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Some Biochemical Studies on Serum of Duodenal Ulcer Patients Infected with *Helicobacter pylori* in Ninevah Governorate

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ABSTRACT

The research was concerned with biochemical studies in serum of duodenal ulcer (DU) patients. The present study included (152) patients (105 males and 47 females), their ages ranged between (15-56) years, attending endoscopy unit of Al-Razzi General Teaching Hospital in Ninevah Governorate.

Blood samples from (152) DU patients, were collected for some biochemical parameters including alkaline phosphatase (ALP), glutamate pyruvate transaminase (GPT), urea, total protein, Immunoglobulins (Igs), and haemoglubin (Hb).

The results predicted that GPT, urea, total protein, IgG and complement C_4 statistically showed no significant difference with *H. pylori* infection, while ALP, IgM, IgA, complement C_3 and Hb showed significant differences.

بعض الدراسات الكيموحيوية لمصل دم المرضى المصابين بقرحة الاثني عشر المسببة عن بكتريا الملوية البوابية في محافظة نينوى

الملخص

تضمن البحث دراسة كيموحيوية لمصل دم المرضى المصابين بقرحة العفج (الاثني عشر) . حيث شملت عينة الدراسة 152 مراجعاً لوحدة الناظور في مستشفى الرازي التعليمي العام في محافظـة نينـوى وتراوحـت اعمارهم بين (15-56) سنة ، وكان عدد الذكور المشمولين بالدراسة (105) وعدد الاناث (47) .

شملت المتغيرات المقاسة : الفوسفاتيز القاعدي ، كلوتاميت بايروفيت ترانس أمنيز ، اليوريا ، البروتين الكلي ، البروتينات المناعية والهيموكلوبين .

اظهرت نتائج التحليل الاحصائي عدم وجود فروق معنوية لكل من الكلوتاميت بايروفيت ترانس أمنيز ، اليوريا ، البروتين الكلي والبروتينات المناعية (C4, IgG) مع الاصابة بالبكتريا الملوية البوابية ، بينمـا أظهـر الفوسفاتيز القاعدي والبروتينات المناعية (C3, IgA, IgM) والهيموكلوبين فروقات معنوية .

INTRODUCTION

Most foodstuffs are ingested in forms that are unavailable to the organism, since they cannot be absorbed from the digestive tract and must be broken down into smaller

molecules. This disintegration of the naturally occurring foodstuffs into assimilable forms constitutes the process of digestion (Murray *et al*., 1996). Diseases of the gastrointestinal tract are major cases of morbidity and mortality. Approximately (10%) of all general practitioner consultations in the United Kingdom are for indigestion (Haslett *et al*., 1999).

The major functions of the stomach are to store food temporarily, continue digestion by chemically reducing food particle size, and regulate emptying into the duodenum (Sperelakis and Banks, 1996).

The term peptic ulcer refers to an ulcer in the lower oesophagus, stomach or duodenum (Haslett *et al*., 1999). Peptic ulcer is the circumscribed loss of tissue that occurs in portions of the digestive tract exposed to chlorhydro-peptic secretion (de-Carvalho, 2000). There are strong evidence that *Helicobacter pylori* is the most important causative factor regarding peptic ulcer over 90% of adults with duodenal ulcer and 70% of those with gastric ulcer are infected (Graham, 1989; Al-Ajeal *et al*., 1996).

Duodenal ulcer is a round, sharply punched - out defect, usually less than 3 cm in diameter in the mucosa of the duodenum. The defect may be superficial or may penetrate through the serosa. 90% of DU occurs in the first portion of the duodenum. Ulcers occurring in the distal antrum and pyloric channel of the stomach are also considered duodenal (Teitz, 1994). The duodenal mucosa is inflamed and ulcerated by abnormally frequent bursts of acidity (Wormsley, 1974).

MATERIALS AND METHODS

Patients

Patients were enrolled in the present study to the Gastroendoscopy unit in Al-Razzi General Teaching Hospital in Ninevah Governorate in the fasting state (no food or liquids for at least 12 hrs). The patients were not permitted to smoke before or during the test.

These patients were presented with upper gastrointestinal dyspeptic symptoms for more than one-month duration and with definite endoscopical finding of peptic ulcer.

In these patients, gastroscopy confirmed the presence of duodenal ulceration and *Helicobacter pylori* infection.

Collection of Blood Samples

Venous blood samples (5) ml were drawn from each patient after endoscopic confirmation of duodenal ulcer, then transferred immediately to a clean dry plain tube. After removing the needle, the blood was allowed to clot for at least (10-15) min. at a room temperature and then centrifuged for (10) min. at (4000xg). Serum was removed for the measurement of ALP, GPT activity and other biochemical tests (Tietz, 1994).

Biochemical measurements: alkaline phosphatase was assayed by using manufactured Kit by bioMerieux (Kind & King, 1954).

GPT was assayed using kit manufactured by RANDOX (Reitman and Frankal, 1957; Varley, 1967), and serum total protein was determined by Biuret method (Plummer, 1978) using kit manufactured by RANDOX (United Kingdom).

Blood urea was determined by the enzymatic method (urease-modified Berthelot reaction) using kit manufactured by Biomerieux (France).

Immunoglobulins (Igs) and other proteins in serum was determined by the method of Single Radial Immunodiffusion (SRID) of Fahey and Mcklerey (1965) was used, using KALLESTAD^{TM} ENDOPLATE^{TM} Single Radial Immunodiffusion Test Kits from Sanofi Diagnostic Pasteur, Inc.

Haemoglobin was determined by Drabkin's cyanomethaemoglobin method (Markarem, 1974) using kit manufactured by RANDOX (United Kingdom).

RESULTS AND DISCUSSION

Figure (1) showed a significant difference ($p \leq 0.01$) in serum alkaline phosphatase between duodenal ulcer patients infected with *H. pylori* and non-infected patients and no significant difference with sex and age ($p > 0.05$).

Alkaline phophatase is usually increased but remained within normal range (2.5–13) King and Kind U./100 ml (Al-Helaly, 2000), as shown in figure (1). Similar finding was reported by other investigators (Abbas *et al*., 1997). On the other hand, (Bouchier *et al*., 1984) recorded that a small rise in alkaline phosphatase is less marked after vagotomy and pyloroplasty for duodenal ulceration.

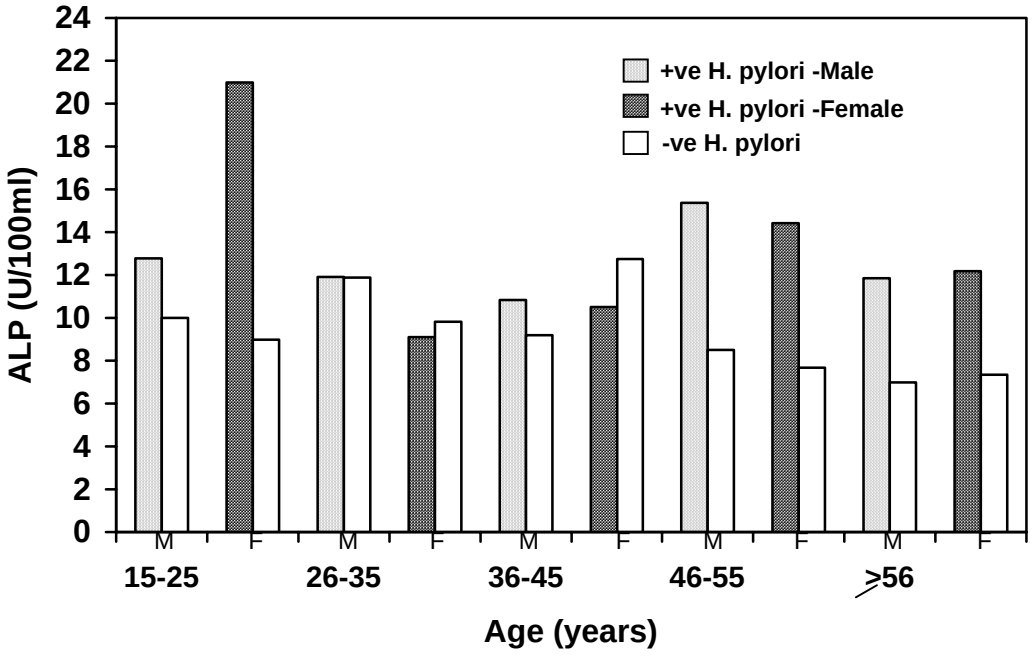

Fig. 1: The relation between age and serum alkaline phosphatase (Kind and King U/100 ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

The results of GPT in duodenal ulcer patients are shown in (fig. 2). The statistical analysis of data showed that sex, age and *H. pylori* infection had no significant effect on serum GPT in duodenal ulcer patient ($p > 0.05$). This observation could be explained on the fact that serum GPT is very rich in heart, liver, skeletal muscle, kidney, pancreas, spleen and lung (Varley, 1967; Tietz, 1994).

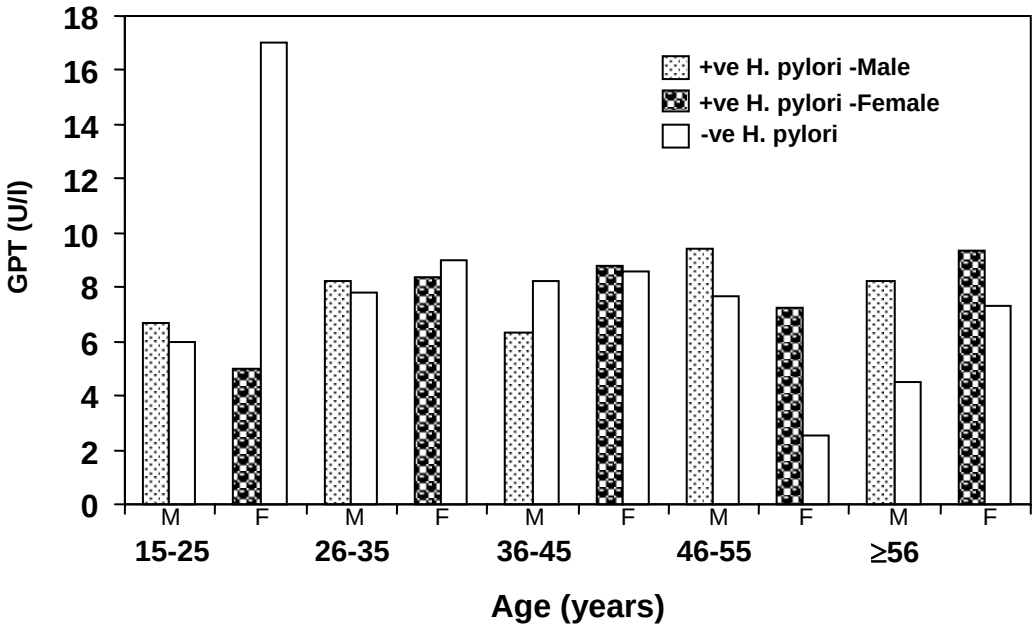

Fig. 2: The relation between age and serum glutamate pyruvate transaminase (U/l) in duodenal ulcer patients of both sexes infected with *H. pylori*.

The results of total protein in duodenal ulcer patients are shown in figure (3). The study found no significant difference ($p>0.05$) in total protein with *H. pylori* infection in duodenal ulcer patients. This result is probably due to presence of PG in blood circulation at the pH of blood which is biologically inactive and has no function (Samloff, 1989). On the other hand, age showed a significant effect on total protein ($p \leq 0.05$) in duodenal ulcer patients. While total protein results showed no significant difference with sex ($p>0.05$).

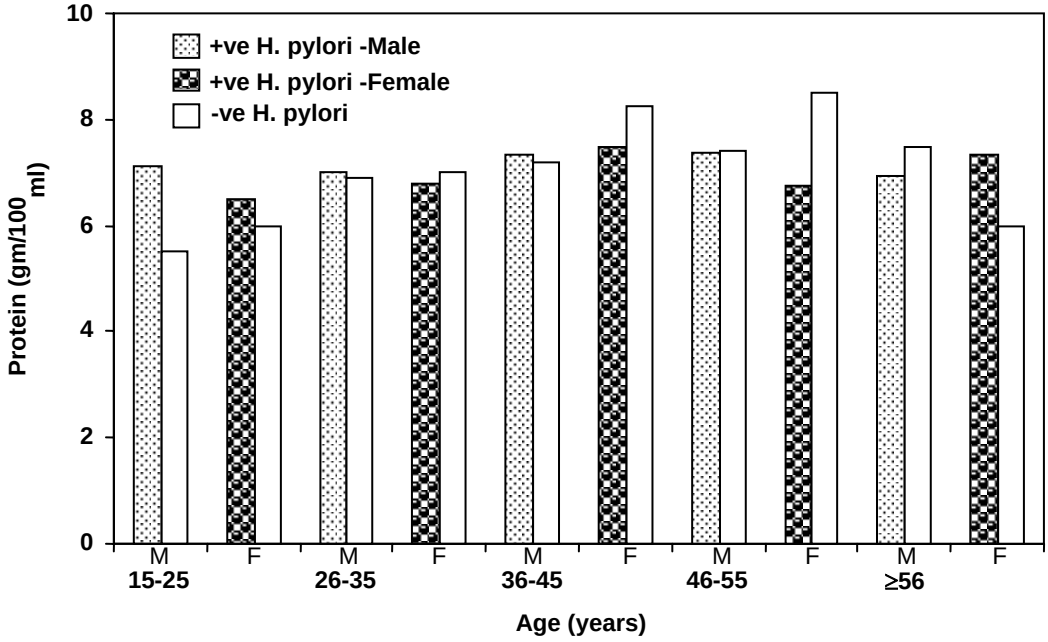

Fig. 3: The relation between age and serum total protein (gm/100 ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

The results of IgG, IgA, IgM, C_3 and C_4 are shown in figures (4,5,6,7,8) respectively. Analysis of variance (ANOVA) showed high significant effect of *H. pylori* infection on Immunoglobulin, IgM, IgA, C_3 ($p \leq 0.01$), and no significant effect on IgG and C_4 ($p > 0.05$).

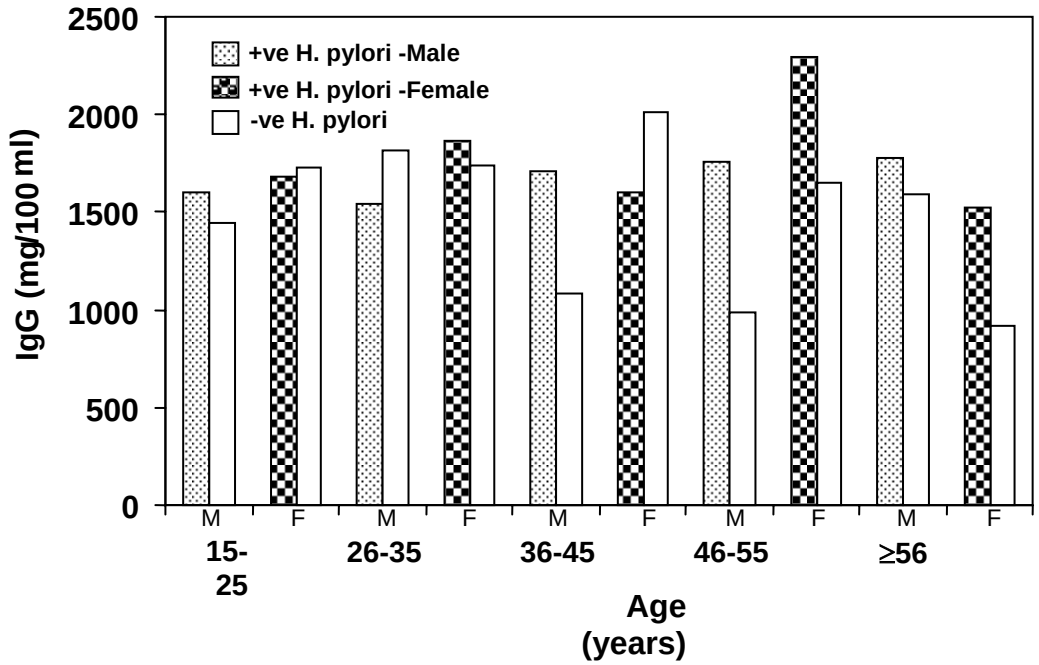

Fig. 4: The relation between age and serum Immunoglobulin G (mg/100ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

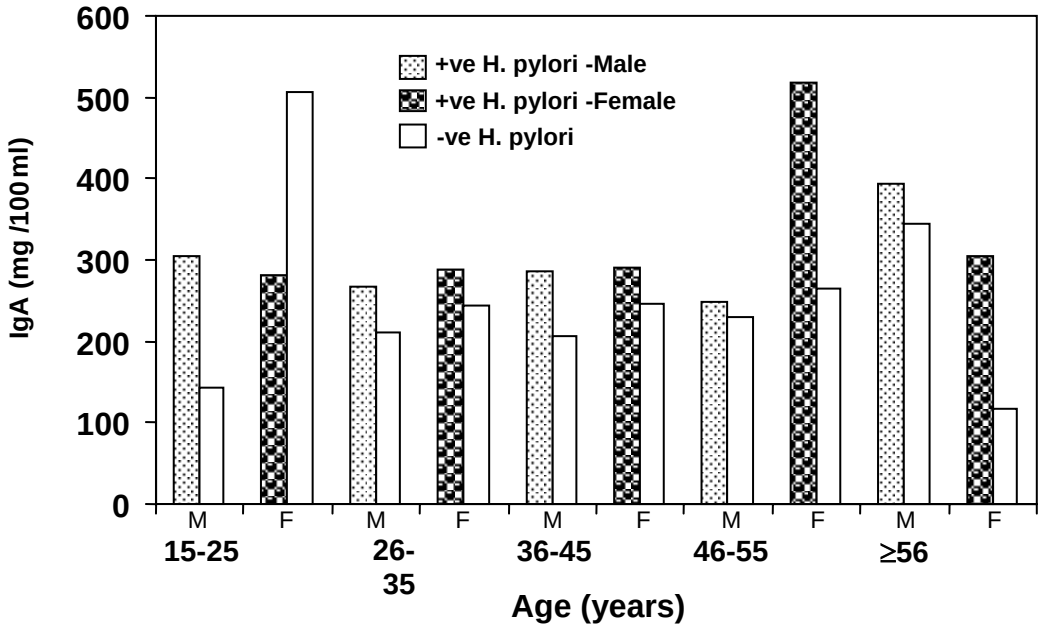

Fig. 5: The relation between age and serum Immunoglobulin A (mg/100ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

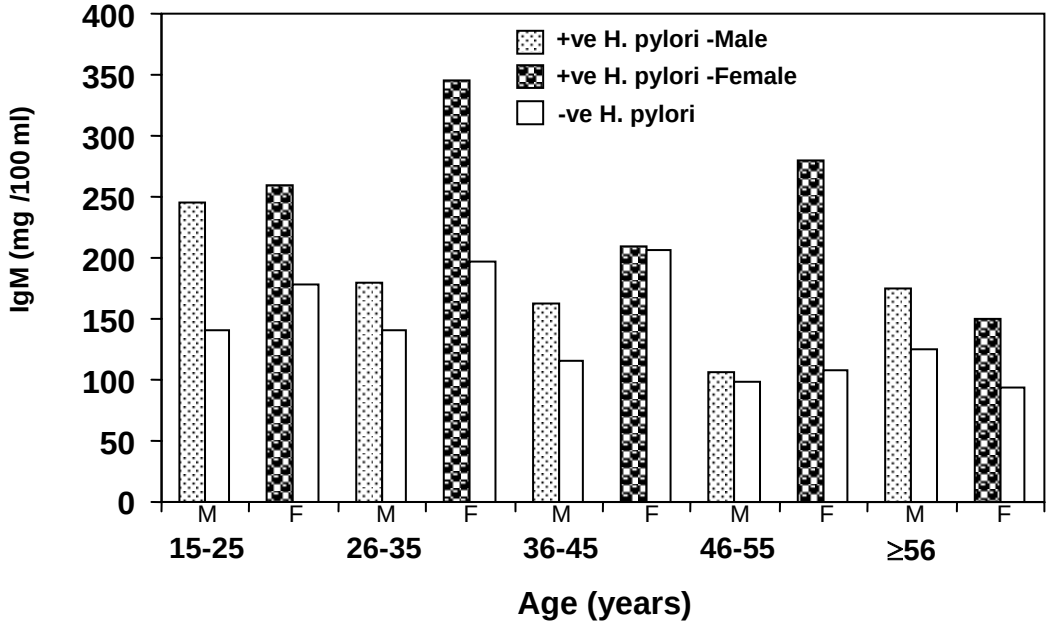

Fig. 6: The relation between age and serum Immunoglobulin M (mg/100ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

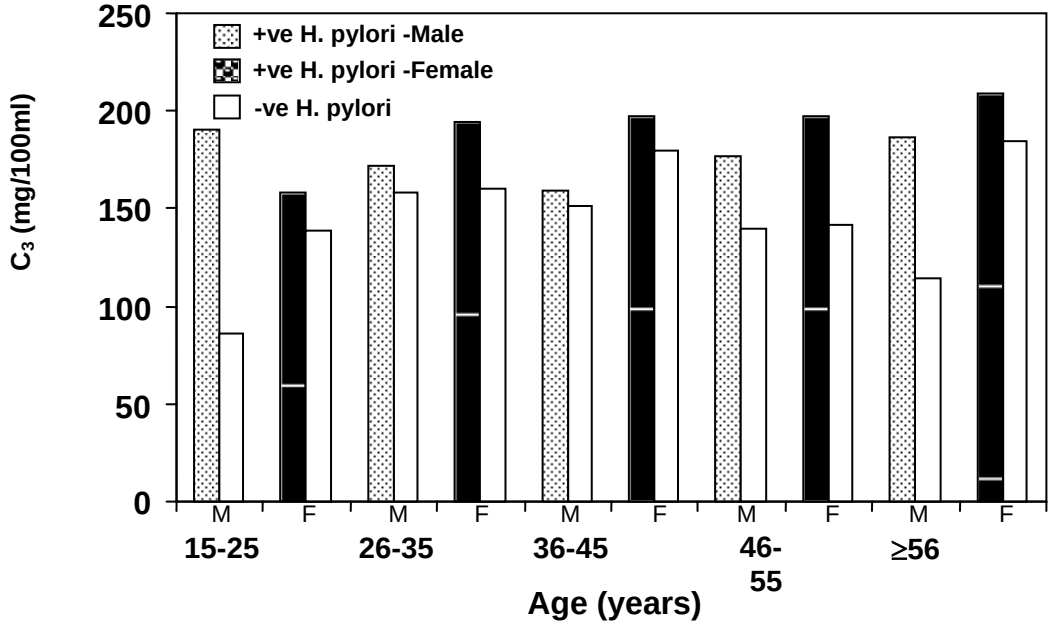

Fig. 7: The relation between age and serum complement protein C_3 (mg/100ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

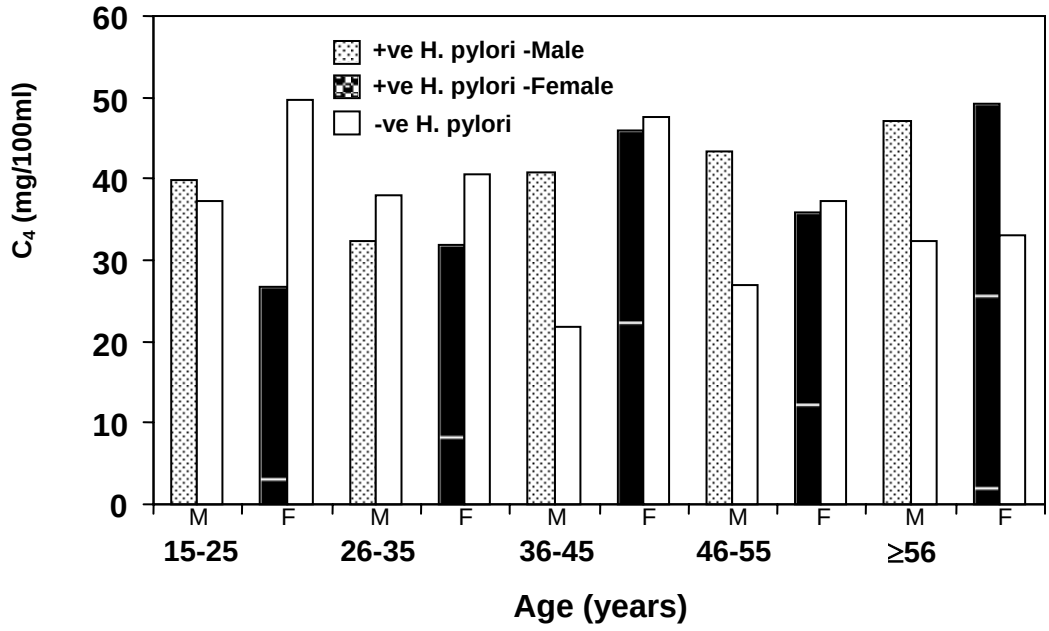

Fig. 8: The relation between age and serum complement protein C_4 (mg/100ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

In addition age and sex had no significant effect ($p > 0.05$) on IgG, IgA, and C_4, while sex has a significant effect on IgM and C_3. This observation is in direct accord with that for patients with gasrities and in control populations where the percentage of subjects with antibodies rise with age and specific antibodies persist for years (Rokkas and Sladen, 1988). These result may be due to binding of C_3 to *H. pylori* which facilitates opsonisation by cells bearing complement receptors, thereby enhancing phagocytosis by neutrophils, monocytes and macrophages (Frank and Fries, 1991). Others showed that it is of considerable interest that activated C_3 was seen to coat *H. pylori* and to be deposited at the apical face of the surface epithelium preferentially in infected patients (Berstad *et al.*, 1997). Therefore, it is possible that the gastric mucus represents a protected niche for *H. pylori* with regard to complement attachment. Antibodies of the IgA, IgG and IgM isotypes have been shown to coat *H. pylori* in vivo, the two latter (the complement activating) are usually in the presence of epithelial neutrophil in filtration (Kazi *et al.*, 1989).

The results of blood urea are shown in figure (9). Duncan test results showed little difference between groups. The lower mean values in blood urea (younger age and 46-55 year non-infected male) differ significantly from the higher value (non-infected younger female).

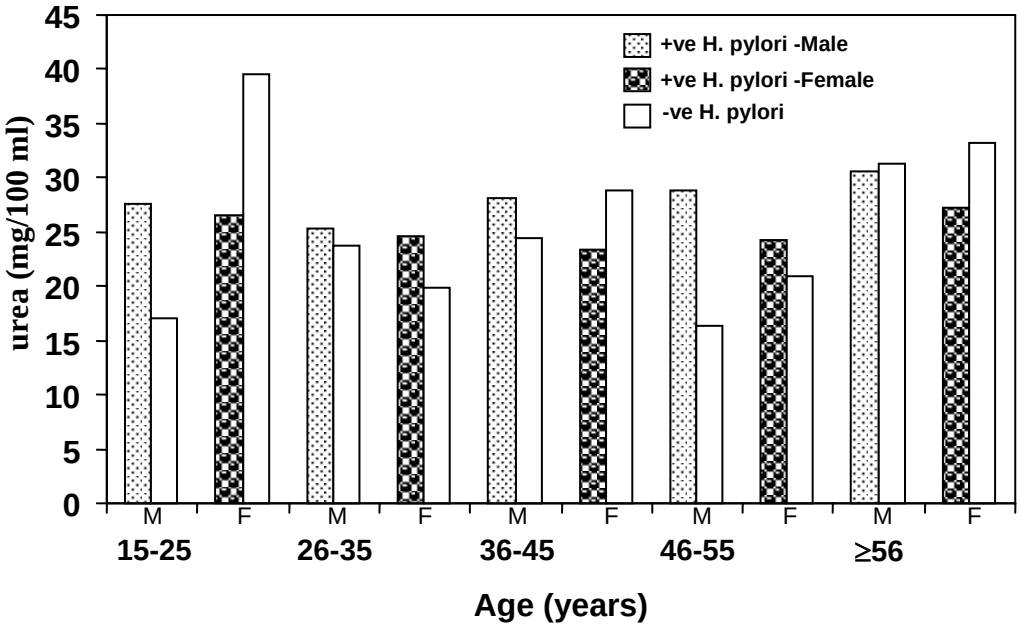

Fig. 9: The relation between age and blood urea (mg/100 ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

Sex and infection of *H. pylori* had a significant effect ($p \leq 0.05$) on blood haemoglobin in duodenal ulcer patients, while age has no significant effect, as show in Figure (10).

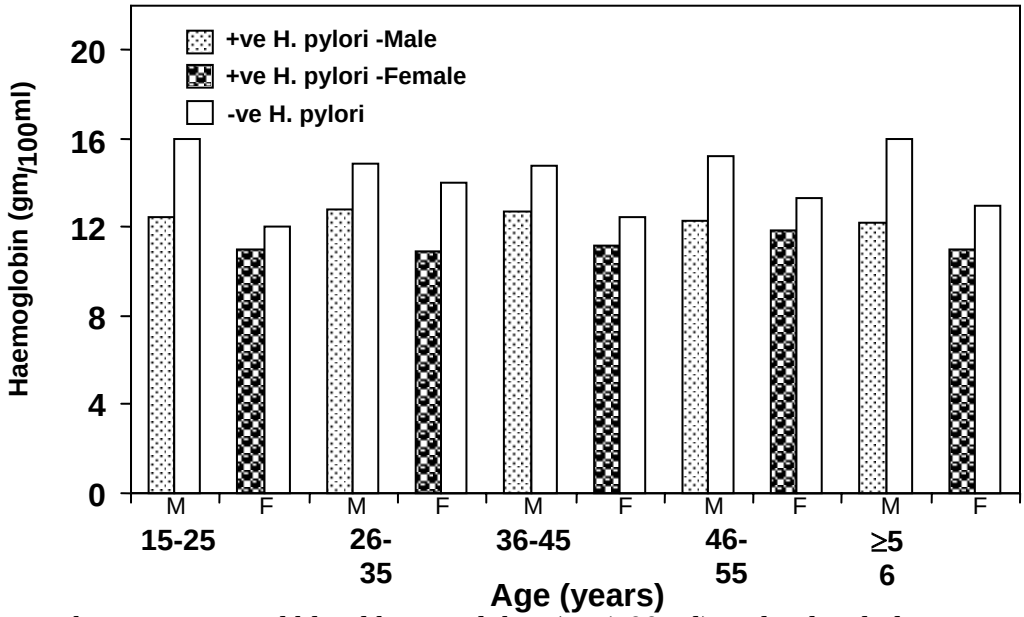

Fig. 10: The relation between age and blood haemoglobin (gm/100 ml) in duodenal ulcer patients of both sexes infected with *H. pylori*.

These results were found to be compatible with the results obtained by other investigators (Soplepmann *et al.*, 1997) where they found that *H. pylori* infection was present in (93%) of the duodenal ulcer patients. Mortality (8%) was related to age, shock, haemoglobin less than (8 g/dl) also found a high prevalence

and the clinical picture of ulcer disease among in patients with iron deficiency anemia and/or occult gastrointestinal bleeding (Wroblewski and Ostberg, 1990).

REFERENCES

Abbas Z., Khan A.H., Jafri S.M., Hamid S.S., Shah S.H., Abid S. and Qureshi J.A., 1997. Duodenal erosions and ulcers in patients with pancreatobiliary obstruction. J. Gastroenterol. Hepatol., 12(11): 703-706.

Al-Ajeal M.D., Lafi A.K. and Awad A.R., 1996. *Helicobacter pylori* incommon upper gastrointestinal disorders in Al-Anbar Governorate. J. of Al-Anbar Univ., 1: 10-14.

Al-Helaly L.A., 2000. Determination and studying the normal reference values for the biochemical parameters in Mosul City and its surrounding area. M.Sc. Thesis, College of Science, University of Mosul, Mosul, Iraq.

Berstad A.E., Hatleback J.G., Maartmann-Moe H., Berstad A. and Brandtzaey P. 1997. *Helicobacter pylori* gastritis and epithelial cell proliferation in patient with reflux oesophagitis after treatment with Iansoprazol. Gut, 41(6): 740-747.

Bouchier I.A., Allon R.N., Hodgson H.J. and Keighley M.R., 1984, Textbook of Gastroenterology. Bailliere Tindall, London.

de-Carvalho T.A., 2000. Peptic ulcer. J. Pediatr (Rioj), 76: 127-34.

Fahey J.L., McKelvey E.M. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol., 94: 84.

Frank M.M. and Fries L.F., 1991. The role of complement in inflammation and phagocytosis. Immunol. Today, 12: 322-326.

Graham D.Y. 1989. *Campylobacter pylori* and peptic ulcer disease. Gastroenterol., 96: 615-625.

Haslett C., Chilvers E.R., Hunter J.A. and Boon N.A. 1999. Davidson's Principles and Practice of Medicine. 18 th ed., Churchill Livingstone, London.

Kazi J.I., Sinniah R., Jaffrey N.A., Alam S.M., Zaman V., Zuberi S.J. and Kazi A.M., 1989. Cellular and hurmoral immune responses in *campylobacter pylori* associated chronic gastritis. J. Pathol., 159: 231-237.

Kind P.R.N. and King E.J., 1954. Estimation of plasma phosphate by determination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol. 7: 322-326.

Makarem A., 1974. Clinical Chemistry-Principles and Techniques. 2 nd ed. R.F. Henry, D.C. Cannon, J.W. Winkelman, Editors. Harper & Row, Hagerstown [MD].

Murray R.K., Granner D.K., Mayes P.A. and Rodwell V.W., 1996. Harper's Biochemistry, 24th Edn., Appleton and Lang, California.

Plummer D.T., 1978. An Introduction to Practical Biochemistry. 2 nd ed., McGraw-Hill Book Company U.K.

Reitman S. and Frankel S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-63.

Rokkas T. and Sladen G.E., 1988. Infection with *Compylobacter pylori*. J. Roy. Coll. Physic. of London. 22(2): 97-100.

Samloff I.M. 1989. Peptic Ulcer: The many proteinases of aggression. Gastroenterol., 96: 586-595.

Soplepmann J., Peetsalu A., Peetsalu M., Tein A. and Juhola M., 1997. Peptic ulcer haemorrhage in Tartu county, Estonia: epidemiology and mortality risk factors. Scand. J. Gastroenterol., 32(12): 195-200.

Sperelakis N. and Banks R.O., 1996. Essentials of Physiology. 2 nd ed., Little, Brown and Company, USA.

Tietz N.W., 1994. Textbook of Clinical Chemistry. W.B. Sanders Company, USA. A Division of Harcourt Brace & Company, Philadelphia.

Varley H., 1967. Practical Clinical Biochemistry. 4 th ed., The White Friars Press Ltd., London and Tonbridge, Great Britain.

Wormsley K.G., 1974. The pathophysiology of duodenal ulceration. Gut, 15: 59-81.

Wroblewski M. and Ostberg H. 1990. Ulcer disease among geriatric in patients with positive fecal occult blood test and / or iron deficiency anaemia. A prospective study. Scand. J. Gastroenterol., 25(5): 489-495.