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PREVALENCE OF *HELICOBACTER PYLORI* IGG AND STOOL ANTIGEN DETECTION FROM DYSPEPTIC PATIENTS IN JOS, NIGERIA

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ABSTRACT

***Helicobacter pylori* is a helical shaped gram negative microaerophilic bacterium, that can inhabit various areas of the stomach. The study was carried out to determine the prevalence of *Helicobacter pylori* infection among dyspeptic patients attending Endoscopy unit, Jos University Teaching Hospital (JUTH). The prevalence of H *pylori*; antibodies in plasma and antigen in stool samples of 80 patients examined was studied using ELISA (DIA PRO, Italy) and ICA (BIOTEST China) respectively. Socio-demographic and clinical information was obtained through the assistance of attending gastroenterology staff. Blood grouping was also performed by slide agglutination test for all patients. *Helicobacter pylori* IgG antibodies were detected in plasma of 28 (35%) patients of which 27 patients out of the sero-positive cases were antigen positive while *Helicobacter pylori* antigen was detected in the stool of 31 (38.8%) patients of which 27 patients out of the antigen positive cases were also seropositve. No significant association was found between *Helicobacter pylori* and age, sex, ABO blood group, economic status, source of water and consumption of alcohol. Thus, *Helicobacter pylori* seropositivity with respect to blood groups was found to be 32.6%, 46.7%, 33.3% and 33.3% in blood groups O, B, A and AB respectively, while *Helicobacter pylori* stool antigen test positive results was found to be 33.3%, 33.3%, 46% and 39.5% in blood groups O, B, A and AB respectively. Hence, no statistical association was found between *Helicobacter pylori* infection and blood groups of the patients (P>0.05). However, marital status was significantly associated with *Helicobacter pylori* antibody test (P<0.05). There is need for government to encourage people about the *Helicobacter pylori* screening test since it is one of the etiologic agent of ulcer.**

Keywords: Helicobacter pylori, Jos, Peptic ulcer, Prevalence.

PREVALENCE D'HELICOBACTER PYLORI IGG ET DETECTION D'ANTIGENE STOOL CHEZ DES PATIENTS DYSPEPTIQUES A JOS, NIGERIA

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ABSTRAIT

Helicobacter pylori est une bactérie microaérophile gram négatif de forme hélicoïdale, qui peut habiter diverses zones de l'estomac. L'étude a été réalisée pour déterminer la prévalence de l'infection à Helicobacter pylori chez les patients dyspeptiques participant à l'unité d'endoscopie, Hôpital universitaire de Jos (JUTH). La prévalence de H pylori; Les anticorps dans le plasma et l'antigène dans des échantillons de selles de 80 patients examinés ont été étudiés en utilisant ELISA (DIA PRO, Italie) et ICA (BIOTEST Chine) respectivement. L'information socio-démographique et clinique a été obtenue grâce à l'assistance du personnel de gastro-entérologie. Le groupage sanguin a également été réalisé par un test d'agglutination sur lame pour tous les patients.

Des anticorps IgG d'Helicobacter pylori ont été détectés dans le plasma de 28 patients (35%) dont 27 patients séropositifs étaient antigènes positifs tandis que l'antigène Helicobacter pylori a été détecté dans les selles de 31 patients (38,8%) dont 27 patients des cas positifs d'antigène étaient également séropositifs. Aucune association significative n'a été trouvée entre Helicobacter pylori et l'âge, le sexe, le groupe sanguin ABO, le statut économique, la source d'eau et la consommation d'alcool. Ainsi, la séropositivité à Helicobacter pylori vis-à-vis des groupes sanguins était respectivement de 32,6%, 46,7%, 33,3% et 33,3% dans les groupes sanguins O, B, A et AB, tandis que les résultats positifs au test antigénique Helicobacter pylori 33,3 %, 33,3%, 46% et 39,5% dans les groupes sanguins O, B, A et AB respectivement. Par conséquent, aucune association statistique n'a été trouvée entre l'infection à Helicobacter pylori et les groupes sanguins des patients (P> 0,05). Cependant, l'état matrimonial était significativement associé au test d'anticorps Helicobacter pylori (P <0,05). Il est nécessaire que le gouvernement encourage les gens au sujet du test de dépistage de l'Helicobacter pylori puisqu'il est l'un des agents étiologiques de l'ulcère.

Mots-clés: Helicobacter pylori, Jos, ulcère peptique, prévalence.

INTRODUCTION

*Helicobacter pylori*is a helical shaped, gram negative, microaerophilic bacterium that can inhabit various areas of the stomach particularly the antrum. Transmission probably occurs mostly by the faecal-oral routes and through contaminated food, water and unclean hands (1). Oral-oral transmission has been identified in the case of African women who pre-masticate foods given to their infants (2). It causes a chronic low level inflammation of the stomach lining and is strongly linked to the development of duodenal, gastric and stomach ulcer (3).

Helicobacter pylori infection is wide spread among human populations and is considered to play a major role in the pathogenesis of several gastroduodenal diseases including gastric ulcer, duodenal ulcer, peptic ulcer, gastric mucosa associated lymphoid tissue (MALT) Lymphoma. Previous sero-epidemiologic studies indicated that about 50% of adults in developed countries and nearly 90% in developing countries are positive for serum antibodies against *Helicobacter pylori* (4, 5). There are puzzles in defining the exact role of *Helicobacter pylori* infection in humans. The bacterium, which is associated with human disease of the upper gastrointestinal tract, may otherwise exist as a commensal with probable symbiotic association in some human hosts (6).

Despite significant advances in the understanding of the biology of the bacterium, the factors that determine the outcome of infection are poorly understood. Epidemiological studies have demonstrated high frequencies of the O blood group among most peptic ulcer patients. Although, the host factors might be important with regard to the outcome of infection by this organism, bacterial factors seem to influence the inflammatory response and the development of a more severe pathology. Cytotoxin associated antigen A (Cag A) is thought to be the major virulence factor involved in the pathogenesis of *Helicobacter pylori* diseases (7). Thus, presently, its role has been established in chronic antral gastritis, duodenal ulcer, chronic gastric ulcer, dyspepsia, gastric cancer and gastric lymphoma. World Health Organization added *Helicobacter pylori* to its list of known carcinogens (8).

The diagnosis of *Helicobacter pylori* gastritis can be made through many laboratory tests. The techniques are divided into two groups the invasive and non-invasive tests (9). Stool antigen tests as a non-invasive test have recently been welcomed with great expectations as they are convenient to the patients and can be easily performed even in small laboratories (10). However, the accuracy of stool antigen tests in different clinical situations is a matter of concern (11). Whereas, serological tests are reported to be unreliable for the diagnosis of *Helicobacter pylori* since they may return false negative results up to 60 days after infection and remain positive for a considerable time after eradication, but serology for IgG, against *Helicobacter pylori* may play an important role in decreasing the need for endoscopy provided the cut-off values must be determined for easy assay based on the prevalence of antibodies in the population (12). However, stool antigen test and serology test methods for H *pylori* are suitable for epidemiological studies, since their performance characteristics has been compared with endoscope-based methods, demonstrating an overall accuracy of 98% and 78% respectively.

In Nigeria, there are considerable controversies over the management of *Helicobacter pylori* infection. There is no national or regional consensus guidelines and very few documented pathological role of the bacteria (13). The updates in Knowledge of the prevalence of *Helicobacter pylori* infection and its associated risks factors is necessary for intervention programs that will reduce the morbidity as well as mortality caused by the bacteria.

The present study was aimed at comparative evaluation of stool antigen test and blood antibody test methods for diagnosis of *Helicobacter pylori* infection in cases of dyspepsia and some risk factors from patients attending the endoscopy clinic at JUTH.

MATERIALS AND METHODS

Study area: The study area of this research work was Jos University Teaching Hospital. .Jos is the Capital City of Plateau State. Plateau State is the twelfth largest State of Nigeria, and is roughly located in the center of the country. With an area of 26,899 square kilometers, the State has a population of 3,178,712 people according to 2006 census. It is located between latitude 80°24'N and longitude 80°32' and 100°38' east.

Study Population and Sample size determination:

A total of 80 patients were recruited for the study due to the few amount of dyspeptic patients that attended the endoscopy clinic within the period of this study. The attending physician interviewed each volunteer who completed a detailed questionnaire. The questionnaire was designed to obtain demographic data, socioeconomic status, and other risk factors.

The sample size was determined using the formula described by IFAD 2003. Thus: $n= t^2 \times p\ (1-p)/m^2$ Where: n=required sample size; t=confidence level at 95%=1.96; p = established local prevalence for serum antibodies = 80% (14) and established local prevalence for stool antigen = 80% (15); m = margin of error at 7.5 = 0.075; n = 109

10% attrition rate = 10.9; Total sample size: 109+10.9 = 119.9.

Ethical approval: Ethical clearance was sought and obtained from the Ethical and Research Committee of Jos University Teaching Hospital (JUTH).

Blood Sample Collection and Procession: The study was carried out between September and November 2016. The clinics were visited between 8am and 9am on Mondays and Wednesday during the week days. Physicians (Gastroenterologist) determined the diagnostic relevance of each volunteer who completed a detailed questionnaire. All the patients had dyspepsia with ulcer which was determined by Endoscopy. After an informed consent was obtained and appropriate pretest counseling conducted, about 5ml of a venous blood was collected in vacutainer tubes. A tourniquet was applied to the upper arm of the patient (to enable the Median cubital veins to be seen and felt) and the patient was asked to make a tight fist (Thus, making the veins prominent). The punctured site was cleaned with sterile alcohol and with the thumb of the left hand held down the skin below the selected punctures site, a vein was punctured with the level of the needle directed upwards in the line of the vein. When sufficient blood is collected the tourniquet is released and the patient was instructed to open his or her fist. The blood was then transported to the laboratory for analysis.

ABO Blood grouping and Rhesus Status

Blood grouping was conducted before plasma was separated, by slide agglutination test using monoclonal anti; A, anti-B anti-D antibodies. A drop of blood was placed on a clean white tile in three places in a vertical row. A drop of anti-A, anti-B, and anti-D was added respectively. Each blood sample was mixed using a glass rod. Blood group was determined on the basis of agglutination of test plasma by the respective antiserum.

Detection of *Helicobacter pylori* IgG Antibodies in Plasma

Plasma was recovered from the blood samples by centrifugation at 2,000rpm for 5 minutes. Serological status of *Helicobacter pylori* was determined using a commercially available *Helicobacter pylori* IgG ELISA Kit (Diagnostic Bioprobes-Italy) according to the manufacturers instruction a value of >1.1µml was taken as positive. The ELISA kit was for the quantitative/qualitative determination of IgG antibodies to *Helicobacter pylori* in human serum and plasma. Samples were diluted at 1:100 into properly defined dilution tubes. The micro wells were placed in the microwell holder, microwell A1 and B1 were left empty for the operation of Blanking. 100µl of calibrators and 100µ/g control serum was dispensed in duplicates. Then 100µl of diluted samples were dispensed in each properly identified well and sealed properly. The microplates were incubated for 60minutes at $+37^0$C. Microplates were washed with an ELISA microplate washer 5 times per cycle. 100µl enzyme conjugate was pipette into each well except A1 + B1 blanking wells, and they were covered with sealer. I checked that the red coloured component has been dispensed in all the wells except A1 + B1. The microplates were incubated for 60minutes at 37^0C. Microwells were washed using ELISA microplate washer.100µl of chromogen/substrate mixture was pipetted into each well, the blank wells A1 and B1 included. The microplates were then incubated at room temperature 24^0C for 20 minutes. 100µl of sulphuric acid was pipette to stop the enzymatic reaction into all the wells the addition of the acid turned the positive calibrators the control serum and positive samples from blue to yellow. The colour intensity of the solution in each well was measured by ELISA reader at 450 nm with the help of the gen5 software.

Detection of *Helicobacter pylori* using Stool Antigen Test (SAT)

Stool samples were collected in clean sample containers from patients whose blood samples was collected and a qualitative immunochromatographic assay was done to determine bacteria antigen according to the detail information provided within the available commercial kit (Biotest Biotech, china): Fecal specimens were collected in sufficient quantity (2g) in a clean dry specimen collection container so as to obtain maximum antigens (if present). The cap of the specimen collection tube was unscrewed, and then the specimen was randomly stabbed with the collection applicator in three different sites so as to collect approximately 50 mg of feces into the extraction buffer. For liquid specimens a dropper was held vertically so as to aspirate fecal specimens, and then two drops approximately 80µl of the specimen was dispensed into the collection tube containing the extraction buffer. The cap of the collection tube was tightened, and then the specimen collection tube was shaken vigorously to mix. The specimen and the extraction buffer in the tube were left alone for two minutes. The specimen collection tube was held upright and the cap of the collection tube was opened, the tube was inverted and two full drops of the extracted specimen (approximately 80µl) was transferred to the specimen well of the test cassette, and the timer was started. Trapping the specimen well(s). Result was read at ten minutes after the specimen was dispensed.

Interpretation of Results: Positive-Two lines appeared, one red line was in the control line region (c) and another apparent red line was in the test line region (T). **Negative-** One red line appeared in the control line region (c) and no line appeared in the test line region (T).

Statistical analysis: Chi-square was used to compare the categorical data. P≤ 0.05 was considered statistically significant.

RESULTS

.The result of the eighty (80) patients that participated in the study which comprised 44 (55%) males and 36 (45%) females shows that 28 (35.0%) were positive for anti-*H. pylori* antibodies, while 31 (38.8%) were positive for *Helicobacter pylori* stool antigen test (SAT).(Table 1). Blood group showed that 79 (98.8%) of the total patients were rhesus positive and 1(1.1%) of the patients was rhesus negative(Table not shown)

TABLE 1: RELATIONSHIP BETWEEN *HELICOBACTER PYLORI* SEROPREVALENCE AND STOOL ANTIGEN TEST IN DYSPEPTIC PATIENTS

No examined	Seroprevalence(%)	Stool antigentest (%)	No. Positive for Abs/Ag
80	28(35.0)	31(38.8)	27
Total	80	28(35.0)	31(38.8)

X^2=0.837df=1

The values for ELISA positive samples ranged from ≥1.1µ/ml to 2.8µ/ml while values for negative samples ranged from ≤1.0.µ/ml to 0.1.µ/ml. Among the participants blood group O was the most common blood group 43 (53.8%) followed by blood group B 15 (18.8%), A 12 (15%) and AB 9 (11.3%) (Table 2).

Helicobacter pylori seropositivity with respect to blood groups was found to be 32.6%, 46.7%, 33.3% and 33.3% in blood groups O, B, A and AB respectively (Table 2). While, *Helicobacter pylori* stool antigen test positive results was 33.3%,33.3%, 46% and 39.5% in blood groups O, B, A and AB respectively. Hence, no statistical association was found between *Helicobacter pylori* infection and blood groups of the patients (P>0.05) (Table 2)..

Table 3 shows the relationship between *Helicobacter pylori* infection and age of the patients. Majority of the patients were young adults aged 20 to 40 years. There was no significant association between *Helicobacter pylori* seropositivity and age of patients since (p>0.05).

TABLE 2: RELATIONSHIP BETWEEN BLOOD GROUPS ABO WITH *HELICOBACTER PYLORI* SEROPREVALENCE AND STOOL ANTIGEN TEST AMONG DYSPEPTIC PATIENTS

Blood group	No examined	Seroprevalence (%)	Stool antigen test(%)	p value
O+	43	14(32.6)	17(33.3)	0.837
A+	12	4(33.3)	4(46.7)	
B+	15	7(46.7)	7(33.3)	
AB	9	3(33.3)	3(39.5)	
O-	1	0(0.0)	0(0.0)	
Total	80	28(35.0)	31(38.8)	

X^2=0.837df=1

The sex specific prevalence was 43.2% in males and 25.0% in females. There was no statistically significant association between *Helicobacter pylori* seropositivity and sex of the patients since (P>0.05) (Table 3). With respect to marital status 24.4% of the married patients were positive, while 48.6% single unmarried patients were positive. There was a statistical significance association between *Helicobacter pylori* seropositivity and marital status of the patients since (P<0.05)(Table 3).

TABLE 3: RELATIONSHIPS BETWEEN SEROPREVALENCE OF H. PYLORI AND GENERAL INFORMATION AMONG DYSPEPTIC PATIENTS.

Factors	No.Examined	No. Positive (%)	Total no. of subjects p=80
Age (years)			
<20	5	3(60.0)	0.700
20-29	29	12(41.4)	
30-39	23	6(26.1)	
40-49	11	3(27.3)	
50-59	9	3(33.3)	
60-69	3	1(33.3)	
Sex			
Male	44	19(43.2)	0.090
female	36	9(25.0)	
Marital status			
Single	35	17(48.6)	0.025
married	45	11(24.4)	

X2=0.700, df=1, X2=0.090, df=1, X2=0.025,df=1

The result of the relationships between sero-prevalence of H. *pylori* infection and risk factors among dyspeptic patients with respect to the economic status of the patients shows that 24.4% were considered average and seropositive, 25.0% were very good and seropositive, while 78.6% were poor and seropositive. However, there was no statistical significance between the patients' economic status and *Helicobacter pylori* seropositivity at P>0.05(Table 4). Seropositivity of anti-*Helicobacter pylori* antibodies was not significantly associated with the patients source of water since (P>0.05). It was also observed that seropositivity of anti-*Helicobacter pylori* antibodies was not significantly associated with alcohol consumption (P>0.05) (Table 4).

TABLE 4: RELATIONSHIPS BETWEEN SEROPREVALENCE OF H. *PYLORI* INFECTION AND RISK FACTORS AMONG DYSPEPTIC PATIENTS

Risk factors	No. examined	No. positive (%)total no. of subjects)	P -value
Economic status			
Average	**41**	**10(24.4)**	**0.081**
Very good	**4**	**1(25.0)**	
Poor	**35**	**17(48.6)**	
Source of water			
Tap	**2**	**0(0.0)**	**0.293**
Sachet	**75**	**26(34.7)**	
Well	**3**	**2(66.7)**	
Alcoholism			
Yes	**2**	**0(0.0)**	**0.293**
No	**78**	**28(35.0)**	

X2 =0.081 df=2, X2=0.293 df=1, X2=0.293 df=1

Table 5 shows the relationship between *Helicobacter pylori* infection and age of the patients. From the result it was observed that there was no association between *Helicobacter pylori* stool antigen and age of the patients since (P>0.05) (Table 5). The sex specific prevalence was 45.5% in males and 30.6% in females. There was no statistically significant association between *Helicobacter pylori* stool antigen and sex of the patients since (P>0.05) (Table 5).

Regarding marital status 31.1% of the patients were positive and married, while 48.6% were positive and single. There was no statistical significance association between *Helicobacter pylori* stool antigen and marital status of patients since (P>0.05) (Table 5).

With respect to economic status of the patients 31.7% were considered average and positive, 25.0% were very good and positive and 48.6% were poor and positive for stool antigen test. There was no statistical association between the patients economic status and *Helicobacter pylori* stool antigen test since (P>0.05) (Table 6).

It was observed that *Helicobacter pylori* stool antigen was not statistically associated with the patients source of drinking water (P>0.05) (Table 6). *Helicobacter pylori* stool antigen was not significantly associated with patients' alcohol consumption. (P>0.05) (Table 6).

TABLE 5: RELATIONSHIPS BETWEEN STOOL ANTIGEN OF H. *PYLORI* INFECTION AND GENERAL INFORMATION AMONG DYSPEPTIC PATIENTS

Factors	No. Examined	No. Positive (%(Total no. of subjects) N=80	p value
Age			
<20	**5**	**3(60.0)**	**0.716**
20-29	**29**	**12(41.4)**	
30-39	**23**	**8(34.8)**	
40-49	**11**	**3(27.3)**	
50-59	**9**	**3(33.3)**	
60-69	**3**	**2(66.7)**	
Sex			
Male	**44**	**20(45.5)**	**0.174**
Female	**36**	**11(30.6)**	
Marital status			
Single	**35**	**17(48.6)**	**0.112**
Married	**45**	**14(31.1)**	

X^2 =0.716 df=5, X^2=0.174 df=1, X^2=0.112 df=1

DISCUSSION

Detection of *Helicobacter pylori* infection with non-invasive methods such as serological tests and stool antigen test are useful and widely available (16). The result of this study revealed that the prevalence of *Helicobacter pylori* among patients attending endoscopy unit in Jos University Teaching Hospital (JUTH) was 35% for seroprevelance and 38.8% for stool antigen test respectively. This finding is lower than the previous report from Keffi showing a prevalence of 56.3% (17). It is well lower than another previous report from a developing country showing a prevalence of 53.5% for *Helicobacter pylori* (18).

Epidemiological studies have demonstrated a higher frequency of the blood group O among patients suffering from peptic ulcer. These findings of this study supports the epidemiological view of the greater sensitivity of those with blood group O to infection by *Helicobacter pylori* which is apparently in line with the conclusion of (18) who demonstrated that the H-antigen in blood group O represents an important receptor expressed in the gastroduodenal mucosa cells to which *Helicobacter pylori* adheres which also enables colonization of the bacterium. Blood group A and AB patients in this study were less prone to *Helicobacter pylori* infection than other blood groups which is not consistent with earlier studies. The present study does not demonstrate any significant difference in *Helicobacter pylori* infection of patients with varying blood groups which is consistent with similar studies from other countries (19).

This result revealed that there was no statistically significant association between *Helicobacter pylori* infection and age of the patients (P>0.05). Therefore, this work is not consistent with previous reports which indicated high frequencies in the elderly (20).

TABLE 6: RELATIONSHIPS BETWEEN STOOL ANTIGEN OF *H.PYLORI* INFECTION AND RISK FACTORS AMONG DYSPEPTIC PATIENTS

Risk Factors	No. Examined	No. Positive (%)	Total no. subjects N=80	p value
Economic status				
Average	**40**	**13(31.7)**		**0.273**
Very good	**4**	**1(25.0)**		
Poor	**35**	**17(48.6)**		
Source of water				
Tap	**2**	**0(0.0)**		**0.325**
Sachet	**75**	**29(38.7)**		
Well	**3**	**2(66.7)**		
Alcoholism				
Yes	**2**	**0(0.0)**		**0.255**
No	**78**	**31(39.7)**		

X2=0.273 df= 2, X2=0.325 df=2,X2 =0.255 df=1

The result of this present study showed that *Helicobacter pylori* infection was higher in males 43.2%, 45.5% than females 25.0%, 30.6% respectively. It was not statistically significant (P>0.05). This is consistent with some studies that have reported higher prevalence of the infection in males which may be attributed to higher exposure of males to potential environment sources of infection (21). It was also observed that *Helicobacter pylori* infection was high among patients whose source of drinking water was sachet water because about 94% of the population depends on sachet water as their source of drinking water. However there was no statistically significant since (P>0.05).

There was no relationship between *Helicobacter pylori* infection and alcohol consumption in this study (P>0.05). This is in line with studies from other countries that have reported no significant association between *Helicobacter pylori* and alcohol consumption. However, history of alcohol (Local alcohol like burukutu) consumption has been shown to be a risk factor for *Helicobacter pylori* infection (17). The reason for this difference may be due to the difference in the type of alcohol beverages consumed or the source of water used in its production. n conclusionthe prevalence of *Helicobacter pylori* among the study group was 35% for blood antibody and 38.8% for stool antigen. This study employed ELISA and ICA to determine the presence or absence of *Helicobacter pylori* infection among the study population. Hence, there is need to employ advance techniques like PCR to identify this organism.

It is necessary for government to encourage people about the *Helicobacter pylori* screening test since it is one of the etiologic agent of ulcer. Also there is need for adopting proper hygienic practices such as

avoidance of eating food prepared in an unhygienic settings, washing hands when visiting hospitals and when leaving the hospital should be a common practice and ensuring that water are obtained from reputable sources and finally, mothers should stop the habit of pre-masticating food before giving their children.

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