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Original Article

# Evaluation of the Immune Response Against *Helicobacter pylori* in Infused BALB/c Mice by pcDNA3.1(+)-*ureA*

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## Abstract

**Background:** The purpose of the present study was to produce a pcDNA3.1(+)-*ureA* recombinant vector and evaluate the capacity of this vector to stimulate the immune response against *H. pylori* infection in infused BALB/c mice.

**Materials and methods:** The pcDNA3.1(+)-*ureA* construct was prepared and transformed into *E. coli*, successfully. The animals we used in the study were allotted into three groups for infusion of 1) recombinant plasmid, 2) pcDNA3.1(+)-*ureA* + nanoparticles, and 3) pcDNA3.1(+). Blood and tissue specimens from each group of mice were collected at days 15, 30, and 45 after the last infusion and the expression levels of cytokines such as TGF-β1, IL-4, and IFNγ genes comparing to GAPDH as well as the expression of *ureA* in the mice's thigh muscle were evaluated.

**Results:** The genes expression analysis showed that the IL4 expression significantly decreased ($p<0.001$) but IFNγ and TGF-β1 expression increased in the blood of infused mice ($p<0.001$). Also, the urea expression level in pcDNA3.1(+)-*urea* and pcDNA3.1(+)-*ureA*+ nanoparticle 15, 30, and 45 days after the last infusion was significantly different ($p<0.001$) and its expressions at days 15 and 30 were significantly different ($p<0.001$), but 45 days after the last infusion it was not significantly different ($p>0.05$).

**Conclusion:** The pcDNA3.1(+)-*ureA* recombinant vector with or without chitosan nanoparticles can stimulate the immune response in animal models against *H. pylori* infection. Also, after combining the recombinant vector with nanoparticles we observed a better immune response was observed. In future studies this recombinant construct can be used as a biomarker and therapeutic approaches in eukaryotic systems.

## Keywords

cytokine genes, *Helicobacter pylori*, pcDNA3.1(+)-*ureA*, recombinant vector

## BACKGROUND

*Helicobacter pylori* is a spiral-shaped microaerophilic Gram-negative bacterium, measuring 2 to 4 μm in length and 0.5 to 1 μm in width inhabiting the gastric epithelium. It was first isolated in 1983. Barry Marshall and Robin Warren were the first to describe the successful isolation and culture of a spiral bacterial species *(Campylobacter pyloridis)* from the human stomach. This helix-shaped bacillus is highly motile due to its unipolar bundle of two to six shelled flagella. It colonizes the mucus layer of the gastric epithelium of humans by producing urease enzyme.[1,2] This bacterium is transmitted through the fecal-oral, gastro-oral, or oral-oral routes. *H. pylori* can lead to a vari-

ety of upper gastrointestinal disorders such as peptic ulcer, chronic gastritis disease, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. While the infection with *H. pylori* is a major cause of stomach diseases, most people infected with *H. pylori* never have the symptoms or develop gastrointestinal disease.[3] Also, considering the drug resistance that has been observed in recent years the metronidazole, tetracycline, and bismuth-metronidazole-tetracycline triple therapy against *H. pylori* is not wholly efficient.[4,5] Thus, infusion combined with antibiotics, could improve the rate of treatment success and designing of proper vaccine against this infectious agent is important, but resistance to antimicrobial agents and patient noncompliance has been observed and there is no efficient vaccine in clinical practice yet.[6]

Due to the clinicians regarding vaccination effects to reduce the gastric cancer rate, especially in developing countries provide an initiative panel to invite researchers for more attempts in this issue.[7] Both antibiotic therapy and probiotics are inadequate strategies in the battle against *H. pylori*. Accordingly, the last available weapon is a vaccine, thus we should search for better vaccine formulations and adjuvants to make vaccines for destroying this rogue chronic infection. It seems to produce and procreate an effective *H. pylori* vaccine only two major topics should be addressed; best adjuvant and best-selected antigen.[7] Urease enzyme is one of the most important *H. pylori* virulence factors that involved in a series of processes that allow bacteria to colonize and induce a strong inflammatory response in the gastric epithelium. This bacterium produces large amounts of urease. This enzyme is regulated by some genes. The genes encoding *H. pylori* urease are detected as a single 6.13-kb gene cluster on the chromosome of the bacterium.[8,9] Seven continuous genes, all transcribed in the same direction, are necessary for the synthesis of an active enzyme. The operon has been included ureABIEFGH and all genes except *ureI* contribution homology with urease genes of other species. Urease enzyme catalyzes the hydrolysis of urea to produce ammonia and carbamate. Therewith liberating ammonia, which can counteract acid, permitting survival of the bacterium and primary colonization of the gastric mucosa.[10] The urease protein is encoded by *ureas* genes. *H. pylori* urease enzymatic is a large protein, consisting of a dodecameric organization of two subunits. The two structural subunits are encoded by *ureA* and *ureB*, the first two genes of the gene cluster. UreA has 729 nucleotides and a forecasted molecular mass of 26.5 kDa, and the predicted masses of UreB are between 60.3 and 61.0 kDa, the sequences differing for various strains. Expression of *ureA* and *ureB* are enough to produce an assembled apoenzyme.[9]

The three things required for developing an effective vaccine against *H. pylori* are appropriate bacterial antigens, safe and effective adjuvants, and a path of delivery. Although most vaccine studies employ urease as the antigen of choice, investigators continue to evaluate potential new antigens and mechanisms to stabilize such antigens.[11,12]

Nanotechnology has had a great influence on all aspects of human life. Using nanotechnology in vaccine development is a new area and the initial results have been so promising. Commonly, nanostructures play two main roles in vaccine formulations: firstly, they are used as a vehicle for the delivery of vaccines, and, secondly, due to their intrinsic adjuvanticity, they could improve the immune responses. Nanoparticles (NPs) have been used more than any other types of nanomaterials in vaccine formulations.[13]

Cytokines are regulatory proteins that play a central role in the immune system by shading cellular responses; including lymphocyte activation, proliferation, differentiation, survival, and apoptosis.[14] Many investigators have examined the relation between *H. pylori* infection and cytokines.[15]

There is no effective treatment method against *H. pylori* infection has been identified yet. Considering to the importance role of urease enzyme in pathogenicity of this pathogen, in this study the cytokine genes expression patterns in infused mice by pcDNA3.1(+)-*ureA* against *H. pylori* infection was investigated.

## MATERIALS AND METHODS

### Animals and ethical approval

Sixty-three female 7-week-old white BALB/c mice obtained from the Pasteur Research Center (Tehran, Iran) were used in the study. Animals were housed, fed and kept under traditional microbe-free situations in the animal care facility of the Islamic Azad University of Shahrekord Branch and treated according to international adjustments for the care of laboratory animals. The Ethics Committee of our university approved all animal experimental methods on November 20th 2016.

### Recombinant by pcDNA3.1(+)-*ureA* preparation

The plasmid pcDNA3.1(+) plasmid with 5428 bp length was prepared by Invitrogen company (Invitrogen, San Diego, CA) and used as an expression vector in eukaryotic systems for injection into BALB/c mice. This plasmid contains ampicillin and neomycin resistance genes and has 4 promoters such as CMV-T7-bla-SV40. In this research, the pcDNA3.1(+) plasmid was injected into the control group (plasmid without target gene) and pcDAN3.1(+)-*ureA* was infused in animal models. For plasmids proliferation first, the lyophilized stock of *E. coli* strain Top10F' was purchased from Pasteur Institute of Iran and was cultured in 5 mL of antibiotic-free Luria-Bertani (LB) broth and then incubated for overnight at 37°C with shaking (2000 rpm/min). Both pcDAN3.1(+)-*ureA* recombinant vector and pcDNA3.1(+) (control plasmid) using $CaCl_2$ and heat shock (42°C for 90 seconds) treatment were transformed

into competent *E. coli*. Then, the bacterial cells were cultured in LB-Agar medium containing 100 μg/mL of ampicillin antibiotic for the screening of the engineered bacterial cells. The colony-PCR was done on selected colonies and positive colonies (containing the target gene) were cultured into LB broth containing ampicillin antibiotic for overnight at 37°C. All plasmids were purified using the GeneJET plasmid Miniprep Kit (Thermo Fisher Scientific, Freiburg, Germany) according to the recommendations of the company. The enzymatic digestions using *XbaI* and *XhoI* restriction enzymes were done on purified plasmids for confirmation of the transformation and the digestion patterns were evaluated on 2% agarose gel electrophoresis using 1-kb DNA ladder (Gibco, Burlington, ON, Canada). As we needed to inject appropriate amounts and concentrations of plasmids into the animals, the large-scale isolation of plasmid was done by MaxiPrep Plasmid Purification Kit (Qiagen; cat. #12163) according to the manufacturer's instructions, but Phosphate-buffered saline (PBS) was used instead of elution buffer in the last step of extraction. The ratio of absorbance of the extracted recombinant vector and pCDNA3.1 plasmid for quantity and quality verification was measured at a wavelength of 260/280 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA) according to the method described by Sambrook and Russell, 2001.[16] All extracted plasmids were kept at -20°C for future experiments.

## Gene proliferation

The presence of *ureA* gene in extracted plasmids was confirmed by conventional polymerase chain reaction (PCR) technique. The specific oligonucleotide primers for *H. pylori ureA* gene were designed using Gene Runner software version 3.05 and the primer sequences was aligned to a query sequence of GenBank data using BLAST (Basic Local Alignment Search Tool) **(Table 1)**. 25 μL of the reaction mixture containing 20 to 50 ng of purified plasmid DNA, 2.5 μL of 10x Taq buffer (100 mM Tris-HCl, pH 8), 100 μM dNTPs, 1.2 μM forward and reverse primers, 1.25 mM $MgCl_2$, and 2.5 U *Taq* DNA polymerase (all Invitrogen, USA) was added to the 0.2 mL micro-tubes. The content was mixed, and the proliferation was performed in a Mastercycler Gradient PCR (Eppendorf, Germany) following the program given below: initial denaturation (94°C, 5 min), followed by 35 cycles, including denaturation (94°C, 1 min), annealing (66°C, 1 min), extension (72°C, 1 min), then the final extension step at 72°C for 10 min and hold at 4°C.

PCR products were visualized by ethidium bromide staining on 2% agarose gel electrophoresis in TBE 1X buffer according to the procedure as mentioned above.

## Chitosan nanoparticles preparation

In this research, the chitosan nanoparticles were prepared based on ionic gelation method using sodium tripolyphosphate (TPP) as cross-linking agent according to Calvo et al., 1997 study.[17] In sum, the 50 grams of pure chitosan powder (Sigma-Aldrich, USA) with low molecular weight was dissolved in 1.0% acetic acid aqueous solution (2 mg/mL) and was stirred using a magnetic stir bar at 1000 rpm at room temperature for 24 hours. The pH of the solution was adjusted to 5.5 using 0.5 M NaOH. This prepared solution and TPP (0.7 mg/mL) by filtering through a 0.45 μm filter and then 20 mL of TPP were slowly dropping off (every 7 seconds, 1 drop) to the dissolving chitosan in a 50 mL of acetic acid solution and was kept at 1000 rpm for an hour at room temperature under magnetic stirring. This solution was centrifuged at 14,000 rpm for 15 min at 4°C and the supernatant was dried and powdered by freeze-dryer (Virtis Advantage Plus freeze-dryer; SP Scientific, Warminster, USA). The chitosan nanoparticles properties were characterized physico-chemically (particle size and zeta potential) with dispersion severity was measured using Malvern Zetasizer Nanoseries Nanos ZS90 (Malvern Instruments, UK). Equal volumes of chitosan solution (1%) and DNA plasmid (2000 μg/mL eluted in PBS) were mixed well and placed at 55°C for 1 hour. Finally, the plasmid mixed with nanoparticles was compared to pure plasmid on agarose gel electrophoresis and the differences in the motions were evaluated.

**Table 1.** Primer sequences used for the qRT-PCR assay

| Primers | Sequence | Annealing temperature (°C) | Product length (bp) | Accession number |
|---|---|---|---|---|
| GAPDH | F: 5′-TCCCGTAGACAAAATGGTGAAGG-3′<br>R: 5′-ATGTTAGTGGGGTCTCGCTCCTG-3′ | 65 | 261 | XM_017321385 |
| TGF-β1 | F: 5′-ACCGCAACAACGCCATCTATGAG-3′<br>R: 5′-GCGTATCAGTGGGGGTCAGCAG-3′ | 66 | 234 | BC013738 |
| IL-4 | F: 5′-TCACAGGAGAAGGGACGCCATG-3′<br>R: 5′-TGGACTTGGACTCATTCATGGTGC-3′ | 67 | 246 | NM_021283 |
| IFNγ | F: 5′-GCCTAGCTCTGAGACAATGAACG-3′<br>R: 5′-GCCAGTTCCTCCAGATATCCAAG-3′ | 64 | 188 | M28621 |
| ureA | F: 5′-TGGGACTAAACTCGTAACCGTGC-3′<br>R: 5′-TGGAAGTGTGAGCCGATTTGAAC-3′ | 66 | 171 | AM997162 |



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## Ethical approval and infusion

The protocol of all animal experiments was approved by the Ethical Committee and Research Deputy of Islamic Azad University of Shahrekord Branch, Iran on July 12th 2016. The experimental animals (63 6-week-old female BALB/c mice) were divided into three groups (each group with 21 mice) including pcDAN3.1(+)-*ureA* recombinant DNA, pcDAN3.1(+)-*ureA* adjuvant by chitosan nanoparticles (recombinant DNA + nanoparticles), and pcDNA3.1(+) without target gene as a control plasmid. The infusion of recombinant DNA with a concentration of 1000 µg of plasmid dissolved in PBS were performed on days 0, 7, and 15 and infused in quadriceps muscle of all three groups of BALB/c mice. The sampling from each group was done in 15, 30, and 45 days after the last injection. Mice were anesthetized with ketamine/xylazine solution (8 and 1.6 mg/kg), the whole blood specimens were obtained from the heart of each mouse (the area under the jaw) at each sampling stage and ethylenediaminetetraacetic acid (EDTA) was used as anticoagulant. The muscle near the infusion site (quadriceps) was removed and all samples were stored at -70°C until use.

## Real-time quantitative reverse transcription PCR (Real-time qRT-PCR) analysis

Total RNA from 100 mg of tissue sample and white blood cells of BALB/c mice in each group was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The optical density (OD) of purified RNA was determined through comparison of a wavelength of 260 to 280 nm. The cDNA samples synthesize was done in final reaction volume of 20 µL by a cDNA synthesis kit (Takara, Kyoto) using specific primers or mix them with an oligo (dT) primers according to the manufacturer's instructions. The temperature program of cDNA synthesize was included 85°C for 5 seconds and then incubated at 42°C for 15 min, and the inactivation of the enzyme for 5 min at 85°C. After designing of specific oligonucleotide primers using GenBank data and Gene Runner software version 3.05 (Hastings Software Inc. Hastings, NY, USA), the expression levels of cytokine genes including *TGF-β1*, *IL-4*, and *IFNγ* were evaluated by q-RT-PCR. In this stage the *GAPDH* and *ureA* genes were used as an internal control and a target gene of recombinant DNA, respectively. Primers used for RT-PCR are given in **Table 1**.

A 1:10 dilution of each cDNA samples (50 ng) was amplified by real-time PCR in a total reaction volume of 20 µL including 2 µL cDNA, 10 µL 1× SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan), 1 µL each of specific forward and reverse (4 pM). The thermal cycling program included one cycle of initial denaturation step of 5 min at 95°C, followed by 45 cycles of denaturation at 95°C for 15 s; primer annealing at optimal temperature for 20 s (according to **Table 1**); extension at 72°C for 30 s. All Reaction was performed in triplicate for each gene. The melt curve analysis was performed by slowly cooling the PCRs from 95°C to 60°C (0.1°C per cycle) that performed by the Corbett Rotor-Gene 6000 machine. Finally, the cycle of threshold (Ct) values for the relative quantification strategy of the target and the reference genes were analyzed by $2^{-\Delta\Delta CT}$ method (Livak method).

## Statistical analysis

The results presented as mean values and standard deviations obtained from three independent experiments. All data for examination of the relationship between infused groups were analyzed by Social Sciences software (SPSS, Inc., Chicago, IL, USA) version 20 using paired T-test.

# RESULTS

## Identification of recombinant clones

After preparation of competent *E. coli strain Top10F'* using the conventional calcium chloride method, the recombinant pcDAN3.1(+)-*ureA* plasmid was transformed successfully into the bacterial cells and the presence of target gene in purified recombinant plasmid was confirmed by PCR and enzymatic digestion. The PCR amplification was confirmed the *ureA* gene in pcDAN3.1(+)-*ureA* recombinant vector. The digestion of extracted recombinant plasmid by *XhoI* and *XbaI* restriction endonucleases and evaluation of the products on 2% agarose gel electrophoresis were revealed two fragments including 5428 (pcDNA3.1(+) vector) and 729 bp (*ureA* gene) (**Fig. 1**).

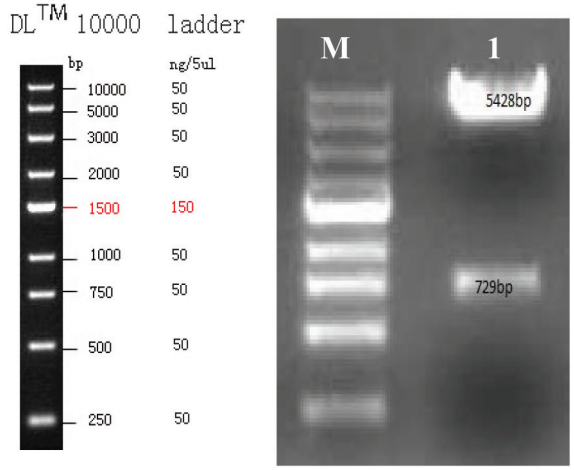


**Figure 1.** The enzymatic digestion of pcDAN3.1(+)-*ureA* recombinant plasmid by *XhoI* and *XbaI* restriction endonucleases on 2% agarose gel electrophoresis to confirm the accuracy of the transformation and the presence of the target gene. (Lane M is 1 kb DNA marker (Thermo Fisher Scientific, Freiburg, Germany), and lane 1 were pcDNA3.1(+) vector and exited *ureA* gene, respectively).

## Chitosan nanoparticles properties

The particle size and zeta-potential of chitosan nanoparticle were studied by dynamic light scattering (DLS) and scanning electron microscope (SEM) and it was shown that 98.6% of these nanoparticle had a spherical morphology and smooth edges with a diameter of 133.4 nm. The nanoparticles were deposited in a polystyrene covet using a Malvern Zetasizer Nano-ZS (ZEN3600) at 633 nm wavelength and the dispersion intensity was measured at 25°C (**Fig. 2**).

## Analysis of cytokine genes expression

The analysis of *urea* gene expression in tissue of infusion site in both groups, including pcDNA3.1(+)-*ureA* and pcDNA3.1(+)-*ureA* + nanoparticle showed the expression of *urea* gene were decreased 15, 30, and 45 days after the last infusion. Also, the expression level of *urea* gene in pcDNA3.1(+)-*urea* (DNA vaccine without nanoparticles) compare to the pcDNA3.1(+)-*ureA* + nanoparticle group in each day of sampling (15, 30, and 45 days after the last infusion) were significantly different ($p$-value <0.001) (**Fig. 3**).

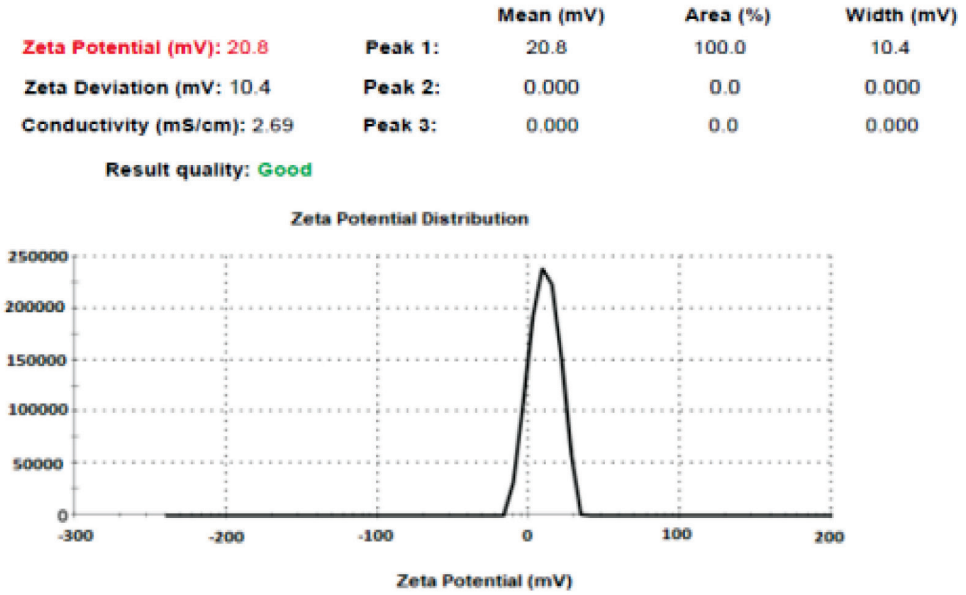


**Figure 2.** Zeta potential distribution of chitosan nanoparticles. The nanoparticles exhibited the zeta potential range from 22.6 to 34.7 mV and had a mean charge of 27 mV.

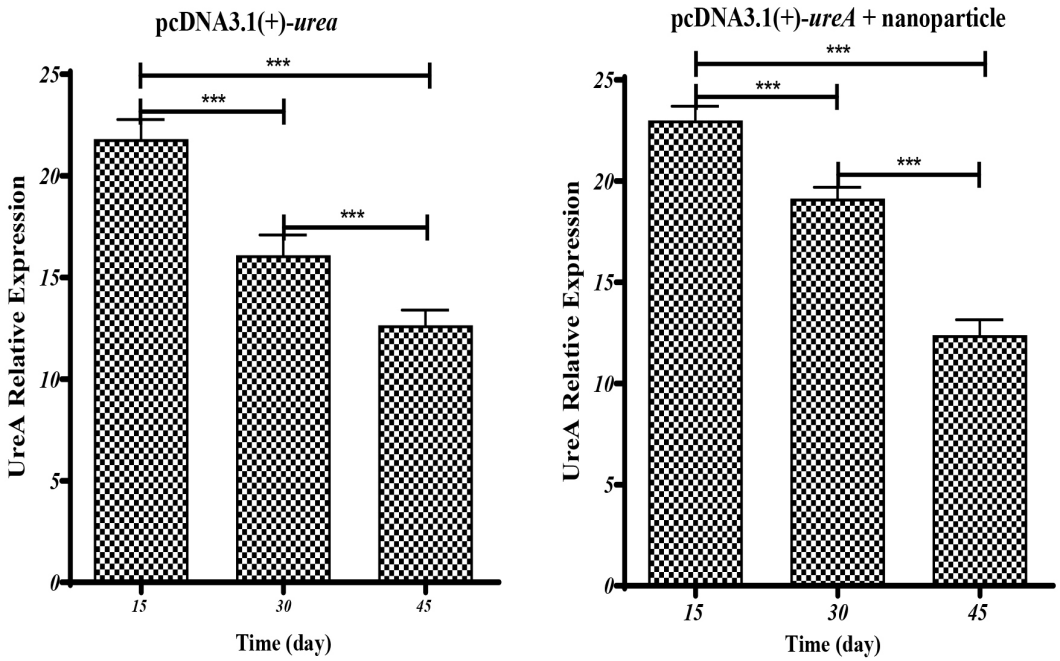


**Figure 3.** Comparison of the expression level of *urea* gene in pcDNA3.1(+)-*urea* (left) and pcDNA3.1(+)-*ureA* + nanoparticle (right) at each day of the sampling.



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The expression level of *ureA* gene in pcDNA3.1(+)-*ureA* and nanoparticle groups in tissue samples of infused mice at day 15 and 30 were significantly disparate ($p$-value <0.001), but 45 days after the last infusion was not significantly different ($p$-value >0.05) (**Fig. 4**).

The expression level of *TGF-β1* and IFNγ cytokine genes were increased in the blood of both groups of infused mice (p-value <0.001). While in both pcDNA3.1(+)-*ureA* and pcDNA3.1(+)-*ureA* + nanoparticle decreasing in the IL-4 expression was observed (p-value <0.001). The *TGF-β1* expression level in the pcDNA3.1(+)-*urea* group was not significantly altered ($p$-value >0.05), but in the pcDNA3.1(+)-*ureA* + nanoparticle group compared to the pcDNA3.1(+)-*urea* group was increased ($p$-value <0.05). Moreover, the expression level of *IFNγ* and *IL4* genes in nanoparticle group compared to the pcDNA3.1(+)-*ureA* group were significantly different ($p$-value <0.001) (**Fig. 5**). Generally, these effects were showed that stimulation of the immune system in mice occurred at a significant level.

## DISCUSSION

According to the worldwide spread of *H. pylori* infection the finding of a suitable vaccine for the prevention and treatment of this pathogen is attractive.[18,19] The *ureA* enzyme acts as an important virulence factor in *H. pylori* infection and in the present work, the recombinant pcDNA3.1(+)-*ureA* is based on this coding gene was designed and together chitosan nanoparticles were used for infusion of BALB/c mice. The efficiency level of this gene constructs against *H. pylori* infection was evaluated by q-real-time RT-PCR. The chitosan nanoparticles were used for better tissue absorption of the recombinant DNA. In order to infusion of BALB/c mice the each recombinant DNA with or without nanoparticles were dissolved in PBS and were injected into a mice's hip muscle. The injections were performed in 3 steps (days 0, 7 and 15). Three groups of mice (each group of 21 mice) were injected. The first group was a pcDNA3.1(+)-*ureA* recombinant plasmid group to-

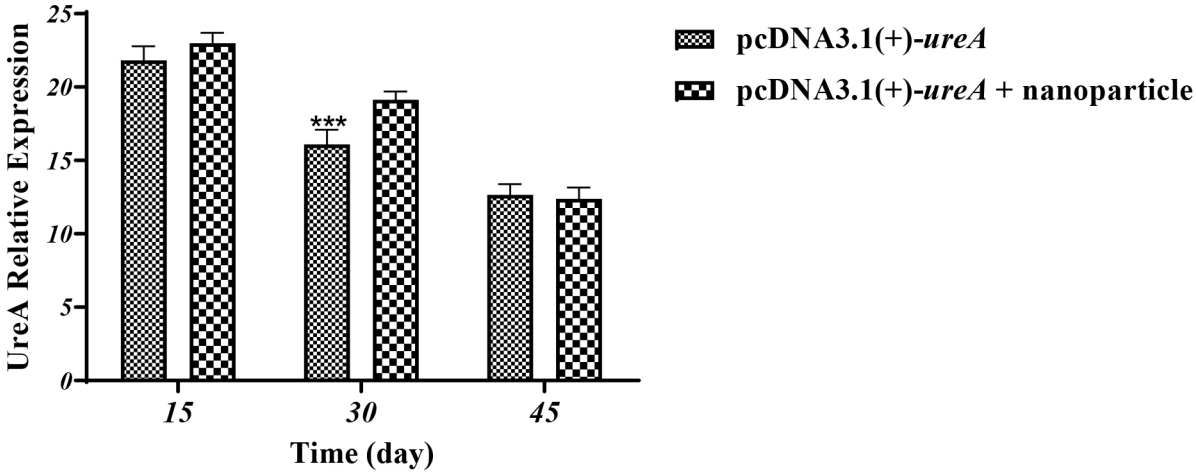


**Figure 4.** Comparison of *ureA* gene expression in the tissue of infused BALB/c mice by pCDNA3.1(+)-*ureA* and pcDNA3.1(+)-*ureA* + nanoparticle groups in 15, 30, and 45 days after the last infusion.

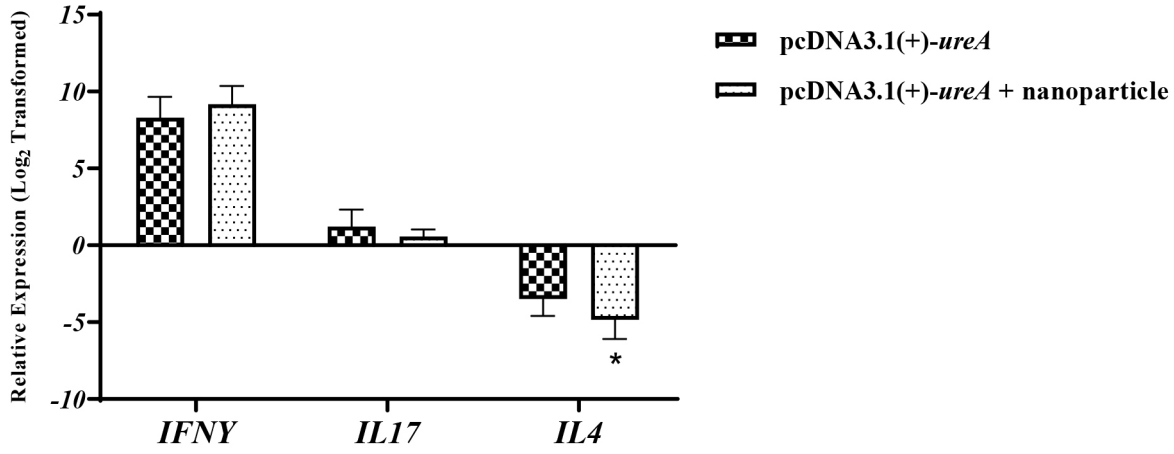


**Figure 5.** Comparison of the expression level of *IFNγ*, *TGF-β1*, and *IL4* cytokines in infused BALB/c mice by pcDNA3.1(+)-*ureA* + nanoparticle and pcDNA3.1(+)-*ureA*.

gether chitosan nanoparticles, the second group was the pcDNA3.1(+)-*ureA*, and the third group was plasmid without gene (control group). After 15 days from the last infusion stage, the animals were surgery for sampling, and then their blood was collected in a tube and as well as the tissue of the infusion site was isolated. The total RNA was extracted from each blood (white blood cells) and tissue specimens and cDNA was synthesized. Finally, the expression of cytokines genes such as *TGF-β1*, *IL-4*, and *IFNγ* compare to *GAPDH* gene as a reference gene and the expression of the *ureA* gene as a target gene of recombinant vector were investigated by q-Real-time RT-PCR method. The analysis of the cytokines genes expression in the blood specimens of pcDNA3.1(+)-*ureA* + nanoparticle and pcDNA3.1(+)-*ureA* groups were showed that the expression of *IL-4* in infused BALB/c mice was decreased ($p<0.001$) while the expression of *IFNγ* and *TGF-β1* genes were significantly increased ($p<0.001$). The molecular findings on the injection site at mice's hip muscle were showed that the expression level of *ureA* gene in both pcDNA3.1(+)-*ureA* and nanoparticle groups at day 15 and 30 were significantly different ($p<0.001$), while at day 45 was not significantly different ($p>0.05$).

In several studies in recent decades, not only the prevented program, but also immunization against *H. pylori* infection has been taken into consideration.[7,20,21] In a study by Hajikhani et al., 2010 a recombinant vector containing a component of beta enzyme urease (332ureb) and an adhesion of *Helicobacter pylori* (HpaA) was built and its expression in expression host (*E. coli BL21*) was investigated. Also the antigenicity as a good candidate for the vaccine against *Helicobacter Pylori* was checked. The hPAA and UreB332 coding genes were isolated from the standard strain genome of *H. pylori* by PCR and digested with enzymes and cloned into the pET28a vector and then entered into colonization and expression membranes. In spite of the results of this study, this antigen combination could be introduced as a suitable candidate for further studies on *Helicobacter pylori* vaccine. After confirmation, the protein composition was analyzed by chromatography and then by the western stains method. The difference between this research and the present study was to determine the type of gene and the method of gene analysis. In this study, the expression of the cytokine genes was also investigated[22]. In another study the *ureB* (*urease B*) gene fused with IL-2 as the mucosal adjuvant and cloned into *Lactococcus lactis* (*L. lactis*) and mice infused by this construct. Their study showed the producing of cytokines such as IFN-γ, IL-4, and IL-17 and as well as anti-UreB antibody in infused mice. These findings implied that this recombinant *L. lactis* expressing UreB–IL-2 had a potential advantage as a vaccine for controlling *H. pylori* infection.[23] However, in our research, the pcDNA3.1(+)-*ureA* recombinant plasmid was able to express the *ureA* gene in BALB/c mice and also the expression level of *IFNγ* and *TGF-β1* cytokine genes were increased but the *IL4* expression was significantly decreased in the blood of infused mice in both groups pcDNA3.1(+)-*ureA* and pcDNA3.1(+)-*ureA* + nanoparticle. In another study the *ureB* gene was cloned and expressed in order to create a recombinant vector against *H. pylori*. In their study, the ureB gene was reproduced from H. pylori genome using PCR method and the amplified products were T/A cloned into pTZ vector and then digested with *XhoI* and *XbaI* enzymes and sub-cloned into the pcDNA3.1(+) vector. The pcDNA3.1(+)-*ureB* final construct transformed into CHO cells using electroporation method. The *ureB* gene expression was shown on the SDS-PAGE gel. Their findings indicated that the pcDNA3.1(+)-*ureB* recombinant vector had a high potential as a candidate for the evaluation of the immunogenicity in the animal model. The difference between their research and the present study was the type of the gene and the method of gene analysis.[24] In our study, after the infusion of animals by pcDNA3.1(+)-*ureA* with or without chitosan nanoparticles the expression level of *TGF-β1*, *IL-4*, and *IFNγ* cytokine genes were also investigated.

In conclusion, in this study the enhancing of cytokine genes like *IFNγ* and *TGF-β1* and decreasing of *IL-4* after infusion of BALB/c mice were observed. Also, the increasing in the expression of interleukins after combination of chitosan nanoparticles were seen in infused mice.

## CONCLUSIONS

In conclusion, the pcDNA3.1(+)-*ureA* that produced in this work can stimulate the immune system of animal model and in future study it was suitable for production of recombinant protein and antibodies against *H. pylori* infection and prevention in human models. Also, this construct can be use in order to the biomarker and drug-discovery in eukaryotic systems.

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## Compliance with ethical standards

The protocol of all animal experiments was approved by the Ethical Committee and Research Deputy of Islamic Azad University of Shahrekord Branch, Iran on July 12th 2016.

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# Оценка иммунного ответа против *Helicobacter pylori* у мышей BALB / c, инфузированных pcDNA3.1 (+) – *ure*A

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## Абстракт

**Введение:** Целью данного исследования было получение рекомбинантного вектора pcDNA3.1 (+) - ureA и оценка способности этого вектора стимулировать иммунный ответ против инфекции *H. pylori* у инфузированных мышей BALB / c.

**Материалы и методы**: Структура pcDNA3.1 (+) - *ure*A была успешно приготовлена и трансформирована в *E.coli*. Животные, которых мы использовали в исследовании, были разделены на три инфузионные группы: 1) рекомбинантная плазмида, 2) pcDNA3.1 (+) - *ure*A + наночастицы и 3) pcDNA3.1 (+). Образцы крови и тканей каждой группы мышей отбирали на 15, 30 и 45 сутки после последней инфузии, и уровни экспрессии цитокинов, таких как гены TGF-β1, IL-4 и IFNγ, оценивали по сравнению с GAPDH (глицеральдегид-3-фосфатдегидрогеназой) , а также экспрессию urea в мышцах бёдер у мышей.

**Результаты:** Анализ экспрессии генов показал, что экспрессия IL4 была значительно снижена ($p$ <0,001), но экспрессия IFNγ и TGF-β1 увеличилась в крови инфузированных мышей (p <0,001). Кроме того, уровень мочевины, экспрессируемой в наночастицах pcDNA3.1 (+) - urea и pcDNA3.1 (+) - *ure*A + в дни 15, 30 и 45 после последней инфузии, значительно различался (p <0,001) и между его экспрессией на 15 и 30 дни наблюдалась значительная разница (p <0,001), но через 45 дней после последней инфузии разница была незначительной (p> 0,05).

**Выводы:** Рекомбинантный вектор pcDNA3.1 (+) - *ure*A с частицами хитозана или без них может стимулировать иммунный ответ на животных моделях против инфекции H. pylori. Кроме того, после объединения рекомбинантного вектора с наночастицами мы наблюдали лучший иммунный ответ. В будущих исследованиях эта рекомбинантная структура может быть использована в качестве биомаркера и для терапевтических подходов в эукариотических системах.

## Ключевые слова

*Helicobacter pylori*, pcDNA3.1(+)-*ure*A, рекомбинантный вектор, гены цитокинов