HELICOBACTER PYLORI AMONG PATIENTS WITH DYSPEPSIA AND CHRONIC SKIN DISORDERS

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INTRODUCTION

Helicobacter pylori has been recognized as a major pathogen of humankind for nearly four decades. However, despite the impact of treatment of infected individuals and the reduced transmission of infection in communities in which socioeconomic living standards have half improved, it continues to be the most common human bacterial pathogen, infecting perhaps of the world's population [1]. As a result, it is still a major cause of morbidity and mortality .worldwide

H. pylori infection invariably causes active chronic gastritis. In most people, this may be

clinically silent throughout life, but in a substantial minority it causes gastroduodenal

-diseases, most importantly peptic ulcer disease, noncardia gastric cancer, and gastric mucosa associated lymphoid tissue (MALT) lymphoma.

ulceration and bleeding in patients who are taking It also increases the risk of gastroduodenal NSAIDs) such as aspirin and is responsible for symptoms (nonsteroidal anti-inflammatory drugs .functional dyspepsia in a subset of patients with

of disease expression are still incompletely understood, including many The determinants pathogen interaction. The pathophysiology of this interaction is complex and –aspects of the host reviewed in detail elsewhere [2,3]. has been

The quest for the most effective, safe, and simple treatment is still a major issue for

and .clinicians, and the problem of antimicrobial resistance to therapy is a significant challenge Some speculate that *H. pylori* infection might .the quest for an effective vaccine is ongoin trigger the production of antibodies by cross-reaction between the bacterium and the gastric parietal cells or by causing inflammation in the gastrointestinal tract, which might facilitate the absorption of antigens. Once this occurs, the production of IgE antibodies responsible for the urticaria symptoms might continue even after the eradication of *H.pylori* [4].

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The purpose of this study is to recognize variations in prevalence and impact of infection and the association of skin disorders. The burden of disease brought by *H. pylori* falls disproportionately on less well-resourced regions, which is the in most parts of our country.

Materials and Methods

This was a case-control study which had been conducted in Khartoum Dermatology Teaching Hospital and Omdurman Teaching Hospital.

One hundred and twenty patients who had gastrointestinal symptoms (heart-burn, nausea, vomiting, pain in upper abdomen) suspected to had H. pylori infection and chronic idiopathic urticaria symptoms (itching, skin-rash), also healthy individuals who have no symptoms of H. pylori infection or chronic idiopathic urticaria (CIU) were included**.**

Data was collected by using direct interviewing questionnaire; the study was based on non probability-simple random technique. Written consent was also obtained from the patients under supervision of physician.

One hundred and nineteen specimens (blood & stool samples) were obtained. Both were collected under strict sterile conditions. 3 ml of whole venous blood was collected after disinfected of skin by 70% alcohol, and then the blood was poured in plain container and centrifuged at 2000 rpm for 5 minutes to obtain the serum. Serum was stored at -20°C until used. Stool was collected in clean container and tested immediately.

Specimens were processed by Serum ICT & Stool Antigen ICT KITS for detection of H. pylori.

Type of serum ICT kits used was commercially available rapid card test (ACCURATE, diagnostic use, china) according to instructions 3drops of the serum dropped on the absorbent pad. When the control line and the test line were visible the ICT test was regarded positive, when the control line only seen it regarded as negative. When the control line absent and the test line seen it was regarded as invalid.

Type of stool antigen ICT kits used was CERTEST BIOTEC S.L. Certest is a colored chromatographic immunoassay for detection of H. Pylori antigen in the stool samples. The strip consists of a nitrocellulose membrane pre-coated with monoclonal antibodies on the test line (T), in the result window, against H. Pylori and with polyclonal antibodies, on the control line (C), against specific protein. The label/sample absorbent pad is sprayed with test label solution (monoclonal antibodies anti-helicobacter pylori) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to green polystyrene latex, forming colored conjugate complexes.

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According to instruction we take out the cape of the stool collection tube and use the stick to pick up sufficient sample quantity. Then introduce the stick into the stool sample and then added to the collection tube. Close the tube with the diluents and the stool sample and shake it to assure good sample dispersion. Then cut the end of the cap and dispense 4 drops in the circle window marked with the letter (S) the result red after 5 min.

When the control line is visible only, it regarded negative. When both the control and test line are visible the test regarded as positive. If the test line was visible alone it's regarded as invalid.

Those who were stool ICT test positive for H. pylori were given eradication therapy consist of amoxicillin 1g/d, metroniadzole 500mg T.D.S (for 10 days), and omeprazole 20mg (for one month). After one month re-examine of these patients by stool antigen ICT kits.

Results

The sample initially consisted of 120 individuals, 56 males and 64 females, and was composed of three groups: urticaria patients (60); gastritis patients (30); and apparently healthy individuals, labled 'Normal', (30). One male participant from the gastritis group, however, was excluded for refusing to take the stool test. The remaining individuals (119) had a mean age of 30.7 (SD: 10.3) years. Their ages ranged between 18 and 60 years. The sample characteristics of those participants are summarized in table 1.

Table 1: Baseline Sample Characteristics

				Sex				Skin Symptoms				GI Symptoms							
Group	H. Plori	N[1]	Age Mean (SD)	Male N	(%)	Female N	(%)	Itching N	(%)	Skin Rash N	(%)	Nausea N	(%)	Vomiting N	(%)	Pain[2] N	(%)	Heart Burn N	(%)
Urticaria	+ve	29	32.6 (11.2)	13	(44.8)	16	(55.2)	29	(100)	28	(96.6)	17	(58.6)	1	(3.4)	12	(41.4)	16	(55.2)
	-ve	31	30.8 (10.0)	10	(32.3)	21	(67.7)	31	(100)	25	(80.6)	17	(54.8)	1	(3.2)	12	(38.7)	17	(54.8)
Total		60	31.5 (10.5)	23	(38.3)	37	(61.7)	60	(100)	53	(88.3)	34	(56.7)	2	(3.3)	24	(40.0)	33	(55.0)
Gastritis	+ve	14	28.2 (9.0)	6	(42.9)	8	(57.1)	1	(7.1)	1	(7.1)	14	(100)	0	(0)	12	(85.7)	11	(78.6)
	-ve	15	31.9 (12.3)	6	(40.0)	9	(60.0)	3	(20.0)	3	(20.0)	15	(100)	3	(20)	11	(73.3)	14	(93.3)
Total		29	30.3 (10.9)	12	(41.4)	17	(58.6)	4	(13.8)	4	(13.8)	29	(100)	3	(10.3)	23	(79.3)	25	(86.2)
Normal	+ve	6	29.6 (8.1)	5	(83.3)	1	(16.7)	1	(16.7)	0	(0.0)	4	(66.7)	1	(16.7)	1	(16.7)	3	(50.0)

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	-ve	24	29.3 (12.8)	15	(62.5)	9	(37.5)	2	(8.3)	2	(8.3)	6	(25.0)	2	(8.3)	2	(8.3)	2	(8.3)
Total		30	29.5 (9.7)	20	(66.7)	10	(33.3)	3	(10.0)	2	(6.7)	10	(33.3)	3	(10.0)	3	(10.0)	5	(16.7)

1: N: Number of individuals; **2**: Abdominal Pain.

Participants in the urticaria group, mainly, presented with itching (100%) and skin rash (88.3%). Those in the gastritis group mainly presented with nausea (100%), heart burn (86.2%) and abdominal pain (79.3). Of the apparently healthy group, 33.3% were found to be suffering from nausea; but no other remarkable complaint.

Prevalence of H. Pylori in the Three Study Groups

H. Pylori stool test was performed at baseline for all 119 participants. The results are shown in table 2 below.

TABLE 2: H. PYLORI STOOL TEST RESULTS IN THE THREE STUDY GROUPS

Group	H. Pylori		Total
	+ve	-ve	
Urticaria	29 (48.3%)	31 (51.7%)	60 (100%)
Gastritis	14 (48.3%)	15 (51.7%)	29 (100%)
Normal	6 (20.0%)	24 (80.0%)	30 (100%)
Total	49 (41.2%)	70 (58.8%)	119 (100%)

In the urticaria group, 29 out of the 60 patients tested positive for H. Pylori. The prevalence of H. Pylori infection in that group was found to be 48.3% (CI: 35.2% to 61.6%). In the gastritis group, 14 out of the 29 patients who took the test, were found to be positive for H. Pylori with an estimated prevalence of also 48.3% (CI: 29.4% to 67.5%). The apparently healthy group had only six individuals who tested positive, with an estimated prevalence of 20% (CI: 7.7% to 38.6%).

To investigate differences in the prevalence of H. Pylori in the three study groups, Pearson's χ^2 test was performed. The test indicated a statistically significant difference between at least

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two of the three estimates of prevalence of H. Pylori in those (p=.002). The Marascuillo procedure for comparing multiple proportions was further used to simultaneously test for differences within the three pairs. Result of that comparison is shown in table 3 below.

Table 3: Comparison of the proportions of H. Pylori infected individuals in the three groups

Pair*	value	Critical range	Significance (5%)
U vs G	0.001	0.126	No
U vs N	0.283	0.114	Yes
G vs N	0.283	0.114	Yes

*U: Urticaria group; G: Gastritis group; N: Normal (apparently healthy) group

It is evident from table 3 that the prevalence of H. Pylori infection in patients with urticaria was not significantly different from that of the gastritis patients, whereas the prevalence of H. Pylori in the apparently healthy sub-sample differed significantly from the prevalence in the two other groups suggesting an association between H. Pylori and both urticaria and gastritis.

Diagnostic test analysis

At baseline, participants were subjected to both stool test and a serum test. The stool test was used as the reference test (The S-ICT is non-invasive, cost effective, and requires less than 15 min to perform. Therefore, it is convenient for patients and can be easily performed even in small laboratories and primary outpatient clinics, unneeded requirement of expensive equipment and medical personnel especially in 1ry infection and follow up. A meta-analysis revealed that the global sensitivity and specificity of stool antigen tests are 94% (95%CI: 93-95) and 97% (95%CI: 96-98), respectively [5] for detecting the presence or absence of H. Pylori infection.

Participants were then classified according to the results of the two tests in each group, as shown in table 4 below.

Table 4: Cross classification of participants by group and type of test

Test	Group			
	Urticaria	Gastritis	Normal	All

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		Stool Test			Stool Test			Stool Test			Stool Test		
		+ve	-ve	Total	+ve	-ve	Total	+ve	-ve	Total	+ve	-ve	Total
Serum Test	+ve	8	9	17	4	6	10	0	5	5	12	20	32
	-ve	21	22	43	10	9	19	6	19	25	37	50	87
Total		29	31	60	14	15	29	6	24	30	49	70	119

Diagnostic accuracy of the serum test was investigated in the three study groups, separately. Result obtained is shown in table 5.

Table 5: Comparison of the serum test performance in the three study groups

	Urticaria	**Gastritis**	**Normal**
Prevalence	48.3% (35.2% to 61.6%)	48.3% (29.5% to 67.5%)	20% (7.7% to 38.6%)
Sensitivity	27.6% (12.7% to 47.2%)	28.6% (8.4% to 58.1%)	0% (97.5% one-sided CI: 0% to 45.9%)
Specificity	71.0% (52.0% to 85.8%)	60% (32.3% to 83.7%)	79.2% (57.9% to 92.9%)
Predictive value of +ve test (post-test likelihood of disease)	47.1% (23.0% to 72.2%) {change = -1%}	40% (12.2% to 73.8%) {change = -8%}	0% (97.5% one-sided CI: 0% to 52.2%) {change = -20%}
Predictive values of -ve test			
(post-test likelihood of no disease)	51.2% (35.5% to 66.7%) {change = -1%}	47.4% (24.5% to 71.1%) {change = -5%}	76% (54.9% to 90.6%) {change = -4%}

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(post-test disease likelihood despite -ve test)	48.8% (33.3% to 64.5%) {change = 1%}	52.6% (28.9% to 75.6%) {change = 5%}	24% (9.4% to 45.1%) {change = 4%}
Likelihood Ratio			
LR (positive test)	0.950 (0.428 to 2.090)	0.714 (0.254 to 1.911)	0 (0 to 2.222)
LR (negative test)	1.020 (0.726 to 1.428)	1.190 (0.68 to 2.127)	1.263 (0.667 to 1.589)

From table 5 above, both urticaria and gastritis patients had the same point estimate of prevalence of H. Pylori infection (48.3%); but the 95% CI was wider in the gastritis group due to the smaller sample size of that group compared with the urticaria group. Prevalence in the apparently healthy group (20%) was less than half the prevalence found in each of the two other groups.

The sensitivity (true positive rate) of the serum test in the urticaria group was only 27.6% (CI: 12.7% to 47.2%). The 95% CI indicated that the true sensitivity of the serum test could be as low as 12.7% where the false negative fraction would have reached a high of 87.3%, while the upper limit of the confidence interval of 47.2% indicated that the serum test, at its best, would miss a minimum of 52.8% of those truly infected with H. Pylori.

In the gastritis group of patients, the sensitivity of the serum test was also low at 28.6% (CI: 8.4% to 58.1%). Again, the 95% CI indicated that the true sensitivity of the serum test could be even lower at 8.4%, where the false negative proportion could have reached a high of 91.6%, whereas the upper limit of the confidence interval of 58.1% indicated that the serum test, at its best, would miss a minimum of 41.9% of those truly infected with H. Pylori.

In the apparently healthy group of patients, the serum test failed to pick out any of the infected individuals, resulting in a sensitivity of 0% (97.5% one-sided CI: 0% to 45.9%). The upper limit of the CI, however, indicated that a sensitivity of 45.9% could also be as plausible – resulting in a false negative fraction of 54.1%.

Compared to its sensitivity, the specificity (true negative rate) of the serum test was generally higher. It was at its highest level in the apparently healthy group 79.2% (CI: 57.9% to 92.9%); less high in the urticaria group 71.0% (CI: 52.0% to 85.8%); and at its lowest level in the

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gastritis group at only 60% (CI: 32.3% to 83.7%). The test would have incorrectly identified 20.8%, 29% and 40% of the non-infected participants in the three groups, respectively, as being HP infected (false positives).

The predictive value of the positive test (post-test likelihood of H. Pylori infection) at 47.1%, 40% and 0% in the urticaria, gastritis and the apparently healthy groups was 1%, 8% and 20%, respectively, less than the pre-test likelihood of H. Pylori infection. Being lower than the pre-test likelihood, the post-test likelihood obtained in the three groups indicated that the serum test was worse than the mere judgement that could have been made depending only on the prevalence of the infection in those groups (48.3% in both urticaria and gastritis groups; and 20% in the apparently healthy group). It was rather clinically useless when applied to the urticaria patients and misleading, to a varying extent, in the gastritis group and in the apparently healthy individuals, having had a change over pre-test likelihood of the infection of (-8%) and (-20%), respectively.

The predictive value of the negative test (post-test likelihood of no H. Pylori infection) was found to be 51. 2% (CI: 35.5% to 66.7%) in the urticaria patients; 47.4% (CI: 24.5% to 71.1%) in the gastritis patients; and 76% (CI: 54.9% to 90.6%) in the apparently healthy group, which was rather uninformative (change of -1% to -4% over the pre-test likelihood of no HP infection).

From table 5, it is also evident that the likelihood ratio (LR) for a positive test was less than unity (<1) in the three study groups, whereas a sensible test is supposed to have a value >1; and the likelihood ratio for a negative test was >1 contrary to what is also expected from a sensible test. This indicates that the serum test seems to have worked in the reverse direction: a positive test result suggested absence of HP infection, while a negative test result suggested the presence of an infection - contrary to expectations.

Treatment Outcome:

Of the 29 patients who initially tested positive for H. Pylori in the urticaria group, 22 were treated with regimen A: which consist of amoxicillin 1g\day, metronadizole 500mg T.D.S both antibiotics used for 10 days and omeprazol 20mg for one month. one patient was treated with regimen B: which contains amoxicillin 1g\d, clarithromycine 500mg B.D and omprazole 20mg, and 3 patients were treated with A followed by B.

The remaining three patients were lost to follow up. One month after completion of treatment, all 26 patients had a stool retest carried out and the treatment outcome assessed. Data on stool retest and treatment outcome (presence/absence of itching) were cross classified as is shown in table 6 below.

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Table 6: Outcome classified by Therapy and the stool re-test result

Therapy	Stool Re-test	Itching		Total
		Disappeared	Persisted	
A	H. Pylori +ve	0	**2**	2
	H. Pylori -ve	11	9	20
Total		11	11	22
B	H. Pylori +ve	0	**1**	1
	H. Pylori -ve	0	0	0
Total		0	**1**	1
A,B	H. Pylori +ve	0	**1**	1
	H. Pylori -ve	0	2	2
Total		0	3	3
Total	H. Pylori +ve	0	**4**	4
	H. Pylori -ve	11	11	22
Total		11	15	26

It can be seen from table 6 that out of the 22 patients who were treated with therapy A alone, 11 (50%, CI: 28.2% to 71.8%) did not attain relief from baseline symptoms, namely itching, together with the single patient who was treated with treatment B only and all of the three patients who were treated with A followed by B.

The eradication rate of treatment A (20/22) amounted to 90.1% (CI: 70.8% to 98.9%). Treatment B alone and the combination (A, B), however, were tested in a too small number of patients that didn't warrant a meaningful analysis.

Overall, persistence of skin symptoms, namely itching was reported for 15 out of the 26 patients (57.7%), of whom only 4 had a positive stool retest while the remaining 11 were found to be H. Pylori free after receiving treatment A only.

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Assuming no difference between the baseline proportion of those who suffered from itching and their proportion after complete eradication of H. Pylori was attained (100% vs 45%, respectively), a paired proportion test was performed. The difference between the two proportions (55%, Score based (Newcombe) 95% CI: 28.7% to 74.2%) was found to be statistically significantly different from zero (Exact two sided P = 0.001). With 95% confidence it can be said that the true population value for the proportion difference lies somewhere between 28.7% and 74.2%. This shows that eradication with treatment A produced a significant reduction in itching suffered by urticaria patients and provides some evidence about the association between H. Pylori and urticaria.

Discussion

The prevalence of H. pylori infection in this study group was found to be in the urticaria, gastritis about 48.3%, 51.7% respectively which shows there is no significant difference between patients of urticaria and those with gastritis.

To find a reliable non-invasive test for H. pylori, we evaluated commercially available conventional serum and stool antigen (ICT) immunochromatographic test. The commercially used serum ICT kits in our study was serum (ACCURATE) rapid card test, it has a very low sensitivity in all three groups tested and moderate spacifity as it was shown in the results, so it cannot be used as reliable test for detection of H. Pylori infection.

Stool antigen testing is a relatively new methodology that detects the presence of H. pylori antigen in the stool with the use of polyclonal anti-H. Pylori antibody the type of test used was (CERTEST, BIOTEC) card test. As this test detects bacterial antigen in ongoing infection, it can be used as a reliable means of diagnosing active infection and also useful for eradication therapy follow-up purposes.

Chronic idiopathic urticaria still remains one of the most difficult skin diseases to treat being, different infections have been implicated in the causation of chronic urticaria but, the concept of H. pylori infection as a possible causative factor is a relative new one. What make this association worthy of investigation are the chronic and asymptomatic nature of H. pylori infection and its highly endemic nature. Although it resides and colonizes in the stomach but can manifest extra-intestinal symptoms such as skin.

A potential association between CIU and H. pylori infection of the upper gastrointestinal tract has been proposed, but the studies so far showed controversial results [6]. Our study was performed to confirm such association and the prevalence of H. Pylori in urticaria group was found 48.3%. Proportions of H. Pylori infection in the urticaria and the gastritis groups were not statistically significantly different.

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It was observed that eradication therapy showed significant results in improvement of symptoms (itching) even if it's for a short period, in those who received treatment regimen (A) attained complete eradication (20 patients) showed statistically significant reduction of 55% in the itching symptom.

In this study which is consistent with the studies performed by many researchers [7] according to them, bacterium eradication results in improvement of urticaria symptoms.
Commonly recommended treatment options for eradication of H. pylori infection, initially included amoxicillin, omeperazole and metronidazole, some patients complained of developing abdominal discomfort (nasua) particulary on taking meteronidazole or they have received the same treatment but was not effective, treatment was changed by taking out metronidazole and give in Clarithromycin unfortunately these patient did not come again for re-assessment. But was proved that patient who received regimen (A) treatment successfully eradicated from H. Pylori infection, the eradication rate amounted to 90.1%.

CONCLUSION AND RECOMMENDATIONS:
Using serum ICT kits (ACUURATE) rapid card test does not seems helpful for detection of H. Pylori in patients. In general serum ICT kits are used because they are less expensive and available, easy to use but this should not be the only reasons. Our aim to have a reliable test for detection of H. Pylori especially in the 1st time prior to medication. Stool antigen kits test have proved to be used as detector for H. Pylori infection and in follow up the progress of treatmen. Association between CIU & H. Pylori is still far to be clear, but a possible hypothesis that chronic H. Pylori infection can trigger extra-digestive immunological response which may cause skin symptoms as in urticaria may exist.

The serum test kit evaluated in this study has been proven to have no clinical value; therefore, we recommend banning its import and distribution and use.

A large randomized controlled trial is needed for an acceptable strong evidence of association between H. Pylori and urticaria.

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