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ORIGINAL ARTICLE

Helicobacter pylori cytotoxin-associated gene A genotype in Egyptian patients with Parkinson's disease: could eradication benefit?

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Abstract Strong growing evidence supports a link between *Helicobacter pylori* (*HP*) infections and Parkinson's disease (PD). The purpose of this study was to explore the association between infection with HP *cytotoxin-associated gene A* (*cagA*) genotype and PD in Egyptian patients and the influence of its eradication on pharmacokinetic and clinical response to L-dopa. This study was performed on 87 idiopathic PD patients and 45 age- and sex-matched controls. HP infection was diagnosed with HP stool antigen test and HP *cagA* genotype was detected by PCR. PD patients were subjected to UCLA parkinsonism scale, Hoehn and Yahr staging, and L-dopa plasma level detection by high-performance liquid chromatography. HP PD patients were divided into eradicated group (received standard anti-HP therapy) and placebo group (received antioxidant therapy). Four weeks after therapy, patients were reevaluated. Among PD patients, frequency of HP infection was significantly higher than controls (55.2 vs 33.3%, $P = 0.02$), especially with HP cagA strain (81.2 vs 40%, $P = 0.002$). PD was more severe in HP infected ($P < 0.001$), especially in HP *cagA*-positive patients ($P = 0.04$) with significant lower L-dopa plasma levels ($P < 0.001$). *cagA* strains were associated with higher risk of increased PD severity (OR, 2.139; $P = 0.029$). Opposing antioxidant therapy, HP eradication treatment improved PD severity (UCLA score decreases by 30%) and increased L-dopa level by 40%. There is a proved link between PD and infection with virulent HP *cagA* strains. HP could affect L-dopa level by mechanisms other than reactive oxygen radical's production which need further evaluation.

Keywords *Cytotoxin-associated gene A* · Eradication · *Helicobacter pylori* · Levodopa · Parkinsonism

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Introduction

Parkinson's disease (PD) is recognized as the second most common neurodegenerative disorder after Alzheimer's disease and affects 1% of the population worldwide after the age of 65 years (Tanner and Goldman 1996; Pringsheim et al. 2014). The most common PD symptoms are related to the motor system: bradykinesia/hypokinesia, rigidity, tremor, and postural abnormality (Berg et al. 2013). *Helicobacter pylori* (*HP*) is a wide spread Gram-negative bacterium found on the luminal surface of the duodenal and gastric epithelium infecting about one half of the world population. While HP infection in developed countries ranges from 25 to 40%, it is as high as 90% in some developing countries as Egypt (Hunt et al. 2011; Khedmae et al. 2013).

HP is responsible for many gastric pathologies such as chronic gastritis, peptic ulcer, and gastric cancer as well as a variety of extra-gastric disorders including neurodegenerative, metabolic, and cardiovascular conditions, as well as hepatobiliary, pancreatic, and colorectal diseases (Roubaud-Baudron et al. 2012; Rizk et al. 2016).

Recently, complex interactions between genetic predispositions and exposure to environmental factors, such as toxins

and infectious agents, have been suggested to underlie the selective but widespread and multisystem loss of neurons in PD (Pfeiffer 2009). The hypothesis of association of gastrointestinal microbiota and PD development had been postulated (Cersosimo and Benarroch 2008; Fasano et al. 2013). Chronic HP infection may trigger inflammatory and autoantibody/molecular mimicry mechanisms, which could consequently lead to the destruction of dopaminergic neurons (Dobbs et al. 1999). Interestingly, HP has also been shown to have a role in the bio-synthetic route of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a known neurotoxic to dopaminergic neurons (Altschuler 1996).

Moreover, it is postulated that HP infection affects levodopa (L-dopa) bioavailability, hence motor fluctuation in PD by disrupting the duodenal mucosa or producing reactive oxygen species, which could inactivate the drug (Kankkunen et al. 2002).

Two putative markers of HP virulence, the *cytotoxin-associated gene* (*cagA*) pathogenicity island and the *vacuolating cytotoxin genes* (*vacA*), have been extensively studied. The highly virulent HP *cagA* strains were directly proportional to the severity of many extra-gastric diseases as well as the risks for ischemic stroke and atherosclerotic stroke (Esmat et al. 2012; Franceschi et al. 2014). However, there are no detailed studies on the distribution and association of HP *cagA* genotype in PD patients yet.

We aimed at studying the frequency of HP *cagA*-positive strains among Egyptian PD patients. Moreover, the impact of its eradication on both plasma level of L-dopa and the clinical response was addressed.

Materials and methods

Study subjects

This is a prospective, randomized, placebo-controlled, parallel-group study carried out from October 2013 to August 2015 on 87 idiopathic PD patients attending outpatient clinic at neurology and neurosurgery center in Mansoura University. Forty-five age- and sex-matched participants who were neither complaining of neurological disease nor relatives to Parkinson's disease patients were included as controls for this study.

Eligible patients for this study were adult whose age was equal or more than 18 years and diagnosed as having idiopathic Parkinson's disease according to UK Parkinson's Disease Society Brain Bank criteria. They were also required to have no associated neurologic diseases other than PD, no use of antiparkinsonian other than L-dopa ± anticholinergics, and no use of any drug potentially affecting gastrointestinal motility. We excluded those with secondary parkinsonism or Parkinson's plus syndrome and those with a recent history of medication with proton pump inhibitors or histamine antagonist (for at least 4 weeks) or antibiotics ((last 30 days prior to inclusion). Patients were evaluated twice as follows:

First visit At the beginning of the study, all PD patients fulfilling the inclusion criteria were subjected to baseline evaluations including demographic characteristics (age and sex), previous antibiotic use, medical and medication history, neurological clinical assessments, diagnosis of HP infection by stool antigen test, and determination of their baseline plasma level of L-dopa. Thereafter, PD patients diagnosed with active HP infection were randomly allocated, using a random computer-generated code in blocks of five, to either eradicated or placebo (antioxidant) group.

Eradicated group Were assigned to receive triple therapy for eradication of H. pylori (omeprazole 20 mg, clarithromycin 500 mg and amoxicillin 1 g) twice daily for 14 days.

Placebo group Were assigned to receive antioxidant therapy (selenium 55 μg, vitamin A 1500 IU, vitamin C 90 mg, and vitamin E 22 IU) twice daily for 14 days.

Second visit Four weeks after the end of intervention therapy, successful eradication of HP was confirmed by absence of H. pylori antigen in stool. Then, all patients in both groups were neurologically re-examined and their plasma levels of L-dopa were measured.

Neurological examinations

Clinical assessment of PD before and after intervention therapy was done by a single examiner to reduce inter-observer variability. PD severity was assessed using UCLA parkinsonism scale, while staging of the PD was performed according to Hoehn and Yahr stages (Markham and Diamond 1981; Hoehn and Yahr 1967). The UCLA parkinsonism scale is of sufficient validity and reliability and is sensitive enough to detect changes within the frame of L-dopa therapy (McDowell et al. 1970). It consists of 21 sub-items, 14 of them are done by clinical examination of the patient and 7 sub-items are determined by questioning the patient or family members. Each sub-item is individually weighted. For example, "masked facies" has the weighting factor 1 and speech or gait has10. This factor is then multiplied by 0 if "absent," 1 for "present," or 2 for "marked." The total disability score is the sum of all subscores and ranges from 0 to 220 (0 = no impairment and 220 = severest impairment) (Markham and Diamond 1981). Clinical fluctuations, dyskinesias, tremors, and rigidity were assessed by using the Unified PD Rating Scale, section III (UPDRS-III) (Ramaker et al. 2002). The motor scoring was done in medication *on* state. Clinical response to L-dopa was assessed by recording the L-dopa onset time and the duration of *on* time in minutes.

Laboratory diagnosis of *H. pylori* infection

H. pylori screening test

We used the noninvasive stool antigen test (SAT) to screen both PD patients and controls for active HP infection and considering the validity of eradication treatment status as recommended by Saha et al. (2016). They proved stool antigen test of having high sensitivity and specificity against gold standard tests such as histology, culture, and [13C] urea breath and considered it the most convenient way for diagnosing the active HP infection.

All enrolled patients and controls were screened by analysis of their stool using an immunochromatographic HP stool antigen test (Cal-Tech Diagnostics, Inc., Chino, CA, USA) according to the manufacturer's instructions. This test was repeated 4 weeks following eradication therapy to confirm successful eradication.

Molecular detection of HP cagA strains (Agha et al. 2013)

All stool samples that showed positivity for HP antigen by the immunochromatographic rapid assay were candidates for detection of the presence of cagA gene as follows:

DNA extraction Done using the QIAamp DNA stool minikit (Qiagen) according to the manufacturer's instructions.

Amplification and detection of *cagA* gene by conventional PCR A 349-bp target sequence from *cagA* gene was amplified using two primers: forward primer P1: 5′-GATA ACAGGCAAGCTTTTGAGG-3′ and reverse primer P2: 5′-CTGCAAAAGATTGTTTGCGAGA-3′. The PCR was performed in a total volume of 50 μL of master mix (EzWay PCR Master Mix, Koma Biotech, Seoul, Korea) containing 1 μL of the extracted DNA and 0.5 μM of each primer.

High-performance liquid chromatography analysis of L-dopa (Muzzi et al. 2008)

One hour after the oral administration of levodopa, 2 mL of venous blood were withdrawn into EDTA tubes from PD patients and were centrifuged (2000*g*, 5 min, 4 °C) immediately. Then protein precipitation was performed by perchloric acid and plasma samples were frozen (−80 °C) until analysis.

L-Dopa concentration was determined by high-performance liquid chromatograph of 1260 series hp chemstation software (Agilent, USA) equipped with UV detector adjusted at 220 nm, autosampler, quaternary pump, and a ready-to-use prepacked C-18 column (15 cm, 4.6 mm I.D.). Mobile phase was 0.05 M potassium dihydrogen orthophosphate with PH adjusted at 3.5. Stationary phase was 5 μm base deactivated silica. Flow rate was 1.0 mL/min, the injection volume was 20 μl at 7 °C autosampler temperature, and the run time was 6.0 min.

Calibration curves were constructed by serial dilutions of stock solution of L-dopa standard (1.97 mg/mL) with distilled water. The range of assay was 0.01–80 mg/L (Fig. 1).

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16. Qualitative data was presented as number and percentage. Quantitative data was presented as mean and standard deviation. The chi-square (χ^2) was used to find the association between variables of qualitative data. Student's *t* test was used to compare the numerical data between two groups. Univariate and multivariate regression tests were used to examine the effects of potential confounding factors on PD severity. *P* value of ≤0.05 indicated a significant result.

Results

Eighty-seven PD patients and 45 healthy individuals fulfilled the inclusion criteria for this study. They were of matched age and gender (*P* = 0.2, *P* = 0.9). We revealed that frequency of HP infection are significantly higher among PD patients (55.2%) than among controls (33.3%), *P* = 0.02. Also, HP *cagA* strains were significantly more frequent among PD patients than control group, 81.2 versus 40%, *P* = 0.002 (Table 1).

Baseline neurological evaluations of PD patients showed that severity of PD had not affected only by HP status of the patients but also by the presence or absence of *cagA* gene (Table 2). PD was more severe in the HP-positive patients as compared to the HP-negative patients (*P* < 0.001). Also, it was significantly more severe in HP *cagA*-positive patients than HP *cagA*-negative patients as shown by their UCLA scales (111.1 ± 26.2 vs 83 ± 23, *P* = 0.04). HP *cagA*-positive patients suffered also more tremors than HP *cagA*-negative patients (*P* = 0.001).

Despite the insignificant difference in the daily dose of L-dopa between HP-positive and HP-negative PD patients, we observed that HP-negative PD patients responded in a better way to L-dopa as evidenced by their faster "on" time (23.5 + 4.2 min) and their significant higher plasma level of L-dopa (0.8 ± 0.26 mg/L) as compared to HP-positive patients (28.1 ± 4.7 min and 0.6 ± 0.28 mg/L).

In spite that HP *cagA*-positive PD patients treated with a significantly higher dose of L-dopa than HP *cagA*-negative PD patients (613.5 ± 213.4 vs 450 ± 112.5, *P* = 0.03), we observed that their onset of response to L-dopa dose did not differ significantly from HP *cagA*-negative patients (*P* = 0.3) and their

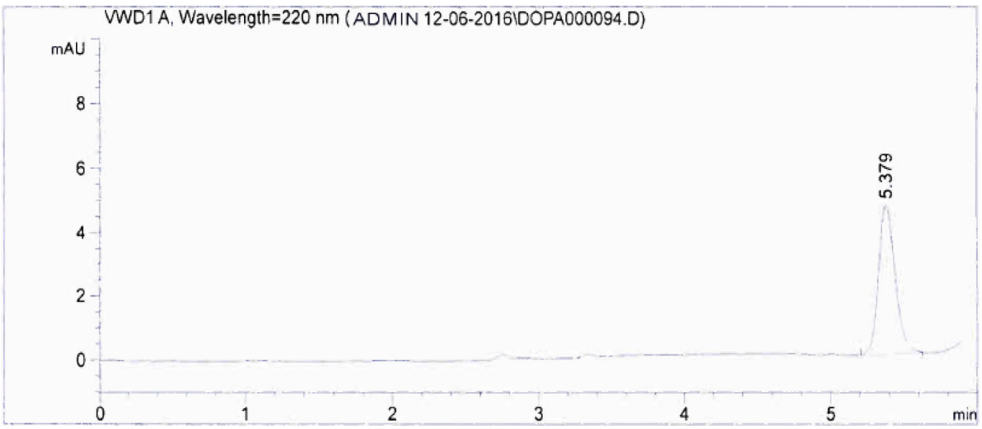

Fig. 1 Chromatograms of an internal standard at concentration 3 mg/L (injection volume 0.3 μL)

plasma level of L-dopa was still significantly lower than HP *cagA*-negative patients (0.5 ± 0.22, 0.9 ± 0.2, $P < 0.001$).

Four weeks after the end of this intervention therapy, all of 27 patients assigned to anti-HP treatment successfully eradicated HP but none of patients received antioxidant therapy changed to HP negative. As shown in Table 3, among eradicated group, the total UCLA score was significantly decreased after successful HP eradication as compared to their baseline score (83.1 ± 19.2 vs 120.2 ± 35.8, $P < 0.001$). Also, their motor symptoms in the form of tremors, fluctuations, and akinesia were improved significantly in comparison to their baseline symptoms. On the contrary, none of the patients randomized to antioxidant therapy showed clinical improvement from their baseline.

After intervention therapy, while plasma level of L-dopa seemed to increase about 40% after anti-HP treatment, it did not change and remained the same after antioxidant therapy (Fig. 2).

Table 1 Demographic data of studied population

	PD ($N = 87$)	Control ($N = 45$)	P value
Age mean ± SD	61.8 ± 8.3	59.8 ± 6.3	0.2
(Range)	(32–75)	(42–71)	
Gender *N* (%)			
Male	69 (79.3)	36(80)	0.9
Female	18 (20.7)	9 (20)	
H. pylori status *N* (%)			
Negative	39 (44.8)	30 (66.7)	0.02
Positive	48 (55.2)	15 (33.3)	
cagA positive	39/48 (81.2)	6/15 (40)	0.002
cagA negative	9/48 (18.8)	9/15 (60)	
Plasma level of L-dopa (mg/L)			
Mean ± SD	0.7 ± 0.3	0.03 ± 0.01	<0.001
(Range)	(0.2–1.5)	(0.01–0.05)	

P significant at ≤0.05

PD Parkinson's disease, *SD* standard deviation, *cagA* cytotoxin-associated gene A

Linear regression analysis was used for prediction of severity in all studied patients using age, sex, L-dopa level, disease duration, and *cagA* as covariates. Female gender, low L-dopa level, disease duration, and *cagA p*ositive were associated with higher risk of increased severity in univariate analysis, while in multivariate analysis, only *cagA* positive was associated with higher risk of increased severity, odds ratio 2.139, 95% CI 1.943–4.852, $P = 0.029$ (Table 4).

Discussion

Helicobacter pylori (*HP*) infection is the most widespread gastrointestinal infection in the world and depends on age and socioeconomic status (Azevedo et al. 2009). Confirming it as a causal agent of many extra-gastric pathologies, we carried out our study on Egyptian Parkinson's disease (PD) and revealed that frequency of HP infection was significantly higher among PD patients than healthy control subjects. This is consistent with findings from previous studies reported 36, 53, 70, and 32% prevalence of HP in PD patients (Pierantozzi et al. 2006; Lee et al. 2008; Dobbs et al. 2010; Tan et al. 2015). Furthermore, a previous case-control study conducted in our locality (Mansoura City, Egypt) on a different cohort of PD patients revealed a prevalence of 46% (Esmael et al. 2016).

Despite that several hypotheses have been postulated to explain the high frequency of HP among PD, it remains unclear why PD patients have higher tendency to develop this infection. HP could produce an inflammatory state, induces autoantibody/molecular mimicry mechanisms, which could consequently lead to the destruction of dopaminergic neurons and/or causing apoptosis of nerve cells via circulating monocytes (Dufek et al. 2015; Hirsch and Hunot 2009).

In addition to the high prevalence, HP infection affected our patients' motor performances adversely. We showed that the severity of parkinsonism was higher in HP-infected patients than HP negative as clarified by clinical scales. This finding is matched with many studies addressing the severity

Table 2 Effect of *H. pylori* and its *cagA* genotype on clinical symptoms and plasma level of L-dopa of Parkinson's disease patients

	HP positive			HP negative ($N = 39$)
	cagA positive ($N = 39$)	cagA negative ($N = 9$)	Total ($N = 48$)	
Disease duration (years)				
Mean ± SD	5.1 ± 3.1	3. 6 ± 0.5	4.8 + 2.8	4.3 ± 2.8
H-Y staging *n* (%)				
Stage 1	15 (38.5)	3 (33.3)[b]	18 (37.5)	6 (15.4)[a]
Stage 2	0	3 (33.3)	3 (6.2)	24 (61.5)
Stage 3	18 (46.2)	0	18 (37.5)	6 (15.4)
Stage 4	6 (15.4)	3 (33.3)	9 (18.8)	3 (7.7)
Stage 5	0	0	0	0
UCLA scale	111.1 ± 26.2	83 ± 23[b]	105.8 ± 18.7	78.9 ± 13.2[a]
Fluctuations (% of time day)	48.1 ± 11	41.7 ± 15	46.88 ± 10.6	36.54 ± 10.8[a]
Rigidity	10.8 ± 3.5	9.3 ± 2.5	10.5 ± 3.5	9.69 ± 3.45
Tremors	8.1 ± 2.5	5 ± 0[b]	7.5 ± 2.52	7.69 ± 2.52
Akinesia	13.2 ± 4.5	12 ± 4.5	12.94 ± 4.51	12.46 ± 4.43
Onset of on time (min)	28.5 ± 4.6	26.7 ± 5.0	28.1 ± 4.7	23.5 ± 4.2[a]
Duration of on time (min)	214.6 ± 62.5	280 ± 30[b]	226.9 ± 23.1	240 ± 22.1
Total dose of L-dopa /day (mg)	613.5 ± 213.4	450 ± 112.5[b]	582.8 ± 27.7	550 ± 22.1
Plasma level of L-dopa (mg/L)				
Mean ± SD	0.5 ± 0.22	0.9 ± 0.2[b]	0.6 ± 0.28	0.8 ± 0.26[a]
(Range)	(0.2–1.1)	(0.8–1.2)	(0.2–1.2)	(0.5–1.5)

All data are expressed as mean ± SD except for staging as percentage

HP H. pylori, *cagA* cytotoxin-associated gene A, *SD* standard deviation, *H-Y* Hoehn and Yahr, *min* minute

[a] Significant difference between HP positive and HP negative at $P \leq 0.05$

[b] Significant difference between cagA positive and cagA negative at $P \leq 0.05$

Table 3 Effect of *H. pylori* eradication and antioxidant therapy on HP-positive Parkinson's disease patients

	Eradicated group ($N = 27$)			Antioxidant group ($N = 21$)		
	Before anti-HP therapy	After anti-HP therapy	*P*	Before antioxidant therapy	After antioxidant therapy	*P*
UCLA scale	120.2 ± 35	83.1 ± 19.2	0.000	87.3 ± 35.8	86.19 ± 34.603	0.110
Fluctuations (% of time day)	47.2 ± 22.3	33.3 ± 12	0.002	46.4 ± 21.3	46.33 ± 21.207	0.162
Rigidity	10.9 ± 3.5	7 ± 0	0.000	10 ± 3.6	9.81 ± 3.356	0.104
Tremors	7.2 ± 2.5	5 ± 0	0.000	7 ± 2.5	7.62 ± 2.397	0.096
Akinesia	14 ± 4.6	9 ± 0	0.000	11.6 ± 4.2	11.24 ± 3.673	0.069
Onset of on time (min)	30 ± 4.2	20.6 ± 1.6	0.000	25.7 ± 4.3	25.67 ± 4.223	0.329
Duration of on time (min)	185.6 ± 50.9	288.9 ± 51.8	0.000	280 ± 26.8	282.86 ± 28.49	0.083
Plasma level of L-dopa (mg/L)			0.000			0.3
Mean ± SD	0.5 ± 0.2	0.7 ± 0.3		0.6 ± 0.2	0.6 ± 0.2	
(Range)	(0.2–1.1)	(0.2–1.5)		(0.2–1.2)	(0.2–1.3)	

All data are expressed as mean ± SD. *P* significant at ≤0.05

HP H. pylori, *min* minute, *SD* standard deviation

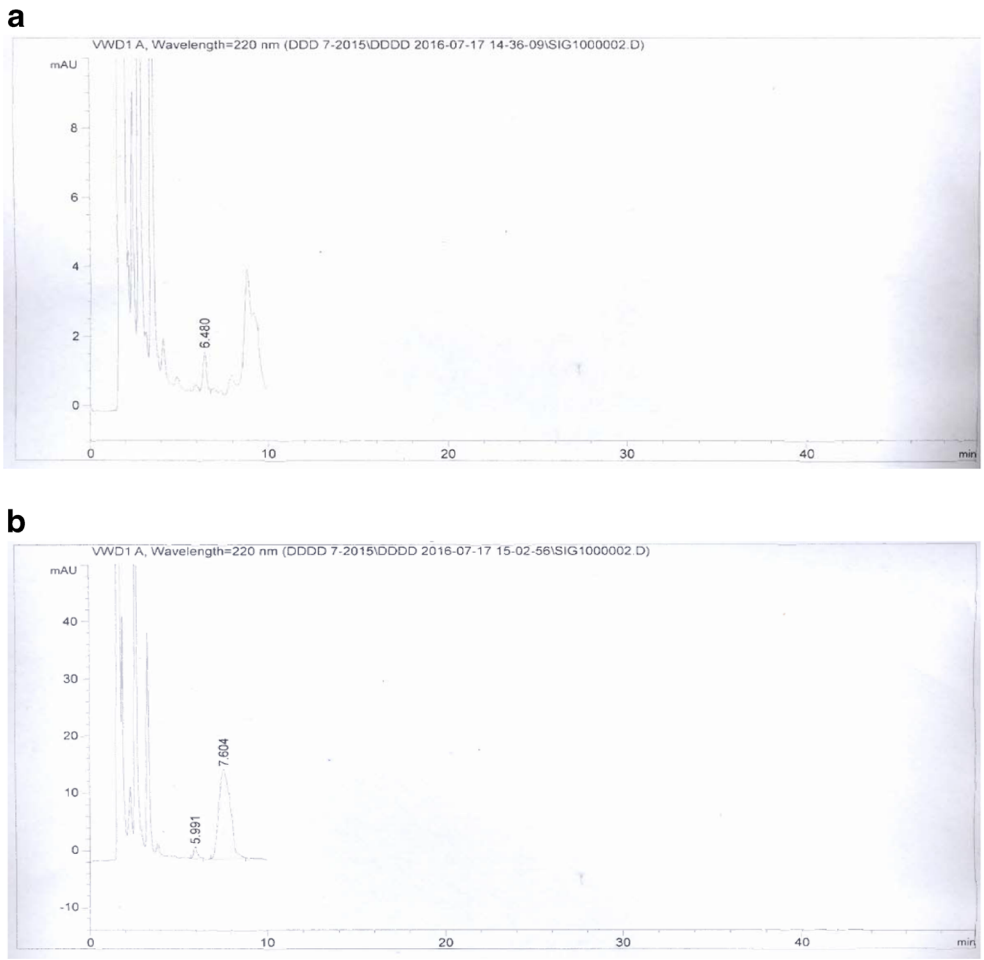

Fig. 2 Chromatograms of a patient sample: **a** before treatment and **b** 2 h after the oral administration of L-dopa (375 mg), showing concentrations of 1.43 and 2.32 mg/L, respectively

of Parkinson's diseases and HP infection (Tan et al. 2015; Tsuboi and Yamada 2008).

Our observation of a lower plasma level of L-dopa in HP infected than non-infected patients despite being matched for the total L-dopa dosage could be attributed to many hypothesis like the following: HP infection affects L-dopa absorption, inactivation of the drug by local production of reactive oxygen species, increased consumption of L-dopa by the bacteria, or delayed delivery into the duodenum (Deretzi et al. 2011; Tan et al. 2014).

To our knowledge, no previous study has assessed the possible role of *cagA* virulent HP strains in PD. Apart from Weller et al. (2005) who explored the extra-alimentary consequence of HP and concluded that persistence of serum *cagA* antibodies was predictive for PD and associated with a poor prognosis.

In the same context, our study showed that infection by the virulent HP *cagA* strains were significantly more frequent among Egyptian PD patients (81%), whereas *cagA*-negative strains have a more prevalence in controls (66%).

Table 4 Prediction of Parkinson's disease severity (UCLA score) in *H. pylori*-positive patients

	Univariate			Multivariate		
	P value	OR	95% CI	*P* value	OR	95% CI
Age	0.150	1.894				
Sex (male/female)	0.001	1.372	1.147–1.642	0.793	1.049	0.735–1.496
Disease duration (year)	0.025	1.088	1.011–1.171	0.265	1.285	0.126–1.466
Plasma level of L-dopa (mg/L)	<0.001	0.607	0.475–0.776	0.089	2.615	0.593–7.467
cagA (positive/ negative)	<0.001	1.741	1.346--2.252	0.029	2.139	1.943–4.852

P significant at ≤0.05

OR odd ratio, *CI* confidence interval, *cagA* cytotoxin-associated gene A

Interestingly, HP *cagA*-positive strains were associated with higher risk of increased severity in PD patients confirming the hypothesis that the association between HP and PD is related to the virulence of this bacterium. Also, HP *cagA*-positive patients suffered more aggressive tremors than HP *cagA*-negative patients. However, fluctuations, rigidity, and akinesia did not differ significantly among HP-infected PD patients according to their *cagA* gene status.

Moreover, in our study, although HP *cagA*-positive PD patients received a significantly higher daily dose of L-dopa than HP *cagA*-negative patients, we found that the onset of response to the daily total dose of L-dopa (*on* time) did not differ significantly between HP *cagA*-positive and HP *cagA*-negative patients and plasma level of L-dopa was significantly lower in *cagA* positive and consequently duration of this "on" time state was significantly more shorter in HP *cagA*-positive patients than HP *cagA*-negative patients.

These findings could raise the possibility that implication of HP in the interference with L-dopa absorbance is due to the presence of *cagA* gene as a virulent factor in this bacteria. Recently, Shimoda et al. 2016 reported that *cagA* is present as exosomes secreted from gastric epithelial cells of infected patients. These exosomes may enter into circulation and deliver *cagA* to distant organs that may provoke more systemic clinical effects that are seemingly unrelated to the primary infection (Shimoda et al. 2016).

Our study was performed with two arms: HP eradication arm and placebo arm who received antioxidants drugs. Eradication of HP clarified a significant overall improvement in the motor severity score (UCLA score decreases by 30%), increased plasma level of L-dopa by about 40%, the mean L-dopa onset time shortened by 10 min (33%), and the mean duration of "on" time increased by 55 min (55%).

Most of publications considering eradication issues agreed with this finding and reported an increase of 21%, 54% in the plasma level of L-dopa after successful cure of HP infection in PD patients ((Pierantozzi et al. 2006; Lahner et al. 2009). Improved hypokinesia following antimicrobials appeared unique to *Helicobacter* eradication while rigidity worsened while antimicrobials for other indications had no effect (Dobbs et al. 2013).

Based on clinical evaluation, the current antioxidant-treated patient results failed to support the hypothesis that HP may affect L-dopa pharmacokinetics by reactive oxygen radical's production (Drake et al. 1998).

Some authors reported an exacerbation of rigidity and increase in tremor associated with antimicrobial exposure and explained it by an altered admixture of intestinal microbiota that drive different pathogenic processes concerning motor manifestation of Parkinson's disease. Those findings gave an idea of paradigm shift from cold neurodegeneration to microbe-triggered/microbe-driven immunoinflammatory processes (Dobbs et al. 2010, 2013; Charlett et al. 2009). Tan et al. (2014) confirmed the hypothesis of neuroinflammation secondary to increased gastrointestinal microbiota (Tan et al. 2014).

Strengths of this study include recruitment of a relatively large number of subjects with a matched healthy control group. Patients underwent blinded evaluations, including objective and quantitative measures of motor function and plasma L-dopa level. Addressing the HP *cagA* strain effect on Parkinson severity and plasma L-dopa level occur for the first time up to our knowledge.

There are several limitations that need to be highlighted. Longer duration of follow up to 6 months post treatment is more convenient. Post eradication improvement in L-dopa absorption can be evaluated by pharmacokinetic sampling for measuring pharmacokinetics indices (area under the curve (AUC) and time to maximum plasma levodopa concentration (Cmax); however, we used only one sample for plasma L-dopa and we evaluated this improvement indirectly via the modification of plasma L-dopa concentration and through clinical scores. It was better to investigate the effect of administrating selenium, vitamin A, vitamin C, and vitamin E on the level of oxidative stress by objective quantitative measurement.

Conclusion

The current results of our study suggest higher frequency of HP infection in PD patients. Moreover, HP infection increase the severity of PD and it affect motor symptoms. Additionally, virulent HP *cagA*-positive infection could play a relevant role in daily clinical variations in PD. Eradication of HP improves L-dopa absorption and the overall severity of PD specifically tremors. Therefore, PD patients should be investigated for HP infection and specific antibiotic therapy against HP should be recommended for HP-positive PD patients (especially those with *cagA* positive) even in absence of dyspeptic symptoms. The relationship between HP and PD should be further clarified by more comprehensive studies in the future, especially the exact mechanism by which chronic HP infection could affect L-dopa absorption in PD patients.

Compliance with ethical standards

Ethical standards Informed consents were obtained from all participants. The local ethics committee, Mansoura University ethical committee (IRB), approved our study with a code number of R/16.07.56 and thus performed in accordance with the Declaration of Helsinki.

Conflict of interest All authors declare that they have no competing interests.

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