UNIVERSITI SAINS MALAYSIA
'GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

THE ROLE OF SPECIFIC HELICOBACTER PYLORI VIRULENCE AND HOST GENETIC FACTORS IN GASTRODUODENAL DISEASE

PENYELIDIK

PROFESOR MADYA DR. HABSAH HASAN

PENYELIDIK BERSAMA

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2016

Project Code :
(for RCMO use only)

RU GRANT
FINAL REPORT FORM

Please email a softcopy of this report to rcmo@usm.my

ROJECT DETAILS

itle of Research: The role of specific Helicobacter pylori virulence and host genetic factors in astroduodenal disease.

Account Number: 1001 / PPSP / 812108

Name of Research Leader: Habsah Binti Hasan

Name of Co-Researcher:

1. Zilfalil Bin Alwi
2. Rapeah Binti Suppian
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4. Nor Aizal Binti Che Hamzah
5. Dr Shashi Kumar Menon a/l Bashkaran
6. Dr Nurzam Suhaila Che Hussein

Duration of this research:

a) Start Date : 15 JULAI 2012

b) Completion Date : 14 JULAI 2015

c) Duration : 3 YEARS

d) Revised Date (*if any*) : .....

ABSTRACT OF RESEARCH

(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

The aim of this study was to determine the distribution of *H. pylori* virulence genes (*cagA, babA2, SabA* and *dupA*) and cagA EPIYA motifs and correlation with clinical outcomes. It also explored the presence of SNPs in genetic variants which may be associated with susceptibility or protective to *H. pylori* infection. *H. pylori cagA,*

babA2, sabA and *dupA* genes in *H. pylori* dyspeptic patients were 69.5%, 41.0%, 43.8% and 22.9% respectively. Majority of Chinese patients were predominantly infected with *cagA* type A-B-D East Asian strain (88.9%) while cagA type A-B-C Western strain (82.8%) was predominantly detected in Indians while Malays have mixed strain. The present study identified SNPs rs3770521 (***P*=1.33 x^{-5}**) of XRCC5 gene, rs7042986 of SMARCA2 (***P*=0.0001**) and rs10860808 (***P*=0.0002**) of DRAM1 gene as susceptible SNPs to *H. pylori* infection among gastritis patients of Indian, Malay and Chinese respectively. This study also identified two protective SNPs rs1809578 (***P*=9.85x 10 $^{-6}$**) of gene BANK1 and rs3776349 (***P*=0.0001**) of gene ARHGAP26 among *H. pylori* gastritis patients of Indian and Malay respectively. In conclusion, the current GWAS study revealed five novel SNPs that may be associated with susceptibility and protection of *H. pylori* gastritis in this population.

BAHASA MALAYSIA

Tujuan penyelidikan ini adalah untuk menentukan taburan gen virulen *H pylori* (*cagA, babA2, Sab A* dan *dupA*) dan corak EPIYA *cagA* serta korelasi dengan hasil klinikal.Ia juga bertujuan mengkaji kehadiran SNPs dalam variasi genetik yang berkemungkinan mempunyai hubungkait dengan sensitiviti atau pertahanan terhadap jangkitan *H pylori*. Gen *cagA, babA2, sabA* dan *dupA H pylori* di dalam pesakit dispeptik *H. Pylori* masing-masing adalah 69.5%, 41.0%, 43.8% dan 22.9%. Majoriti pesakit Cina dijangkiti dengan *cagA* jenis A-B-D strain Asia Timur (88.9%) manakala *cagA* jenis A-B-C strain Barat (82.8%) dikesan lebih tinggi dalam kalangan bangsa India. Bangsa Melayu mempunyai strain bercampur. Kajian ini menunjukkan bahawa SNP rs3770521 (**P=1.33 X10^{-5}**) gen XRCC5, rs7042986 (**P=0.0001**) gen SMARCA2, dan rs10860808 (**P=0.0002**) gen DRAM1 adalah SNP yang cenderung kepada jangkitan *H. pylori* dalam kalangan pesakit gastrik berbangsa India, Melayu dan Cina. Kajian ini turut mengenalpasti dua SNP yang protektif, iaitu rs1809758 (**P=9.85X 10^{-6}**) gen BANK1 dan rs3776349 (**P=0.0001**) gen ARHGAP26 dalam kalangan pesakit gastirk India dan Melayu. Sebagai kesimpulan, kajian GWAS terkini menunjukkan lima SNP unggul yang boleh dikaitkan dengan kecenderungan dan ketahanan terhadap *H. pylori* gastritis dalam populasi ini.

C BUDGET & EXPENDITURE

i

ii **Equipment Purchased Under Vot 35000**

No.	Name of Equipment	Amount (RM)	Location	Status
	NIL			

 # Please attach the Asset/Inventory Return Form (Borang Penyerahan Aset/Inventori) – Appendix 1

RESEARCH ACHIEVEMENTS

Project Objectives *(as stated/approved in the project proposal)*

No.	Project Objectives	Achievement
1	To determine the genotype distribution of *cagA*, *dupA*, *babA* and *sabA* of *H. pylori* genes in *H. pylori* infected patients and to correlate with clinical outcome.	ACHIEVED
2	To determine the variability of *cagA* EPIYA motifs among *H. pylori* infected patients	ACHIEVED
3	To determine the SNP profiles between patients infected with *H. pylori* and non-infected healthy controls	ACHIEVED
4	To determine the genotype and phenotype association between SNP profiles of patients infected with *H. pylori*	ACHIEVED

Research Output

a) **Publications in ISI Web of Science/Scopus**

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
1.	Hussein Ali Osman, **Habsah Hasan**, Rapeah Suppian, Syed Hassan, Dzulkarnaen Zakaria Andee, Noorizan Abdul Majid, Bin Alwi Zilfalil Prevalence of Helicobacter pylori cagA, babA2 and dupA Genotypes and Correlation with Clinical Outcome in Malaysian Patients with Dyspepsia. Turkey Journal of Medical sciences, 2015	published
2	Hussein Ali Osman, **Habsah Hasan**, Rapeah Suppian, Norhaniza Bahar, Nurzam Suhaila Che Hussin, Amry Abdul Rahim, Syed Hassan, Dzulkarnaen Zakaria Andee, Bin-Alwi Zilfalil. Evaluation of the Atlas *Helicobacter pylori* Stool Antigen Test for Diagnosis of Infection in Adult Patients. Asian Pacific Journal of Cancer Prevention, 2014, **15 (13)**, 5245-5247	published

3	HusseinAli Osman, **Habsah Hasan**, Rapeah Suppian, Nor Aizal Che Hamzah, Sharifah Emilia Tuan Sharif, Noorizan H A Majid, Bin Alwi Zilfalil. Characteristics and Helicobacter pylori distribution in upper gastrointestinal bleeding in elderly. Journal of Gastroenterology and Hepatology, 2013, Volume 28, Issue Supplement S3.	published

b) Publications in Other Journals

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
1	Hussein Ali Osman, **Habsah Hasan** , Rapeah Suppian, Nor Aizal Che Hamzah, Sharifah Emilia Tuan Sharif, Noorizan Abdul Majid and Bin-Alwi Zilfalil. The Characteristics, *Helicobacter Pylori* and Clinical Outcomes of Patient with Upper Gastrointestinal Bleeding Admitted at Hospital Universiti Sains Malaysia. World applied science Journal, 2014, 32 (5): 747-751	published
2	Hussein Ali Osman, Habsah Hasan, Rapeah Suppian, Saravanan Arjunan, Zilfalil BA. Genotyping of Helicobacter pylori cagA Gene from a Patient Who Failed Eradication Therapy: A Case Report and Review of the Literature. International Medical Journal, 2015, 22(2), 1 - 2	published

c) Other Publications
(book,chapters in book,monograph,magazine,etc.)

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)

d) Conference Proceeding

No.	Conference (conference name,date,place)	Title of Abstract/Article	Level (International/National)

 # Please attach a full copy of the publication/proceeding listed above

iii Other Research Ouput/Impact From This Project
(patent, products, awards, copyright, external grant, networking, etc.)

Networking – Ministry of Health (Hospital Kuala Lumpur)

HUMAN CAPITAL DEVELOPMENT

a) **Graduated Human Capital**

Student	Nationality (No.)		Name
	National	International	
PhD		1	1.Hussein Ali Osman 2.
MSc			1. 2.
Undergraduate			1. 2.

b) **On-going Human Capital**

Student	Nationality (No.)		Name
	National	International	
PhD			1. 2.
MSc			1. 2.
Undergraduate			1. 2.

c) **Others Human Capital**

Student	Nationality (No.)		Name
	National	International	
Post Doctoral Fellow			1. 2.
Research Officer			1. 2.
Research Assistant	1		1.Siti Nurain Osman 2.
Others (.....)			1. 2.

COMPREHENSIVE TECHNICAL REPORT

Applicants are required to prepare a comprehensive technical report explaining the project. The following format should be used (this report must be attached separately):

- Introduction
- Objectives
- Methods
- Results
- Discussion
- Conclusion and Suggestion
- Acknowledgements
- References

G	PROBLEMS/CONSTRAINTS/CHALLENGES IF ANY
	(Please provide issues arising from the project and how they were resolved) The main challenge in this study is to get sample from Hospital Kuala Lumpur and to use their laboratory facilities. However after a few meetings with the head of gastroenterologist as well as head of Microbiology Department and the specialist involved we managed to get good cooperation from them. We also appoint them as co researcher in the project
H	**RECOMMENDATION**
	(Please provide recommendations that can be used to improve the delivery of information, grant management, guidelines and policy, etc.) The online system is a very useful monitoring system however sometime it could not be accessed especially the e statement. The e statement (budget) is confusing especially to me who is not familiar with account. The most important thing for me to know is just how much we have spent and how much is left to be spent.

COMMENTS, IF ANY/ENDORSEMENT BY PTJ'S RESEARCH COMMITTEE

Hasil penyelidikan secara amat baik. Penyelidik telah dapat menghasilkan pelajar PhD dan 3 ISI indexed journal beserta penerbitan lain

Diperakui untuk tutup.

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG UNIVERSITI PENYELIDIKAN (RU)
PENYATA PERBELANJAAN SEHINGGA 30 JUN 2015

	RM		
Jumlah Geran :	202,641.00	Ketua Projek :	PROF. MADYA DR. HABSAH HASSAN
Peruntukan JULAI 2012 : (Tahun 1)	77,590.00	Tajuk Projek:	THE ROLE OF SPECIFIC HELICOBACTER PYLORI VIRULENCE AND HOST GENETIC FACTORS IN GASTRODUODENAL DISEASE.
Peruntukan JULAI 2013 : (Tahun 2)	102,070.00	Tempoh :	3 Tahun (15/07/2012-14/06/2015)
Peruntukan JULAI 2014 : (Tahun 3)	22,981.00	No. Akaun :	1001/PPSP/812108

Kwgan	Akaun	PTJ	Projek	Peruntukan Projek	Perbelanjaan Terkumpul sehingga Tahun lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
1001	11000	PPSP	812108	36,000.00	70,424.33	(34,424.33)	-	4,649.59	4,649.59	(39,073.92)
1001	14000	PPSP	812108	-	-	-	-	-	-	-
1001	15000	PPSP	812108	-	1,000.00	(1,000.00)	-	-	-	(1,000.00)
1001	21000	PPSP	812108	10,000.00	8,992.81	1,007.19	-	-	-	1,007.19
1001	22000	PPSP	812108	1,500.00	-	1,500.00	-	-	-	1,500.00
1001	23000	PPSP	812108	450.00	3.71	446.29	-	-	-	446.29
1001	24000	PPSP	812108	-	-	-	-	-	-	-
1001	25000	PPSP	812108	-	-	-	-	-	-	-
1001	26000	PPSP	812108	-	-	-	-	-	-	-
1001	27000	PPSP	812108	144,351.00	86,443.80	57,907.20	3,564.00	19,610.00	23,174.00	34,733.20
1001	28000	PPSP	812108	-	-	-	-	-	-	-
1001	29000	PPSP	812108	10,340.00	7,443.00	2,897.00	-	-	-	2,897.00
1001	32000	PPSP	812108	-	-	-	-	-	-	-
1001	35000	PPSP	812108	-	-	-	-	-	-	-
				202,641.00	174,307.65	28,333.35	3,564.00	24,259.59	27,823.59	509.76

UNIVERSITI SAINS MALAYSIA

BORANG PENYERAHAN ASET / INVENTORI

==========

A. BUTIR PENYELIDIK

1. NAMA PENYELIDIK :HABSAH BINTI HASAN
2. NO STAF :1001/10
3. PTJ :PUSAT PENGAJIAN SAINS PERUBATAN
4. KOD PROJEK :1001/PPSP/812108
5. TARIKH TAMAT PENYELIDIKAN :14 JULAI 2015

B. MAKLUMAT ASET / INVENTORI

BIL	KETERANGAN ASET	NO HARTA	NO. SIRI	HARGA (RM)
	TIADA			

C. PERAKUAN PENYERAHAN

Saya dengan ini menyerahkan aset/ inventori seperti butiran B di atas kepada pihak Universiti:

☐ Lengkap
☐ Rosak
☐ Hilang : Nyatakan.....
☐ Lain-lain : Nyatakan .....

Diperakukan Oleh :

.....

Tandatangan
Pegawai Aset PTJ

Nama : .....
Tarikh : .....

*Nota : Sesalinan borang yang telah lengkap perlulah dikemukakan kepada Unit Pengurusan Harta, Jabatan Bendahari dan Pejabat RCMO untuk tujuan rekod.

PUBLICATION

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

İBİTAK

Research Article

Turk J Med Sci
(2015) 45: 940-946

doi:10.3906/sag-1409-77

Prevalence of *Helicobacter pylori cagA, babA2,* and *dupA* genotypes and correlation with clinical outcome in Malaysian patients with dyspepsia

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kground/aim: The severity of disease outcome in dyspepsia has been attributed to *Helicobacter pylori* virulence genes. The aim of s study was to determine the distribution of *H. pylori* virulence genes (*cagA*, *babA2*, and *dupA*) and to determine whether or not there es a significant correlation with clinical dyspepsia outcomes.

terials and methods: *H. pylori* genotypes *cagA*, *babA2*, and *dupA* were identified by polymerase chain reactions from gastric biopsy ıples in 105 *H. pylori*-positive patients.

ults: The positive rates for *cagA*, *babA2*, and *dupA* genes in *H. pylori* dyspeptic patients were 69.5%, 41.0%, and 22.9%, respectively. *A* was more prevalent in Indians (39.7%), *babA2* was more prevalent in Malays (39.5%), and *dupA* detection occurred more quently in both Indians and Malays and at the same rate (37.5%). The Chinese inhabitants had the lowest prevalence of the three es. Nonulcer disease patients had a significantly higher distribution of *cagA* (76.7%), *babA2* (74.4%), and *dupA* (75.0%). There was apparent association between these virulence genes and the clinical outcomes.

nclusion: The lower prevalence of these genes and variations among different ethnicities implies that the strains are geographically l ethnically dependent. None of the virulence genes were knowingly beneficial in predicting the clinical outcome of *H. pylori* infection our subjects.

y words: *Helicobacter pylori*, *cagA*, *babA2*, *dupA*, ethnicity, virulence genes

Introduction

licobacter pylori affects more than half of the world's pulation and over 70% of those inflicted reside in veloping countries (1). *H. pylori* colonizes the gastric cosa, causing chronic gastritis, peptic ulcers, gastric enocarcinoma, and gastric mucosa-associated lymphoid sue lymphoma (2,3). The clinical outcome linked to se diseases has been associated with host genetic tors, environmental factors, and pathogen virulence tors (4). A number of proteins, including vacA, cagA, bA, dupA, SabA, and iceA, have been inferred to play ital role in the virulence of *H. pylori* by increasing the verity of the disease outcome (5-8).

The cytotoxin-associated gene (*cagA*) is most mmonly associated with cytotoxin production and the duction of interleukin 8 (IL-8) by gastric epithelial cells (9). The cag pathogenicity island (PAI), in which the *cagA* gene is localized at one end, is involved in the induction of gastric IL-8 production, though most reports have demonstrated that the cagA protein is not involved in IL-8 induction (10,11). However, one study has verified that *cagA* participates in IL-8 induction in a strain-dependent and time-dependent manner (12). *cagA* is deemed to be one of the most imperative virulence factors in the pathogenesis of *H. pylori*. *cagA* belongs to a cag PAI that codes a type IV secretion system and this secretion system is responsible for the translocation of *cagA* into host cells (13). In Western countries, *cagA*-positive strains are reported to be linked with severe clinical outcomes, but in East Asian countries, it remains abstruse when trying to find this link because almost all *H. pylori* strains possess *cagA* (14).

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babA is a blood-group antigen-binding adhesin encoded by the *babA2* gene, which has been shown to bind *H. pylori* to human Lewis b blood-group (Leb) antigens on gastric epithelial cells (15). Although three *bab* alleles have been identified (*babA1*, *babA2*, and *babB*), only the *babA2* gene product is functional for Leb binding activity (16). Some studies discovered a significant relation between *babA2* positive genotypes and the occurrence of peptic ulcer diseases (12), while others failed to find these relationships (17,18).

The duodenal ulcer (DU)-promoting gene (*dupA*) was initially described by Lu et al. in a study examining 14 *vir* gene homologs and their association with gastroduodenal disease, and especially with DU; hence, the gene was named *dupA* (6). *dupA* has been linked to an increased risk of DU and protection against gastric atrophy, intestinal metaplasia, and gastric cancer in Japan and Korea (6). Although some researchers have supported Lu et al.'s theory, others have found no such association. A study conducted within the Iraqi population reported that *dupA* is associated with peptic ulcers (19). In comparison, Argent et al. did not discover any correlation between *dupA* and DU in populations from Belgium, South Africa, China, and the United States (20).

The Malaysian population is divided into three ethnic groups (Malay, Chinese, and Indian) and these groups reflect differences in *H. pylori* infection. There are variations regarding the association between *H. pylori* virulence markers and *H. pylori*-associated diseases from one geographic area to another. Therefore, the aim of the present study was to assess the distribution of *cagA*, *babA2*, and *dupA* in *H. pylori* strains from Malaysia's multiethnic population and to determine its association with clinical outcomes.

2. Materials and methods

This was a prospective study conducted on 226 patients who underwent routine endoscopies from July 2012 to January 2014 in the endoscopy units of Hospital University Sains Malaysia and Hospital Kuala Lumpur. Patients were excluded from the study if they had received treatment with antibiotics, proton pump inhibitors, H2 receptor antagonists, or bismuth compounds within the 4 weeks prior to the study. After the endoscopic examination, the gastric biopsy specimens from the antrum were examined for the presence of *H. pylori* by rapid urease tests, culture, and histology.

This study was approved by the Human Research Ethics Committee, University Sains Malaysia, Kubang Kerian, Kelantan, Malaysia, and the National Medical Research Registry. Written informed consent was obtained from each patient prior to enrollment in the study.

2.1. Rapid urease test

Gastric antral biopsies were collected for the rapid urease test (RUT). The diagnosis of infection was based on the RUT, culture, and histology. All 105 biopsy samples tested positive by RUT. Culture was performed on 81 samples, out of which 33 samples tested positive for *H. pylori*. Out of 30 samples diagnosed by histology, only 20 samples proved positive for *H. pylori*. RUT was performed with a solution of 1 mL of distilled water, one drop 1% of phenol red, and 100 mg of urea. One antral biopsy sample was placed in the solution immediately after endoscopy and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 h (21).

2.2. Transport of samples

Biopsy samples for polymerase chain reaction (PCR) were placed in 500 µL of *Brucella* broth with 20% (v/v) glycerol and kept at −80 °C until processing (22).

2.3. Culture and identification of *H. pylori*

Gastric biopsy specimens were inoculated onto Columbia agar base (Oxoid, UK) supplemented with 7% laked horse blood and *H. pylori* Dent's selective (containing 5.0 mg/mL vancomycin, 2.5 mg/mL trimethoprim, 2.5 mg/mL cefsulodin, and 2.5 mg/mL amphotericin B), and the plates were incubated for 5–7 days at 37 °C under microaerophilic conditions. Organisms were identified as *H. pylori* by Gram stain and oxidase, catalase, and urease tests.

2.4. DNA extraction

Genomic DNA was extracted from a gastric biopsy using the QIAamp DNA tissue extraction kit (QIAGEN, Germany) according to the manufacturer's instructions and the DNA was stored at −20 °C until analysis.

2.5. PCR amplifications and conditions

PCR amplifications of *cagA*, *babA2*, and *dupA* were carried out with the use of the primers listed in Table 1 (23–26). The PCR reaction mixtures were prepared using the TopTaq Master Mix Kit (QIAGEN) in a final volume of 25 µL containing 1.25 U of TopTaq DNA polymerase, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each primer, 10 µL of molecular grade water, and 2.5 µL of DNA. The mixtures were placed in a PCR thermocycler (Eppendorf, Germany).

The PCR conditions for *cagA* included an initial denaturation of target DNA at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, with final extension at 72 °C for 15 min. As for the *babA2* and *dupA* genes, the PCR conditions were: 35 cycles of denaturation at 94 °C for 45 s, primer annealing at 52 °C for 45 s, and extension at 72 °C for 45 s. PCR products were run on 1.5% agarose gels containing red gel in the TBE buffer according to the manufacturer's instructions.

Table 1. Primers used for PCR amplification of *cagA*, *babA2*, and *dupA* genes.

Primer	Primer sequence (5′-3′)	Size base pairs	References
cagA-D008	GGTCAAAATGCGGTCATGG	297	(23,24)
cagA-R008	TTAGAATAATCAACAAACATCACGCCAT		
babA2	CCAAACGAAACAAAAAGCGT	271	(25)
	GCTTGTGTAAAAGCCGTCGT		
*dupA*1	CGTGATCAATATGGATGCTT	197	(26)
*dupA*2	TCTTTCTAGCTTGAGCGA		

5. Data analyses

ata Version 11 (StataCorp, USA) was used for analysis. e chi-square test or Fisher's exact test was applied in der to analyze variances in *H. pylori* virulence genes ong gastric ulcer (GU), DU, and gastritis. The statistical nificance was set at $P < 0.05$.

Results

1. Patients and *H. pylori*

at of 226 patients, 105 (46.5%) were confirmed to be fected with *H. pylori* by RUT. The infected patients 7 males and 48 females) ranged from between 26 to 86 ars old (mean age: 54.48 ± 12.94 years). Based on the doscopic findings, 77 patients were diagnosed with nulcer dyspepsia (NUD) or gastritis, 9 had GUs, 5 had Js, and 13 were normal.

2. Frequency of *cagA*, *babA2*, and *dupA*

gA was detected in 73 (69.5%) of the biopsy samples. e distribution of *cagA* among the three groups (Indian, alay and Chinese) was 29 (39.7%), 26 (35.6%), and 18 4.7%), respectively (Table 2). The Indian population hibited the highest distribution of *cagA* compared to the ers.

The *babA2* gene was observed in 43 (41.0%) patients rived from biopsy samples. The distribution of e *babA2* gene among Indian, Malay, and Chinese pulations was 14 (32.6%), 17 (39.5%), and 12 (27.9%), spectively (Table 2).

The *dupA* gene was also found in 24 (22.9%) biopsy samples. The distribution of the *dupA* gene among Indian, Malay, and Chinese inhabitants was 9 (37.5%), 9 (37.5%), and 6 (25.0%), respectively (Table 2).

3.3. *cagA*, *babA2*, and *dupA* genes and clinical outcome

The prevalence of *cagA* was higher in the NUD group (76.7%) than in the GU (11.0%), DU (4.1%), and normal groups (8.2%). Similarly, *babA2* was most prevalent in NUD (77.1%) patients. *dupA* was more frequent in the NUD group (75.0%) than in the others (Table 3). The endoscopic findings (NUD, DU, GU, and normal group) were higher in male patients (54.3%) than in females (45.7%). Overall, there was no significant difference between *cagA*, *babA2*, and *dupA* genes and clinical outcomes (Table 3).

A combination of *cagA*, *babA2*, and *dupA* was detected in 15 biopsy samples and a combination of *cagA* and *babA2* was noted in 38 biopsy samples. Twenty-one patients had a combination of both *cagA* and *dupA*. A total of 16 biopsy samples tested positive for *babA2* and *dupA*, as indicated in Table 4. There was no significant variance observed between the combinations and clinical outcomes.

4. Discussion

The clinical development of *H. pylori* infection depends on a combination of many factors pertaining to both the host and the bacteria. Among the bacterial factors, studies have revealed that certain *H. pylori* genotypes cause more severe pathologies (27).

Table 2. Distribution of *cagA*, *babA2*, and *dupA* gene by ethnicity.

Ethnic group (n)	*cagA*		*babA2*		*dupA*	
	Positive	Negative	Positive	Negative	Positive	Negative
Indian (37)	29 (39.7)	8 (25.0)	14 (32.6)	23 (37.1)	9 (37.5)	28 (34.6)
Malay (42)	26 (35.6)	16 (50.0)	17 (39.5)	25 (40.3)	9 (37.5)	33 (40.7)
Chinese (26)	18 (24.7)	8 (25)	12 (27.9)	14 (22.6)	6 (25.0)	20 (24.7)

Table 3. Distribution of *cagA*, *babA2*, and *dupA* and clinical outcome in *H. pylori*-infected patients.

Sex	NUD n (%)	GU n (%)	DU n (%)	Normal n (%)	*P-value
Male	44 (56.4)	4 (44.4)	4 (80.0)	5 (38.5)	0.371
Female	34 (43.6)	5 (55.6)	1 (20.0)	8 (61.5)	
Virulence genes					
cagA+	56 (76.7)	8 (11.0)	3 (4.1)	6 (8.2)	0.146
cagA -	22 (68.8)	1 (3.1)	2 (6.2)	7 (21.9)	
babA2+	32 (74.4)	5 (11.6)	3 (7.0)	3 (7.0)	0.290
babA2-	46 (74.2)	4 (6.5)	2 (3.2)	10 (16.1)	
dupA+	18 (75.0)	1 (4.2)	2 (8.3)	3 (12.5)	0.700
dupA-	60 (74.1)	8 (9.9)	3 (3.7)	10 (12.3)	

NUD = Nonulcer disease (gastritis); GU = gastric ulcer; DU = duodenal ulcer.
*Fisher's exact test was applied.

Table 4. Combined *cagA*, *babA2*, and *dupA* genotypes and clinical outcome.

Virulence genes	NUD n (%)	GU n (%)	DU n (%)	Normal n (%)	*P-value
cagA+/babA2+/dupA+	11 (32.4	1 (20.0)	1 (33.3	2 (66.7)	0.678
cagA-/babA2-/dupA-	23 (67.6)	4 (80.0)	2 (66.7)	1 (33.3)	
cagA+/babA2+	28 (82.4)	5 (100)	2 (66.7)	3 (100)	0.549
cagA-/babA2-	6 (17.6)	0 (0.0)	1 (33.3)	0 (0.0)	
cagA+/dupA+	16 (47.1)	1 (20.0)	2 (66.7)	2 (66.7)	0.565
cagA-/dupA-	18 (52.9)	4 (80.0)	1 (33.3)	1 (33.3)	
babA2+/dupA+	12 (35.3)	1 (20.0)	1 (33.3)	2 (66.7)	0.654
babA2-/dupA-	22 (64.7)	4 (80.0)	2 (66.7)	1 (33.3)	

NUD = Nonulcer disease (gastritis); GU = gastric ulcer; DU = duodenal ulcer.
*Fisher's exact test was applied.

In this study, we determined the frequency of *cagA*, *babA2*, and *dupA* in dyspeptic patients. The prevalence of *cagA* differs in every part of the world. The prevalence of *cagA* is lower in Western countries (28,29) when compared to East Asian countries, where *cagA* is present in more than 90% of cases irrespective of clinical presentation (30).

The prevalence of *cagA* in this study was 69.5%; this is slightly lower than reports from East Asian countries. Studies conducted locally have reported differing percentages. According to Ramelah et al., the prevalence of *cagA* was 94% (31), while Amjad et al. reported 43% (32). This divergence within the same country may well be due to differences in sample size, primer sets, or the variety of strains within the same country. In addition, the results of this study did not elaborate on conclusive evidence linking *cagA* with NUD patients; our results are in agreement with supplementary studies in Asian countries (31,33) that failed to find any association. However, various studies have reported that *cagA* was statistically concomitant with peptic ulcers (34,35).

babA2 attaches *H. pylori* to these cells, enabling delivery of vacA and *cagA* toxins near the gastric epithelium and therefore increasing gastric tissue damage (15). The prevalence of the *babA2* genotype in our study was 41.0%.

ur results are consistent with a study conducted in China, ich reported a prevalence rate of 38.9% in dyspeptic atients (36), but slightly lower than studies from Turkey 3.8%) (34). Oliveira et al. discovered that *babA2* was more quently found in patients with DU and gastric cancer (35). ne current study did not include a gastric cancer case and U accounts only for 4.8% of the studied population, so this ght have contributed to the low prevalence of *babA2* in r study. Gerhad et al. discovered that *babA2* was associated ith peptic ulcer disease in Western populations (15). Our udy failed to find any substantial associations between *abA2* and the clinical outcome. This is in agreement with a evious conducted study (18).

During the present study, 24 (22.9%) *dupA*-positive *H. lori* strains were observed in the patients' biopsy samples. ur data are in line with a study of Japanese patients (21.3%)) and slightly lower than the findings of a study conducted China (35.3%) (37). The only other study performed in alaysia also unearthed a comparable prevalence of 21.3% 8). *dupA* has been linked to an increased risk of DU and decreased risk of gastric cancer (6). In contrast, Lu et al.) did not observe an association between *dupA* and DU in ast Asia. These differences in results might be due to strain ariations moving from one region to another. Furthermore, e DU patients used in our study account for only 4.8% of e study group, which prevents us from drawing a definitive nclusion.

Combinations of two or three of the virulence genes were not noticeably diverse among the NUD, GU, DU, and normal groups in our study, although a study conducted in Cuba reported a significant association between a combination of *cagA* and *babA2* genotypes (39).

In our study, NUD patients tended to have a higher distribution of *cagA* (76.7%), *babA2* (74.4%), and *dupA* (75.0%) compared to peptic ulcer disease. Our result is in agreement with a study from Iran, which found an obviously much higher prevalence of the *cagA* gene (73%) in NUD patients (40). Others have found a higher presence of *cagA* in peptic ulcer disease patients than in NUD (31,41). This dissimilarity might be due to an imbalance in NUD and peptic ulcer disease cases.

In conclusion, although there is no association between virulence genotypes and clinical outcomes in our study, the lower prevalence of these genotypes in *H. pylori*-positive patients and variations among different ethnicities indicates that there is strain variation among countries and ethnic groups.

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RESEARCH ARTICLE

Evaluation of the Atlas *Helicobacter pylori* Stool Antigen Test for Diagnosis of Infection in Adult Patients

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Abstract

Background: *Helicobacter pylori* (*H.pylori*) is one of the most important causes of dyspepsia and gastric cancer and diagnosis can be made by invasive or non-invasive methods. The Atlas *Helicobacter pylori* antigen test is a new rapid non-invasive method which is simple to conduct. The aim of this study was to determine its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Materials and Methods: This prospective study was conducted between July 2012 and December 2013. Stool samples of 59 dyspeptic patients who underwent upper endoscopy were evaluated for *H. pylori* stool antigen. Results: From the 59 patients who participated in this study, there were 36 (61%) males and 23 (39%) females. *H. pylori* was diagnosed in 24 (40.7%) gastric biopsies, 22 (91.7 %) of these being positive for the Atlas *H. pylori* antigen test. The sensitivity, specificity, PPV, NPV and accuracy were 91.7%, 100%, 100%, 94.6% and 96.6% respectively. Conclusions: The Atlas *H. pylori* antigen test is a new non-invasive method which is simple to perform and avails reliable results in a few minutes. Thus it can be the best option for the diagnosis of *H. pylori* infection due to its high sensitivity and specificity.

Keywords: *Helicobacter pylori*- sensitivity - specificity - Atlas *H. pylori* antigen test

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Introduction

Helicobacter pylori(*H. pylori*) is a Gram-negative microaerophilic bacterium and one of the most common bacterial pathogens of humans that infects more than half of the world's population (Amjad et al., 2010; Zhang et al., 2014). The bacteria has worldwide distribution and the prevalence ranges from 25% in developed countries to more than 90% in developing areas, but not all infected individuals eventually developed the disease (Miernyk et al., 2011; Ghotaslou et al., 2013).

The prevalence of *H. pylori* infection varies widely by geographic area, age, race, and socioeconomic status (Brown et al., 2002). *H. pylori* infection is associated with chronic gastritis, gastric or duodenal ulcer, gastric cancer and MALT-lymphoma (Ben Mansour et al., 2010; Zhao et al., 2012). *H. pylori* was classified as a class I carcinogen in humans by a working group of the World Health Organization International Agency for Research on Cancer (IARC) based on various epidemiological studies (Khalilpour et al., 2013)

There seems to be no firm agreement as to which method should be used as gold standard for the detection of *H. pylori* infection (Redeen et al., 2011). Gastric biopsy based tests which include culture, histology and the rapid urease test (RUT) are considered the standard diagnostic tests (Al-Humayed et al., 2008; Kalem et al., 2010). However, these tests necessitate an upper gastrointestinal endoscopy and are considered invasive tests.

Non-invasive tests include the urea breath tests (UBT) and serology and stool antigen test (Bhewa et al., 2007; Redeen et al., 2011). Urea breath tests and stool antigen test can detect active infection while serology test does not differentiate between active infection and exposure to *H. pylori* (Ricci et al., 2007; Peng et al., 2009).

The choice of a given testing strategy is influenced by sensitivity, specificity, the clinical circumstances and the cost-effectiveness of the test (Peng et al., 2009). In the last years, many studies have focused on noninvasive methods; *H. pylori* stool antigen test provides a simple alternative to the urea breath test and is appropriate for diagnosis and follow-up of infection (Gisbert and Pajares,

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2004; Shimoyama et al., 2009).

The aim of this study was to assess the efficacy of new Atlas *Helicobacter pylori* Antigen Test for the detection of *H. pylori* infection in dyspeptic patients and find its sensitivity, specificity and negative and positive predictive values and accuracy in the diagnosis of *H. pylori* infection.

Materials and Methods

Patients

This is a prospective study conducted at Hospital Universiti Sains Malaysia and Hospital Kuala Lumpur among 59 adult dyspeptic patients between July 2012 to December 2013. The patients were selected from patients who presented with gastrointestinal symptoms at the endoscopy unit of Universiti Sains Hospital, Kubang Kerian, Kelantan and Hospital Kuala lumpur, Malaysia. After receiving a full explanation of the purpose of the study, each patient gave informed consent and was enrolled into the study.

Gastric antral biopsies were collected for rapid urease test as well as stool sample for the detection of *H. pylori* antigen from stool. The diagnosis of infection was based on the RUT. Patients were considered *H. pylori*-positive when the results of RUT were positive. This test was performed with a homemade solution with 1 mL distilled water, one drop 1% phenol red, and 100 mg urea. One antral sample were placed in the solution and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 hours (Pourakbari et al., 2011).

Inclusion and exclusion criteria

Patients were excluded from the study if they had received treatment with antibiotics, proton pump inhibitors, H2 receptor antagonists and bismuth compounds within the last four weeks. Patients with previous gastric surgery, long-term use of corticosteroid and immunosuppressant, a history of bleeding or active gastrointestinal bleeding and diarrhoea were also excluded from the study.

This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia and National Medical Research Resgistry (NMRR).

Detection of H. pylori by Atlas H. pylori antigen test (Atlas Medical, UK)

Atlas *Helicobacter pylori* Antigen Test (Atlas medical, UK) is a rapid immunoassay using a monoclonal anti-*H. pylori* antibody on a strip for the detection of *H. pylori* infections in stool specimens. The *Helicobacter pylori* antigen reacts with the conjugate-Pink Red latex particles sensibilized with anti-*H. pylori* monoclonal antibody coated to the membrane of the strip. The formed H. pylori-conjugate complex, which migrates upward the membrane by capillarity, binds to the specific antibody molecules fixed to the reaction zone.

The stool is collected in a clean container and the test done as soon as possible or stored at 2-8°C for a longer period of time. The test device and sample are put at room temperature (15-30°C) prior to testing. The test was performed according to the manufacturer's instructions. By using the applicator stick of the provided sample diluent vial, a small portion of stool specimen is transferred into the sample diluent and mixed well by shaking gently. The tip of the vial was broken off and four drops were added to the sample well in the test device.

The test was read after 5 minutes of incubation. A positive test result is indicated by appearance of green band at (control line) and red band in the zone marked T (result line). The sample is considered negative when only one green band (control line) appears in the white central zone of the strip. If no colored bands appear or only one band appears in the T zone the result is regarded as invalid and if an inconclusive result is obtained, the test is repeated with a new strip.

Table 1. Sensitivity, Specificity, Positive and Negative Predictive values, and Accuracy of Atlas *Helicobacter pylori* Antigen test in the Detection of *Helicobacter pylori* Infection Atlas *Helicobacter pylori* Antigen Test (n=59)

	Atlas *Helicobacter pylori* antigen test
True positive	22
True negative	35
False positive	0
False negative	2
Sensitivity (95% CI)	91.7% (72.9-98.7)
Specificity (95% CI)	100% (89.9-100)
Positive predictive value (PPV) (%)	100% (84.4-100)
Negative predictive value (NPV) (%)	94.6% (81.8-99.2)
Accuracy	96.6

*95%CI=95% confidence interval

Statistical analysis

The sensitivity, specificity and positive and negative predictive values of the Atlas *Helicobacter pylori* antigen test were calculated against the gold standard for diagnosis of *H. pylori* infection by two by two standard method. Calculations of 95% confidence intervals (CI) were conducted for proportions of these values.

Results

A total of 59 patients, who consisted of 36 (61%) males and 23 (39%) females with a mean age of 51.2±13.3 years and ranging from 26-80 years were recruited into the study.

Out of the total, 24 patients were *H. pylori* positive and 35 were *H. pylori* negative by the gold standard method. Atlas *Helicobacter pylori* antigen test was positive in 22 patients and negative in 35. Thus the sensitivity, specificity, PPV and NPV of Atlas *Helicobacter pylori* Antigen Test were 91.7%, 100%, 100% and 94.6% respectively. The diagnostic accuracy was 96.6% (Table 1).

Discussion

H. pylori is acquired in childhood and survives in the human stomach, the only niche known to date (Tan and Wong, 2011; Valliani et al., 2013). Noninvasive testing for *H. pylori* has been strongly recommended as it is less expensive and more patient-friendly than invasive testing

that requires endoscopy (Manes et al., 2001) and does not need a very complicated laboratory facility.

To the best of our knowledge, this is the first prospective study to find out the efficacy of Atlas *Helicobacter pylori* antigen test in the diagnosis of *H. pylori* infection.

The most important finding of this study was that the new stool antigen test showed high sensitivity (91.7%) and specificity (100%) (Table 1). Our result is similar to other studies done on monoclonal stool antigen test based on immune chromatography, study done In Brazil, found the sensitivity and specificity as 88.0% and 87.5%, respectively (Silva et al., 2010). Similar study done in Turkey showed the sensitivity and specificity of HpSA test as 68.9% and 100% respectively (Ceken et al., 2011). In addition to this, study done in Korea, found the sensitivity and specificity of *Helicobacter pylori* stool antigen immunochromatographic assay (S-ICT test as 84.5% and 96.2% respectively (Jekarl et al., 2013).

The advantages of Atlas *Helicobacter pylori* antigen test over stool antigen test especially HpSA test which has been validated and widely used is that it is easy and takes less than 10 minutes. HpSA test based on enzyme immunoassay tests takes over 1 hour to avail the result, Therefore the new Atlas *Helicobacter pylori* Antigen test is more convenient and saves patient time.

The limitations of this study were small sample size and the fact that we only investigated the performance of the test during initial diagnosis of *H. pylori* infection and not in the post treatment setting.

In conclusion, the Atlas *H. pylori* antigen test is a new non-invasive method which is simple to perform and avails the result in few minutes. Our results have shown that, it has high sensitivity, specificity and diagnostic accuracy and can be used as an alternative method in the diagnosis of *H. pylori* infection in adults. However, there is a need for further studies with a greater number of different patients and to find also its effectiveness in the post treatment setting.

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The Characteristics of *Helicobacter pylori* infection and Clinical Outcomes of Patient with Upper Gastrointestinal Bleeding Admitted at Hospital Universiti Sains Malaysia

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Abstract: Upper gastrointestinal bleeding (UGIB) remains one of the most common clinical life threatening emergencies which are associated with a high morbidity and mortality. The main aim of this study was to determine the cause of *Helicobacter pylori* (*H. pylori*) infection and the use of non-steroidal anti-inflammatory drugs (NSAID) in upper gastrointestinal bleeding patients. A retrospective record review study was conducted among UGIB confirmed patients from January 2009 and December 2012 at Hospital Universiti Sains Malaysia. All patients who were admitted in hospital were recruited. Data collection included age, gender, *Helicobacter pylori* positivity, associated symptoms and Endoscopic findings. There were 46 patients with a mean age of 62 years. *H. pylori* was detected only in 2 (4.3%) both in Male among UGIB patients by Campylobacter-like organism (CLO) test. The prevalence of UGIB was higher in men than women 27(58.7%). The most common cause of UGIB was peptic ulcer (56.5%) and especially high amongst male patients (59.2%).The second common cause of UGIB was gastritis (19.6%). The majority of the patients are NSAID users 25 (54.3%). In conclusion, Peptic ulcer disease is the leading cause of UGIB and mainly common among males and *H. pylori* infection in upper gastrointestinal bleeding patients was low.

Key words: *Helicobacter pylori* · Campylobacter-Like Organism Test · Gastritis · Peptic Ulcer · Upper Gastrointestinal Bleeding

INTRODUCTION

Upper gastrointestinal bleeding (UGIB) is a common medical emergency that requires hospitalization leading to higher patient morbidity and medical care [1]. The overall mortality rate associated with UGIB is nearly 10-15% [2]. The incidence rates of UGIB reveal a large geographic variation ranging from 100 to 150 cases per 100 000 population, with regular reports of higher incidences among men and elderly people [3, 4].

The most common cause of UGIB is Peptic ulcer bleeding [PUB], accounting for 31%-67% of all cases, followed by erosive disease, variceal bleeding, oesophagitis, malignancies and Mallory-Weis tears

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[5]. In PUB patients, bleeding from duodenal ulcers is slightly more frequent than from gastric ulcers [6].

A study done in Malaysia among 128 UGIB patients found that Peptic ulcer is the main cause of UGIB [7]. *H. pylori* infection and Non-steroidal anti-inflammatory drugs (NSAID) use are independent risk factors for UGIB, especially PUB [8].

Helicobacter pylori (*H. pylori*), a Gram-negative microaerophylic bacterium, is associated with gastrointestinal diseases such as chronic gastritis, gastric and duodenal ulcers and gastric cancer [9-11]. *H. pylori* infection can be diagnosed by invasive techniques requiring endoscopy and biopsy (histological examination, culture, Polymerase chain reaction (PCR) and rapid urease test) and by non-invasive tests (serology, urea breath test, detection of *H. pylori* antigen in stool specimen) [12, 13]. The prevalence of *H. pylori* infection in UGIB patients varies between 24.4% (Serbia) to 92.4% (Spain) [14, 15].

UGIB has high morbidity and mortality rate worldwide and to the best of our knowledge only one study addressed these issues in Malaysia [7] However, it did not study the contribution of *H. pylori* to the morbidity and mortality rate of UGIB in the local population. Therefore, the aim of this study is to find out the causes of UGIB and incidence of *H. pylori*, use of NSAID in upper gastrointestinal patients.

MATERIALS AND METHODS

This retrospective study was conducted at Hospital Universiti Sains Malaysia. The study involved 46 patients who were hospitalized for UGIB with clinical complaints, black tarry stool (melena),coffee-ground vomiting or hematemesis and who underwent endoscopy between January 2009 and December 2012. Cases with UGIB were identified from the records department for the period under study. Case files were then individually analysed to collect data according to the scopes of the current hypothesis.

Collected demographics data (age, gender, race and place of residence), clinical characteristics, cause of UGIB (peptic ulcer, gastritis, duodenitis,varices, erosions and others), *H. pylori* infection and predisposing factors (NSAID). In addition, endoscopy reports were individually examined to ascertain endoscopic findings and confirm the underlying aetiology of bleeding. Patients who had received treatment with antibiotics or proton pump inhibitors were excluded in order to avoid false negative *H. pylori* results.

Helicobacter pylori presence in UGIB patients was confirmed by the invasive method Campylobacter-like organism (CLO) test.

This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan, Malaysia.

Statistical Analysis: Statistical Package for Social Science (SPSS 20) was used to perform the analysis.Numerical variables are given as means and standard deviation (SD) while Categorical variables were expressed as frequency and percentages. Categorical data were analysed by Chi- square test and Fisher's exact test. The results were considered significant if the P value was less than 0.05.

RESULTS

A total of 46 patients with diagnoses of UGIB were admitted to Hospital Universiti Sains Malaysia between the year 2009 and 2012 and comprised of 27 (58.7) males and 19 (41.3) females. The mean age of patients was 62.3% (range 12 to 83 years) (Table 1). Our study shows that the incidence of UGIB is more common in males than in females.

The ethnic distribution were 41 (89.1%) Malay, 4 (8.7%) Chinese and 1(2.2%) Indian (Table 1). Malay represents the highest ethnic group in this study, probably because they are the highest inhabitant of this state (95%).

The most common presenting complaints were melena (22) and epigastric pain (16) followed by haemetemesis and coffee ground (Table 2). Most of the patients had more than one symptom.

The leading causes of upper gastrointestinal bleeding were Peptic ulcer 26 (56.5%), gastritis 9 (19.6%) and duodenitis 7 (15.2%). Other less common causes included gastric erosion, gastric polyp and oesophageal varices (Table 3).

Peptic ulcers were the most common cause of UGIB 26 (56.5%) and males being with highest percentage 16 (59.2%) as compared with females 10 (52.6%).

Helicobacter pylori infection was found in 2(4.3%) of upper gastrointestinal patients by Campylobacter-like organism (CLO) test (Table 1).