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Original Article

The Plausibility of Helicobacter Pylori and CagA Strains Related Infertility Among Males in Alexandria, Egypt

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Abstract

Background: *Helicobacter pylori (H. pylori)*, especially the strains expressing cytotoxin-associated gene A (CagA), besides causing gastric diseases, may also involve other systems including the reproductive system leading to infertility. In males, antibodies produced against *H. pylori* flagella may cross react with spermatozoa flagella; due to antigenic mimicry between them. Infected males have decreased sperm count, motility and viability, reduced numbers of normally shaped sperms and augmented systemic levels of inflammatory cytokines.
Objective(s): to detect *H. pylori*–related infertility prevalence among males; and to address the possibility that such infection may play a detrimental role in their semen quality.
Methods: One hundred infertile male patients attending a private hospital in Alexandria were screened for *H. pylori* by enzyme linked immunosorbent assay (ELISA). CagA strains were further identified using CagA IgG ELISA. Semen analysis was performed to assess semen quality as regards sperm count, motility, vitality and morphology.
Results: *H. pylori* seropositivity was 73% (73 out of 100) among screened cases. Sixty out of the 73 positive cases for *H. pylori* IgG (82.19%) were CagA strains. *H. pylori* prevalence was significantly higher among the group of patients with idiopathic infertility (79.7%) than among those who had one or more diagnosed causes of infertility; p value= 0.024. CagA status significantly influenced the quality of semen among infected cases compared to uninfected ones. (p value<0.001).
Conclusion: *H. pylori* infection; specially by CagA strains can be responsible for cases of idiopathic infertility in males through its negative effect on semen quality.

Keywords: *H. pylori*, CagA protein, serodiagnosis, ELISA.

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INTRODUCTION

Infertility is a global problem that affects one couple out of each six couples and is defined as failure to conceive after twelve months of regular contraceptive-free unprotected intercourse in the reproductive age.[(1)] Primary infertility affects about 15% of couples; with male factor infertility responsible for 50% of cases. In more than 20% of cases, the cause of infertility stays behind unexplained.[(2)] Earlier, only the physiological causes of infertility were considered but gradually the focus shifted to infectious and immunological causes behind it.[(3)]In many cases, infections like those caused by Ureaplasma urealyticum and Chlamydia trachomatis may lead to hypofertilityand if treated successfully, the problem of infertility is solved[(4)]Normally, in males; the blood-testis barrier protects the antigenic spermatozoa from the circulating immune cells. However, in about 2% of males, auto antibodies called antisperm antibodies (ASAs) which reduce the ejaculate quality and hence fertility, are produced.[(5)] The prevalence of such autoantibodies is greatly increased in infertile males with unexplained and persistent infertility; ranging from 7-26 %.[(3)]

Antisperm antibodies interfere with sperm function through inhibition of motility, viability, and acrosome reaction, blocking the fertilization of oocytes at a certain stage and interfering with sperm binding to the oocyte.[(6)] Researchers set forth an explanation that being the only flagellated human cells, spermatozoa may share homology with bacterial flagella and therefore may cross-react with antibodies produced against flagellated organisms.[(7)]

Molecular mimicry between spermatozoa and some microorganisms as Candida albicans, Ureaplasma urealyticum, Chlamydia trachomatis, Streptococcus viridans, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Salmonella Typhi and Helicobacter pylori (H. pylori) was reported.[(7,8)] H. pylori is a microaerophilic, gram-negative, spiral-shaped bacterium that infects more than half of the world's

humans.[9] In developed countries, prevalence increases about 1% per year of age and reaches 70% in the seventh decade. Meanwhile, in developing countries, more than 50% of children acquire the infection by the age of 10 years and more than 80% of the population gets infected by the age of 20 years. Prevalence of H. pylori infection varies from 31% to 84% in asymptomatic individuals.[10]

The possible outcome of H. pylori infection may not be restricted to the gastroduodenal tract. The list of disorders related to H. pylori infection extended to encompass heart and vessels, skin, oropharynx and multiple systems, such as the endocrine, respiratory, haemopoietic, immune and central nervous systems.[9]

Figura et al., (2002), reported for the first time that H. pylori infection could be involved in the development of infertility; increasing the risk of reproductive disorders and aggravation of their clinical expression. A linear homology was observed between β-tubulin (abundant in the tails and the pericentriolar area of human spermatozoa) and three H. pylori proteins: flagellin, vaculating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA).[7]The cytotoxin-producing strains of H. pylori contain the CagA gene; that codes for CagA protein. This protein is immunodominant and is recognized immunologically early following infection with H. pylori CagA-positive strains both by gastric mucosal IgA and serum IgG responses.[11]

Infected idiopathic infertile males, especially those with serum antibodies to CagA, have reduced sperm motility and a greater number of necrotic and apoptotic sperms in their ejaculates.[9] Simultaneously, such cases have increased systemic level of interleukin-8 (IL-8), IL-1 b, IL-6 and tumour necrosis factor-alpha (TNF-α); that may cause sperm damage.[12]

The study objectives were to (i) detect the prevalence of H. pylori infection (specially by CagA strains) in male patients with reproductive disorders,(ii) to confirm the presence of anti- H. pylori and anti CagA antibodies(IgG) in serum samples of cases of infertility using serodiagnosis by means of ELISA kits and (iii) to assess the effect of H. pylori infection; specially CagA strains on the quality of semen samples of infected cases.

METHODS

Study design and setting

The current study was conducted in an infertility clinic of a private hospital in Alexandria over a period of 7 months (February to August 2018). It included100 infertile male patients suffering from primary or secondary infertility for ≥ 1 year and attending the clinic for routine semen analysis. Diagnosed cases of infertility suffered from: varicocele, cryptorchism, local infections or hormonal imbalance. Diagnosis was based on radiological and laboratory investigations and clinical examination performed by specialists and all reports were documented. The enrolled patients had none of the following exclusion criteria: fertility problems in the female partner, history of diabetes, radiotherapy, chemotherapy, chronic illnesses or autoimmune disorders.

For each of them a questionnaire covering demographic data, socioeconomic data, lifestyle and dietary habits was completed and they were assured about confidentiality of collected data.

Samples collection and processing

a. Blood samples

Peripheral blood samples were collected by intra-venous puncture and aspiration from the cubital vein. The blood was centrifuged, and the obtained sera were stored at -20°C until examined at the Medical Laboratory Department of Faculty of Allied Medical Sciences, Pharos University, for detection of serum *H. pylori* and CagA IgG antibodies. Repeated freezing and thawing of sera were avoided.

H. pylori IgG status was determined serologically using a commercial enzyme linked immunosorbent assay kit (Accu Bind ELISA micro wells, product code: 1425-300, Monobind Inc, Lake Forest, CA 92630, USA). The reagents were stored closed at 4°C and the assay procedure was carried out according to the manufacturer's instructions. The color change was measured spectrophotometrically at a wavelength of 450nm±2nm.

Quantitative results: Positive results were expressed in units (U), the optical density (OD) values of the 5 calibrators, supplied with the kit, were interplotted as a reference curve on a linear graph paper and the value of the sample was compared to this curve.

Qualitative results: The presence of IgG antibodies to *H. pylori* was considered when the serum level exceeded 20 U/ml (according to manufacturer's recommendations). Specimens with concentrations higher than 100 U/ml were additionally diluted 1:5 or 1:10 with the supplied serum diluent and the final result was recorded after multiplication by the dilution factor.[13]

The presence of CagA IgG antibodies was confirmed using CagA Ig G ELISA Kit (Product Code: GD033, Genesis Diagnostics Ltd, UK). Test procedure steps were performed in order; according to the manufacturer's guidelines.

Quantitative results: The OD of each standard was plotted against its concentration and a curve was drawn through the points. Values above 100 were re-assayed at a higher dilution. The concentration of CagA in the samples was then determined by comparing the OD of the samples to the standard curve.

Qualitative results: Values above the 6.25 U/ml standard were regarded as having significant levels of anti-CagA antibodies.[14]

b. Semen analysis:

Semen samples were collected by masturbation after 4 days of sexual abstinence and examined after liquefaction for 30 min at 37°C. Volume, pH, sperm concentration, and motility were evaluated according to World Health Organization (WHO) guidelines.[15]

Sperm concentration was determined using a Burker counting chamber. Samples were diluted in natrium chloride (0.9% in distilled water), pre-warmed at 37°C and the sperms were counted in 20 square fields under the light microscope. During scoring, the sperm motility was assessed. Reference value for motility indicated by WHO is expressed by the percentage of spermatozoa that are 50% or more motile or 25% or more with progressive motility. The normal values that had been established by the WHO are: sperm concentration > 20 million/ml, and progressive motility > 50%. [15]

Sperm vitality was assessed in semen samples showing a progressive motility <40%. The specimens were stained with 10 µL of 0.5% eosin Y (CI 45380) in a 0.9% aqueous sodium chloride solution. A few minutes after staining, the samples were examined using a light microscope under magnification of 400 X. The stained (dead) cells and unstained (living) cells were scored.[6]

Sperm morphology was assessed by the Papanicolaou (PAP) staining modified for spermatozoa following the WHO guidelines. Morphology was considered normal if 30% or more of sperms were normally shaped. [15]

Statistical analysis:

Collected data were revised and checked for completeness. Data analysis was done using IBM SPSS software package version 20.0.[16] Qualitative data were presented in number and percent. Comparison between various groups regards categorical variables was tested using Chi-square test. When more than 20% of the cells had expected count below 5, Fisher's exact test or Monte Carlo tests were used. Significance of the reported results was calculated at the 5% level ($p< 0.05$).

Ethical considerations:

The study protocol was reviewed and approved by the Ethics Committee of the High Institute of Public Health, Alexandria University. The International Guidelines for Research Ethics and that of the declaration of Helsinki were followed. Informed verbal consent was obtained from were taken from all participants to collect blood samples to investigate their H. pylori infectious status, after explanation of the objectives and benefits of the research. Anonymity and confidentiality of the participants' data were ensured.

RESULTS

One hundred male participants suffering from infertility for ≥ one year were recruited in the current study. The age of the participants ranged from 20 to 75 years old with a mean of 37.46 ± SD 8.33. H. pylori seropositivity among all the participants was 73%. Out of the 73 positive cases for H. pylori IgG, only 60 (82.19%) were CagA positive, while 13 out of the 73 cases (17.81%) were CagA negative. All the 27 cases that were negative for *H. pylori* IgG were also negative for CagA. Although the prevalence of *H. pylori* was higher among participants ≤ 35 years old (74.3%) than among those older than 35 years (35.6 %); yet no statistically significant difference between both categories was reported (p=0.832) (Table 1). A cause that explains the reason for infertility was previously diagnosed in 31 % of cases, while 69 % of cases were idiopathic. *H. pylori* prevalence was higher among the group of patients with idiopathic infertility (79.7%) than among those who had one or more diagnosed causes of infertility (58.1%).The difference was statistically significant; p value= 0.024 (Table 1).

In the present work, 66 % of cases suffered from primary infertility, while 34 % suffered from secondary infertility. Duration of infertility ranged from 1.5 to 23 years with a mean of 8.42 ± SD 4.34. No statistically significant difference between both groups regards their *H. pylori* status was recorded. (Table1).

Residence in rural areas was highly significantly associated with higher prevalence of *H. pylori* among the current cases (85.2% vs. 53.8 %, p= 0.001) (Table 1).

Fifty two percent of the examined patients were classified as of high socioeconomic class, 42% were of average class and only 6% belonged to the low socioeconomic class according to the modified score for social leveling of families.[17]There was no significant association between the socioeconomic standard of the patients and the prevalence of *H. pylori* among them.(Table1).

There was no significant association between the prevalence of *H. pylori* among the participants and some factors as: family history of *H. pylori* infection, smoking (≥ 10 cigarettes/day), drinking coffee and tea, skipping meals, level of education of patients and awareness of *H. pylori* transmission routes (Table 1).

Eating spicy food showed a significant correlation with the prevalence of *H. pylori* among patients. Sixty seven out of the 86 cases who frequently ate spicy food were positive for *H. pylori* (77.9%) compared to 6 cases out of 14; who didn't eat such food (42.9%) (x^2=7.504, p= 0.019) (Table1).

Normal semen profile was recorded in only 19% of the screened samples in the present work, while 81% showed alteration of one or more of the parameters. Sperm count among participants ranged from 0 to 176 x 10^6, with a mean of 37.24± SD 42.10. No statistically significant difference as regards *H. pylori* prevalence was recorded between those having normal semen profile and those with abnormal profile nor between those having different altered parameters of semen analysis. (Table 2 and Table 3). Semen samples were considered as abnormal if one or more parameters as sperm concentration, motility or morphology were altered.

Fifty six out of the 60 CagA positive cases (93.3%) versus 4 out of the 9 CagA negative cases had abnormal semen profile. A high statistically significant difference was recorded between both groups (p<0.001) (Table 4).

Table (1): Relationship between seropositivity for H. pylori and different parameters

	Total	H. pylori				Test of significance	*p*
		+ve (n =73)		-ve (n =27)			
		No.	%	No.	%		
Age (years)							
≤35	35	26	74.3	9	25.7	$\chi2$= 0.045	0.832
>35	65	18	27.7	47	72.3		
Min. – Max.		20.0 – 75.0		22.0 – 52.0			
Mean ± SD.		37.41 ± 8.53		37.59 ± 7.90		t=0.096	0.923
Median		38.0		39.0			
Cause of infertility							
Known	31	18	58.1	13	41.9	$\chi2$= 5.085*	0.024*
Unknown	69	55	79.7	14	20.3		
Type of infertility							
Primary	66	46	69.7	20	30.3	$\chi2$= 1.074	0.300
Secondary	34	27	79.4	7	20.6		
Duration of infertility (years)							
1 – 5 years	31	23	74.2	8	25.8		
6 – 10 years	32	24	75.0	8	25.0	$\chi2$= 1.100	MCp= 0.818
11 – 15 years	35	25	71.4	10	28.6		
>15 years	2	1	50.0	1	50.0		
Min. – Max.		1.50 – 23.0		1.50 – 16.0			
Mean ± SD.		4.27±8.31		4.60±8.70		U= 916.50	0.591
Median		8.0		10.0			
Residence							
Urban	39	21	53.8	18	46.2	$\chi2$= 11.900*	0.001*
Rural	61	52	85.2	9	14.8		
Socio-economic standard							
High	52	38	73.1	14	26.9		
Middle	42	31	73.8	11	26.2	$\chi2$= 0.349	0.877
Low	6	4	66.7	2	33.3		
Family history of H. pylori infection							
Yes	29	21	72.4	8	27.6	$\chi2$= 0.007	0.933
No	71	52	73.2	19	26.8		
Smoking							
Yes	48	35	72.9	13	27.1	$\chi2$= 0.000	0.986
No	52	38	73.1	14	26.9		
Dietary Habits							
Drinking coffee							
Yes	80	61	76.3	19	23.8	$\chi2$= 2.144	0.143
No	20	12	60.0	8	40.0		
Drinking tea							
Yes	92	69	75.0	23	25.0	$\chi2$= 2.334	FEp= 0.206
No	8	4	50.0	4	50.0		
Eating spicy food							
Yes	86	67	77.9	19	22.1	$\chi2$= 7.504*	FEp= 0.019*
No	14	6	42.9	8	57.1		
Skipping meals							
Yes	94	69	73.4	25	26.6	$\chi2$= 0.130	FEp= 0.660
No	6	4	66.7	2	33.3		
Education							
Illiterate	12	10	83.3	2	16.7		
Primary school	17	13	76.5	4	23.5	$\chi2$= 1.363	MCp= 0.727
High school	36	24	66.7	12	33.3		
University	35	26	74.3	9	25.7		
Awareness of transmission routes							
Yes	76	56	73.7	20	26.3	$\chi2$= 0.075	0.784
No	24	17	70.8	7	29.2		

χ^2: Chi square test
FE: Fisher Exact
MC: Monte Carlo
U: Mann Whitney test
p: p value for comparing between the two groups
*: Statistically significant at p≤ 0.05

Table (2): Distribution of the studied cases according to the results of their semen analysis versus their H. pylori status

	N	H. pylori test				Test of Significance	*p*
		+ve (n=73)		-ve (n=27)			
		No.	%	No.	%		
Semen analysis							
Asthenoteratozoospermia	6	1	16.7	5	83.3	$\chi2$=10.277	FEp=8.95
Oligoasthenozoospermia	24	21	87.5	3	12.5	$\chi2$=3.369	0.066
Oligozoospermia	5	2	40.0	3	60.0	$\chi2$=2.908	FEp=0.120
Normal seminal profile	19	13	68.4	6	31.6	$\chi2$=0.250	0.617
Asthenozoospermia	36	28	77.8	8	22.2	$\chi2$=0.651	0.420
Azoospermia	10	8	80.0	2	20.0	$\chi2$=0.276	0.725
Sperm count x10^6							
Min. – Max.		0.0 – 175.0		0.0 – 176.0			
Mean ± SD.		37.41±32.89		51.72±49.0		U=845.00	0.275
Median		21.0		34.0			

χ^2: Chi square test
FE: Fisher Exact
U: Mann Whitney test
p: p value for comparing between the two groups

Table (3): Relationship between the results of semen analysis and H. pylori status of the screened cases

H. pylori status	Semen profile				Total (n = 100)		$\chi2$	FEp
	Normal		Abnormal					
	No.	%	No.	%	No.	%		
+ve	13	17.80	60	82.20	73	73.0	0.250	0.617
-ve	6	22.22	21	77.78	27	27.0		
Total	19	19	81	81	100	100.0		

χ^2: Chi square test
FE: Fisher Exact
p: p value for comparing between the two groups

Table (4): Relationship between the results of semen analysis and CagA status of the screened cases

H. pylori (+ve cases)	Semen profile				Total (n = 73)		$\chi2$	FEp
	Normal		Abnormal					
	No.	%	No.	%	No.	%		
CagA +ve	4	6.67	56	93.33	60	82.2	28.574*	<0.001*
CagA –ve	9	69.23	4	30.77	13	17.8		
Total	13	17.8	60	82.2	73	100.0		

χ^2: Chi square test
FE: Fisher Exact
p: p value for comparing between the two groups
*: Statistically significant at p≤ 0.05

DISCUSSION

H. pylori infection is prevalent throughout the world and more than half of the world population harbors this organism. The prevalence of infection remains >80% in developing countries, while it dramatically declined in the developed countries.[11, 18]

Infection is usually acquired during childhood and is related to socio-demographic factors such as low socio-economic status, poor hygiene, and dietary habits.(19) The most probable mode of transmission is person-to-person spread but oral-oral and fecal-oral transmissions have also been reported. [20]

H. pylori infection is putatively associated with extra-digestive disorders and may also play a role in development of autoimmune diseases. H. pylori can directly or indirectly cause extragastric manifestations through the release of inflammatory mediators and

cytokines, molecular mimicry and systemic immune response.[8, 12]

H. pylori infection, specially by strains expressing the CagA protein, has been proposed as a possible concomitant cause of hypofertility and sperm alterations because it has been associated with reduced motility and an increase in unviable sperms.[7]

Serology is one of the first diagnostic methods for H. pylori infection. Serum ELISA is a rapid, cheap, easy noninvasive screening test for H. pylori infection in absence of endoscopy indication. Unlike other diagnostic methods, its sensitivity is not affected if the patient is under antisecretory therapy.[18]

Because of acceptable sensitivity and specificity rates reported; many commercial IgG-based tests exist and have been validated in recent years.[13,19-21]

The highlighted problem with the serologic approach is its weak distinguishing power to discriminate between active and between asymptomatic colonization and past and current H. pylori infection.[20]

In the present work, serodiagnos is using ELISA technique was the method of choice for screening 100 cases of male infertility. H. pylori seropositivity among all the current participants was 73%. Out of the 73 positive cases for H. pylori IgG , only 60 (82.19%) were CagA positive, while 13 out of the 73 cases (17.81%) were CagA negative. All the 27 cases that were negative for H. pylori IgG were also negative for CagA.

Residence in rural areas was highly significantly associated with higher prevalence of H. pylori among the current cases (85.2% vs. 53.8 %, p= 0.001). This could be attributed to inadequate sanitary conditions and to absence or poor personal hygiene in such areas. This finding is in line with previous studies as those carried out by EL-Kady(2018)[21], Abdallah et al., (2014)[22], Lim et al., (2013)(23), Vilaichone et al., (2013)[24] and Hanafi and Mohamed,(2013).[25] On the other hand, Mohamed et al., (2016)[26], Laszewicz et al., (2014)[27] and Almehdawi and Ali (2016)[28], reported no significant association between residence and prevalence of H. pylori infection.

Currently, there was no significant association between the socioeconomic standard of the patients and the prevalence of H. pylori. This result is coincident with that reported by Mclaughlin et al., (2003)[29] who reported no significant association between the prevalence of H. pylori and the socioeconomic standard in Zambia. On the other hand, this is contradictory to previous studies carried out in Egypt and other countries which proved that the prevalence of H. pylori was higher among those who belonged to the low socioeconomic class.[21,30-33]

Family history of H. pylori infection among the current participants didn't significantly influence the prevalence rate of H. pylori. This is opposite to the previous reports by several investigators who emphasized the role of family history and intrafamilial transmission of H. pylori infection.[21, 33-41] Smoking (≥ 10 cigarettes/day) showed no significant association with H. pylori infection rate among the cases of this study. Similarly, in most studies, no significant association between smoking and H. pylori infection was reported.(28,42-44) Meanwhile, other authors reported that smokers were significantly at higher risk of acquiring H. pylori infection.[21, 25, 45,46] Regards the dietary habits of the participants in the current work; drinking coffee and tea and skipping meals were not significantly implicated to increase the risk of H. pylori infection. Unlikely, these factors were previously reported to have a significant association with H. pylori infection rates.[21, 28,46]

Eating spicy food showed a significant correlation with the prevalence of H. pylori among patients in the present study. Sixty seven out of the 86 cases who frequently ate spicy food were positive for H. pylori (77.9%) compared to 6 cases out 14; who didn't eat such food (42.9%) (x^2=7.504, p= 0.019). This is in line with the findings of Bakka et al., (2009)[47] and opposite to those reported by El-Kady, (2018)(21) and Almehdawi and Ali (2016).[28] No significant association between level of education of patients and H. pylori prevalence rate was found. On the other hand, an inverse association between the level of education and H. pylori infection was reported in previous studies.[21, 48, 49]

Awareness of H. pylori transmission routes by the current participants didn't show a significant association with the prevalence rate of *H. pylori* among them. This is coincident to El-Kady report in 2018[21] and opposite to the previous report by Alebie and Kaba, (2016).[46]Awareness about good personal hygiene and environmental sanitation is the first recommended step towards the control of *H. pylori* contamination of food and water sources .

In general, the seropositivity for *H. pylori* among infertile males in the current work was high (73%) compared to previous reports as these carried out by Moretti et al., (2012) (34.6%)[50], Moretti et al., (2014) (50.8%)[9], Figura *et al*., (2002) (51.8%)[7] and Berwary et al., (2017) (58.9%)[1]. This may be attributed to the fact that most of these previous studies were carried out in developed countries with higher socioeconomic standard of residents and with better sanitary conditions which limit the spread of faecal oral infections in general. *H. pylori* prevalence rate among cases of idiopathic infertility in the present work was 79.7%; which is relatively high in comparison to previous reports as those of Collodel et al., (2010) (45%),[12] Figura et al., (2002)[7] and Dimitrova-Dikanarova et al., (2017),[8];(66.6%) each. A high prevalence rate of 79.4 % was recorded for *H. pylori* among cases with secondary infertility in the current work vs. 69.7% among those with primary infertility. On the other hand, Berwary et al., (2017) [1] reported much lower rates among cases of primary and secondary infertility: 24.03% vs. 20.93%, respectively. This could simply be attributed to the variance in sensitivity and specificity of the diagnostic tools applied in these different studies.

CagA antibodies may be detected in patients who have a negative H. pylori serologic tests since CagA antibodies

can remain positive for a longer period of time than the anti H. pylori antibodies. A negative H. pylori serologic test does not rule out the possibility of a previous infection with H. pylori and anti-CagA antibody alone is not a superior biomarker to the anti-H. pylori antibody alone.[51] Therefore, in the present study all the 100 cases were simultaneously screened for H. pylori and CagA IgG antibodies.

CagA strains were detected in 60% of cases in the current work; and represented 82.2% of all seropositive cases for H. pylori (60 out of 73 cases), while all seronegative cases for H. pylori IgG were simultaneously negative for CagA antibody test.

In earlier studies CagA strains represented relatively lower percentages of H. pylori strains detected: Moretti et al., (2012)(50) (40.7%) and Collodel et al., (2010) [12] (47%). This can be attributed to the fact that those earlier studies were carried out in Italy (developed country); in which CagA strains are significantly less prevalent than in our developing nations.

In previous surveys, it was reported that H. pylori was more prevalent among the infertile population and played a negative influence on sperm motility, viability and morphology; either through increasing the systemic and the semen levels of inflammatory cytokines or by promoting autoimmunity.[8]

In the current work, H. pylori infection didn't significantly affect the quality of semen profile in seropositive cases in comparison to seronegative ones. Meanwhile CagA seropositivity significantly affected the seminal profile. This finding is in line with that reported by Collodel et al., (2010).[12]

Several studies carried out on infertile males emphasized the fact reported in the current work that H. pylori infection specially with CagA strains significantly reduced the semen quality in patients compared to uninfected cases of infertility.[8,12,50]

Detection of anti-H. pylori and anti-CagA antibodies by earlier researchers in the seminal fluid of infected individuals and the existence of cross mimicry between H. pylori and sperm epitopes supported the hypothesis that immune reaction phenomena could take place in semen specimens, with the consequent injury of spermatozoa.[12, 50]

CONCLUSION AND RECOMMENDATIONS

Detection of anti-H. pylori and/or anti CagA IgG antibodies, in serum samples of male cases suffering from Detection of anti-H. pylori and/or anti CagA IgG antibodies, in serum samples of male cases suffering from primary and secondary infertility; especially idiopathic cases, supports the hypothesis that the cross reactivity between spermatozoa antigens and microbial antigens is one of the causes of infertility. It is recommended to conduct further analytical case-control studies to verify the findings on a wider scale and it is also recommended that individuals with reproductive disorders be examined for *H. pylori* infection; with CagA strains in specific.

***Conflict of Interest*:** None to declare

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