Library (2)

Date 27 Dec '87

REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. P.K. Bardhan

Trainee Investigator (if any)

Application No. 87-027

Supporting Agency (if Non-ICDDR,B) S

Title of Study: Campylobacter pylori: its association with human gastroduodenal diseases in Bangladesh.

Project status:
(√) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects — Yes No
   (b) Non-ill — Yes
   (c) Minor — Yes
   under
2. Does the
   (a) Physical risks to the subjects
   (b) Social
   (c) Psychol to subjects
   (d) Discomfort to
   (e) Invasion of priv
   (f) Disclosure o tion damag ject or ot
   Does the study
   (a) Use of record hospital, medical, death, birth or other) — (Yes) No
   (b) Use of fetal tissue or abortus / — Yes (No)
   (c) Use of organs or body fluids — (Yes) No
   Are subjects clearly informed about:
   (a) Nature and purposes of study — (Yes) No
   (b) Procedures to be followed including alternatives used — Yes No
   (c) Physical risks — Yes (No)
   (d) Sensitive questions — Yes (No)
   (e) Benefits to be derived — (Yes) No
   (f) Right to refuse to participate or to withdraw from study — (Yes) No
   (g) Confidential handling of data — (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes (No)

5. Will signed consent form be required:
   (a) From subjects — (Yes) No
   (b) From parent or guardian (if subjects are minors) — Yes (No)

Will precautions be taken to protect anonymity of subjects — (Yes) No

Check documents being submitted herewith to Cttee:
- Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
- Protocol (Required)
- Abstract Summary (Required)
- Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
- Informed consent form for subjects
- Informed consent form for parent or guardian
- Procedure for maintaining confidentiality
- Questionnaire or interview schedule

If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

(PTO)

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

DEC 31 1987

Trainee

87-027

17-25-12-87

SECTION I: RESEARCH PROTOCOL

Title	:	Campylobacter pylori: its association with human gastroduodenal diseases in Bangladesh.
Principal investigator	:	Dr P. K. Bardhan
Collaborative PIs	:	Dr S. K. Azad Khan, BIRDEM
		Dr Mahmud Hassan, IPGM&R
		Dr E. Beglinger, Basel University Hospital, Switzerland
Co-investigators	:	Dr S. Q. Akhtar
		Dr S. I. Ally
		Dr P. P. L. Van Loon
		Dr I. Kabir
Date of starting	:	January 1988
Date of completion	:	December 1988
Total direct cost	:	US Dollar 19,950.00
Source of fund	:	
Scientific programme	:	This protocol has been approved by the Laboratory Sciences Division.

Signature of the Associate Director, LSD

Date: Dec, 27, 1987

Abstract summary

Gastric acidity, an important host defence barrier against enteric pathogens, is dependent upon the integrity of gastric mucosa. Recent reports suggest that a strong association exists between the present of campylobacter pylori and gastritis. This study will attempt to detect the presence (if any) of campylobacter pylori from gastroduodenal biopsies obtained from Bangladeshi patients, and to correlate with clinical, endoscopic and histological findings. A total of 150 patients referred for routine upper GI examinations in three centres (ICDDR,B, BIHDRM, and IPGMR) will be included in the study. Biopsies obtained from these patients will be examined in the ICDDR,B lab., microbiological methods to isolate and identify campylobacter pylori, and histopathological methods to assess state of gastric mucosa. Similarly sera will be obtained to check specific antibodies against campylobacter pylori by ELISA technique. The results of this study may be useful to better define gastro duodenal diseases in Bangladesh.

Reviews:

(i) Research Review Committee: _____

(ii) Ethical Review Committee: _____

(iii) Director's signature: _____

SECTION II: RESEARCH PLAN

A. INTRODUCTION

1. Objective

(a) To investigate the occurrence of campylobacter pylori (CP) in gastric biopsies from Bangladeshi patients.

(b) To assess the connection between CP and gastritis and peptic ulcer.

2. Background

Among the several host defence factors against diarrhoeagenic organisms, gastric acid, which reduces the number of viable organisms reaching the small intestine, is particularly important (1). Inadequate concentration of gastric acid, as in achlorhydria, increases the proliferation of non-pathogenic bacteria in the upper gut (2), and also contributes in the pathogeneses of several forms of diarrhoea including clinical cholera (3,4), salmonellosis (5), shigellosis (6), giardiasis (7), and strongyloidiasis (8). Gastric acid output, both basal and maximal, is correlated with gastric mucosal histology (9, 10, 11), as also is gastric pepsin output (12). This correlation is inverse, i.e. the more severe and extensive the mucosal damage, the lower the maximal acid output (13). Patients with atrophic gastritis have marked hyposecretion, and those with gastric atrophy showed anacidity - this gastric acid response is not only low, but is also delayed in onset (12).

Campylobacter pylori (CP) is a recently discovered curved bacilled colonizing the human gastric mucosa, first described in Australia (14, 15). Interest in the relationships between those bacteria and gastritis and peptic ulcer disease has been greatly

enhanced by the numerous reports of frequent findings and positive correlation between the presence of CP in the inflamed gastric mucosa and the occurrence of gastritis and/or gastric duodenal ulcers (14, 15-27). A possible pathogenetic role is further supported by the fact that the patients with gastritis and peptic ulcer disease have significantly higher serum titers against CP than that of controls (28, 29). Moreover, ingestion of live CP in a volunteer with normal gastric histology resulted in gastritis and moderate clinical symptoms (30).

Though there is a general consensus about the association between chronic gastritis and CP, it is still not clear how the organisms causes gastritis. One suggestion is that some patients are unable to clear the organism, and develop chronicity (30). Others have suggested that the hydrolysis of urea at intercellular junctions facilitating H^+ back-diffusion causes hypochlorhydria predisposing to ulcer formation (31).

CP is a microaerophilic curved, gram negative rod, which under suitable conditions could be easily cultured and isolated (32). It can be histologically detected on the gastric mucosa as S-shaped organisms in close contact with epithelial cells. Haematoxylin-eosin (H-E) stains CP only faintly but Warthin-Starry silver stain (14), modified Giemsa stain (21), or fluorescent stain labeling with acridine orange (33) are reliable methods to demonstrate these bacteria in gastric mucosa. Usually, there is a good correlation between the microscopic detention of CP and the cultural results (34).

Systemic and local antibodies, directed against both the body and the flagella of this organism, are produced in response to this

infection (35). Raised IgG and IgA antibodies were detected in colonized patients with gastritis, as well as elevated IgA and IgM antibodies in gastric juice in the same patients (28). These antibodies are probably not protective for gastritis, although they may prevent systemic invasion.

CP has been shown to be sensitive in vitro to ampicillin, tetracycline, cephalothin, metronidazole, erythromycin, and bismuth substrate. It seems to be resistant to vancomycin polymixin and SXT and there is relative insensitivity to cimetidine carbenoxolone, and sucralfate (16, 36).

3. Rationale

Gastric acid is an important barrier to enteric infections, and impaired gastric acid production is associated with higher risks of gastrointestinal infections including acute infectious diarrhoea. Gastric acid production is dependent upon the integrity of the gastric mucosa - both structural and functional. The strong association between CP and gastritis, prompts to suggest that by properly controlling the bacteria, impairment of this important host defence factor may be corrected leading to broder protection from enteric infections.

SPECIFIC AIMS

1. To correlate the presence of CP with clinical, endoscopic and histological findings.
2. To quantify circulating CP antibodies and to relate to the endoscopic, microbiological and histological findings.

METHODS OF PROCEDURE

Sample size

Quoting data from Price et al. 1985 (38), CP was found in 81% of duodenal ulcer patients, whereas 36% of patients with normal endoscopy had CP. The following formula was used to calculate sample size:

$$n = (Z_{\alpha} + Z_{\beta})^2 (P_1q_1 + P_2q_2)/(P_2-P_1)^2$$

With 95% confidence limit and 80% power, $(Z_{\alpha} + Z_{\beta})^2$ is 7.9.

Substituting the values, the sample size in each group is -

$$\frac{81 \times 19 + 36 \times 64}{(36 - 81)^2} \times 7.9 = 15 \text{ patients}$$

Expecting 11.9% of patients undergoing upper GI endoscopy to have duodenal ulcers (39), the total number of patients becomes 126. Anticipating 20% experimental failures, the total number is 150 (approx.).

Patients: One hundred and fifty (150) patients referred for routine upper GI endoscopic examination will be investigated for histopathological and microbiological evaluation of the gastric mucosa. Patients under 18 years of age or patients suffering from acute haemorrhage will not be included. Those admitted will be questioned about previous gastric surgery and recent (last 6 months) intake of drugs including antibiotics, non-steroidal inflammatory agents (NSAIDS), cytotoxic and immunosuppressive drugs,

antacids, H_2-blockers, sucralfate, and bismuth containing compounds from every patient before inclusion into the study. Patient recruitment, examination, and biopsies will be performed by the investigators at their respective centres. Biopsies obtained by Drs Azad Khan and M. Hassan will be transferred to the ICDDR,B lab. within 24 hours of collection. There will be no separate target number of patients to be reached by the three collaborating centres, rather suitable patients will be admitted into the study as they present. Recruitment of patients will be stopped when total patient number reaches 150 (continuously updated records will be maintained by the PI). Written consent will be obtained from each patient before inclusion into the study.

Gastroduodenoscopy and biopsy: After endoscopic examination of the esophagus, stomach and duodenum, biopsy specimens will be taken in duplicate from the duodenal bulb, gastric antrum, and gastric body. In patients with gastric ulcer, two samples each will be taken from the margins of the ulcer, 1-2 cm from the ulcer, and from a non-ulcerated region. In patients with duodenal ulcer, the third pair of specimens will be taken from the gastric antrum. Immediately after biopsy, one tissue sample from each location will be fixed with formaldehyde and the other transferred to Cary-blair transport medium (BBL 11 101). Relevant clinical features and endoscopic findings will be recorded, and will be related later with histological and cultural findings. Before reuse, endoscope and forceps will be washed and cleaned thoroughly.

Histopathology: Sections will be stained with H-E to assess the degree of gastritis. For detection of CP, tissue sections will be treated with Warthin-Starry sliver stain. Gastritis will be graded 'blind' on a 5-point scale. Normal-0, superficial gastritis-1, mild atrophy-2, moderate atrophy-3,

and severe atrophy-4 (37). The incidence of inflammatory cell types (lymphocytes, mononuclear cells, neutrophilic granulocytes and eosinophilic granulocytes), degree of foveolar hyperplasia and metaplasia will also be graded (25, 37).

Isolation and identification of CP (32): Biopsy specimens will be transferred to the laboratory in Cary-Blair transport medium kept at 4°C and processed within 24 hours. Tissues will be homogenized in 0.1 ml normal saline using Griffith ground glass homogenizers and streaked on brain-heart infusion agar supplemented with 7% sheep blood, 1.0% isovitalex, vancomycin 6 mg/l, nalidixic acid 20 mg/l, and amphotericin 2 mg/l. The plates will be incubated at 37°C in microaerophilic atmosphere using gaspak anaerobic system. Growth will be checked 4 and 6 days later. Small colourless colonies consisting of spiral shaped gram negative bacteria which are positive for catalase and oxidase, show a very fast urease reaction (38), and do not grow at 42°C will be considered to be CP.

Serological tests: Five ml of blood will be collected from the study patients and 50 healthy volunteers. Sera will be separated as soon as possible and then will be stored at -20°C. The samples will be sent to Basel, Switzerland, packed in dry ice, where serum antibody levels will be assayed by a specific ELISA test. The results will be expressed as antibody titres representing the highest reciprocal serum dilutions obtained at the intercepts of the dilution curves with the base line. The base line will be determined by the absorbance measured in a standard high-titre antiserum pool diluted 1:9720 and run as a control in every plate. The specificity of the antibodies will be assessed by multiple absorptions of rabbit antiserum to CP, and a high titre human sera pool with a variety of

bacteria including C. jejuni, C. fetus and E. coli.

Data analysis

The results will be evaluated statistically with the Mann-Whitney test, the Chi-square test and Spearman's rank correlation test. Statistical significance will be accepted at the level of 0.05.

SIGNIFICANCE

The results of this study is expected to examine the presence of CP in Bangladesh and evaluate their association with gastroduodenal diseases in Bangladeshi patients. This information will be useful not only to better understand the peptic ulcer diseases and other common vague chronic gastrointestinal symptoms like non-ulcer dyspepsia and flatulent dyspepsia in Bangladeshi patients but may also stimulate newer studies to develop methods to broaden protection against enteric pathogens.

FACILITIES REQUIRED

1. Hospital facilities of ICDDR,B will be utilized.
2. Laboratory facilities of ICDDR,B will be used.

COLLABORATIVE ARRANGEMENTS

This will be a collaborative study among ICDDR,B, BIRDEM, IPGM&R, and Basel University Hospital, Switzerland.

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ABSTRACT SUMMARY FOR RESEARCH REVIEW COMMITTEE

1. The subject population will be only adult patients being referred for routine upper G.I. endoscopic evaluation. There will be no special population.

2. The potential physical risks are gastro-intestinal haemorrhage and perforation. G.I. haemorrhage occurs during biopsy but is very little in quantity and stops within 2-3 minutes. Perforation is a very unlikely event. Obtaining biopsies during endoscopic examination is an universally accepted routine procedure with minimal risks when performed by trained personnel. There are no psychological, social, legal risks or other alternate method suitable for the study.

3. As the procedures will be performed on patients referred for endoscopic examinations, the patients will be routinely screened for general condition, bleeding disorders, or any other contra-indication for performing endoscopy.

4. There will be no personal identification of the patients. Only hospital registration numbers will be used.

5. Signed consent will be obtained from the patients.

   (a) Does not apply.

   (b) No information will be withheld from the patient.

   (c) It has been stated in the consent form that every precaution will be taken during the collection of biopsy.

6. Except clinical history no other interviews will be required.

7. This study is important for etiological investigation and better understanding of pathophysiology of acid peptic diseases.

8. This study requires several small bits (0.5 mg approx.) of tissues from gastric and duodenal mucosa. 5 ml of venous blood will be obtained for examining anti-campylobacter pylori antibodies.

SECTION III - BUDGET

1. Personnel Services	Position	% effort	Rate/m	Total requirement
Dr. P.K. Bardhan	P.I.	15%	915	1647
Prof. A.K. Azad Khan	"	-	-	-
Dr. Mahmud Hassan	"	-	-	-
Dr. C. Beglinger	"	-	-	-
Dr. S.Q. Akhter	Co-P.I.	10%	970	1164
Dr. S.A.I. Ally	"	10%	1100	1320
Dr. F.P.L. Van Loon	"	5%	-	-
Dr. I. Kabir	"	5%	915	550
Secretary		10%	425	510
			Sub Total=	5191

2. Supplies and Materials		
Hospital supplies, chemicals and stationeries		394
Non-stock supplies		100
Cary-Blair media	500X0.13	65
	Sub Total=	559

3. Inter-departmental services	
(a) Local transport (Dhaka)	300
(b) Laboratory tests:	
Campylobacter pylori $ 9X150ptsx3 biopsies Isolation and identification	4050
Histopathylogy (H.E. & Masthin X 3 biopsies stain)	8100
Xerox & Mimeography	300
Data Analysis	500
Sub-Total =	13350

4.	Travel international	-
5.	Capital expenditure (Griffith ground glass homogenizer)	350
6.	Others: Printing & Publication	300
7.	International transport	200
	Sub total	850

GRAND TOTAL = US $ 19,950.00

CONSENT FORM

Campylobacter pylori: its association with human gastroduodenal diseases in Bangladesh.

The International Centre for Diarrhoeal Disease Research, Bangladesh has undertaken a research programme to examine the presence of a bacteria called Campylobacter pylori in the gastric mucosa of Bangladeshi patients. This bacteria is suspected to be involved in producing inflammation in stomach. The findings will help us to understand the mechanism of inflammation produced in peptic ulcer diseases.

During endoscopy, three small bits of tissues will be obtained by biopsies from your stomach and duodenum. This is a safe procedure, routinely done for diagnostic purposes, and you will not feel any pain during obtaining biopsies. We will also obtain 5 ml of blood from your veins to examine the presence of antibodies to this bacteria. We hope that the results will help us to develop better guidelines in helping patients suffering from peptic diseases.

If you do not agree to join in this study, we will still perform your routine endoscopic examination without any reservation.

If you agree to join in this study, then please put your signature/thumb impression on this consent form.

Signature of the Investigator

Signature/thumb impression of the patient.

Date:_____

CONSENT FORM

Campylobacter pylori: its association with human gastroduodenal diseases in Bangladesh.

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র বর্তমানে ক্যাম্পাইলোব্যাক্টর পাইলোরি নামক একটি জীবাণুর উপর গবেষণা করছে। ধারণা করা হয় যে এই জীবাণুটি পাকস্থলীর প্রদাহের সাথে জড়িত রয়েছে কিন্তু এই জীবাণুটি পেপটিক আলসার পাকস্থলীর ঝিল্লীতে প্রদাহের সৃষ্টি করে। এই গবেষণাটি রোগটি আমাদের বুঝতে সাহায্য করবে।

আপনার এন্ডোস্কোপি পরীক্ষার সময়ে পাকস্থলীর ও ক্ষুদ্রান্ত্রের ঝিল্লী থেকে তিনটি ছোট অংশ বায়োপসি দ্বারা পরীক্ষার জন্য নেয়া হবে। এতে ভয়ের কারণ নেই অথবা আপনি কোন ব্যথা পাবেন না। তবুও আমরা সকল সতর্কতামূলক ব্যবস্থা অবলম্বন করব। আমরা আপনার শিরা থেকে ৫ মি: লি: পরিমাণ রক্ত নেব - যাতে এই জীবাণুর এ্যান্টিবডি আছে কিনা তা দেখা হবে। আমরা আশা করি এসব পরীক্ষার দ্বারা আপনার বা অন্যান্য লোকের ভবিষ্যতে পেপটিক আলসারের রোগীদের সুচিকিৎসাতে সাহায্য করবে।

আপনি এ গবেষণায় স্বেচ্ছায় গ্রহণ না করলেও আমরা কিছু মনে করব না এবং আপনার এন্ডোস্কোপি পরীক্ষা যথাযথভাবে করা হবে।

আপনি যদি এ গবেষণায় স্বেচ্ছা প্রদানে রাজী থাকেন তবে নিচে স্বাক্ষর বা বৃদ্ধাঙ্গুলের ছাপ দিন।

গবেষকের স্বাক্ষর

রোগীর স্বাক্ষর / টিপসই

তারিখ: