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ABSTRACT:

Helicobacter pylori (H. pylori) are gram negative bacteria that are strongly associated with gastro-duodenal disorders and some extra-intestinal manifestations, such as chronic bacterial infection. Dental plaque has been implicated as possible reservoir for H. pylori in individuals with and without gastric or duodenal disorders. Early detection and management of H. pylori can effectively reduce the prevalence of their pathologic effects and frequency of transmission. Non-invasive and inexpensive methods for detection of H. pylori can be used for screening of those at risk in the population. The major objective of this study was to assess the prevalence of H. pylori in saliva and plasma samples of residents in the National Capital District, Papua New Guinea. Subjects for this prospective cross-section study were selected from patients and their relatives attending dental clinics in Port Moresby General Hospital and St John's Hospital Gerehu. Saliva and blood samples were collected from randomly selected subjects after obtaining their signed informed consent. Solid phase Enzyme-Immuno-Assay (EIA) commercial kits were used for the estimation of IgG antibodies against H. pylori in human saliva and plasma. The guidelines and cut-off index indicated by the manufacture of the EIA kits were used for interpretation of the data. The results indicated that of the 204 saliva samples 183 (89.7%) were negative, 15 (7.4%) were equivocal and 6 (2.9%) were positive for H. pylori IgG. Subjects in the 40 – 49yrs age group had the highest positive (2.0%) prevalence for H. pylori IgG. Results obtained for 44 plasma samples collected, indicated that 19 (43.2%) were negative, 11 (25.0%) were equivocal and 14 (31.8%) were positive for H. pylori IgG. Comparison of the data indicated statistically significant difference ($p = 0.01$) between the results obtained for the plasma and corresponding saliva samples. A statistically significant positive linear correlation was obtained between the H. pylori IgG in saliva and plasma samples (Spearman $\rho = 0.514$, $p = 0.01$). The results indicated higher sensitivity of EIA in detecting H. pylori IgG in plasma compared to saliva samples.

KEYWORDS: H. pylori, Saliva, Plasma, IgG, EIA

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INTRODUCTION:

Helicobacter pylori (H. pylori) are strongly associated with gastro-duodenal diseases, including chronic active gastritis, peptic and duodenal ulcers, gastric cancer, distal gastric adenocarcinoma, and gastric mucosal lymphoproliferative disease [1- 5]. H. pylori can also cause extra-intestinal manifestations; they have been identified in some patients with chronic bacterial infection without any clinical signs or symptoms [6]. Dental plaque (DP) has been implicated as possible reservoir for H. pylori in individuals with and without gastric or duodenal disorders [7, 8]. The prevalence of H. pylori infection worldwide is very high, with the highest rates reported among children and adults in the developing countries [6-11].

The exact modes of transmission of H. pylori are not clearly understood, but some researchers have suggested person-to-person via saliva, fecal-oral, oral-oral and gastro-oral routes [6 -12]. Different methods are available for the diagnosis of H. pylori [4, 11, 15, 16]. The invasive methods involve obtaining biopsies by endoscopy for culture and histology, the serology and Rapid Urease Test (RUT). These tests are not suitable for large scale screening or epidemiological studies in resource limited countries [4, 11, 15, 16]. The non-invasive tests include the detection of H. pylori antibodies or antigens in urine, saliva or feces samples using Enzyme Immunoassay (EIA) technique [11, 15, 16]. This technique is relatively simple, reproducible and inexpensive; it can be used for large scale screening and in epidemiological studies.

Recent studies have indicated saliva as a non-invasive sample for detection of antibodies (IgA and IgG) to H. pylori [4, 11, 14, 15]. Collection and testing of salivary specimen is non-invasive, painless, convenient, and fast and carries no risk of needle stick injury [11]. Assay for the detection of H. pylori antibodies in saliva is a useful and noninvasive way to identify infection, permit selective use of endoscopy, and monitor the response to antimicrobial therapy [17]. In addition, the use of EIA technique for early detection of H. pylori infection can reduce the prevalence of transmission and pathologic effects of the bacteria, especially among the population in resource limited countries like Papua New Guinea (PNG).

There are no published data on the prevalence of H. pylori infection among the population in PNG. The aim of this study was to assess the prevalence of H. pylori among residents in the National Capital District (NCD) in PNG using saliva and serum samples.

SUBJECTS AND METHODS:

This prospective cross-sectional study was carried out in the NCD, which is the incorporated area around Port Moresby, the capital of PNG. The dental clinics in the School of Medicine and Health Sciences (SMHS) in University of Papua New Guinea (UPNG), Port Moresby General Hospital (PMGH) and St John's hospital in Gerehu a major suburb in NCD were the selected study sites. These sites were selected mainly because of the difficulty in obtaining blood samples from health individuals in NCD. Calculation of sample size was based on a design effect of one, relative precision of 10%, assumed prevalence rate of 25%, with confidence interval of 95% and non-response rate of 20%. All individuals, both patients and relatives that visited the clinics during the study period were enrolled in the study. However, individuals with gastric disorders, those on antibiotic medications, beetle-nut chewers, and those not residing in NCD were excluded from the study. Final selection of subjects was by simple random sampling.

About 3.0 – 5.0ml of saliva was collected in clean plastic vial, which was then put in a cool-box kept at 4 – 8^{o}C in the field. About 3.0ml of blood was collected in Heparinized vacutainer, which was put in a cool-box kept at 4 – 8^{o}C in the field. Both samples were transported to the Micronutrient Laboratory (MNL) in SMHS, UPNG.

The blood samples were centrifuged and aliquots of plasma stored in dark vials. The vials containing the untreated saliva and plasma samples were kept frozen at – 70^{o}C till required for analysis. Demographic data of each consented subject was obtained using a pretested self-designed questionnaire.

All reagents used in the assay were of analytical grade and were components of the IBL commercial Enzyme-Immunoassay (EIA) Kits. Before analysis, unlike the saliva samples, each plasma sample was diluted 101 times as recommended by the manufacturer. Samples were analyzed using Solid Phase EIA for qualitative and quantitative determination of IgG antibodies against H. pylori in human serum and plasma [18]. The same EIA Kits were used after modification of the protocol for assay of the saliva samples as approved by the manufacturer [18]. Automated 96-wells Microplate Washer and Reader were used for processing and analysis of the Microplates.

Ethical clearance and permission for the study were obtained from SMHS Ethics and Research Grant committee, CEO PMGH, and CEO of the St John's Hospital Gerehu. Both saliva and blood samples were collected from subjects only after obtaining their signed informed consent.

The Statistical Package for Social Sciences (SPSS) version 13 for Windows was used for Statistical analysis of the data. The guidelines (Table 1) indicated by the manufacturer were used for interpretation of the data [18].

Table 1: Guideline for interpretation of the data [18]

Cut-off Index (U/mL)	Interpretation
< 0.8	Negative
0.8 – 1.2	Equivocal (Borderline)
>1.2	Positive

RESULTS:

A total of 216 subjects were randomly selected from the over 1000 that visited the clinics during the duration of this study. However, informed consent was obtained from 204 subjects (consent rate 94.4%). This gave a non-response rate of 5.6%, which was lower than the predicted non-response rate of 20% used for calculating the sample size. The mean age of all the consented subjects was 30.5 ± 13.7 years. Saliva sample was collected from each of the 204 consented subjects. Analysis of the data using the cut-off index indicated that 183 (89.7%) saliva samples were negative, 15 (7.4%) were equivocal and 6 (2.9%) were positive for H. pylori IgG. There was no significant difference in the results obtained for the dental and non-dental patients. Thus the data was pooled for all further analysis.

The data was separated and analyzed according to age groups of the subjects. The results are presented in Table 2. Those in the 40 – 49 years of age group had the highest positive (2.0%) prevalence. Equivocal was highest (2.9%) among those in the 20 – 29 years of age group followed by 1.5% of those in the 30 – 39yrs and 40 – 49 years of age groups. Gender distribution of the 204 subjects indicated 83 (40.7%) males and 121 (59.3%) females. The mean age for the males was 32.1 ± 14.3 years and for the females 29.4 ± 12.0 years. The prevalence of H. pylori IgG in the saliva samples of the male and female subjects is presented in Table 3. Both male and female subjects had low positive prevalence of H. pylori IgG in their saliva samples. There was no statistically significant difference between the results for the male and female subjects.

Of the 204 consented subjects a total of 50 were randomly selected and requested to donate blood for the analysis of H. pylori IgG in plasma. Signed informed consent for the collection of blood was obtained from 44

subjects (consent rate 88.0%). The mean age of the consented subjects was 31.7 ± 10.2 years. The results of the H. pylori IgG in the 44 plasma samples indicated that 19 (43.2%) were negative, 11 (25.0%) were equivocal and 14 (31.8%) were positive.

Table 2: Distribution of the 204 subjects according to age groups and prevalence of H. pylori IgG in saliva samples

Age Range (years)	Negative % (n)	Equivocal % (n)	Positive % (n)
< 10	2.0% (4)	0.5% (1)	0
10 – 19	17.2% (35)	0.5% (1)	0
20 – 29	30.9% (63)	2.9% (6)	0
30 – 39	18.6% (38)	1.5% (3)	0
40 – 49	12.7% (26)	1.5% (3)	2.0% (4)
50 – 60	6.4% (13)	0.5% (1)	0.5% (1)
>60	2.0% (4)	0	0.5% (1)
Total	89.7% (183)	7.4% (15)	2.9% (6)

Table 3: Distribution of male and female subjects according to the cut-off index and prevalence of H. pylori IgG in saliva

Interpretation	Males (n = 83)	Females (n = 121)
Negative	91.6% (76)	88.4% (107)
Equivocal	6.0% (5)	8.3%) (10)
Positive	2.4% (2)	3.3% (4)

Gender distribution of the 44 plasma samples indicated 19 (43.2%) males and 25 (56.8%) females. The mean ages of the male and female subjects were 32.2 ± 14.3 years and 31.3 ± 12.0 years respectively. The interpretation of the results indicating prevalence of H. pylori IgG in the plasma of the male and female subjects is presented in Table 4. There was no statistically significant ($p>0.05$) difference between the results for the male and female subjects. Comparison of the results for H. pylori IgG in the saliva and plasma samples of the 44 subjects indicated that both data were not normally distributed as indicated in the box-plot in Fig. 1. The data spread was greater for the plasma compared to the saliva samples. The results obtained for prevalence of H. pylori IgG in the saliva and plasma samples of the 44 subjects are presented in Table 5.

Table 4: Distribution of male and female subjects according to prevalence of H. pylori IgG in their plasma

Interpretation	Males (n = 19)	Females (n = 25)
Negative	36.8% (7)	48.0% (12)
Equivocal	31.6% (6)	20.0% (5)
Positive	31.6% (6)	32.0% (8)

Fig. 1: Box-plots of the results for H. pylori IgG in saliva and plasma of the 44 subjects

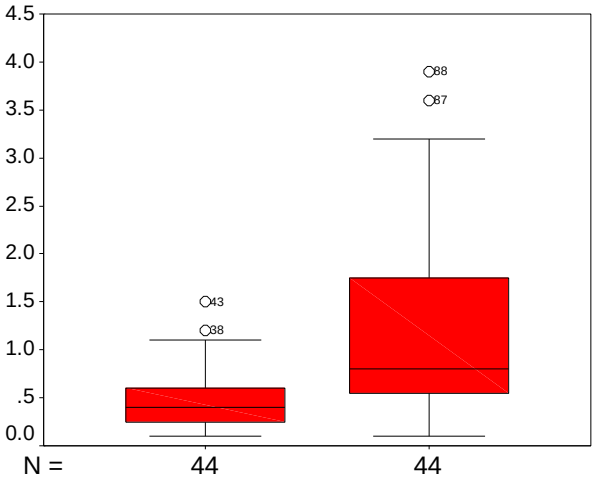
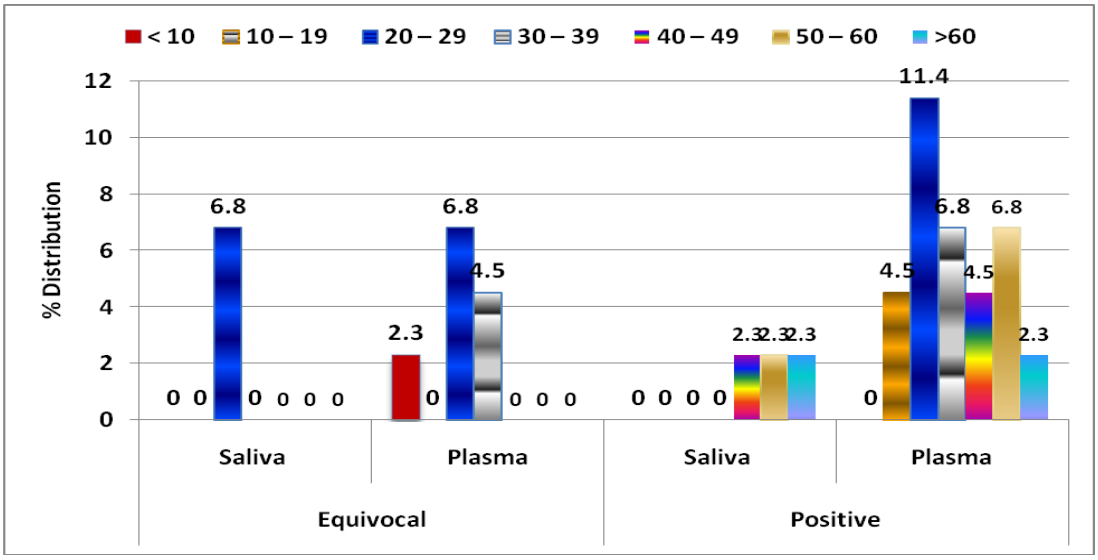


Table 5: Distribution of the subjects according to the prevalence of H. pylori IgG in their saliva and plasma samples

Interpretation	Saliva (n = 44)	Plasma (n = 44)
Negative	84.1% (37)	43.2% (19)
Equivocal	11.4% (5)	25.0% (11)
Positive	4.5% (2)	31.8% (14)

Analysis of the data using the Mann-Whitney and Wilcoxon Signed Rank tests indicated statistically significant difference (p = 0.01) between the saliva and plasma results. This indicates higher sensitivity of EIA in detecting H. pylori IgG in plasma samples compared to saliva samples. However, statistically significant positive linear correlation was obtained between the H. pylori IgG in saliva and plasma samples (Spearman ρ = 0.514, p = 0.01). The H. pylori IgG data in the saliva and plasma samples of the 44 subjects was distributed according to age groups. Fig. 2 shows the percent distribution of the equivocal and positive H. pylori IgG results for saliva and plasma samples in the various age groups. The prevalence of H. pylori IgG was highest (11.4%) in the plasma of subjects in the 20 – 29 years of age group, compared to the zero in their saliva samples. Compared to the saliva samples, H. pylori IgG was also prevalent in the plasma of subjects in the 10 to 19 years of age group (4.5%) and the 30 to 39 years of age group (6.8%).

Fig. 2: Percent distribution of subjects according to age groups and H. pylori IgG in saliva and plasma samples (Equivocal and Positive distributions are shown)



DISCUSSION:

The 5.6% non-response rate of the subjects during the collection of saliva samples in the present study was lower than the predicted non-response rate of 20% used for calculating the sample size. This high response rate supports the observation by other researchers that non-invasive methods are less problematic for collection of biological samples from subjects [10, 11].

It is true that this procedure offers definite advantages, such as, avoiding the risk of needle-stick injury, ease of sample collection and better comfort of consented subjects.

In the present study H. pylori was prevalent in 2.9% of all the subjects. This was higher than the 1.6% prevalence reported in the saliva of dentate patients, but lower than the 3.5% prevalence reported in saliva of edentulous patients [7]. The prevalence of H. pylori IgG was highest in the saliva of subjects in the 40 – 49 years of age group compared to the other age groups. These findings were different from those of other authors, who reported highest prevalence among patients in the 25 – 35 years and over 60 years of age groups [19, 20]. These findings indicated that prevalence of H. pylori IgG does not increase with age, especially in patients without gastric disorders [19, 20]. Some authors have reported significantly lower prevalence of H. pylori infection in females compared to males, while others reported higher prevalence among males compared to females [19, 20]. In the present study there was no difference in the prevalence of H. pylori IgG in the saliva of male and female subjects.

The high prevalence (31.8%) of H. pylori IgG in the plasma samples of the 44 subjects compared to 4.5% prevalence in their saliva samples was comparable with other similar studies [17, 21], but different from others [10 – 12]. Some studies indicated that the concentration of H. pylori IgG in oral fluid is between 500 – 1500 times lower than in plasma or serum [21]. In our study the mean amount of H. pylori IgG in the saliva samples was 497 times lower than the mean amount in the plasma samples. However, the sensitivity and specificity of the EIA kits used for the assay of H. pylori IgG in the saliva samples were not determined. This represents one of the limitations in our present study.

The prevalence of H. pylori IgG was highest in the plasma of subjects in the 20 – 29 years of age group compared to the other age groups. These findings further indicate that in patients without gastric disorders the prevalence of H. pylori IgG may not be age dependent [19, 20].

There was no statistically significant difference between the prevalence of H. pylori IgG in the plasma samples of the male and female subjects. This finding was similar to the result obtained in the saliva samples for the male and female subjects.

The statistically significant positive linear correlation between the levels of salivary and plasma H. pylori IgG antibodies, tend to indicate that the EIA test for saliva can be considered as a viable alternative when there are problems in obtaining plasma samples.

CONCLUSION:

H. pylori IgG was prevalent in the saliva of 2.9% of all the subjects. Prevalence of H. pylori IgG in plasma was significantly higher than the prevalence in saliva. There were no significant differences in the gender distribution of both salivary and plasma H. pylori IgG. The statistically significant positive linear correlation between the levels of salivary and plasma H. pylori IgG antibodies, tend to indicate that the EIA test for saliva can be considered as a viable alternative when plasma samples cannot be obtained.

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