

Prevalence of Adherent-Invasive *Escherichia coli* with *fimH* Gene Isolated from Iranian Patients with Ulcerative Colitis

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Abstract

Background: Ulcerative colitis is a kind of inflammatory bowel disease that is considered as immunological response to commensal bacteria colonizing gut lumen. Adherent-invasive *Escherichia coli* strains are pathogens responsible for ulcerative colitis disease. These bacteria have special virulence factors, including type 1 fimbriae, which could be involved in inflammatory bowel disease.

Objectives: The present study was conducted to determine the prevalence of adherent-invasive *E. coli* with *fimH* gene isolated from Iranian patients with ulcerative colitis.

Methods: Sixty intestinal biopsy samples of 30 patients with ulcerative colitis and 30 individuals without inflammatory bowel disease were examined. Biopsies from rectum, descending, ascending, terminal ileum, and colon were taken during colonoscopy.

Results: All biopsy samples were cultured for isolation of *E. coli* strains. Using polymerase chain reaction assay, the invasive plasmid antigen H and invasion-association locus genes were detected from both isolated bacteria and tissue specimens to confirm the presence of adherent-invasive *E. coli*. The frequency of adherent-invasive *E. coli* with type 1 fimbriae was much higher in patients with ulcerative colitis than control subjects. Among isolated bacteria, type 1 fimbriae of adherent-invasive *E. coli* were detected in 53.3% and 13.3% of ulcerative colitis patients and control subjects, respectively. In addition, from 60 biopsy samples, type 1 fimbriae were detected in 56.7% of ulcerative colitis patients but in 10% of healthy subjects.

Conclusions: Subjects without inflammatory bowel disease had a high rate of *E. coli* strains than patients with ulcerative colitis via cultivation detection. We found a high rate of type 1 fimbriae of adherent-invasive *E. coli* in ulcerative colitis patients by polymerase chain reaction assay. It appears that the presence of adherent-invasive *E. coli* with type 1 fimbriae in the gastrointestinal tract of patients with ulcerative colitis is more likely than previously supposed.

Keywords: *Escherichia coli*, Colitis, Ulcerative, Inflammatory Bowel Diseases, PCR

1. Background

Ulcerative colitis (UC) is known as a chronic immune-mediated disease, causing inflammation and ulcers in the colon (1). The first symptom of active disease is diarrhea mixed with blood. Many parts of the body outside the intestinal tract are affected by ulcerative colitis. In fact, the initiation of UC disease is in intestinal zones with high bacterial counts (2, 3). The pathogenesis of UC is complicated even though its cause is still unknown. Genetic background could be a risk factor for UC disease; however, microbiota and immune system may have roles in the occurrence of the disease (4). A wide range of bacterial species is involved in the inflammation of the colon, including *Enterobacteriaceae*, especially *Escherichia coli* (5). *Escherichia*

coli strains living in the gut are not considered to be harmful. However, numerous studies have indicated that the number of adherent-invasive *E. coli* (AIEC) strains in inflammatory bowel disease (IBD) patients is significantly higher than previously estimated (6).

Adherent invasive *E. coli* can adhere to epithelial cells and invade cytoplasmic eukaryotic infectious cells owing to type 1 fimbriae (*fimH*), invasive plasmid antigen H (*ipaH*), and invasion-association locus (*ial*). Also, it can replicate into macrophages. Accordingly, it should be considered a separate pathogenic cohort triggering intestinal disease in human. Subsequently, studies proposed that AIEC can be associated with pathogenicity of IBD (7). In adherent-invasive *E. coli*, the *fimH* gene, in particular, helps in adhering and colonizing epithelial cells.

The ability to invade intestinal cells is acquired by *ipaH* and *ial* genes. The *ipaH* gene exists in multiple copies located on both chromosomes, and the plasmid is in charge of release in epithelial cells (8). Recently, there are no data about the prevalence of adherent-invasive *E. coli* in the large bowel of patients with inflammation (9). Adherent invasive *E. coli* pathotype has been gradually involved in the etiopathogenesis of ulcerative colitis (10). A number of culture-based and molecular-based studies support the theory that adherent-invasive *E. coli* especially those with *fimH* gene are a microbiological factor effective in IBD (11-13).

2. Objectives

The main aim of the present study was to determine the prevalence of adherent-invasive *E. coli* with *fimH* gene isolated from Iranian patients with ulcerative colitis.

3. Methods

3.1. Patients and Tissue Samples

In the present study, a group of biopsy samples of 60 subjects comprising 30 UC patients (12 males, 18 females, median age 36.9 years, age range 16 - 75 years) and 30 individuals without IBD (14 males, 16 females, median age 48.31 years, age range 19 - 76 years) were taken during colonoscopy. The study was approved by the ethics committee of Tehran University Medical Sciences.

3.2. Microbial Identification

Samples obtained during colonoscopy were transferred immediately into sterile vials containing either thioglycolate broth or saline (Sigma-Aldrich, Hi Media) and stored at -20°C. The biopsy specimens were homogenized and inoculated into Hi Chrome *E. coli* agar (Sigma-Aldrich, Hi Media) and incubated for 18 - 24 hours at 35 ± 2°C. The bacteria were stored in TSB broth containing 30% glycerol at -70°C until further analysis.

3.3. DNA Extraction from Biopsies for PCR

Biopsies were crushed and DNA was extracted by RTP® Mycobacteria kit (Berlin, Germany).

3.4. DNA Extraction from Culture for PCR

Isolated bacteria were prepared for PCR amplification. The bacterial colonies were harvested and centrifuged. The sediments were suspended in 500 µL of sterile deionized water, and boiled for 10 minutes. After centrifugation of the boiled samples at 19000 g for 5 minutes, the supernatant was used as DNA template in PCR assay (14).

3.5. PCR Assay

All isolates were tested for the occurrence of *ipaH*, *ial*, and *fimH* genes using PCR. The nucleotide sequence of primers (Macrogen, Pishgam) and size of product (base pairs) for amplification of *fimH*, *ipaH*, and *ial* genes are displayed in Table 1 (15, 16). PCR was performed in 25 µL solution comprising 5 µL of master mix (Amplicon, Pishgam), 1 µL of each primer (10 pm/µL), 2 µL of DNA template (50 ng), and 16 µL of ddH₂O. Subsequently, the following thermal cycling conditions were used: 5 minutes at 94°C and 36 cycles of amplification consisting of 30 seconds at 95°C, 30 seconds at 56°C, and 1 minutes at 72°C, with 5 minutes at 72°C for final extension. PCR products were investigated by electrophoresis on a 1% agarose gel in 1X TBE buffer [10.8 g Tris and 5.5 g Boric acid, 0.5 M Na₂EDTA (pH 8.0)] (17).

3.6. Statistical Analysis

The data were evaluated by Pearson Chi-Square test. A P value < 0.05 was considered statistically significant.

4. Results

Escherichia coli strains were detected from biopsy samples of 24 patients with UC (80%) and 26 healthy controls (86.7%). All isolated bacteria were confirmed as AIEC by biochemical tests and PCR assay. The *ipaH*, *ial*, and *fimH* genes were amplified by utilizing particular primers and became visible at approximate bands of 619, 320, and 150 bp on polyacrylamide gel, respectively (Figures 1, 2 and 3). Among isolated bacteria, the presence of *fimH* was confirmed in 53.3% (n = 16) of specimens from UC patients, while 46.7% (n = 14) were negative. Also, this gene was detected in 4 control subjects (13.3%) while the remaining 86.7% (n = 26) lacked this gene (Table 2). In addition, amongst 60 biopsy samples taken during colonoscopy, the *fimH* gene was detected in 17 (56.7%) patients with UC and 3 (10%) control subjects (Figure 3). Accordingly, 43.3% (n = 13) of UC patients and 90% (n = 27) of control subjects did not yield any amplicon in PCR assay (Table 3). Therefore, *fimH* gene in *E. coli* strains isolated from UC patients was more frequent than that of control population in PCR assay. Moreover, PCR assay was more reliable than cultivation. Based on the results, the association of *fimH* gene presence in adherent-invasive *E. coli* with UC patients was statistically significant (P < 0.05). Also, all positive amplified fragments were sequenced.

5. Discussion

The link between ulcerative colitis disease and the presence of adherent-invasive *E. coli* carrying *fimH* gene has

Table 1. Primers and Annealing Temperature for Amplification of the AIEC Genes

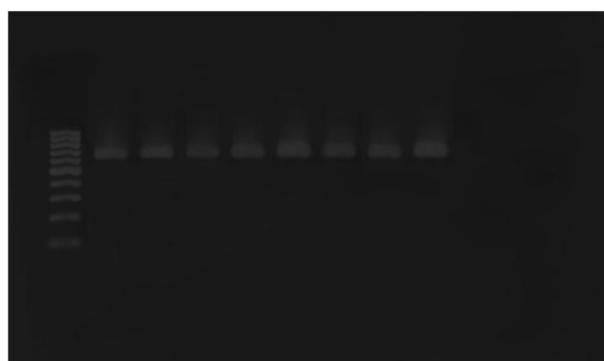
Primers	Nucleotide Sequences (5' - 3')	Size of Product, bp
<i>fimH</i>	TATGGCGCGTGTATCTAG	150
	CACAGGCGTCAAATAAGCG	
<i>ipaH</i>	GTTCTTGACCGCCTTCCGATACCGTC	619
	GCCGGTCAGCCACCCTCTGAGAGTAC	
<i>ial</i>	CTGGATGGTATGGTGAGG	320
	GGAGGCCAACAAATTATTCC	

Table 2. The Presence of the *fimH* Gene of AIEC in Isolated from Two Groups (UC Patients and Control Subjects) by PCR Assay

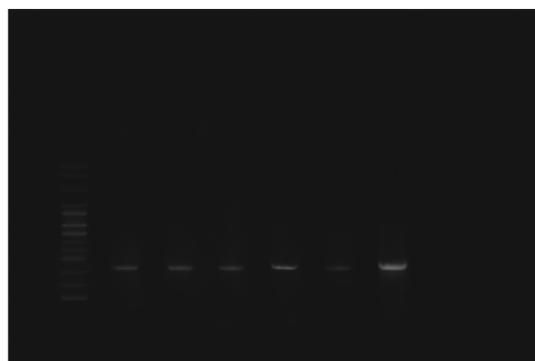
Population	<i>fimH</i>		
	Positive, No.%	Negative, No.%	P Value
UC, No. = 30	16 (53.3%)	14 (46.7%)	0.001
Control, No. = 30	4 (13.3%)	26 (86.7%)	0.001

Table 3. The Presence of the *fimH* Gene of AIEC in Biopsy Samples from Two Groups (UC Patients and Control Populations) by PCR Assay

Population	<i>fimH</i>		
	Positive, N.%	Negative, N.%	P Value
UC, No. = 30	17 (56.7%)	13 (43.3%)	0.000
Control, No. = 30	3 (10.0%)	27 (90.0%)	0.000

Figure 1. Polymerase Chain Reaction Amplification of the *ipaH* Gene

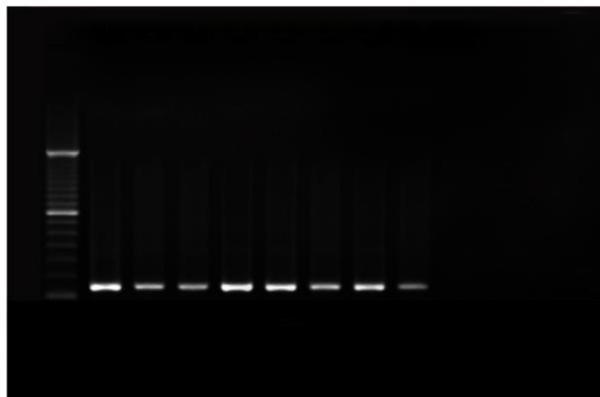
M, DNA molecular size marker (100-bp ladder); lanes 1-8, *E. coli* clinical isolates containing the *ipaH* gene (619 bp); lane 9, negative control (without DNA template).

Figure 2. Polymerase Chain Reaction Amplification of the *ial* Gene

M, DNA molecular size marker (100-bp ladder); lanes 1-6, *E. coli* clinical isolates containing the *ial* gene (320 bp); lane 7, negative control (without DNA template).

been studied in recent years. The pattern of epidemiological characteristics and risk factors related to geographical and regional variations are still unknown (18-20). This study was carried out to determine the prevalence and possible role of AIEC with *fimH* gene in Iranian patients with UC by PCR assay since limited data are currently available

on this subject. To clarify the possible colonization of AIEC in intestinal tract, we screened tissue samples for *ipaH* and *ial* genes by PCR assay as these genes have been reported in many cases to be involve in AIEC invasiveness (21). However, there are other studies proposing that these genes are significant virulence determinants in many extra intestinal

Figure 3. Polymerase Chain Reaction Amplification of the *fimH* Gene

M, DNA molecular size marker (100-bp ladder); lanes 1-8, *E. coli* clinical isolates containing the *fimH* gene (150 bp); lane 9, negative control (without DNA template)

infections in human, especially infections of urinary tract caused by *E. coli* (22).

Most studies indicate that modified microbial components and function in IBD can lead to the increased immune response. However, genetic, phenotyping, and microbial diversity within UC patients indicate that this disease is the heterogeneous cohort of different disorders, which probably has predictable natural histories (23). However, recent studies demonstrated that *Enterobacteriaceae*, *E. coli*, and *Mycobacterium paratuberculosis* strains are associated with IBD (24). The current study showed that there was a higher rate of *fimH* gene in UC patients than control subjects; a difference that was statistically significant. Despite the results of this study, some investigators did not observe any increase in AIEC among UC patients (25, 26).

In this regard, Raso et al. reported that AIEC was not detected in patients with UC and control subjects (27) and likewise, other studies found a higher prevalence for fimbriae I, encoded by the gene *fimA*, in UC patients compared to healthy individuals (28, 29). However, in our study, 56.7% of the patients with UC were positive for AIEC, in particular for those with *fimH* gene; a rate that was significantly higher than the rates reported by previous studies. Martin et al. indicated that *E. coli* is more common in patients with IBD in comparison with control people (30). It can be inferred that *fimH* gene of AIEC may trigger UC disease. But the absence of *fimH* gene in AIEC isolated from UC patients may indicate the point that other bacterial genera may also involve in this disease.

In many cases, several studies indicated that invasive *E. coli* strains are associated with Crohn's disease (31). Based on previous studies, AIEC strains were identified as true in-

vasive strains involved in the pathogenesis of IBD. The invasive ability of AIEC, acquired by *fimH* gene, helps its translocation to pass human intestinal barriers and move inside deep tissues (32, 33). In spite of the interesting debate, with the exception of assessing AIEC strains with *fimH* gene, this gene has been obtained in other strains of *E. coli*. It can be present in 68% of uropathogenic *E. coli* (UPEC) strains which are pathogenic in urinary tract (34). The evidence gathered proposes that adhesive and invasive *E. coli* strains could be involved in ulcerative colitis pathogenesis.

Also, limited data concerning the relation of adherent-invasive *E. coli* and *fimH* gene with UC disease in Iran are available. Therefore, we evaluated the presence of *fimH* gene of AIEC and its relation with UC disease. This study suggests that there is a possible role for this bacterium in pathogenesis of UC disease.

5.1. Conclusion

Several factors have been implicated in the progression of inflammatory bowel disease. In the present study, detection of AIEC with *fimH* gene from UC patients demonstrated that AIEC with *fimH* may be a predisposing factor in UC patients. This study provides the first clinical investigation about the relationship between AIEC, especially those with *fimH* gene, with ulcerative colitis disease in Tehran, Iran.

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Footnotes

Authors' Contribution: Mohammad Mehdi Feizabadi designed the research; Akram Sarabi Asiabar, a PhD student, did laboratory works; Hamid Asadzade Aghdaei and Mohammad Reza Zali conducted the clinical examinations and prepared the clinical specimens for culture and PCR; Azar Sabokbar was co-adviser of PhD thesis.

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