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Original Article

Study on the immunogenicity of pcDNA3.1(+)-*cagT* recombinant vector against *Helicobacter pylori* in BALB/c mice

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Abstract

Background and aims: The role of *Helicobacter pylori* in the development of gastric ulcer and gastrointestinal cancer was identified in this study. More precisely, the study focused on the creation of a DNA vaccine based on the *cagT* gene of this bacterium and the investigation of its immunogenicity against *H. pylori* in infused BALB/c mice.

Materials and Methods: To this end, the pcDNA3.1(+)-*cagT* was prepared and transformed into *Escherichia coli*. Then, animals were injected with recombinant pcDNA3.1(+)-*cagT* plasmid, pcDNA3.1(+)-*cagT* + nanoparticles, and pcDNA3.1(+). After the plasmid purification and confirmation of the transformation by digestion and polymerase chain reaction (PCR), chitosan nanoparticles were synthesized using the ionic gelation method. Next, the animals were classified into three groups each including 21 mice. The injectable solutions including pcDNA3.1(+)-*cagT*, pcDNA3.1(+)-*cagT* + nanoparticles, or empty pCDNA3.1 (as a control group) were injected into the quadriceps muscle of mice, separately. Finally, the blood and tissue samples of each mouse were collected 15, 30, and 45 days after the last injection, and the expression levels of transforming growth factor-beta (TGF-β1), interleukin-4 (IL-4), and interferon-gamma (IFNγ) were evaluated by real-time PCR.

Results: The IFNγ and TGF-β1 expression increased in the infused mice ($P<0.01$) while the IL4 expression represented a significant decrease ($P<0.01$). Moreover, the IFNγ and IL4 expression level in pcDNA3.1(+)-*cagT* + nanoparticle significantly altered ($P<0.01$) compared to the pcDNA3.1(+)-*cagT* group although the TGF-β1 expression was not significantly different ($P=0.075$). Contrarily, the *cagT* gene expression in the tissue samples of both groups was significantly different 15, 30, and 45 days after the last injection ($P<0.01$). Eventually, the expression of the *cagT* gene in the infused mice by pcDNA3.1(+)-*cagT* and in the nanoparticle group was not significantly different 45 days after the last injection ($P=0.105$).

Conclusion: In general, the decrease of IL-4 expression was observed in the injected mice by pcDNA3.1(+)-*cagT* and indicated that the immune system work by a Th1 pattern. The findings showed that a pcDNA3.1(+)-*cagT* construct combined with chitosan nanoparticles can increase the stimulation of the immune system in an animal model and thus it can be used as an appropriate method for controlling *H. pylori* infection.

Keywords: *H. pylori*, pcDNA3.1(+)-*cagT*, Cytokine genes, Chitosan nanoparticles

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Introduction

Helicobacter pylori is a spiral-shaped microaerophilic Gram-negative bacterium that colonizes the luminal surface of the gastric mucosa (1,2). The infection with this human pathogen causes chronic inflammation, duodenal and gastric ulcers, and gastric cancer (3). This pathogen is present in more than half of the world's population. In developing countries, *H. pylori* incidence and prevalence vary from one country to another and depends on awareness and prevention programs and the standards of health quality (4,5). The prevalence of *H. pylori* infection gradually increases with age in developed countries while, in developing countries, more people are infected in childhood and a large percentage of young people also become infected after adulthood (6,7). Several effectors and toxins released by *H. pylori* in the host play an important role in its pathogenicity. This human pathogen can escape from stomach acid and colonize the gastric epithelium by the possession of polar flagella, encoding cag pathogenicity island (cag PAI), vacuolating cytotoxin (Vac), and the urease enzyme thus causing tissue damage (8-10). The role of the cag PAI in this bacterium is to encode a type IV secretion system (T4SS). The entire length of the cag PAI is approximately 37 kb and contains 28 genes (11,12). The CagT of pathogenicity island is needed to deliver CagA (cytotoxin-associated gene A) into

gastric epithelial cells (13,14).

Although several drugs have been evaluated for the treatment of *H. pylori* infection, no drug has so far been effective in treating this microorganism. The usual treatment of *H. pylori*, consisting of triple therapy using metronidazole, tetracycline, and bismuth, is not extremely costly but has a series of side effects such as oral metallic flavor associated with metronidazole consumption and increased sensitivity to light due to tetracycline and other complications such as a temporary grainy feeling of oral mucosa and teeth, constipation, diarrhea, and stool coloration (15,16). Another method of treatment against this pathogen is the use of omeprazole, bismuth, and 2 antibiotics (e.g., metronidazole and tetracycline) for 2 weeks. This method of treatment is not only extremely costly but also the use of these antibiotics can cause a bad taste, diarrhea, and itching. In addition, seizures and polyneuropathy may be observed by the prolonged use of metronidazole (17,18). Further, *H. pylori* can easily become resistant to clarithromycin and metronidazole, and it is impossible to use these antibiotics again after a course of treatment. Nowadays, the antimicrobial effects of many plant species such as garlic on *H. pylori* infection have received special attention. The association between garlic consumption and the reduction of *H. pylori* infection was emphasized (19) although, this relationship was not observed in another study (20). Vaccination is one of the most powerful health interventions and a way for increasing the function of the immune system against microbial infections such as *H. pylori* in humans (21,22). Live-attenuated or killed vaccines, subunit vaccines such as DNA vaccines and recombinant vaccines have been used against *H. pylori* infection. Each kind of vaccine has some advantages and disadvantages. For example, traditional vaccines including live-attenuated or killed vaccines can transmit infectious pathogens into the vaccine recipient. Further, the production and purification of recombinant vaccines are costly, provide primarily humoral immunity, and usually require refrigeration (23,24). However, DNA immunization by using genetically engineered DNA is a cost-effective strategy that can be used to efficiently stimulate humoral and cellular immune responses. It can be easily prepared, highly specific, cheap, stable at room temperature, and prevents the transmission of pathogens (25,26).

Given that the *cagT* gene of *H. pylori* cag PAI is an important virulence factor in the pathogenicity of this pathogen and can stimulate the immune system of the host, the present study was performed to evaluate the immunogenicity of pcDNA3.1(+)-*cagT* recombinant plasmid against *H. pylori* in the infused BALB/c mice by the real-time polymerase chain reaction (RT-PCR).

Materials and Methods

Recombinant vector preparation

The *cagT* gene was synthesized and colonized into the pcDNA3.1(+) vector by Generay Biotech Company (Ltd., Shanghai, China), and pcDNA3.1(+)-*cagT* recombinant vector (6244 bp) was prepared accordingly. A pcDNA3.1(+) vector (Invitrogen, San Diego, CA) with a length size of 5428 bp was used as a control plasmid for injection into BALB/c mice (Figure 1). Moreover, the Top10F' *Escherichia coli* was purchased from the Pasteur Institute (Tehran, Iran) and the lyophilized stock was cultured in a Luria-Bertani (LB) agar for obtaining a single colony. Then, a single colony was cultured in a 5 mL of LB broth by overnight incubation at 37°C and used as a host of transformation.

pcDNA3.1(+)-cagT transformation

In this study, the pcDNA3.1(+)-*cagT* recombinant vector was transformed into competent *E. coli* Top10F' cells using $CaCl_2$ (0.1 M) treatment, along with heat shock for 90 seconds at 42°C in order to obtain sufficient plasmids for future experiments. The verity of vector transformation was confirmed by colony-PCR and then the chosen colonies containing the recombinant vector were cultured overnight in a LB broth containing 100 µg/mL of ampicillin antibiotic with 180 rpm shaking at 37°C. The plasmid purification was performed using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, Freiburg, Germany), and PCR, sequencing, and enzymatic digestion with *XhoI* and *XbaI* restriction enzymes were done on purified plasmids for transformation confirmation. A large scale of plasmids was extracted using the MaxiPrep Plasmid Purification Kit (Qiagen; cat. #12163) according to the manufacturer's protocol. To verify the quality and quantity of the extracted plasmid and pCDNA3.1 (empty

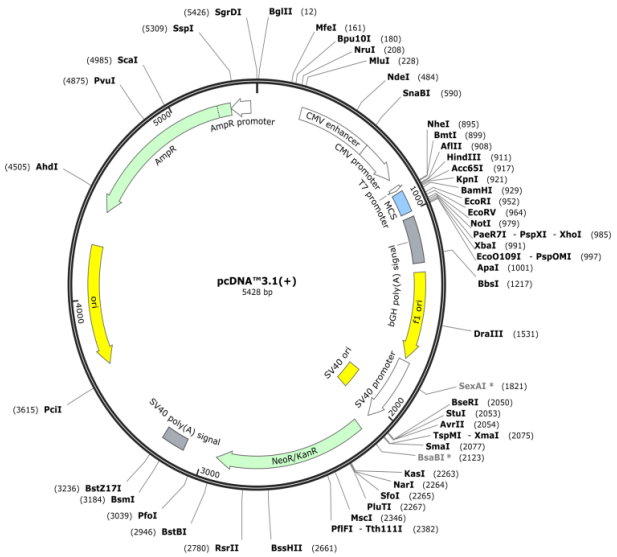

Figure 1. The map of pcDNA3.1(+) vector.

vector), the electrophoresis on 2% agarose gel was done at a constant voltage of 80 V using a 1 kb molecular ladder, and the quantity and quality of the extracted vectors was measured at a wavelength of 260/280 nm by Thermo Scientific™ NanoDrop 2000 (Wilmington, DE, USA) according to the method described by Sambrook and Russell (27). After electrophoresis, the gel was stained by Gel-Red and photographed under an ultraviolet light using UVIdoc gel documentation systems (Uvitec, UK).

cagT gene amplification

For the amplification and confirmation of the presence of the *cagT* gene in the extracted plasmids, the conventional PCR assay was done in a final volume of 25 µL in 0.2 mL micro-tubes. Specific oligonucleotide primers were designed using Gene Runner software, version 3.05, and the basic local alignment search tool (BLAST) from the NCBI GenBank data was used to compare the sequence similarity in the GenBank data (Table 1). The reaction mixture containing 1 µM of each forward and reverse primer, 20-50 ng of purified plasmid DNA, 0.5 µL dNTPs, 2.5 U Taq DNA polymerase, 1.5 mM $MgCl_2$, and 2.5 µL 10× buffer (all Invitrogen, USA) was used for gene amplification. Proliferation was carried out in a Mastercycler Gradient PCR (Eppendorf, Germany) with 5 minutes of initial denaturation at 94°C, followed by 35 cycles including 1 minute of denaturation at 94°C, 1 minute of primer annealing at 63°C, 1 minute of elongation at 72°C, and then a final elongation at 72°C for 10 minutes with a final hold at 4°C. The PCR products were visualized by agarose gel electrophoresis through a 2% agarose gel in TBE 1X buffer according to the above-mentioned procedure.

Chitosan nanoparticles formation by the ionic gelation technique

In this study, chitosan nanoparticles were used for better absorption of the plasmid DNA in the infusion site of the animal. These nanoparticles were prepared according to the study by Calvo et al using an ionic gelation technique (28). The chitosan powder (Sigma-Aldrich, USA) was dissolved in a 1.0% acetic acid aqueous solution (2 mg/mL) under stirring with a magnetic stirrer at 1000 rpm for 24 hours at room temperature. The pH was adjusted to 5.5 with 0.5 M NaOH. After the filtration of this solution, as well as the sodium tri-polyphosphate (TPP) solution (0.7 mg/mL) through a 0.45 µm filter, 20 mL of TPP was added extremely slowly and drop-off (1 drop every 7 seconds) to 50 mL of an acetic acid solution containing chitosan and was kept at 1000 rpm for 1 hour on a magnetically stirred at room temperature. The nanoparticle suspension was centrifuged for 15 minutes at 14000 rpm at 4°C and the supernatant was powdered after drying by a freeze-dryer (Virtis Advantage Plus freeze-dryer, SP Scientific, Warminster, USA). The physicochemical properties of chitosan nanoparticles, such as particle size and zeta-potential via dispersion severity were measured using Malvern Zetasizer NANO Series NANO ZS90 (Malvern Instruments, UK). Finally, the equal value of the plasmid (2000 µg/mL in PBS) and chitosan nanoparticle solution (1%) were mixed well and placed at 55°C for 1 hour.

Animal Groups for the Injection of the Recombinant Vector

A total of 63 6-week-old female BALB/c mice were classified into three groups for injections (each group 21 mice) including the injections of recombinant pcDNA3.1(+)-*cagT* plasmid (recombinant vector), recombinant plasmids with chitosan nanoparticles (recombinant plasmid + nanoparticles), and the empty plasmid (pcDNA3.1(+) as a control group). For injections, 100 µL of each infused solution including pcDNA3.1(+)-*cagT* + nanoparticles with a concentration of 1000 µg, 1000 µg per mL of pcDNA3.1(+)-*cagT* dissolved in PBS, and empty pcDNA3.1(+) as a control group (1000 µg per mL of pcDNA3.1(+) vector) were prepared and injected into the quadriceps muscles of the animals of each group. The infusions were performed on days 0, 7, and 15 in all three groups of BALB/c mice. Seven mice of each group were killed and sampled 15, 30, and 45

Table 1. The applied primer sequences for q-RT-PCR

Primers	Sequence	Annealing temperature (°C)	Product length (bp)	Accession number
GAPDH	F: 5′-TCCCGTAGACAAAATGGTGAAGG-3′ R: 5′-ATGTTAGTGGGGTCTCGCTCCTG-3′	65	261	XM_017321385
TGF-β1	F: 5′-ACCGCAACAACGCCATCTATGAG-3′ R: 5′-GCGTATCAGTGGGGGTCAGCAG-3′	66	234	BC013738
IL-4	F: 5′-TCACAGGAGAAGGGACGCCATG-3′ R: 5′-TGGACTTGGACTCATTCATGGTGC-3′	67	246	NM_021283
IFNγ	F: 5′-GCCTAGCTCTGAGACAATGAACG-3′ R: 5′-GCCAGTTCCTCCAGATATCCAAG-3′	64	188	M28621
cagT	F: 5′-TGAGAGCAAGTGTTTTAATCGGTG-3′ R: 5′-TGAATGGTGTGTCTTTGAGTTTGTC-3′	63	177	JQ685147

Note. q-RT-PCR: Quantitative real-time polymerase chain reaction; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; TGF-β1: Transforming growth factor-beta; IL-4: Interleukin-4; IFNγ: Interferon-gamma;

days after the last injection. After anesthesia, the whole blood specimens (1.5 mL) were taken from the heart of each mouse at each sampling stage and transferred into ethylenediaminetetraacetic acid as an anticoagulant. Then, the quadriceps muscle of the mice was removed and the injection site of muscle tissues was isolated as well.

RT-PCR assay

Total RNA was isolated from 100 mg of each tissue sample or 100 µL of a buffy coat of blood specimens, immediately using the TRIzol reagent (Invitrogen, USA) according to the manufacturer's specifications. The absorbance of the extracted RNA samples was measured at a wavelength of 260-280 nm, and cDNA samples were synthesized by a cDNA synthesis kit (Takara, Kyoto, Japan) using specific primers or mixing them with an oligo (dT) primer according to the manufacturer's instructions. The mixture was heated to 85°C for 5 seconds and then incubated at 42°C for 15 minutes, and the inactivation of reverse transcriptase was done for 5 minutes at 85 °C.

Specific oligonucleotide primers were designed using Gene Runner software, version 3.05 (Hastings Software Inc. Hastings, NY, USA) and the BLAST of GenBank data (Table 1). The expression levels of cytokine genes, including TGF-β1, interleukin-4 (IL-4), and IFNγ were evaluated by comparing to *GAPDH (*glyceraldehyde 3-phosphate dehydrogenase) as an internal control and *cagT* as a target gene of the manipulated vector in q-RT-PCR. The cDNA samples (50 ng) in appropriate dilutions (1:10) were added in a final volume of 20 µL, containing 1× SYBR Green PCR Master Mix (Toyobo, Japan) and 1 µL of each primer (2 µM). The temperature cycling was including initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, primer annealing at the optimal temperature according to Table 1 for 20 seconds, and extension at 72°C for 30 seconds. All reactions were done in triplicate, and q-RT-PCR was monitored by measuring the fluorescence at the end of the annealing phase of each cycle. The relative levels of gene expression were revealed by the standard curve method, and the cycle of the threshold (Ct) values of the target and the reference genes was analyzed by the comparative Ct ($2^{-\Delta\Delta Ct}$) method. The DNA melting-curve analysis was performed by the Corbett Rotor-Gene 6000 machine by holding the reaction mixtures at 95°C for 60 seconds and decreasing it to 40°C at a transition rate of 0.1°C/second for the detection of fluorescence absorption at a wavelength of 640 nm.

Statistical analysis

The results were analyzed by Social Sciences software (SPSS, Inc., Chicago, IL, USA), version 20 using the independent t-test to examine the relationship between groups and the significance of the data. All data were considered statistically significant at a $P<0.05$.

Results

Verifying the Inserted Gene in the Recombinant Vector

The recombinant pcDNA3.1(+)-*cagT* vector was successfully transformed into competent *E. coli*, and the accuracy of the transformation on the extracted plasmids and formation of the final construct were determined by enzymatic digestion and PCR. The PCR showed the presence of the inserted gene in the vector, and the digestion of the pcDNA3.1(+)-*cagT* recombinant vector by *XhoI* and *XbaI* restriction endonucleases on 2% agarose gel electrophoresis revealed two fragments including 5428 and 855 bp as pcDNA3.1(+) and the *cagT* gene, respectively (Figure 2).

Physical characteristics of chitosan nanoparticles

The chitosan-DNA nanoparticle characteristics, including the particle size and zeta-potential, were determined using the dynamic light scattering method and Malvern Zetasizer Nano-ZS (ZEN3600), respectively, showing that 98.6% of these particles had a 133.4 nm diameter. Additionally, the velocity distribution of the nanoparticle movement was measured by the dynamic fluctuations of light scattering intensity at 25°C and a wavelength of 633 nm (Figure 3).

The investigation of the appearance of chitosan nanoparticles by the SEM showed that the nanoparticles had a homogeneous spherical shape with smooth edges (Figure 4).

Gene expression analysis

The gene expression analysis by RT-PCR using REST 2009 software indicated that IFNγ and TGF-β1 expression levels with the recombinant vector increased in the infused group ($P<0.01$) while the IL4 expression demonstrated

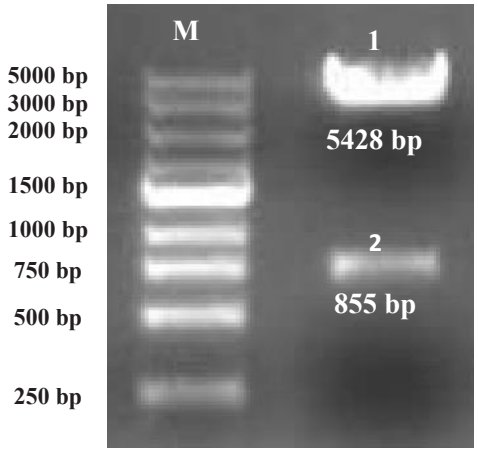

Figure 2. The restriction digestion of pcDNA3.1(+)-*cagT* recombinant plasmid constructs on 2% agarose gel electrophoresis. *Note*. Lane M is the 1 kb DNA ladder (Thermo Fisher Scientific, Freiburg, Germany). Fragments 1 and 2 were pcDNA3.1(+) vector and the exited *cagT* gene, respectively.

Figure 3. Zeta-potential Distribution of Chitosan Nanoparticles

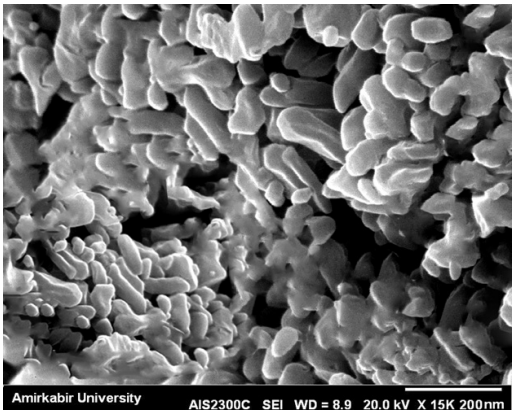

Figure 4. The image of chitosan nanoparticles by SEM.
Note. SEM: Scanning electron microscope.

a significant decrease ($P<0.01$). The comparison of the expression level of cytokine genes in the blood of infused BALB/c mice represented that the expression level of IFNγ and IL4 genes in the pcDNA3.1(+)-*cagT* + nanoparticle group significantly differed compared with the injected BALB/c mice by the pcDNA3.1(+)-*cagT* group ($P<0.01$) although the expression of the TGF-β1 gene in these groups displayed no significant difference ($P=0.075$), the related data of which are illustrated in Figure 5.

Based on the comparison of *cagT* gene expression in the tissue samples of the infused animal by pcDNA3.1(+)-*cagT* + nanoparticle in different time intervals after the last injection (Figure 6), the expression of the *cagT* gene was significantly different at days 15, 15, and 30 compared to days 30, 45, and 45, respectively ($P<0.01$). In addition, the *cagT* gene expression level in pcDNA3.1(+)-*cagT* (without nanoparticles) on day 15 compared with days 30 and 45, and on day 30 compared to day 45 was significantly different ($P<0.01$).

Moreover, the expression level of the target gene in the tissue samples of infused mice by pcDNA3.1(+)-*cagT* in each day was significantly different ($P<0.01$) after the last injection (Figure 7).

Similarly, the expression level of the *cagT* gene in the infused BALB/c mice by pcDNA3.1(+)-*cagT* and pcDNA3.1(+)-*cagT* + nanoparticle groups on days 15 and 30 was significantly different ($P<0.01$) although at day 45 it was not significantly different ($P=0.105$) after the last injection (Figure 7).

Discussion

Helicobacter pylori infection is highly common worldwide, and the prevention of primary gastrointestinal infections by a suitable vaccine, especially using DNA vaccine is attractive (29,30). One of the effective virulence factors in *H. pylori* infection is the *cagT* gene thus the recombinant pcDNA3.1(+)-*cagT* DNA vector was created based on this gene in this study. After the vaccination of BALB/c mice, its immunogenicity against this pathogen was evaluated by the RT-PCR. The BALB/c mice were classified into three groups of pcDNA3.1(+)-*cagT*, pcDNA3.1(+)-*cagT* + nanoparticle, and controls (pcDNA3.1(+), the empty vector). Injections into the quadriceps muscle of mice were performed after the preparation of chitosan nanoparticles by the ionic gelation method. The mice were killed 15, 30, and 45 days after the last injection, and the blood and tissue samples of infused mice were collected. Then, the total RNA was extracted from each sample, and cDNA was synthesized as well. The expression level of cytokines including TGF-β1, IL-4, and IFNγ, as well as *cagT* (as a target gene of the recombinant vector) against *H. pylori* infection was evaluated using the RT-PCR method. The results of cytokine gene expression showed that the expression of IFNγ and TGF-β1 genes in the infused animals increased ($P<0.01$) but the IL4 expression decreased significantly ($P<0.01$). The comparison of the gene expression levels

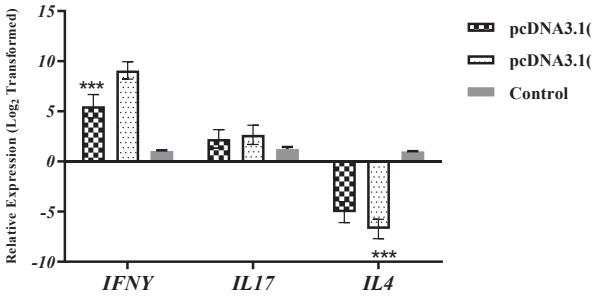

Figure 5. Comparison of the expression pattern of all three cytokines (i.e., IL4, TGF-β1, and IFNγ) in injected BALB/c mice by pcDNA3.1(+)-*cagT* + nanoparticle and pcDNA3.1(+)-*cagT* compared with the control (infused by empty pcDNA3.1(+) vector).

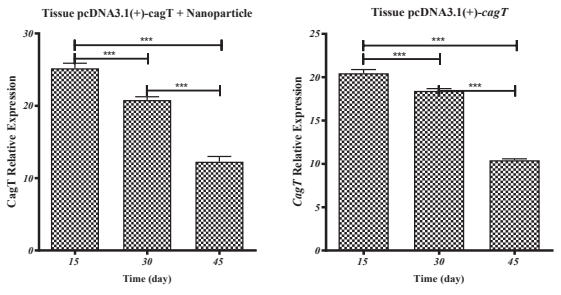

Figure 6. The comparison of the *cagT* gene expression in the infused BALB/c mice tissue by pcDNA3.1(+)-*cagT* + nanoparticle (left) and pcDNA3.1(+)-*cagT* (right) in different time intervals.

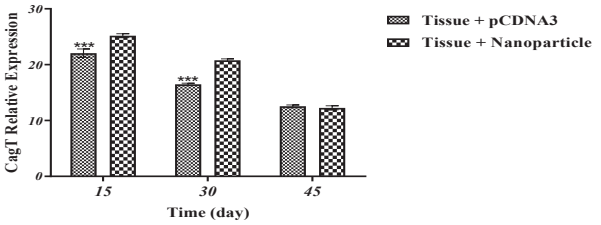

Figure 7. The comparison of the *cagT* gene expression in injected groups by pcDNA3.1(+)-*cagT* and pcDNA3.1(+)-*cagT* + nanoparticle at different times after the last injection.

of these cytokines in blood specimens revealed that IFNγ and IL4 genes in the pcDNA3.1(+)-*cagT* + nanoparticle group were significantly different ($P<0.01$) compared to the pcDNA3.1(+)-*cagT* group although the expression level of the TGF-β1 gene was not significantly different ($P>0.075$). In tissue samples, the comparison of *cagT* gene expression in both pcDNA3.1(+)-*cagT* + nanoparticle and pcDNA3.1(+)-*cagT* groups were significantly different at different time intervals after the last injection ($P<0.01$). Further, the expression level of the *cagT* gene in infused BALB/c mice by a recombinant plasmid with and without nanoparticles at 45 days after the last injection was not significantly different ($P>0.105$).

In recent decades, various studies have focused on finding an appropriate vaccine to immunize and prevent *H. pylori* infection (24,31). In a study by Sun et al, an *H. pylori* neutrophil-activating protein gene was cloned into the pBT vector and then sub-cloned into the pIRES eukaryotic expression vector. After transformation into live attenuated *Salmonella typhimurium*, the vector was used as an oral recombinant DNA vaccine against *H. pylori* infection. Their findings indicated that pIRES-NAP recombinant plasmid induced a specific immune response in animal models (32). Their method was somewhat similar to the present study, but in our study, the pcDNA3.1(+)-*cagT* construct was created and its efficiency for inducing the immune system by the investigations of TGF-β1, IL-4, and IFNγ cytokine genes was observed using molecular techniques against *H. pylori* pathogen in an animal model. Najar Peerayeh et al examined the colonies and *HpaA* gene expression of *H. pylori*, and this gene was amplified and cloned into the PET28a propagating vector and transformed into BL21DE2 *E. coli*. The PET-HPa-BL21 was created and the expression of the recombinant protein was confirmed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis and the western blot. Then, the immunogenic activity of the *HpaA* gene against *H. pylori* cells was detected by the anti-polyclonal antibody. The prokaryotic expression system PET-HPa-BL21 that expressed the HPaA fusion protein showed a satisfactory immunogenic activity that could be an appropriate candidate against *H. pylori* (33). In their research, the PET28a vector expressing the *HpaA* gene was created while, in the present study, the pcDNA3.1(+)-*cagT* construct with or without nanoparticles was prepared and used against *H. pylori*. Finally, the expression of TGF-β1, IL-4, and IFNγ genes, as well as *cagT* (as a target gene) was investigated by the RT-PCR after the infusion into the BALB/c mice, and the appropriate expression with sufficient immune stimulation was observed accordingly. In another study by Gu et al, the expressing recombinant urease subunit B by transforming the urease subunit B of *H. pylori* into *Lactococcus lactis* was prepared and used as an oral vaccine in mice against *H. pylori*. Based on their results, a specific immunoglobulin G of urease B was produced in the injected mice which stopped the infection of *H. pylori* in the stomach (34). Our work was similar to their study, except that the pcDNA3.1(+)-*cagT* recombinant vector, alone or in combination with chitosan nanoparticles, was infused into the quadriceps femoris muscle.

In a study by Doosti the *Omp31* gene of *Brucella melitensis* was cloned into pcDNA3.1 to create an Omp31-pcDNA3.1 construct. After the immunization of BALB/c mice, the Th-1 cell reaction was observed in mice (35). In a different work by Doosti et al, the heavy chain of *Clostridial botulinum* neurotoxin was cloned into *E. coli* by the T/A cloning method and its validity was confirmed by the PCR. Based on their results, this construct gene could be used to produce a *botulinum* neurotoxin vaccine (36).

The antigenic region of the *cagA* gene of *H. pylori* was amplified by the PCR and subcloned into the PET32a prokaryotic vector. The PET32a-*cagA* construct was transferred into *E. coli* BL21 (DE3) PLYSS. The expressed protein was purified by the chromatography column, enzymatic digestion, the PCR, and sequencing and showed that the antigenic region of the recombinant CagA protein could be selected as the suitable candidate for the gene vaccine. The difference between their research and the present study was the kind of applied gene and vector for vaccination (37). In a study by Farjadi et al, the PLYSS strain BL21 (DE3) was used and PET32a-*cagA* was produced and applied for vaccination while, in the present work, the pcDNA3.1(+)-*cagT* was created and transferred into *E. coli* Top10 strain as a host, and then this recombinant vector was infused into the mice, suggesting that pcDNA3.1(+)-*cagT* could be used as a suitable candidate for vaccination against *H. pylori*.

In another study, the *UreB* (urease B) gene of *H. pylori* was fused with IL-2 as the mucosal adjuvant and cloned into *Lactococcus lactis* and used for the injection of mice. Their findings represented the production of anti-UreB antibody and more cytokines such as IFN-γ, IL-4, and IL-17 in the infused mice and revealed that this recombinant *L. lactis* expressing UreB–IL-2 can be potentially used as an edible vaccine for controlling *H. pylori* infection (38). In the present study, the expression level of IFNγ and TGF-β1 genes increased after the injection of mice by the pcDNA3.1(+)-*cagT* recombinant vector although the IL4

expression indicated a significant decrease.

Conclusion

In conclusion, the expression of IFNγ and TGF-β1 significantly increased in the blood specimens of the infused BALB/c mice by pcDNA3.1 (+)-*cagT* + nanoparticle and pcDNA3.1(+)-*cagT*, but the expression of IL-4 represented a significant decrease. In addition, a decrease was observed in the *cagT* gene expression in the tissue specimens of mice 45 days after the last injection. Considering that the IL-4 is the most prominent cytokine of Th2, a reduction of IL-4 can indicate the non-response of Th2 in BALB/c mice, which confirms the response of the immune system as a Th1 pattern. Further, the findings demonstrated that the application of chitosan nanoparticles together with recombinant plasmid increased the expression of ILs in injected mice. In future studies, the produced pcDNA3.1 (+)-*cagT* construct could be used as a suitable marker for biological and pathological investigation and drug-discovery in eukaryotic systems, biomarker discovery, and vaccination and prevention in human models.

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This study was approved by the Research Ethics Committees of the Deputy of the Research and Technology of Islamic Azad University of Shahrekord Branch, Shahrekord, Iran on August 30th, 2016 (The ethics code: IR.IAU.SHK.REC.1395.334).

Authors Contribution

Conception and design, acquisition of data, analysis and interpretation of data done by AD. Drafting of the manuscript performed by AD and AB. Critical revision of the manuscript for important intellectual content and statistical analysis done by AD. AB and AD contributed in all microbial tests, animal injections and molecular techniques. The final draft approved by AD.

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