Molecular Detection of β-Giardin Gene of *Giardia lamblia* in Peptic Ulcer Patient in Babylon Province

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Abstract

background: *Giardia lamblia* is one of the commonest human intestinal protozoa, it cosmopolitan and is endemic throughout the world with a wide range of mammalian hosts.
Objective: This object aimed to focus on molecular detection of β-Giarin gene of *Giardia lamblia* in patient with peptic ulcer to investigate an association between *Giardia lamblia* and *Helicobacter pylori* infection.
Methods: a total of 64 stool sample were collected from patients attending to endoscopy unit (specialized Marjan Hospital for Internal and Cardiac Diseases in Babyoln province) for their peptic ulcer symptoms And were positive for *H. pylori*. Polymerase chain reaction was achived by primers that targeted the (β-Giardin) gene (218pb) of *Giardia lamblia*.
***Results*:** in this study 64 stool sample were collected from patients with peptic ulcer, *Giardia lamblia* infection was found in 12 (18.7%) according to direct examination of stool sample, while 21 (32.8%) was positive to giardiasis when used PCR for detection of β-Giardin gene (218pb) ,also the percentage of infection in male was significantly ($p < 0.05$) higher than female with giardiasis infection isolated from peptic ulcer patients and the most frequent symptom was heartburn (15.6%) and diarrhea (9.3%), followed by abdominal pain (7.8%).
***Conclusion*:** from the results of this study we conclude that *Giardia lamblia* is high in patients with peptic ulcer. To investigate the causes of peptic ulcer, it should be considered the *Giardia lamblia* infection as an etiologic factor. This approach will be useful in diagnosis and management of peptic ulcer

Keywords*: Giardia lamblia* , polymerase chain reaction, β-giardin gene.

Introduction

Giardia lamblia consder the most common protozoan parasite isolate from gastrointestinal tract, worldwide incidence is belived to range from 20% to 60% and responsible for an estimated 2.0 million case per year[1]. The incidence of this parasite is high in developing countries as poor sanitary condition favors the contamination of water and food with infective cysts.

Giardiasis ordinarily occurs when eating contamenated foods with fecal material containing cysts, the infected person can by asymptomatic or may have extraintestinal symptoms, such as fever, diarrhea, abdominal pain, bloating and weight loss resulting from malabsorption [2]. most of this symptoms are similar with

presenting in patients with dyspepsia, also recently studies found that *Giardia lamblia* infection has been found to trigger abdominal symptom or dyspepsia[3]. The diagnosis of *Giardia lamblia* has been classically on detection of cysts or trophozoite in stool and duodenal aspirates specimen by direct microscopic examination and duodenal endoscopic biopsy specimen by histologically[4].recently, molecular detection methods depended on polymerase chain reaction have been developed to investigate *Giardia labmlia* in stool sample, these methods have advantages in sensitivity and specificity in compared with conventional methods[5].

Gastric giardiasis was reported by many authors[6], changes in gastric environment are essential prerequisites for occurrence of such colonization such as increase in gastric pH or intestinal metaplasia of the gastric mucosa , both conditions are well known complication of *H pylori* infection. Moreover, concomitant *H. pylori* and *Giardia lamblia* infection is common for their similar route of transmission and there is a strong association to socioeconomic levels [6].

Helicobacter pylori is a Gr^{-ve} microaerophilic bacterium, which infects more than 50% of human population, it cause of acute and chronic gastritis, peptic ulcer and atrophic gastritis [7]. A virulent of *H. pylori* would be more likely to cause atrophic gastritis during the childhood period . many results [6,7]show that co-infection of H. pylori with Giardia lamblia infection, they confirm a correlation between *H. pylori* infection and the precence of *G. lamblia* in stool sample. Also [8] found that urease activity was significantly elevate in the patients infected with (*Giardia lambila* and *H. pylori*) in compare with the patients infected with *Giardia lamblia* alone , so this co-infection may be due to an increase risk of *Giardia lamblia* colonization upon the presence of *H. pylori* in patients or alternatively, *H. pylori* colonization may be facilitated by a previous establishment of *Giardia lamblia*.

So the present study design to focus on molecular detection of β-Giardin gene of *Giardia lamblia* in patient with peptic ulcer to investigate an association between *Giardia lamblia* and *Helicobacter pylori* infection.

Material and Methods

1-Sample collection:

During the period from February to August 2015, sixty four (43males/21females) stool sample were collected from patients attending to endoscopy unit(specialized Marjan Hospital for Internal and Cardiac Diseases in Babyoln province) for their peptic ulcer symptoms (epigastric pain, heartburn and diarrhea), And were positive for *H. pylori*. samples were examined immediately after collection using normal saline and lugol's iodine wet mount preparation. Then all samples were stored at (–20)°C until used for molecular study.

2-DNA extraction and amplification:

Stool samples were washed three time in sterile distilled water (D.W) according to the manufacturer's prescript, a modified QIA amp DNA stool Mini Kit (Qiagen, USA) was used to detecte DNA from the stored stool specimens.

Polymerase chain reaction was done with specific primers targeted the β-Giardin gene (218pb) of *Giardia lamblia* (5- CATAAGGACGCCATCGCGGCTCTCAGG3- and 5- TTTGTGAGCGCTTCTGTCGTGGCAGCGCTA3- . The amplification were performed in a 25μL volume that contained 12.5 μL 2x PCR master mix, 2 μL of each primer, 5 μL of genomic template DNA and the volume was complete by added DDH2O.

The following PCR cycle was used: an initial denaturation at 95°C for 5 minute, 35 subsequent cycle of denaturation at 94°C for 30second, annealing at 55°C for 30 second and extenstion at 72°C for 7 minute. PCR products then visualized by electrophoresis on a 2% agarose gel containing ethidium bromide.

Results and Discussion

Giardiasis is a common infection in worldwide, the prevalence rate was reported 2-7% as in developed and 40% in developing countries, respectively [1]. The prevalence was higher in the countries that lack proper sanitation and hygienic conditions than other parts of the world. Also peptic ulcer was distinct clinical entity which affected a very large population worldwide.

In this study, microscopical and molecular methodes were done on stool samples collected from patients with peptic ulcer. *Giardia lamblia* was recorded in 12 (18.7%) of the 64 patients with peptic ulcer according to direct examination of stool sample table (1), while 21 (32.8%) was positive to giardiasis when used PCR for detection of β-Giardin gene (218pb) of *Giardia lamblia* (figure 1).

Molecular methods have high sensitivity against conventional methods, many studies [9] showed that PCR technique is sensitive method can be used in diagnosis of *Giardia lamblia*, also conventional methods using microscopy, biochemistry and immunological approaches have serious restrictions such as essential need to a high degree of experience in the diagnosis of *Giardia lamblia* . Other study[10] confirmed that giardiasis was high significant correlation with *H. pylori* infection, may be the two organisms have a number of risk factors, this correlation may have numerous clinical implications in regard to the transmission route [11], the possibility of a synergy in metronidazole resistance and the experiment evidence of a common pathogenesis scenario, leading to gastrointestinal metaplasia[6].

The results of this study show the percentage of infection in male was significantly ($p < 0.05$) higher than female with giardiasis infection isolated from peptic ulcer patients (table 1). This may be due to that males are mostly outside their houses and mostly exposed to transmitted source, This results agree with [12. 13] who recorded that males have more rate of infection with giardiasis than female. *Giardia lamblia* infection in human has a wide range of clinical manifestation from asymptomatic to serious diarrhea, in this study, the most frequent symptom was heartburn (15.6%) and diarrhea (9.3%), followed by abdominal pain (7.8%) table(2). The results of our study was agree with previously [14] published studies. In the present study heartburn was found in 22 out of 64 patients, this possibly due to the coexistence of *Giardia lamblia* and *H. pylori* infection in the same host. urase produce from *H. pylori* have an important role by producing ammonia that neutralize the gastric acidity

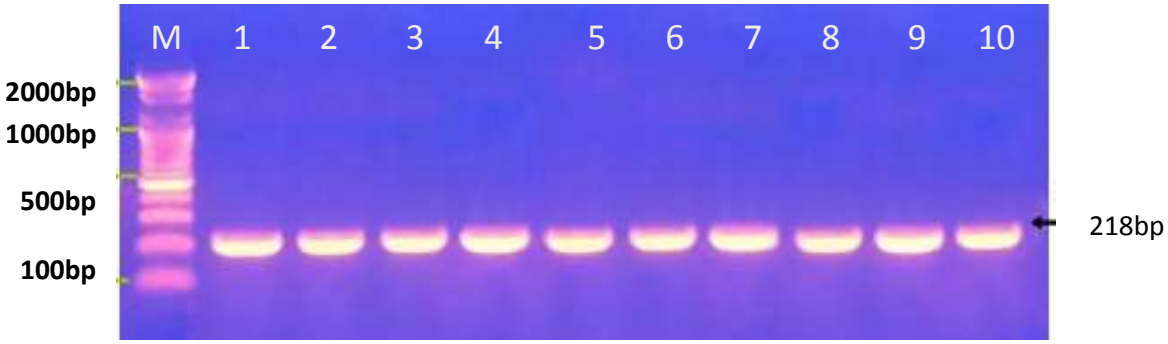
[15], in addition to the alkaline pH conseder a suitable environment to *G. lamblia* presence. So, the combination between *G. lamblia* and *H. pylori* infection indicates a significant action, they both predispose to one another [8].

Table(1): Distribution of *Giardia lamblia* infection according to sex.

Sex	No. of patients	No. of positive	
		Direct exam. (%)	PCR (%)
Male	39	8 (12.5)	14(21.8)
Female	25	4(6.2)	7(10.9)
Total	64	12(18.7)	21(32.8)

Table(2): Distribution of *Giardia lamblia* infection according to symptoms.

Symptoms	No. of patients	No. of positive	
		Direct exam.(%)	PCR(%)
Abdominal pain	13	2(3.1)	5(7.8)
Heartburn	22	4(6.2)	10(15.6)
Diarrhea	29	6(9.3)	6(9.3)
Total	64	12(18.7)	21(32.8)



Figure(1): Agarose gel electrophoresis for PCR product for detected β- Giardin gene. Electrophoresis was performed on 2 % agarose gel and run with a 5volt/cm for 1.30 hr. M 100bp ladder. Line (1) control positive. Line (2,3,4,5,6,7,8,9 and 10) positive results.

In conclusion, the incidence of *Giardia lamblia* is high in patients with peptic ulcer. To investigate the causes of peptic ulcer, it should be considered the *Giardia lamblia* infection as an etiologic factor. This approach will be useful in diagnosis and management of peptic ulcer.

Conflict of Interests.
There are non-conflicts of interest .

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الخلاصة

الخلفية: الجيارديا اللامبليا واحدة من اكثر الطفيليات المعوية للانسان انتشارا فهي عالمية الانتشار وبشكل وبائي في جميع انحاء العالم مع مجموعة واسعة من المضائف الثدية.

الهدف: الكشف الجزيئي لجين بيتا–جياردين في مرضى القرحة الهضمية للتحقق من وجود علاقة بين الاصابة بالجيارديا اللامبليا مع بكتريا الملوية البوابية.

طرائق العمل: ما مجموعة 64 عينه براز جمعت من مرضى القرحة الهضمية المراجعين لوحدة الناضور الداخلي (مستشفى مرجان التخصصي لامراض القلب والباطنية في محافظة بابل),ممن لديهم اعراض القرحة الهضمية. اجري تفاعل البلمرة المتسلسل باستخدام البادئ المستهدف للبيتا– جياردين جين(218pb) للجيارديا اللامبليا.

النتائج: في هذه الدراسة تم جمع 64 عينه براز من مرضى القرحة الهضمية، تم العثور على الاصابة بالجيارديا اللامبلية في 12 (18.7%) باستخدام الفحص المباشر لعينه البراز.في حين 21(32.8%) عينه كانت موجبة للجيارديا اللامبيليا عند استخدام تفاعل البلمرة المتسلسل للكشف عن جين البيتا جياردين. كما ان نسبة الاصابة بالجيارديا لامبليا في الذكور كانت اعلى معنويا ($p < 0.05$) من الاناث، واكثر الاعراض شيوعا هي الحرقة بنسبة 15.6% ، اسهال بنسبة 9.3 % تلتها الام في البطن بنسبة 7.8%.

الاستنتاج: من نتائج هذه الدراسة نستنتج ان نسبة الاصابة بالجيارديا اللامبيليا عالية في مرضى القرحة الهضمية. للكشف عن القرحة الهضمية ينبغي الاخذ بنظر الاعتبار الاصابة بالجيارديا اللامبليا كعامل مسبب للقرحة الهضمية وهذه الطريقة مفيدة في تشخيص وعلاج القرحة.

الكلمات الدالة: الجيارديا اللامبلية، تفاعل البلمرة المتسلسل، بيتا–جياردين جين.