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The Seroprevalence and Eradication Success of *Helicobacter pylori* in Indigenous People of Seletar in Southern Malaysia

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Authors' contributions

This work was carried out in collaboration between all authors. The study was designed by author AFM who wrote the protocol, managed the literature searches, interviewed and conducted the study at the indigenous people's settlements, and wrote the first draft of the manuscript. Author MNMY assisted in conducting the research collected and tabulated the data. Author SAR analysed the histopatopathology of the gastric specimens from OGDS and author RBN performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: *Helicobacter pylori* prevalence rate varies widely from one geographical area to another and marked differences have been noted among different ethnic groups. While several studies have been carried out to review the prevalence of *H. pylori* among the major races in Malaysia, only one is available to study the indigenous *Penan* community in East Malaysia.
Aims: The aims of this study are to determine the seroprevalence of *H. pylori* in the indigenous Seletar community in Southern Malaysia and the effectiveness of the eradication program.
Methods: A seroepidemiological study was done to determine *H. pylori* infection by serological assay of *H. pylori* antibodies and the demographic pattern. Serological assay

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was carried out using the Visual *H. pylori* Quickpac Test. All serological positive patients were then subjected to an endoscopic study and the *Campylobacter*-Like Organism-test (CLO-test). Positive patients were then offered for eradication using triple therapy of Omeprazole 20mg, Amoxycillin 1gm and Metronidazole 400mg twice daily for one week. Subjects will be required to perform a Urea Breath Test (UBT) four weeks after therapy to determine the success of eradication. When attending for UBT, the medication diary will be assessed. UBT was conducted using a 13C IRIS (Infra Red Isotope Analyzer) breath test kit.

Results: We studied a total of 298 subjects with a mean age of 34.9 years. The seroprevalence was 37.9% and CLO-test positivity on Oesophago-gastro-duodenoscopy (OGDS) was 98.9%. Histopathological examination showed evidence of gastritis in 97% of the positive patients. Majority of subjects (96%) showed evidence of acute or chronic inflammation. The follow-up of patients that came back for the UBT after completing the eradication process was 73.5%. Out of these, 63.9% showed negative UBT. However, only 48.6% complied with medication based on medication diary. Since only two subjects out of 35 who complied remains positive, the eradication success rate was 94.3%.

Conclusion: Previous studies have shown that the prevalence of *H. pylori* in a Malaysian cohort ranges from 26.4% to 55% with the highest in Indians of about 35.6% out of the three major races, followed by Chinese (28.6%) and Malays (28.5%). The indigenous people of *Penan* in East Malaysia showed 37.7% positivity, which is almost similar to our study that showed 37.9%. The prevalence is supported by the CLO-test that proved that the sensitivity of the serological assay was 98.9%. However, our study also proved that the eradication process was very efficient if subjects were compliant on medication. This study shows that despite the very low socio-economic status of the indigenous community, this is not the sole factor in determining *H. pylori* infection. This may also be due to genetic factors and probably a recent arrival of *H. pylori* in this isolated and remote community.

Keywords: Helicobacter pylori; seroprevalence; Oesophago-gastroduo-denoscopy (OGDS); disease eradication.

1. INTRODUCTION

One of the most important discoveries in modern medicine was probably on *Helicobacter pylori* by Warren and Marshall and its association with peptic ulcer disease and gastric cancer. It is also a known fact that the prevalence varies widely from one geographic area to another. It shows a higher incidence rate in developing countries and lower rates in developed countries [1]. This has led to the known conclusion that the transmission of the organism is associated with low socio-economic status and is also compounded by inferior hygienic conditions and high-density living [2,3].

Several studies conducted in Malaysia known for its multiracial society have shown a higher prevalence of *H. pylori* infection among non-Malays as compared to Malays [4-11]. Indian has the highest rate of *H. pylori* infection (68.9-75%) followed byChinese (45-60%) and lowest in Malays (8-43%) [8,12]. The differences in prevalence has been attributed to what is known as the “racial cohort theory” [8].

H. pylori is thought to have migrated with its host out of Africa [9]. Analysis of *H. pylori* isolates by multilocus sequence typing (MLST) revealed the *H. pylori* ancestral populations. The Malaysian *H. pylori* isolates have been differentiated into three populations using MSLT,

namely hpEastAsia, hpAsia2 and hpEurope [13]. The *H. pylori* isolates from the Chinese and Indians were divided along their ethnic origins. The high prevalence in these two communities as compared to the native inhabitants of Malaysia – the Malays – has been attributed to the high reservoir of infection from the country of origins [14,15]. Chinese and Indians have migrated in significant numbers from these regions during the last century.

However, the prevalence of *H. pylori* is known to be low in the Malay population. While it may have been attributed to an improved hygiene standard as in the West, this explanation may not be true in the Malaysian context [8,12]. Therefore, the Malay population was probably initially free from *H. pylori* and it was only recently acquired. Based on the MLST analysis, the majority of Malay isolates were found to be grouped together with Indian isolates. As has been stated earlier, studies have shown that *H. pylori* follows human route of migration that reflects human ancestry but there was no anthropological data to support such a notion. Hence, the most plausible explanation is that the Malay population was initially free of *H. pylori* and the *H. pylori* in the current Malay population have beenacquired recently from the Malaysian Indian community [13].

Another potential source of *H. pylori* would be from the indigenous people or *Orang Asli* who originated from early human migration out of Africa. It is thought that the *Orang Asli* would have taken the "Southern Route" into South East Asia to reach Malaysia by travelling along the Indian Ocean Coast Line about 50 to 65000 years ago [16-18]. Therefore, the *Orang Asli H. pylori* may share the common ancestry with the Indian *H. pylori* leading to the apparent similarity between the Malay and Indian isolates.

However, considering the fact that the indigenous Maori and Red Indian *H. pylori* populations can be readily identified [19], the *H. pylori* of the *Orang Asli* would also be unique after such a long period of separation. Hence, the acquisition of *H. pylori* from the Indian population is more plausible [13]. Unfortunately, so far there has never been any attempt to isolate and study the alleles of *H. pylori* from the *Orang Asli.*

This is the second study looking into the prevalence of *H. pylori* in *Orang Asli* but into a sub-population of a unique group of *Seletar* people in the southern part of Peninsular Malaysia categorized as *Proto Malays.* It must be noted that the first study being conducted on an indigenous people of Malaysia was carried out on the *Penan* community in East Malaysia [20]. However, it was restricted to the prevalence of *H. pylori* based on detection of *H. pylori* antigen using commercially available enzyme-linked immunosorbent assay (ELISA) on stool samples. Our study was the first to not only look at the prevalence of *H. pylori* in a specific Peninsular Malaysia subpopulation of *Orang Asli* but also to ascertain the sensitivity of the serological technique by doing oesophagogastroduodenoscopy (OGDS) and *Campylobacter*-Like Organism-test (CLO-test) to be followed by eradication therapy. Subsequently, the success of eradication therapy was ascertained by conducting the urea breath test using the 13C Infrared Isotope Analyzer (IRIS) test kit (13C UBT).

2. MATERIALS AND METHODS

This first part of the survey was conducted together with the Cardio-metabolic team of Monash University Malaysia. The team visited five *Orang Asli* settlements in Johor, the Southern-most state in Peninsular Malaysia namely Simpang Arang, Bakar Batu, Kuala Masai, Sungai Selangi and Telok Jawa. The settlements are mainly in rural areas close to the sea with very basic amenities of electricity and water supply. Most of the inhabitants are fishermen and only a few are skilled workers.

Blood samples were taken apart from conducting a demographic and cardio-metabolic survey after consent was obtained. Blood was taken back to the lab and spinned for supernatant. Serological assay was done using the Visual *H. pylori* Quickpac Test. Those found to be positive on serological assay were instructed to follow-up at Hospital Sultanah Aminah (HSA), the General Hospital of Johor Bahru, capital of the state of Johor. Transport was provided by Monash University Malaysia to fetch the subjects to the hospital and later to take them back.

Positive subjects on serology will then be subjected to OGDS by surgeons from the Surgical Department of HSA. A CLO-test rapid urease test was done immediately on-site on the biopsy and positivity was determined by the change in colour of the gel sealed inside the plastic slide from yellow to magenta. The biopsy was then fixed with 10% formalin to be transported back to Kuala Lumpur to be examined by a pathologist who is a member of the study. The histological sections were stained with haematoxylin-eosin and Wright-Giemsa to look for *H. pylori* density, neutrophil activity and evidence of acute or chronic inflammation.

Subjects found to be positive on OGDS and CLO-test were then subjected to eradication therapy. The protocol was a triple therapy regime with a proton pump inhibitor, Omeprazole 20mg twice daily, Amoxycillin 1gm twice daily and Metronidazole 400mg twice daily for one week. Compliance was determined by giving each subject a booklet to be filled every time the medicine as taken.

After four weeks upon completion of treatment, a final visit was carried out at the different settlements accordingly. Subjects who have been treated with the triple therapy were determined whether *H. pylori* have been successfully eradicated by subjecting them to the 13C Urea Breath Test (UBT) (Graf Medical System). Before conducting the test, the booklet will be examined for compliance to the triple therapy.

For the UBT analysis, four breath samples were needed. The initial breath sample was before ingestion of the urea tablet. After ingestion of the urea tablet, another three subsequent breath samples were taken at twenty minutes interval. The breath bags were then sent to the laboratory for assessment.

Results of serological study by Quickpac test, OGDS finding, CLO-test, histopathological examination (HPE), booklet compliance and 13C UBT were tabulated and analysed.

Fig. 1 shows the flow chart of this study.

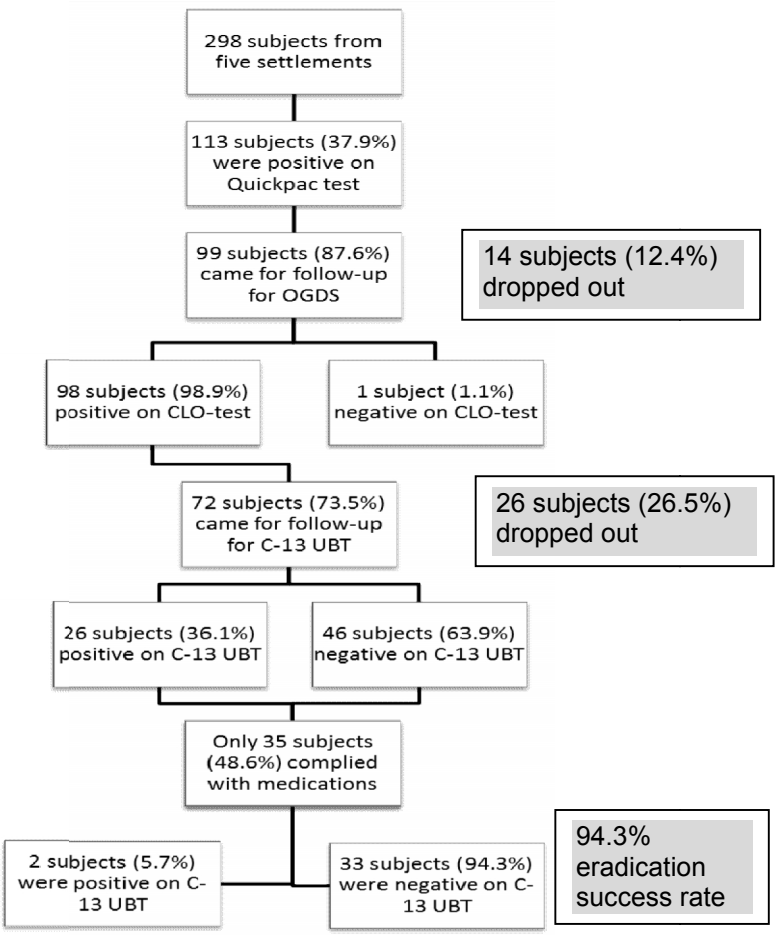

Fig. 1. Flow chart of study

3. RESULTS

Two hundred ninety eight (298) subjects were studied from five different settlements in the southern state of Johor. The male to female composition was 34.9% to 65.1%. Majority were Muslims (57.7%) followed by Christians (20.8%), Buddhists (3.7%) and the rest were Animists. The mean age was 34.93 (SD: 13.62) years. The median age was 31.5 (21.0) with a range of 15 to 80 years. None of the subjects complained of any symptoms of dyspepsia and none of them are on any medications (Table 1).

The seroprevalence based on Quickpac test was 37.9%. Only 99 subjects out of 113 came for follow-up at HSA for OGDS, which means 12.4% of them dropped out. Of the 99 subjects, 98 of them were found to be positive on CLO-test, which means 98.9% was positive. Although all the subjects found positive on OGDS and CLO-test were given the course of triple therapy, only 72 out of 98 turned for the last follow-up for C-13 UBT (drop-out of 26.5%). Of the 72 subjects who turned up for the final test, only 35 subjects complied with the booklet given. UBT was found to be negative in 46 out of 72 subjects or 63.9%. However, after taking into consideration that only 35 patients complied with the booklet

which indicated that those who complied had completed the one-week course of triple therapy, we found that only two subjects out of thirty-five had a positive 13C UBT. Hence, it is safe to assume that the eradication success rate was 94.3% (Table 2).

On reviewing the OGDS results, only 63 out of the 99 subjects were found to have abnormal mucosa. However, subsequent laboratory assessment with HPE detected *H. pylori* in 96 of the 99 specimens. This accounts for 96.9% of the specimens. HPE also revealed that all of the 96 specimens showed abnormal mucosal layer. The majority of them displayed evidence of acute chronic inflammation (97.9%). All the specimens only showed inflammation of the mucosal layer without any involvement of the sub-mucosal layer. Most of the affected mucosa was of moderate severity (53.5%), while the rest were either mild (18.2%) or severe (28.28%) (Table 3).

Table 1. Demographic characteristics of 298 subjects

Demographic characteristics	No. (%)*
Age (years)	
• Mean (SD)	34.93 (13.6)
• Median (IQR)	31.50 (21.0)
Gender	
• Male	104 (34.9)
• Female	194 (65.1)
Religion	
• Islam	172 (57.7)
• Christian	62 (20.8)
• Buddha	11 (3.7)
• Animism	53 (17.8)
Occupation	
• Fisherman	245 (82.2)
• Skilled worker	53 (17.8)

** For gender, religion and occupation only*

Table 2. Laboratory tests for *H. pylori*

Laboratory tests	No. (%)
Quickpac test (N=298)	
• Positive	113 (37.9)
• Negative	185 (62.1)
CLO-test (N=99)	
• Positive	98 (98.9)
• Negative	1 (1.1)
Histopathological examination (HPE) (N=99)	
• Positive	96 (96.9)
• Negative	3 (3.1)
Urea breath test (13C UBT) (N=72) including non-compliance to medications	
• Positive	26 (36.1)
• Negative	46 (63.9)
Urea breath test (13C UBT) (N=35) of fully compliant subjects	
• Positive	2 (5.7)
• Negative	33 (94.3)

Table 3. HPE: Degree of severity of mucosal inflammation

Degree of severity	%
• Mild	18.2
• Moderate	53.5
• Severe	28.3

4. DISCUSSION

We managed to highlight a few issues concerning this specific study on *H. pylori* in an indigenous population of *Seletar* or Proto Malays community in southern peninsular of Malaysia. First and foremost is that the prevalence rate is at 37.9%. This rate was no higher than the overall prevalence rate in Malaysia that ranges from 26.4% to 55.0%.[8] As has been said before, probably the overall prevalence rate may not be as meaningful since there exists a large variation in prevalence rate between the different races.

Acknowledging the fact of the existence of such variation, the prevalence rate is still low and approximates that of the Malay race. If *H. pylori* thrive within low socio-economy, poor hygiene and over-crowding, then the low occurrence in such a primitive community does not resonate well. A more cogent explanation is needed than merely the environmental factors and sociocultural practices.

It is also interesting to note that a low prevalence rate of *H. pylori* was also found in the indigenous *Penan* community of Malaysian Borneo. Studies conducted on 275 *Penans* showed a prevalence rate of 37.7% [20]. It was almost similar to our prevalence of 37.9% for 298 *Seletars*.

As highlighted by Huang et al. [20], there is no prospective study that tracked *H. pylori* prevalence in isolated communities with low infection rate but it is possible for the number to grow if there is no improvement in the living condition. Studies elsewhere have shown that there was transmission of *H. pylori* within families [21,22].

Our study has also proved the reliability of the Visual *H. pylori* Quick-pack Test. The positivity in seroprevalence was confirmed with HPE results that proved the sensitivity to be approximately 98.9%. Similarly, CLO-test has a specificity of 99% with a 1% false-positivity. We have also established the effectiveness of the eradication of *H. pylori* with triple therapy regime of Omeprazole-Amoxycillin-Metronidazole. The success rate was 94.3%. Taking into consideration that this is a remote community with very limited education, the eradication rate was quite modest considering the published success rate of triple therapy was between 88-97% [23] depending on the triple regime used for eradication.

It is of note that the seroprevalence in this study was done by blood test considering the fact that it is the simplest, non-invasive and reliable method of diagnosing *Helicobacter pylori* infection. However, the eradication of the bacterium was determined by using the 13C UBT. 13C UBT was chosen to confirm eradication and not the same serological examination because from the literature study, we found that 13C UBT is considered the best non-invasive test to validate *Helicobacter pylori* eradication. Serology is considered unreliable for this purpose due to the slow and unpredictable decline in the antibody titre.

A follow-up research on the subjects that have been proven negative after eradication therapy would give us an indication of the rate of re-infection. A more thorough study on the subjects' social interaction would probably allow us to learn more about the natural history of *H. pylori* infection in this remote community.

Finally, having discussed about the racial cohort theory during introduction, we feel that the only plausible explanation to our finding of relatively low prevalence of *H. pylori* among the indigenous *Seletar* people could only be explained by the recent introduction of the organism into the community. The relatively low infection rate in the *Orang Asli* population was probably due to a low cross-infection from other populations.

It would be interesting to know the strain of *H. pylori* in the *Orang Asli* population. Perhaps future studies should look into this matter. There appears not to be a grossly significant direct interaction between *Orang Asli* and other races, hence the prevalence of *H. pylori* remains low in this community. The study of *H pylori* strains in *Orang Asli* would definitely shed some light on whether the *H. pylori* follows the human route of migration and thus reflects human ancestry since they were the first to migrate out of Africa to this side of the world.

5. CONCLUSION

The study on *Helicobacter pylori* remains intriguing ever since its discovery by Warren and Marshall years ago. There were not many research conducted on the marginalized communities to ascertain the prevalence of this bacteria in the low socio-economic groups. Apart from the study conducted on the *Penan* community in Borneo, there was no data whatsoever on the indigenous people of the Malayan peninsular. This study has shown that the prevalence of *H. pylori* in the *Seletar* community is almost similar to those of the *Penan* community in Borneo of less than forty percent infection rate that challenges the long-held theory that this bacterium is more common in the low socio-economic groups.

CONSENT

Written informed consent was obtained from all subjects for publication of this report.

ETHICAL APPROVAL

All authors hereby declare that all procedures have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. In this particular study, the approval was from the Medical Research and Ethics Committee, Ministry of Health Malaysia as of NMRR-09-283-3913.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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