**EFFECTS OF *HELICOBACTER PYLORI* INFECTION ON THE IMMUNOGLOBULINS AND INTERLEUKINS OF INFECTED PATIENTS AT NNEWI NORTH, NIGERIA**

**ABSTRACT**

The debilitating nature of infectious diseases has drastically reduced the health standard of individuals in the society. This study was conducted to evaluate the effects of *Helicobacter pylori* infection on the immunoglobulins and interleukins of infected patients at Nnewi North, Nigeria. Two hundred (200) blood samples were aseptically collected from different age groups in the sampled communities (Umudim, Uruagu, Otolo, and Nnewichi) at Nnewi North using well-capped sterile EDTA bottles. The levels of immunological biomarkers (IgG and IgM) were evaluated in both infected and non-infected individuals using serological technique while Enzyme Immunoassays were used to determine the Concentrations of blood cytokines (interleukin 6 and interleukin 10)**.** The results revealed that 43.5% of the individuals screened were positive to IgG biomarker while 81.5% showed positive to IgM biomarker. Meanwhile, 12.5% of the screened individuals showed normal interleukin 6 while 5.5% showed normal interleukin 10. Therefore, it is obvious that the level of IgG and IgM increased in the serum of individuals that tested positive to the infection while the level of interleukin 6 and interleukin 10 decreased among individuals infected. This finding has shown that *H. pylori* possesses immunomodulatory potential.

1. **INTRODUCTION**

Infectious disease is a major source of threat to man globally due to ubiquitous nature of the causative agents (Bello *et al*., 2018; Marginea *et al.,* 2022). One of the infectious diseases is stomach ulcer caused by a Gram-negative, microaerophilic, and spiral-shaped bacterium that affects more than half of the human population worldwide, and is more predominant in developing countries (Alfarouk *et al.,* 2019; Alexandra *et al.,* 2022).

Research had revealed that *H. pylori* is the major bacterium responsible for stomach ulcer, which produces unbearable clinical manifestations to the infected individuals (Borka *et al.,* 2022; Cheok *et al*., 2022; Che *et al.,* 2023).The infection is generally acquired during childhood but can remain asymptomatic with long-term clinical signs and symptoms such as gastric reflux, abdominal pain, intestinal bleeding, occasional fevers, and loss of weight which if not treated well can result to ulceration and perforation (Narayanan., 2018; Chukwuma *et al.,* 2020). The mode of transmission of *H. pylori* has not been fully elucidated, but studies had revealed that person-to-person transmission, fecal-oral and oral-to-oral routes can lead to the transmission of this infection (Che *et al.,* 2023; (Charitos *et al.,* 2021).

 It is worthy to note that the functions and the number of immune cells have been influenced among individuals infected by *H. pylori* as documented by several researchers (Moyat and Velin, 2014; Lina *et al.,* 2014; Milic *et al*., 2019). The role of immune cells in tackling infectious agent cannot be overemphasized because no individual can thrive comfortably without vibrant immunity (Lina *et al*., 2014). Some of the virulent factors possessed by the pathogen are capable of circumventing immune response, and also produce toxins that are capable of retarding the functionality and counts of the immune cells such as interleukin -6 and IL-10 (Akdogan *et al.,* 2014; Nwachukwu *et al*., 2020) while the number of immunoglobulins increases when a pathogen invades the host in order to arrest the infectious condition provided that the infected individuals are immunocompetent (Moyat and Velin, 2014).

Several researchers have worked on immunology of *H. pylori* infection such as Akdogan *et al. (*2014), Lina *et al.* (2014), Milic *et al*. (2019) and Nwachukwu *et al*. (2020) but few studies are available on the effects of *Helicobacter pylori* infection on the immunoglobulins and interleukins of infected patients at Nnewi North, Nigeria. Hence, the aim of this study is to evaluate the effects of *Helicobacter pylori* infection on the immunoglobulins and interleukins of infected patients at Nnewi North, Nigeria.

**2.0: MATERIALS AND METHODS**

**2.1 Study Area**

The study was carried out at Nnewi North Local Government metropolitan commercial city in Anambra State, Nigeria. Nnewi is located in the South-East zone in Anambra state, Nigeria. It is the second largest commercial city in Anambra State in south-eastern Nigeria with two local government areas, Nnewi North and Nnewi South. Nnewi North is commonly referred to as Nnewi central and is the center of commercial activities.

**Research Design**

This cross-sectional research evaluated the effect of *Helicobacter pylori* infection on the immunoglobulins and interleukins of infected patients at Nnewi North, Nigeria. It was a hospital/school/Laboratory-based study.

**2.2 Ethical Approval**

This was obtained from the ethical committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka, Anambra State, Nigeria (COOUTH/CMAC/ETH.C/Vol.1/FN:04/220).

**2.3 Sampling Technique**

Random sampling technique was used to recruit participants in the study, comprising those who presented with or without the symptoms and signs that were suggestive of ulcers or gastritis. They were educated about the study and those willing to participate gave their consent in writing, and that of their parents/guardian until the required sample size was attained.

**2.4 Collection of Specimen**

Two hundred (200) blood samples were aseptically collected from different age groups in the sampled communities (Umudim, Uruagu, Otolo, and Nnewichi) using well-capped sterile EDTA bottles. Before the collection, oral and written consent were obtained from the participants. The children that participated in the study were from private and public schools within Nnewi North metropolis while the adults that participated were drawn randomly from the area and private diagnostic centres (Cheesbrough, 2010).

**2.5**  **Immunological / Serological Assessment**

Before proceeding with the assay, all reagents, serum references and serum samples were brought to room temperature (27oC). The microplates well for each serum reference control and serum specimen to be assayed were formatted in duplicate. The serum was diluted 1:10 for both kits (IgG and IgM). Any unused microwell strips were replaced back into the aluminum bag, seal and store at 2-8oC. The diluted serum specimen 0.025 ml (25 ul), serum reference, control was pipetted into the assigned microtiter wells for IgG and for IgM, 0.050 ml (50ul) of diluted serum specimen, serum reference, control was also pipetted accordingly. Approximately 0.10 ml of *H. pylori* Biotin reagent solution was added. The microplate was gently swirled for 20-30 sec to mix and then covered (the *H. pylori* IgM or IgG-specific antibody, if present, binds to the antigen). The plates were incubated for 60 min at room temperature. The contents of the micro plate were decanted and while decanting it was tapped and blotted dry with absorbent paper. Then, 300 ul of wash buffer was added and was used to wash the microplate for four times. About 0.10 ml of *H. pylori* enzyme reagent was added to all wells, following same order of addition to minimize reaction time differences between wells. It was not shaken after enzyme addition. The plate was covered and incubated for 30 min at room temperature. Exactly 0.10 ml of single substrate solution was added to all wells and reagents always added in same order in order to minimize time differences between wells. It was incubated at room temperature for 15 min. Approximately 0.050 ml (50 ul) of stop solution was added to each well and the microplate was swirled gently for 15-20 sec to mix. The absorbance was read at 450 nm in a microplate reader compared in a parallel manner with calibrator and controls. The result was read within 30 min of adding the stop solution (Wang *et al*., 2022).

**2.6 Enzyme Immunoassays for the Determination of Blood Cytokines Concentrations**

The human ELISA test kit from U-Cy Tech Biosciences (Cat No CT209A; Lot No 23-32-12-29) was used for the *in vitro* quantitative determination of IL-6 and IL-10 in human fluids such as cell culture supernatant, plasma or serum. All the prepared reagents were thoroughly mixed before use. One hundred micro litre (100 µl) of blank, diluted standard and samples were added to each well. The plates were sealed and incubated for 2 hours at 37oC. After incubation, the plates were washed five times with the Wash buffer using the automated microplate Washer. Then, 100 µl of diluted detection antibody solution was added to each well and the plate was sealed and incubated for 1 hour at 37oC. After incubation, the plates were washed five times with Wash buffer as done previously. Hundred microlitre (100 µl) of diluted SPP conjugate was added to each well and the plates sealed and incubated for 1 h at 37oC. After incubation, the plates were washed six times with Wash buffer. Afterwards, 100 µl of TMB substrate solution was added into each well and left in the dark for 20 minutes at room temperature. After substrate incubation, the reaction was stopped by adding 100 µl of Stop solution (resulting in a yellow solution) and it was read at 450nm within 30 minutes using the Microplate reader (Wang *et al*., 2022).

**2.7 Statistical Analysis**

The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) version 25. The significance of differences in mean values between groups was analyzed using T-test, while significance of the differences in mean values among different groups was evaluated using one-way ANOVA. P value < 0.05 was considered statistically significant.

**3.0 RESULTS**

The result of comparison of mean ± SD of Immunological values of subjects with or without *H. pylori* in the villages sampled is presented in Table 1. In relation to villages, comparative mean ± SD of all Immunological values of subjects across the 4 villages sampled at Nnewichi showed a significant difference in IgM mean ± SD value in total values across the villages (P = 0.035) whereby values of those positive for IgM at Uruagu village was higher than other villages though a non-significant difference was observed across the infection status (P > 0.05). Positive IgG mean ± S.D value status was highest at Umudim village (73.05 ± 12.02); Abnormal IL -6 was higher at Nnewichi village (16.55 ± 24.19) and IL-10 at Uruagu (3.52 ± 4.10) (P > 0.05) at villages sampled.

The result of comparison of mean ± SD of immunological values between male and female gender is presented in Table 2. It revealed that the comparative analysis between mean ± SD of IL-6, IL-10, and IgM was higher and more affected in females (13.88 ± 21.43; 3.28 ± 2.92; and 66.60 ± 22.65) than in males, while IgG was higher in males than females (43.57 ± 31.72) and differed significantly (t = 2.153; P = 0.033).

The result of the comparison of immunological values among the age group is presented in Table 3. The result revealed that the age group ranging from 57 – 67 years recorded the highest immunoglobulin M (79.11±14.47), followed by the age group ranging from 35 – 45 years (75.30±14.81) while the least IgM was recorded among the age group ranging from 13 – 23 years (56.85±23.99). The age group ranging from 57 – 67 years recorded the highest IgG value of 58.71±28.97, followed by age group ranging from 46 – 56 years (44.74±28.73) while the least IgG was recorded by the age group ranging from 2- 12 years (33.97±29.56). The highest value of IL-10 was detected among the age group ranging from 13 – 23 years (3.41±3.73), followed by the age group ranging from 2 – 12 years (2.98±0.95) while the least IL-10 was recorded the age group ranging from 57 – 67 years (2.66±0.27). The highest 1L-6 was detected among the age group ranging from 57 – 67 years (18.02±13.94), followed by the age group ranging from 2- 12 years (12.99±20.58) while the least IL-6 was recorded by the age group ranging from 35 – 45 years (10.14±17.49).

Table 1: Comparison of mean ± SD of immunological values of subjects across the villages sampled

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Villages |  |  |  |
| Immunological marker  | Infection state | Size  | Uruagu | Otolo | Umudim | Nnewichi | Grand Total  | F  | p-val. |
| IgG | Positive | 87 | 70.69±15.16 | 71.96±15.79 | 73.05±12.02 | 66.38±15.18 | 70.93±14.37 | 0.717 | 0.544 |
| Negative | 99 | 12.26±8.46 | 10.69±7.06 | 11.28±8.75 | 10.25±7.07 | 11.03±7.38 | 0.315 | 0.814 |
| Equivocal | 14 | 34.48±3.34 | 34.57±4.15 | 34.85±3.18 | 32.87±4.53 | 34.26±3.36 | 0.184 | 0.905 |
| Total | 200 | 44.31±30.73 | 34.46±31.19 | 43.97±31.65 | 30.93±28.14 | 38.72±30.87 | 2.385 | 0.070 |
| IgM | Positive | 163 | 69.38±17.17  | 72.31±15.92 | 76.47±17.95 | 69.61±15.29 | 72.07±16.77 | 1.552 | 0.203 |
| Negative | 17 | 23.45±3.46 | 24.30±1.84 | 13.65±14.09 | 19.38±10.42 | 19.09±10.33 | 0.612 | 0.619 |
| Equivocal | 20 | 34.47±2.92 | 37.80±0.42 | 33.70±2.98 | 36.74±1.12 | 35.10±2.76 | 2.323 | 0.114 |
| Total | 200 | 63.58±21.01 | 69.13±18.99 | 65.88±26.68 | 55.60±25.12 | 63.87±23.41 | 2.919 | 0.035\* |
| IL-6 | Normal | 25 | 5.84±0.53 | 6.10±0.75 | 5.83±0.77 | 6.30±0.43 | 6.01±0.62 | 0.775 | 0.521 |
| Abnormal  | 175 | 16.06±24.69 | 11.31±16.30 | 8.05±7.82 | 16.55±24.19 | 12.81±19.38 | 1.940 | 0.125 |
| Total  | 200 | 14.68±23.20 | 10.71±15.41 | 7.79±7.38 | 15.15±22.72 | 11.96±18.26 | 1.839 | 0.141 |
| IL-10 | Normal | 11 | 5.40±0.00 | 5.43±0.65 | 5.40±- | 6.08±0.45 | 5.65±0.55 | 1.384 | 0.325 |
| Abnormal  | 189 | 3.52±4.10 | 2.69c0.43 | 2.55±0.33 | 2.69±0.42 | 2.88±2.16 | 2.134 | 0.097 |
| Total  | 200 | 3.59±4.04 | 2.91±0.86 | 2.60±0.51 | 2.99±1.07 | 3.02±2.19 | 1.875 | 0.135 |

*\*Statistically significant at p<0.05.*

**KEYS:** IL-6: Interleukin 6 (pg/ml); IL-10: Interleukin 10 (pg/ml); IgG: Immunoglobulin G; IgM: Immunoglobulin M.

**Table 2: Comparison of Mean ± SD of Immunological Values between Male and Female Gender**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Immunological parameters | Male (N=93) | Female (N=106) | t-value | p-value |
| IL-6 | 9.91±13.69  | 13.88 ± 21.43 | -1.532 | 0.127 |
| IL-10 | 2.75 ± 0.72 | 3.28 ± 2.92 | -1.718 | 0.087 |
| IgG | 43.57 ± 31.74 | 34.20 ± 29.57 | 2.153 | 0.033\* |
| IgM | 60.36 ± 23.76 | 66.60 ± 22.65 | -1.896 | 0.059 |

*\*Statistically significant at p<0.05.*

**KEYS**: IL-6: Interleukin 6 (pg/ml); IL-10: Interleukin 10 (pg/ml); IgG: Immunoglobulin G; IgM: Immunoglobulin M.

 Table 3: Comparison of immunological parameters of subjects in relation to *H. pylori* infection status across age groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Age-group | IL-6 | IL-10 | IgG | IgM |
| 2-12years | 12.99±20.58  | 2.98±0.95 | 33.97±29.56 | 64.81±21.74 |
| 13-23years | 10.84±15.12 | 3.41±3.73 | 41.17±30.26 | 56.85±23.99 |
| 24-34years | 11.85±20.91 | 2.75±0.78 | 36.37±33.34 | 64.95±28.85 |
| 35-45years | 10.14±17.49 | 2.55±0.29 | 44.12±34.46 | 75.30±14.81 |
| 46-56years | 4.98±4.74 | 2.88±0.39 | 44.74±28.73 | 67.72±11.57 |
| 57-67years | 18.02±13.94 | 2.66±0.27 | 58.71±28.97 | 79.11±14.47 |
| F | 0.465 | 0.677 | 1.381 | 2.777 |
| P-value | 0.802 | 0.641 | 0.233 | 0.019\* |

*\*Statistically significant at p<0.05.*

**KEYS:** IL-6: Interleukin 6 (pg/ml); IL-10: Interleukin 10 (pg/ml); IgG: Immunoglobulin G; IgM: Immunoglobulin M.

**DISCUSSION**

Research has shown that infectious diseases are capable of interfering with the physiology and proliferation of immune cells and cytokines especially when the causative agent can circumvent immune defense mechanisms (Akdogan *et al.,* 2014). Any alteration in the number and physiology of the immune cells and cytokines can predispose the host cells to infectivity. The results obtained in this study showed that level of IgG and IgM increased in the serum of individuals that tested positive to the infection. The increase in the number of the immune cells could be attributed to the presence of the bacterial pathogens, which have been identified as unself. This finding corroborates to the observation of Moyat and Velin (2014) who reported that the levels of immunological markers increased during *H. pylori* infection. Also, the number of IgG in male was greater than the number in female while reverse was the case in the number of IgM. The difference in the number of immunological biomarkers in male and female could be attributed to difference in the immunocompetent of the patients. It was also observed in this study that older patients recorded higher levels of the immunological biomarkers compared to the younger patients. The difference recorded could be attributed to the severity of the infection and immunocompetency of the patients. This observation corresponds to the finding of Nwachukwu *et al*. (2020).

In this study, there was a significant difference in the number of IL-6 recorded in this study compared to the observations made by other researchers (Yang *et al.,* 2017; Jaroenlapnopparat *et al.,* 2022). These findings were contrary to the findings of Milic *et al.,* (2019) where IL-6 values were significantly higher in infected *H. pylori* compared to non-infected and also Cichoz-Lach *et al* (--) who demonstrated that *H. pylori* infected with erosive gastritis have higher values of IL-6 compared to matched healthy controls. The difference in the present study could be that the subjects investigated were non-gastritis patients but randomly selected healthy individuals who were in good immune status. The low secretion of IL-6 in this study could also be due to some factors such as immunomodulation by *H. pylori,* suppressing the production of IL-6 as a strategy for survival, eremitic factors, chronic infection such as prolonged *H. pylori* exposure may lead to immune system exhaustion, resulting to reduced IL-6 secretion over time. The etiology of gastritis is a complex interplay of genetic predisposition and environmental pro-inflammatory cytokines and variations in gastric acid secretion due to genetic factors significantly influence gastritis onset and progression.

The pronounced roles of IL-6 in exacerbating gastric inflammation had been reported by researchers (Yang *et al.,* 2017; Jaroenlapnopparat *et al.,* 2022). Over the years, a growing body of evidence has demonstrated that higher levels of inflammatory markers such as serum IL-6 and C-Reactive Protein (CRP) were associated with a high risk of developing Coronary Heart Disease (CHD). One systematic review showed that circulating IL-6 was associated with a high risk of cardiovascular diseases (Nakagawa *et al*., 2013). Although the subjects investigated in this study had serum IL-6 levels ≤ 4 pg/mL, which is within standard values, many cohort studies had shown a significant association between IL-6 levels and the risk of CHD when IL-6 levels were within standard values. IL-10, an anti-inflammatory cytokine is known to inhibit T-cell synthesis and inflammatory cytokine secretion suggesting its role in suppressing anti-tumour activity (Jaroenlapnopparat *et al.,* 2022) where the values were higher in non-infected than infected subjects. The decrease could be that inflammatory response triggered by *H. pylori* in the stomach mucosa may suppress the production of IL-10, leading to lower levels. Immunomodulation by *H. pylori* can manipulate the host immune response to favour its survival by down-regulating the production of anti-inflammatory cytokines such as IL-10. Other factors that could be responsible for the decrease are genetic factors, microenvironment in the stomach which can be influenced by factors such as diet, microbiota composition, and the presence of other infections that can affect production of IL-10 and other cytokines. Moreover, duration and severity of *H. pylori* infection may lead to dysregulation of the immune response, resulting to lower IL-10 levels over time.

 **Conclusion**

This study has shown that *H. pylori* infection causes an increase in the levels of immunological biomarkers such as IgG and IgM, while the levels of interleukin 6 and interleukin10 drastically reduced in the serum of individuals infected, especially in older male.

 **REFERENCES**

Akdogan, R. A., Ozgur, O., Gucuyeter, S., Kaklikkaya, N., Cobanoglu, U., Aydin, F. A (2014). Pilot Study of *Helicobacter Pylori* Genotypes and Cytokine Gene Polymorphisms in Reflux Oesophagitis and Peptic Ulcer Disease. *Bratislavske Lekarske Listy* **115:** 221–228.

Alexandra, L. C., Adriana, M., Dana, C. Z., Ovidiu, P., Luminita, E., Florina, M. and Simona, C. (2022). Evolution of Diagnostic Methods for *Helicobacter pylori* Infections: From Traditional Tests to High Technology, Advanced Sensitivity and Discrimination Tools. *Diagnostics* **12**(2): 508.

 Alfarouk, K. O., Bashir, A. H. H., Aljarbou, A. N., Ramadan, A. M., Muddathir, A. K., AlHoufie, S. T. S., Hifny, A., Elhassan, G. O., Ibrahim, M. E., Alqahtani, S. S., AlSharari, S. D., Supuran, C. T., Rauch, C., Cardone, R. A., Reshkin, S. J., Fais, S. and Harguindey, S. (2019). The Possible Roles of *Helicobacter pylori* in Gastric Cancer and its Management. *Frontiers in Oncology* **9**: 75.

 Borka, B. R., Meliț, L. E., and Mărginean, C. O. (2022). Worldwide Prevalence and Risk Factors of *Helicobacter pylori* Infection in Children. *Children (Basel, Switzerland)* **9**(9):1359.

Charitos, I.A., D’Agostino, D., Topi, S., and Bottalico, L. (2021). 40 Years of *Helicobacter pylori*: A Revolution in Biomedical Thought. Gastroenterology Insights **12**(2): 111 – 135.

Che, T.H., Nguyen, T.C., Vu, V.N.T., Nguyen, H.T., Hoang, D.T.P., Ngo, X.M., Truong, D.Q., Bontems, P., Robert, A. and Nguyen, P.N.V. (2023). Factors Associated with Helicobacter pylori Infection Among School-Aged Children from a High Prevalence Area in Vietnam. International Journal of Public Health **68**: 1605 – 1908.

Chukwuma, O.M., Chukwuma, G.O., Manafa, P.O., Akulue, J.C. and Jeremiah, Z.A., (2020). Prevalence and Possible Risk Factors For *Helicobacter Pylori* Seropositivity Among Peptic Ulcerative Individuals in Nnewi Nigeria. *Biomedical Research Journal* **4**(1): 166 – 172.

Cheesbrough, M. (2010). District Laboratory Practice in Tropical Countries (2nd ed.). Cambridge University Press: Cambridge, England. pp. 45–70.

Cichoz-Lach, H., Slomka, M., Celinski, K., and Baczek, A. (2001). Level of Serum Cytokines in Biliary Gastritis and Erosive Gastritis with *Helicobacter pylori* Coinfection. *Annales Universitatis Mariae Curie-Sklodowska Medicals* **56**: 271 – 274.

Jaroenlapnopparat, A., Bhatia, K. and Coban, S. (2022). Inflammation and Gastric Cancer. *Diseases* **10**: 35.

Lina, T. T., Alzahrani, S., Gonzalez, J., Pinchuk, I. V. and Beswick, E. J. (2014). Immune Evasion Strategies used by *Helicobacter Pylori*. *World Journal of Gastroenterology* **20**: 12753 – 12766.

Milic, L., Karamarkovic, A. and Popadic, D. (2019) Altered Cytokine Expression in *Helicobacter pylori* Infected Patients with Bleeding Duodenal Ulcer. *BMC Research Notes* **12**: 278.

Moyat, M. and Velin, D. (2014). Immune Responses to *Helicobacter Pylori* Infection. *World Journal of Gastroenterology* **5**: 5583 – 5593

Narayanan, M., Reddy, K. M. and Marsicano, E. (2018). Peptic Ulcer Disease and *Helicobacter Pylori* Infection. *Missouri Medicine* **115**(3): 219 – 224.

Wang, L., Cao, Z. M., Zhang, L. L., Dai, X. C., Liu, Z. J., Zeng, Y. X., Li, X. Y., Wu, Q. J. and Lv, W. L. (2022). *Helicobacter Pylori* and Autoimmune Diseases: Involving Multiple Systems. *Frontier of Immunology* **13**: 83 – 134.

Yang, X. (2017). Increased CD177 Expression is Associated with *Helicobacter Pylori*-Related Gastritis. *International Journal of Clinical and Experimental Pathology* **10**: 5924 – 5928