INTRODUCTION

H. pylori are fastidious, microaerophilic, helical shaped, Gram-negative bacteria. During infection, flagellum-associated proteins and adhesins facilitate the adherence of H. pylori to gastric epithelial cells [1,2]. H. pylori produce lipopolysaccharide O-antigen units that can post-translationally produce H. pylori adhesins. The H. pylori strains can be classified as types I and II, depending on the expression of the cytotoxin associated gene product A (cagA) and vacuolating cytotoxin A gene product (vacA).

H. pylori infection has been implicated in chronic immune thrombocytopenia (cITP), rheumatoid arthritis and autoimmune thyroiditis [5-9]. Chronic ITP affects mostly adults with thrombocytopenia lasting usually for at least 12 months. Acute ITP normally lasts less than six months and is more common among children. Both disorders can be characterised by a purpuric rash, an increased tendency to bleed. Both conditions caused by increased platelet destruction in which anti-platelet antibodies sensitise circulating platelets marking them for removal by splenic macrophages bearing Fcγ receptors.

Some studies have proposed an association between H. pylori infection and ITP and in some cases infection eradication has returned the platelet count within the reference range. This has been especially the case in studies reported from Japan and Italy [10-13]. Just how H. pylori infection leads to cITP in some is not known. Approximately 90% of Japanese and East Asian strains and 60% of isolates from the Western world express the cagA virulence protein [8]. The cagA protein has been reported to be expressed in some H. pylori infected cITP patients and it has been proposed that anti-cagA might cross-react with platelet membrane glycoproteins [14]. Another proposed mechanism follows P-selectin-dependent platelet aggregation induced by H. pylori. Antibodies form a complex with the platelet FcγRIIA receptor leading to platelet activation and the release of P-selectin and von Willebrand factor which promotes platelet aggregation and apoptosis [15]. In the third proposed mechanism, H. pylori could invoke an IR producing antibody with Lewis blood group system specificity. In these examples it has been proposed that anti-Le^a auto-antibody promotes adhesion of H. pylori to the gastric epithelium leading to atrophy and secondary binding to circulating platelets in those with an appropriate genetic background [8, 16].

RESULTS

Of the 165 patients enrolled in the study, 24 were CLO-test positive for H. pylori infection. The mean platelet counts for the H. pylori infected and uninfected groups were 247 and 282 x 10^9/L respectively. Of the infected group, cagA positive and cagA negative infections gave a mean platelet count of 237 and 261 x 10^9/L respectively. The mean platelet count for those undergoing eradication treatment was 258 x 10^9/L which increased to a mean of 268 x 10^9/L post treatment.

CONCLUSIONS: This study showed some difference between the mean platelet counts of H. pylori infected and uninfected patients (p = 0.0473). Patients infected with cagA positive strains, tended to have a lower mean platelet count compared to those with cagA negative strains. Eradication of H. pylori infection failed to make a significant difference to the platelet count in this study.

KEYWORDS: Helicobacter pylori, thrombocytopenia, immune thrombocytopenia purpura, eradication, platelet counts.
The effect of eradication of *H. pylori* and the normalisation of the platelet count in cITP patients was first reported by Gasbarrini *et al.* in 1998 [11]. In that study, *H. pylori* eradication led to an increase in the mean platelet count from 75 to 180 x 10^9/L (p < 0.05) in eight cITP patients [11]. Similar studies reported more variable platelet count responses with possible causes reported to be related to environmental factors, differing geographical regions, the genetics of the host and *H. pylori* strain variation [2, 10, 17].

Little work into the association between *H. pylori* infection and the platelet count in non-cITP patients has been conducted. This study investigated whether the platelet counts of people positive for *H. pylori* infection were lower than those without the infection. In addition the study sought to establish whether infection with cagA positive strains affected the platelet count more than cagA negative strain infections. The study also investigated whether eradication of *H. pylori* correlated with an increase in the platelet count.

**MATERIALS AND METHODS**

**Patient recruitment**

Patients referred for gastro-endoscopy to Middlemore Hospital (MMH) and the Manukau Surgical Centre (MSC) in South Auckland between the 7th of August 2012 and 30th of July 2013 were invited to participate in the study. Patients taking medication likely to affect the platelet count were excluded as were cancer patients and those with infectious, haematological or immunological related medical conditions. Patients below the age of 16 and those mentally unfit to provide informed consent were also not accepted. Patients who had been admissions to hospital in the previous two months, those with a history of transfusion within the previous three months and women who were pregnant were also excluded.

Approximately 500 patients who visited the MMH and MSC for gastro-endoscopy were interviewed for the research and 180, who met the acceptance criteria, were enrolled in the study over a 12 month period. Seventeen patients were later excluded after a review of their recent laboratory data. From the 163 patients, 24 were confirmed to be infected with *H. pylori* by CLO testing and/or histological biopsy.

**CLO test**

All patients underwent gastro-endoscopy and biopsy samples taken by the gastro-endoscopy team from the antrum of the patient’s stomach. Biopsied tissue was used for the Kimberly-Clark CLO-test and histological examination. Tissue immersed in the CLO-test urea gel medium was left at room temperature for 4 to 24 hours. The presence of the urease enzyme produced by *H. pylori*, hydrolyses urea to ammonia, increasing the pH causing a colour change of the medium from yellow (negative) to red (positive).

**Histology**

Gastric biopsy specimens were processed at MMH laboratory. Samples were first fixed, embedded into tissue sections, cut and then stained with haematoxylin & eosin (H&E). Samples positive by H&E were confirmed by Giemsa staining.

**Platelet counts**

Ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood was collected by venipuncture from patients at the time of tissue biopsy collection. Platelet counts were performed at the MMH laboratory using a Sysmex XE5000 Haematology Analyser. The platelet count reference range for the region served by the Counties Manukau DHB population had been previously established from population studies. A mean platelet count of 275 x 10^9/L ± 2 standard deviations (SD) provided a platelet reference range of 150-400 x 10^9/L with a confidence interval of 95%.

CagA immunoblots

Serum from CLO-test positive patients was used for the immunoblot method (*H. pylori* - IgG ViraStripe) for anti-cagA. The presence of plasma anti-cagA and a positive immunoblot indicated infection with a cagA positive strain of *H. pylori*.

**H. pylori stool antigen test**

The Immunocore (Meridian Bioscience Inc.) Helicobacter Pylori Stool Antigen (HPSA) test was used to detect *H. pylori* antigens in the faeces of infected patients following eradication therapy.

**H. pylori eradication treatment**

*H. pylori* infected patients were prescribed the standard *H. pylori* triple antibiotic therapy regimen of amoxicillin, omeprazole and clarithromycin for one to two weeks.

**Statistical analysis**

The platelet count mean, median, ranges, 25th percentiles, 75th percentiles, and the lower and upper 95% confidence limits for infected and uninfected groups were calculated. The Kolmogorov-Smirnov test (KS-test) was used to compare the platelet counts of the two groups and a p-value of <0.05 used to indicate statistical significance. The KS-test was also used to compare the cumulative distributions of pre and post eradication platelet counts for the infected subgroups (anti-cagA positive and negative patients).

The biological significance of the difference in platelet counts among infected and uninfected, anti-cagA positive and negative groups was also assessed according to the ISO Manual for Laboratory Reference Ranges in Different Biochemical Analytes [17]. Based on the manual the range of the platelet counts for both the test and control groups would be considered biologically different if the larger standard deviation was at least 1.5 times or greater than the smaller standard deviation [18]. Small subgroups of data lacking sufficient robustness for analysis were not analysed for statistical significance.

**Validity rules**

1. A significantly lower (p < 0.05) mean platelet count in *H. pylori* infected patients as compared to non-infected patients, or an increase in the post-eradication platelet count would demonstrate an association between *H. pylori* infection and the platelet count.

2. A lower mean platelet count (p value < 0.05) in anti-cagA positive patients versus the anti-cagA negative infected patients control group would implicate anti-cagA or cagA protein expression in the reduction of the platelet count in patients.

**Ethical approval**

This project was approved by the Northern X Regional Ethics Committee. Ref: NTX/12/04/025, the Counties Manukau District Health Board ref: #1227 and the Massey University Human Ethics Committee.

**RESULTS**

The ranges of the platelet counts for *H. pylori* infected and non-infected patients were 154-394 x 10^9/L and 168-580 x 10^9/L respectively. The mean platelet counts for *H. pylori* infected and non-infected groups were 247 x 10^9/L (SD: 56 x 10^9/L) and 277 x 10^9/L (SD: 68 x 10^9/L) respectively. Approximately 12.5% of infected and 33% of non-infected patients had platelet counts above 300 x 10^9/L, while 37.5% of infected patients and 10% of the non-infected patients had platelet counts of less than 200 x 10^9/L.

Figure 1 shows the distribution and mean of the platelet counts with infected patients showing a lower mean platelet count and platelet count range as compared to non-infected patients.
Eighteen *H. pylori* participants were tested for serum anti-cagA and eight patients were positive. The mean platelet counts for cagA-positive infections was $237 \times 10^9/L$ (SD: $37 \times 10^9/L$; range: 190 - 298) and for cagA negative infections, $261 \times 10^9/L$ (SD: $69 \times 10^9/L$; range: 154 - 394). Figure 2 presents the box plot results of the platelet counts for the three patient groups: uninfected, infected (cagA neg) and infected (cagA pos). The SD of both cagA negative and the non-infected group was 1.8 fold of the SD for the cagA positive group. This indicates a biologically significant difference in the platelet counts of the anti-cagA positive group compared to anti-cagA negative or non-infected patients [17]. Due to the small sample size, the platelet count results from the cagA positive and negative patient groups did not reach statistical significance.

In the follow-up to *H. pylori* eradication treatment, the HPSA test was performed on 20 of the 24 infected patients. Most patients completed treatment but four patients were lost to follow-up and of the remaining twenty; three did not take the prescribed antibiotics. Of the 20 patients, 16 completed the full treatment and tested negative in the HPSA test with one testing positive. Three patients who did not take the *H. pylori* eradication treatment were also positive for *H. pylori* infection. The mean platelet count for anti-cagA positive *H. pylori* infected patients increased slightly from 241 to 254 $\times 10^9/L$ following eradication of the infection. For the anti-cagA negative *H. pylori* infected group, the mean platelet count rose from 273 to 287 $\times 10^9/L$ following eradication of the infection.

Overall the mean pre and post eradication platelet counts were 258 and 268 $\times 10^9/L$ respectively and are shown in Figure 2. Seven patients showed an increase, while eight showed a decrease in the platelet count. The overall results showed an increase in the platelet count of 10 $\times 10^9/L$ in fifteen of the previously infected, now infection-free patients.

**Table 2.** Pre and post eradication platelet counts for cured cagA positive and negative infections.

<table>
<thead>
<tr>
<th>Patient</th>
<th>cagA positive</th>
<th>cagA negative</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre treatment</td>
<td>Post treatment</td>
</tr>
<tr>
<td>11</td>
<td>200</td>
<td>325</td>
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<td>24</td>
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<td>179</td>
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<td>298</td>
<td>321</td>
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<tr>
<td>140</td>
<td>262</td>
<td>231</td>
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<tr>
<td><strong>Mean</strong></td>
<td><strong>241</strong></td>
<td><strong>254</strong></td>
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</tbody>
</table>

**DISCUSSION**

Approximately 500 patients who visited the MMH and the MSC for gastro-endoscopy were interviewed for the research and 180 who met the acceptance criteria, were enrolled in the study over a 12 month period. Seventeen patients were later excluded after a review of their recent laboratory data. From the 163 patients, 24 were confirmed to be infected with *H. pylori* by CLO testing.

Several hypotheses relating to the platelet count in *H pylori* infected patients, the effect of cagA positive strains on the platelet count and the effect of *H. pylori* eradication on the platelet count were tested in this study. In the first, the hypothesis that the platelet count for *H. pylori* infected patients differed from that of non-infected patients was supported. Results showed a lower overall platelet count in infected patients than in those not infected with *H. pylori* ($p=0.0473$). The second hypothesis tested whether infection with cagA positive *H. pylori* strains was associated with lower platelet counts as compared to cagA negative *H. pylori* strains. This was supported with the SD for the platelet counts from the cagA negative group more than 1.5 times greater than the SD of the cagA positive group.
When the platelet counts of non-infected, cagA positive and cagA negative groups were considered together, the data showed that the mean platelet count and its overall range was higher and wider in the uninfected group as compared to the results from the infected cagA negative group. The infected cagA positive group produced the lowest overall results for the platelet counts. The mean platelet counts for cagA positive and negative patients were 237 and 261 x 10^9/L respectively. The overall spread of the platelet counts for cagA negative patients was 154 - 394 x 10^9/L which was close to the MMH reference range for the platelet count (150-400 x 10^9/L). The highest platelet count for a patient infected with cagA positive *H. pylori* was 298 x 10^9/L. The data showed that infections *H. pylori* expressing cagA protein are associated with a lower platelet count as compared to those infected with cagA negative strains.

The third hypothesis that the platelet counts for infected patients increase after eradication of *H. pylori* infection was tested by repeating the platelet counts of infected patients following *H. pylori* antibiotic eradication treatment. Although the follow up number were few, the mean platelet count for the cured participants increased from 258 to 286 x 10^9/L. The eradication of *H. pylori* infection, irrespective of cagA status, was not associated with an increase in the platelet count with the hypothesis not proven.

The effect of *H. pylori* infection on the platelet count of non-cITP individuals has not been well studied. The studies of Matsukawa et al and Samson et al on non-cITP patients showed little linkage between *H. pylori* infection, the platelet count and eradication of infection [19, 20]. In Australia, Sivapathasingan et al. assessed the effectiveness of *H. pylori* eradication therapy on the platelet counts of cITP patients and concluded that *H. pylori* eradication was a possible therapeutic option for treating cITP [21]. Other studies have demonstrated a causal association between *H. pylori* infection and thrombocytopenia in adult cITP patients [13, 22]. According to Campuzano-Mayà et al, cITP patients can be classified into *H. pylori* dependent cITP, *H. pylori* independent cITP and conventional cITP [8].

In our study the platelet counts for patients with cagA negative *H. pylori* strains revealed that cagA positive strains may be associated with an overall reduction in the platelet count.

In Japan, *H. pylori* eradication has been used as a treatment for cITP and been shown to be effective at increasing the platelet count in some, while in others there has been no improvement in the platelet count. In our study the mean platelet count for patients cured of *H. pylori* infection increased only marginally by 10 x 10^9/L from the pretreatment count. The mean platelet counts of patients with cagA positive and cagA negative *H. pylori* strains increased by 13 x 10^9/L and 14 x 10^9/L respectively. Eradication of cagA positive *H. pylori* strains was not associated with an increased platelet count; in fact the platelet counts for 50% of the cured patients reduced after eradication treatment.

The platelet count increases seen in some patients after eradication of *H. pylori* in our study should be interpreted with caution as spontaneous platelet count increases without treatment can occur in one third of all childhood and 5% of adult cITP patients [23, 24]. This together with small numbers of untreated and uncured patients limited statistical analysis of some results in our study.

Little information about the effect of *H. pylori* infection on the platelet count among the normal population is available [19, 20]. Our study looked at whether *H. pylori* infection affected the platelet count, whether cagA positive infections had a greater impact on the platelet count than cagA negative strains, and whether *H. pylori* eradication therapy increased the platelet count.

Our study showed a statistically significant difference (p<0.05) in the platelet counts of *H. pylori* infected and non-infected patients. This finding receives support from Samson et al. who also reported lower platelet counts among *H. pylori* infected patients or subgroups of infected patients as compared to non-infected patients [20]. The mean platelet count difference between infected and non-infected groups in our study showed a seven-fold greater association as compared to that reported by the Samson et al. study.

**CONCLUSIONS**

Although there has been a high prevalence of *H. pylori* sero-positivity (49% in Pacific Islanders) reported in Auckland high school students [4] the prevalence of active *H. pylori* infection in this study was only 14.7% among all enrolled patients. New Zealand Europeans, the majority ethnic group in South Auckland, have been previously reported to have *H. pylori* sero-positivity rates of 14% [7]. More than three quarters of the potential participants in this study were hospital in-patients with advanced illness (e.g. gastrointestinal bleeding) and failed to meet the inclusion criteria and so may have contributed to the low incidence of *H. pylori* infection in this study.

Our study looked to establish whether *H. pylori* infection was associated with low platelet counts, and if so, did cagA strains have a greater impact on the platelet than cagA negative strains. Our study also looked at the effect of *H. pylori* eradication on the peripheral blood platelet count. Patients enrolled in our study were referrals to either the MMH Gastroenterology Clinic or the MSC in South Auckland and biopsy results divided participants into *H. pylori* infected (cagA positive and negative) and non-infected groups. Infected patients were followed up after 3 months to establish if eradication therapy led to an increased peripheral blood platelet count.

Our study showed a tendency for *H. pylori* infected participants to have a mean platelet count lower than that of non-infected patients. No evidence of thrombocytopenia caused by infection was demonstrated in our study. Those infected with cagA positive strains showed a mean platelet count lower than patients infected with non-cagA positive *H. pylori* strains. There was little evidence to suggest that *H. pylori* eradication therapy led to a statistically significant increase in the platelet count.

The sample size for this study was small. Further studies on the effects of *H. pylori* infection on the platelet count of patients in New Zealand, and in particular on cITP patients is indicated by our study.

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REFERENCES


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