse gastritis (44%), GERD grade A (38%), duodenitis (26%) and antral gastritis (22%). This was different from other studies done for similar group of patients. The study of *Thomson et al.* (2003) revealed that 43% of patients had reflux esophagitis, 9.8% had gastric erosions and 5.3% had peptic ulcer. *Okello* (2006) found that the most common endoscopic findings were duodenal ulcer (14.8%), gastritis (12.6%) and bile reflux (5.2%). These differences could be attributed to different study populations.

In the current study, a highly significant decrease of serum zinc levels was observed in different gastric endoscopic findings compared to controls ($P<0.01$). In addition, a significant stepwise decrease was observed in serum zinc levels as the severity of gastric mucosal injury increases. This was in agreement with the study of *Bandyopadhyay et al.* (1995).

Zhang et al. (2012) carried out a study on 45 patients with gastric cancer, 44 with peptic ulcer, 52 with gastritis and 64 healthy controls. They found that serum zinc level was significantly lower in gastritis patients than in control group and more significantly decreased in those with peptic ulcer and gastric cancer.

Mei et al. (2012) reported that Zn(II) – curcumin in complex had notably and dose-dependently protected the gastric mucosa against ethanol-induced injury. Consistent results were reported by *Salga et al.* (2012) who mentioned that zinc complexes had anti-ulcer activity and can be used as treatment for gastrointestinal disorders. A novel compound dichlorido-zinc (II)-4-(2-(5-methoxybenzylidene aminoethylpiperazin-1-iumphenolate (ZnHMS) was synthesized, characterized and evaluated for its gastro-protective activity against ethanol-induced ulcer in rats. The authors found that ZnHMS significantly enhanced the protection of gastric epithelia and it was suggested that the gastro-protective activity of ZnHMS might contribute in adjusting the inflammatory cytokine-mediated oxidative damage to the gastric mucosa.

In the current study, there was a significant decrease in serum zinc level with increased severity of duodenal endoscopic findings ($P<0.05$). The protective role of zinc on duodenal mucosal membrane was also proved by the study of *Troskot et al.* (1997).

H. pylori infection is a well-known cause of gastritis, peptic ulcer and even gastric cancer. The relation between *H. pylori* infection and positive endoscopic findings was evident in previous studies. However, in our study, there was no significant difference between *H.pylori* positive and negative infection and endoscopic findings in esophagus, stomach and duodenum ($P>0.05$). Also, there was no significant difference between presence of gastro esophageal reflux disease (GERD) in relation to *H. pylori* infection ($P>0.05$).

This was in agreement with *Chung et al.* [21], who suggested that *H. pylori* positive patients were less likely to have GERD. Previous epidemiological studies demonstrated a negative association between *H.pylori* infection and GERD and its complications. This protective effect could be explained by the tendency of *H. pylori* infection to lower gastric acid secretion with advancing age (Delaney *et al*., 2005; Hiyama *et al*., 2008).

Severe zinc deficiency depresses immune function, and even mild to moderate degrees of zinc deficiency can impair macrophage and neutrophil functions, natural killer cell activity, complement activity and the body requires zinc to develop and activate T-lymphocytes (Wintergerst *et al*., 2007). However, we found no significant relation between serum zinc level and *H.pylori* infection (P> 0.05).This was in agreement with *Zhang et al.,* and Janjetic *et al.* (2010).

However, Sempértegui *et al.* (2007) reported that the degree of inflammation in *H. pylori*-induced gastritis was affected by gastric tissue zinc concentrations. *H. pylori*-infected patients with non-atrophic chronic gastritis had lower concentrations of zinc in gastric mucosa and higher polymorphnuclear (PMN) cell infiltration than uninfected patients with the same type of gastritis.

In conclusion, zinc may play a protective role against gastric and duodenal mucosal membrane injury. Serum zinc cut-off level of 87µmol/L could predict positive gastric and duodenal endoscopic findings. However, no significant difference was observed in serum zinc level as regard *H. pylori* infection.

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