MINERVA BIOTEC 2014;26:175-82

Recombinant plasmid KMP-11 gene of *Leishmania major* (pcKMP-11): production, characterization and sequencing

E. BANDANI [1], S. SOFLAEI [2], F. KHALILI [3], M. A. AGHEI AFSHAR [4], H. KOOSHKI [3] A. ABDOLI [2, 6], M. KAMALI [3], S. SARVI [5], V. NASIRI [2], A. A. JAFARI [7], M. ABKAR [8]

[1]*Department of Pharmaceutical Biotechnology Faculty of Pharmacy Shahid Beheshti University of Medical Sciences Tehran, Iran*
[2]*Department of Parasitology, Faculty of Medical Sciences Tarbiat Modares University, Tehran, Iran*
[3]*Nanobiotechnology Research Center Baqiyatallah University of Medical Sciences, Tehran, Iran*
[4]*Department of Nanotechnology Azad University of Pharmaceutical Sciences, Tehran, Iran*
[5]*Department of Parasitology and Mycology Mazandaran University of Medical Sciences, Sari, Iran*
[6]*Department of Parasitology Kashan University of Medical Sciences, Kashan, Iran*
[7]*Young Researchers and Elite Club, Parand Branch Islamic Azad University, Parand, Iran*
[8]*Department of Genetics, Faculty of Basic Sciences Tarbiat Modares University, Tehran, Iran*

Aim. **Kinetoplastid membrane protein-11 expresses life cycle stages of all kinetoplastidae parasites. Previous studies have demonstrated that kinetoplastidae KMP-11 gene is highly conserved and may be useful for vaccine strategies against Leishmaniasis. In this study, we isolated *Leishmania major* (MRHO/IR/75/ ER) KMP-11 gene and formulated a pcKMP-11 recombinant expressing plasmid as a candidate DNA vaccine against cutaneous Leishmaniasis.**

Methods. **After gene amplification, KMP-11 fragments were cloned into pTZ57R/T standard cloning vector and transformed in *E. coli*, then subcloned into pcDNA3 eukaryotic expression vector and pcKMP-11 recombinant plasmid was transfected to CHO eukaryotic cells. Amplification, sequencing, cloning and transfection of gene were performed successfully. mRNA transcription of KMP-11 gene in CHO cells was confirmed by RT-PCR methods. Results. Sequence results were compared with other records of kmp-11 in gene bank and a 97-99% identity was showed. Comparison of KMP-11 protein with other records showed that this protein have 92 amino acids. Additionally, a silico analysis of 3D structures of the wild type and double mutant KMP-11 proteins show that the mutations in position 16 and 41 have led to a change in structure conformation and stability.**

Conclusion. **Present results show that KMP-11 can be an excellent candidate for immunization against leishmaniasis.**

KEY WORDS: Kinetoplastid membrane protein-11 (KMP-11) - Leishmania major - Leishmaniasis, cutaneous - Vaccines, DNA.

Leishmaniasis is a neglected tropical disease with an important public health problem in different parts of the world.[1] Leishmaniasis presence is reported in 88 countries around the world, and the prevalence of this disease is estimated to be approximately 12 million annually and about 350 million people are at the risk of catching the disease.[2] Cutaneous leishmaniasis is prevalent in many areas of the world and recently many studies indicated that the number of cutaneous leishmaniasis is increasing.[2] Unfortunately, there are no effective vaccines for leishmaniasis. Also, treatment of leishmaniasis is associated with various complications such as several adverse side effects and leishmaniasis drug resistance.[3] Hence, searching for new drugs and vaccines to overcome these complications is needed.

Studies have shown that KMP-11 gene expresses in all stages of the parasite's life cycle and proteins

Corresponding author: S. Soflaei, Parasitology Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
E-mail: s.soflaee@hotmail.com

produced by this gene have common characteristics in most of the kinetoplastidae. This protein has 11 KD molecular weight, an isoelectric point 4.8 and is expressed in 1-2×10^6 copies per parasite.[4, 5] KMP-11 was isolated and characterized at first in *L. donovani* by Jardim.[5] In the beginning, this molecule was called "associated protein of lipophosphoglican" and only after named 11 KD of kinetoplastid membrane.[5, 6] The biological function of KMP-11 protein is unknown yet but immune fluorescence studies suggested that the KMP-11 is located mainly along the flagellum and flagellar base of the parasites and it was found in parasites such as *L. infantum*, *T. congolense* and *T. brucei*. Immunoreactivity of KMP is primarily along the flagellum and flagellar pocket.[7] These findings suggest that KMP-11 gene may be useful for prevention strategies against leishmaniasis or immunotherapy.

Present study was performed for characterization of Iranian *Leishmania major* strain KMP-11 gene and protein and comparison between this strain and other parasites and also formulation of a recombinant expressing plasmid pcKMP-11 as immune stimulator against cutaneous leishmaniasis for further studies.

Materials and methods

Parasites preparation

L. major (MRHO/IR/75/ER) promastigotes were purchased from a pasture institute in Iran. Promastigotes were cultured in RPMI1640 medium supplemented with 10% FCS (Fetal Calf Serum, Sigma) and incubated at 25 °C.

KMP-11 fragments amplification

Genomic DNA was extracted from *L. major* promastigotes by DNA genomic extraction kit (Bioneer, Korea) according to manufacturing protocol. The PCR reaction was performed in 25 µL volume containing: 5 µL of DNA genomic as template, 1 µL of each primer, 2 µL of PCR master mixes, and 16 µL of distilled water. Forward and reverse primers were designed based on DNA sequences of KMP-11 that was recorded in Gene bank.

Forward primer: **AAGCTT**ATGGCCACCACGTACGAGGAG and

Reverse primer: **GAATTC**TTACTTGGATGGGTACTGCGCAGC.

Forward primer contains a HindIII restriction site and Reverse primer contains an EcoRI restriction site enzyme. PCR procedure design included: an initial denaturation at 94 ºC for 2 min, 35 cycles at 94 ºC for 30 s, at 59 ºC for 30 s, at 72 ºC for 30 s and then at 72 ºC for 7 min as final extension. Finally, the PCR products were analyzed by electrophoresis on 2% agarose gel. KMP-11 gene fragments were purified from gel by using gel purification kit (Bioneer, Germany).

Cloning in pTZ57R

Ligation of KMP-11 gene into pTZ57R/T standard cloning vector was performed by using InsT/A clone ™ PCR product cloning Kit (Fermentas®). Ligation reaction was prepared in 30 µL volume containing: 5 µL of template (DNA extraction product), 3 µL of pTZ57R plasmid, and 5 µL of 5 x ligation buffer, 1 µL of T4 ligase and 15 µL of distilled water. This reaction was incubated at 22 °C for 1 hour and then at 4 °C for 16 hours. The competent cells were prepared from *TOP10* strain of *E. coli* bacteria by calcium chloride method.[8] 100 µL of *E. coli* was cultured in 50 mL of new LB broth and was incubated at 37 °C for overnight. The next day 400 µL of these cells were cultured in 40 mL of LB broth and incubated at 37 °C for 2 hours. After incubation cells was centrifuged at 7000 rpm, at 4 °C for 5 minutes. The supernatant was discharged and then 1 mL of cold calcium chloride (100 mM) was added to pellet and mixed gently and then incubated on ice pieces for 30 minutes. This suspension was centrifuged at 7000 rpm, at 4 °C for 3 minutes. The supernatant was discharged and 670 µL of cold calcium chloride (100 mM) added to pellet and mixed gently and then incubated on ice pieces for 30 minutes. This suspension was centrifuged at 7000 rpm, at 4 °C for 3 minutes. The supernatant was discharged and 200 µL of cold calcium chloride (100 mM) added to pellet and mixed gently and then incubated on ice pieces for 3 minutes. At this step competent cells were ready for transfection; 10 µL of ligation product was added to 100 µL of this suspension and incubated on ice pieces for 30 minutes. Then the suspension for heat shock was incubated at 42 °C for 90 seconds and then immediately transferred on ice pieces for 5 minutes. 1 mL LB broth without any antibiotic was added and incubated at 37 °C for 1.5 hour. These cells were cultured in plates of Luria-Bertani (LB) agar

medium contain: 100 mg/mL of ampicillin, 200 mg/mL of IPTG and 20 mg/mL of X-Gal. The plates were incubated at 37 °C for 16 hours. Recombinant plasmids (pTKMP-11) were extracted from white colonies by a plasmid extraction kit according to manufacturing protocol (Bioneer, Korea). Then extracted plasmids were sequenced and compared with previous records in gene bank. The recombinant plasmid (pTKMP-11), pTZ57R (negative control) and pcDNA3 (expression vector) were digested by EcoRI and HindIII enzymes. The double digestion reactions were prepared in same way for each of them in 220 µL volumes containing of 80 µL of plasmid extraction product, 88 µL of water, 44 µL of R buffer, 4 µL of EcoRI and 4 µL of HindIII enzymes. These reactions were incubated at 37 °C for 16 hours. The products of digestion were analyzed by electrophoresis on 0.8% agarose gel. The bands belong to KMP-11 fragment and digested expression plasmids (pcDNA3) were purified with a gel purification kit (Bioneer, Korea). KMP-11 fragment was purified from gel and was sent to Gene Fanavaran Company for sequencing.

Tertiary structure prediction

3Dpro software at http://scratch.proteomics.ics.uci.edu/index.html was employed for prediction of KMP-11 (our construct) tertiary structure. 3Dpro uses predicted structural features, and PDB knowledge based statistical terms in the energy function. The conformational search uses a move set consisting of fragment replacement (using a fragment library built from the PDB) as well as random perturbations to the model. Moves are selected or rejected based on a simulated annealing method with linear cooling. Multiple models are constructed using random seeds and the model with the lowest energy is selected as the final prediction. 3Dpro is currently a *de novo* method (structural templates are not used).

Evaluation of structural stability and validation

3D structural stability of the wild type and double mutant KMP-11 were evaluated by Swiss-PdbViewer software for energy minimization. The stereochemical validation of the modeled structures was carried out by structural analysis and verification server (SAVES) at http://nihserver.mbi.ucla.edu/SAVES that validates the models using the programs like Procheck, What-check, verify 3D, Errat and Prove.

Sub cloning in pcDNA3 expression vector

KMP-11 gene ligated into digested pcDNA3 expression vector by using of T4 DNA ligase enzyme. Recombinant plasmids pcKMP-11 were transformed into TOP10 strain of *E. coli* competent cells and recovered in antibiotic free LB broth medium at 37 °C for 1.5 hour and then subcultured on new plates of LB agar containing 100 mg/mL of ampicillin and incubated at 37 °C for 16 hours. The Recombinant plasmids (pcKMP-11) were extracted from transfected *E. coli* cells.

Transfection of KMP-11 gene in CHO eukaryote cells

For transfecting in this study, CHO (Chinese hamster ovary) eukaryote cells were used as host cells for recombinant expression plasmids (pcKMP-11). The CHO cells were cultured under sterile conditions in DMEM (Gibco) medium supplemented with 5% FCS at 37 ºC with 5% CO_2 conditions. Transfection in this study was carried out by fugene 6 transfection reagent kit (Roche). At first, cells should be plated 18 to 24 hours before transfection because the monolayer cell density reaches the optimal 70-80% confluence. CHO cells were cultured in 6-well plate using 3:1, and 6:1 ratios of transfection reagent (94-97 µL) to plasmid (0.5-50 µL). Then these cultures were supplemented with freshly medium containing serum and antibiotics added to each 1-2 hours before transfection and allowed to grow overnight (14-16 hours) according to manufacturing protocol.

RNA extraction and RT-PCR procedure

In the current study for confirmation of gene transcription at the level of mRNA we have used the Reverse Transcriptase-PCR kit. For our study total RNA was isolated from CHO eukaryote cells by RNX ™ (-plus) Isolation RNA kit and RT-PCR was performed using RT-PCR kit (Fermentas ®) according to manufacturing protocol.

Results

Band of *L. major* (MRHO/IR/75/ER) KMP-11 gene was observed by electrophoresis on 2% agarose gel that has a size in range of 279bp (Figure 1). Sequences results of KMP-11 gene in our research were compared with other records in gene bank

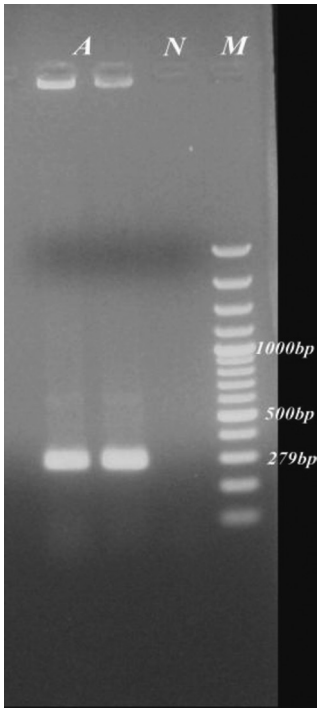

Figure 1.—Electrophoresis of PCR product for KMP-11(279bp) fragment of *L. major* amplification. A: the band KMP-11 fragments of *L. major*; N: control negative; M: 100bp ladder as a marker.

and different nucleotides were determined. These comparison results highlighted the presence of two different nucleotides within the KMP-11 gene of *L. infantum*; in a previous study (accession number: JF422108) it had 1 different nucleotide with another *L. major* (AY490814) and no difference with *L. major* (XM-838234) (Figure 2). The sequence results indicated a 97-99% similarity between KMP-11 genes of *L. major* with other organisms (Table I). These sequence results confirmed this gene encodes KMP-11 protein contained 92 amino acids. We found no difference between results in the present study and two *L. major* with accession number XM_838234 and AY490814, while there was a difference between KMP-11 protein of *L. major* in present report and *L. infantum* with accession number JF422108 (Figure 3).

Results in Figures 2 and 3 show that this protein is much conserved in members of *Kinetoplastidae*. Table II shows the comparison of KMP-11 protein presence in our results and recorded results in gene bank.

The phylogenic tree for comparison between KMP-11 genes of *L. major* in this research with other organisms and strain that were recorded in gene bank is presented in Figure 4.

```
L. major    ATGGCCACCACGTACGAGGAGTTCTCGGCGAAGCTGGACCGCCTGGATGAGGAGTTCAAC 60
XM_838234   ............................................................ 60
AY490814    ............................................................ 60
JF422108    ............................................................ 60
            ************************************************************

L. major    AGGAAGATGCAGGAACAGAACGCCAAGTTCTTTGCGGACAAGCCGGATGAGTCGACGCTG 120
XM_838234   ............................................................ 120
AY490814    ............................................................ 120
JF422108    ............................................................ 120
            ************************************************************

L. major    TCGCCCGAGATGAAGGAGCACTACGAGAAGTTCGAGCGCATGATCAAGGAGCACACAGAG 180
XM_838234   ............................................................ 180
AY490814    ............................................................ 180
JF422108    C...........................G................................ 180
             ******************************* ************************

L. major    AAGTTCAACAAGAAGATGCACGAGCACTCGGAGCACTTCAAGCAGAAGTTCGCTGAGCTG 240
XM_838234   ............................................................ 240
AY490814    ............................................C............... 240
JF422108    ............................................................ 240
            ************************************************** ******

L. major    CTCGAGCAGCAGAAGGCTGCGCAGTACCCGTCCAAGTAA 279
XM_838234   ..................................... 279
AY490814    ..................................... 279
JF422108    ..................................... 279
            *************************************
```

Figure 2.—Sequences results of KMP-11 gene in our research were compared with other records in Gene bank. L. major as sequence in present study, XM-838234, AY490814: *L. major* and JF422108: *L. infantum* in previous study.

TABLE I.—*Similarity and length of KMP-11 sequences were compared with 16 sequences of other organisms that were recorded in gene bank.*

Name of organism	Strain	Length of gene	Accession NO.	Similarity
L. major	-	279bp	(This study)	100%
L. major	-	279bp	(AY490814)	100%
L. major	Friedlin	279bp	(XM-838234)	100%
L. infantum	JPCM5	279bp	(XM_001468995)	99%
L. infantum	LEM78 MON-1	279bp	(X95627.1)	99%
L. donovani	BPK282A1	279bp	(FR799622.2)	99%
L. tropica	-	279bp	(AJ000078.1)	99%
L. mexicana	MHOM/GT/2001/U1103	279bp	(FR799587.1)	99%
L. amazonensis	IFLA/BR/67/PH8	279bp	(AF193432.1)	99%
L. panamensis	M/HOM/PA/71LS94/6/7	279bp	(U93582.1)	99%
L. panamensis	M/HOM/PA/71LS94/5/7	279bp	(U93578.1)	99%
L. guyanensis	MHOM/BR/75/M-4147	279bp	(AF026141.1)	98%
L. braziliensis	MHOM/BR/75/M-2903	279bp	(AF026136.1)	98%
T. cruzi	CL Brener	279bp	(XM_803773.1)	81%
T. brucei	TREU927	279bp	(XM_822498.1)	81%
T. rangeli	5048	279bp	(DQ194343.1)	81%
Crithidia sp.	CJP-2005	279bp	(DQ194339.1)	81%

```
L. major     MATTYEEFSAKLDRLDEEFNRKMQEQNAKFFADKPD
XM_838234    ************************************
AY490814     ************************************
JF422108     ************************************

L. major     ESTLPPEMKEHYEKFERMIKEHTEKFNKKMHEHSEH
XM_838234    ************************************
AY490814     ************************************
JF422108     ********************G***************

L. major     FKQKFAELLEQQKAAQYPSK
XM_838234    ********************
AY490814     ********************
JF422108     ********************
```

Figure 3.—Amino acids sequences of KMP-11 protein were compared between our results and results in Gene bank. *L. major* (our report), XM_838234 and AY490814 (*L. major)* and JF422108 (*L. infantum*).

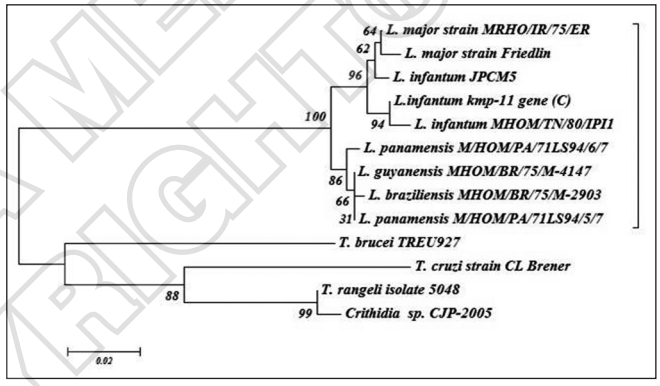

Figure 4.—Phylogenic tree for comparison between KMP-11 genes of *L. major* in this research with other organisms that were recorded in Gene bank.

Tertiary structure prediction

Three dimensional structures of the wild type double mutant KMP-11 were successfully modeled using 3Dpro software. The models were uploaded to the server to draw the tertiary structural illustrations with Accelrys Discovery Studio Visualizer 2.0 software for detecting the final structure of the protein. The results of tertiary structure prediction show that the mutation in position 16 and 41 have led to change in structure conformation (Figure 5).

Evaluation of structural stability and validation

The profile of energy minimization was computed by spdbv (Swiss-PdbViewer) (-1061.732 against -913.333 Kcal/mol) showing that double mutant KMP-11 is more stable than wild type protein. The results of SAVES server showed 95.7% and 90.32% of residues of double mutant and wild type KMP-11 at an average 3D-1D score >0.2, respectively. This shows the predicted structures were further validated.

The extracted plasmids from white colony were digested by EcoRI and HindIII enzymes and electrophoresis on 0.8% agarose gel (Figure 6).

At the further step this gene cloned successfully

TABLE II.—*The protein encoded by KMP-11 gene of L. major in this research was compared with the other records in Gene bank from different country. This protein is containing 92 amino acids in all of them.*

Name	Protein ID	Amino acids	Country
Leishmania major	XP_843328	92	USA
Leishmania major	XP_843327.1	92	USA
Leishmania major	AAR84616.1	92	Tunisia
Leishmania infantum	AEK80413.1	92	Iran
Leishmania infantum	XP_001469032	92	England
Leishmania infantum	CAA64883	92	Spain
Leishmania donovani	AAB33127	92	England
Leishmania donovani	S53442	92	Canada
Leishmania tropica	CAA03902	92	Spain
Leishmania panamensis	AAC61837	92	Colombia
Leishmania amasonensis	AAG32958	92	Brazil
Leishmania guyanensis	AAB94115	92	Colombia
Tryponosoma rangeli	ABA42053	92	Colombia
Crithidia sp.	ABA42050	92	Colombia

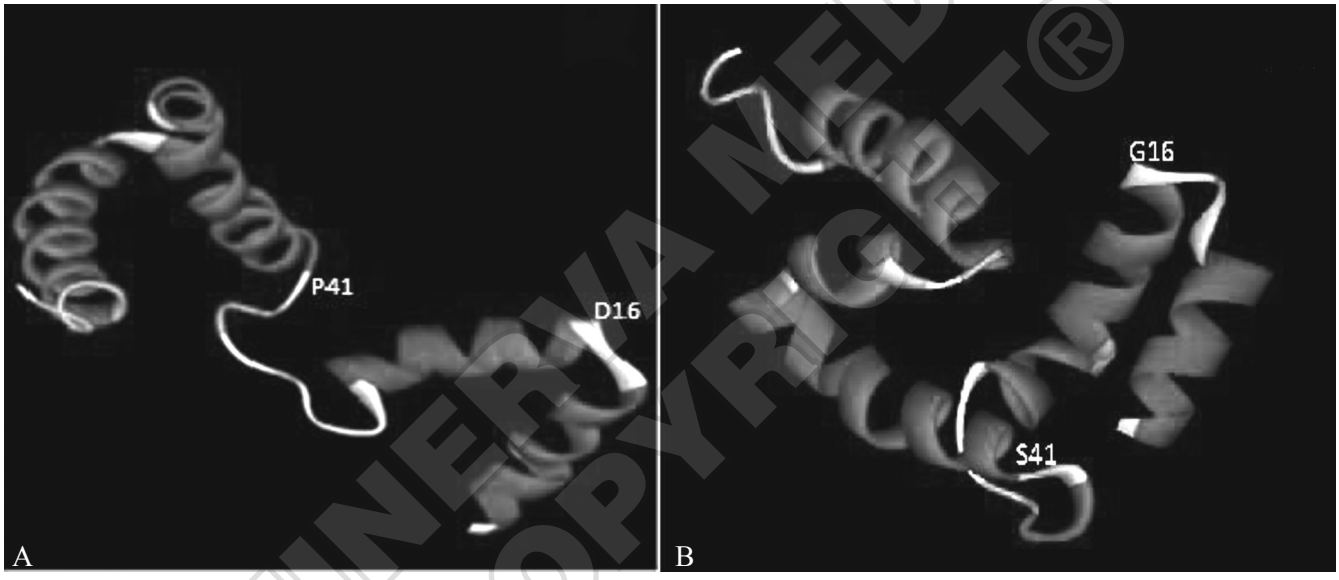

Figure 5.—A) 3Dpro modeling software was employed to predict the 3D structure of the double mutant; B) wild type KMP-11. The results were viewed by Accelrys Discovery Studio Visualizer 2.0 software. The mutation sites have been displayed in yellow color.

into pcDNA3 expressing eukaryote vector and transformed to *TOP10* strain of *E.coli* bacteria. For confirmation, the extracted expression plasmids digested by EcoRI and HindIII enzymes and the band of KMP-11 fragment were observed (Figure 7).

The pcKMP-11 plasmids were transfected successfully in CHO cells. RNA transcription was confirmed by reverse transcriptase-PCR method and then these results were electrophoresis on 2% agarose gel. The band of KMP-11 fragment was observed (Figure 8).

Discussion

KMP-11 is found in all kinetoplastidae protozoa [9] and is highly conserved (>95% homology) in all *Leishmania* species, suggesting an essential role of this protein in the biology of the parasite.[6] The findings in present study show that from a sequence of this gene KMP-11 protein with 92 amino acids can be produced. The transcription of KMP-11 genes gives in a variable sized mRNA with 0.5 kb in *T. cru-*

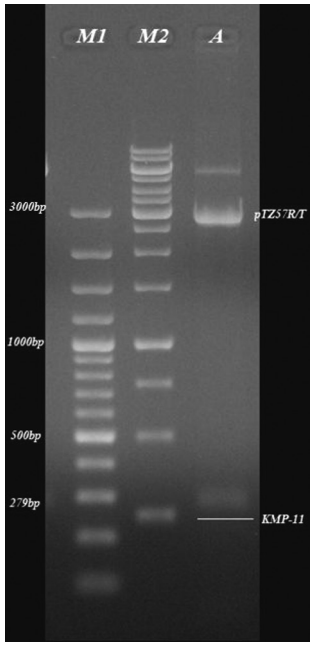

Figure 6.—Recombinant plasmid (pTKMP-11) was digested by EcoRI and HindIII enzymes and electrophoresis on 0.8% agarose gel. M1: 100bp ladder M2: 1kb ladder as marker, A: the bands of KMP-11 fragment (279bp) and plasmid after digestion.

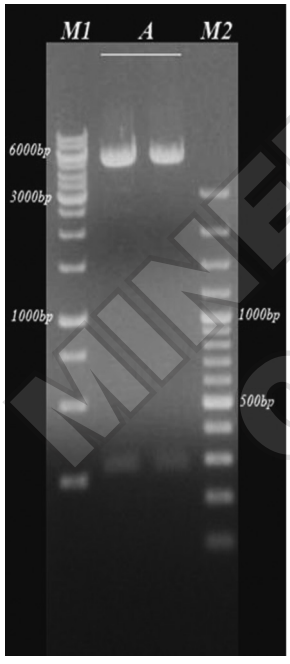

Figure 7.—Electrophoresis of recombinant vector (pcKMP-11) after digested by EcoRI and HindIII enzymes. M1: 1kb ladder, M2: 100bp ladders as marker, A: the bands of KMP-11 fragment (279bp) and plasmid (pcDNA3) after digestion.

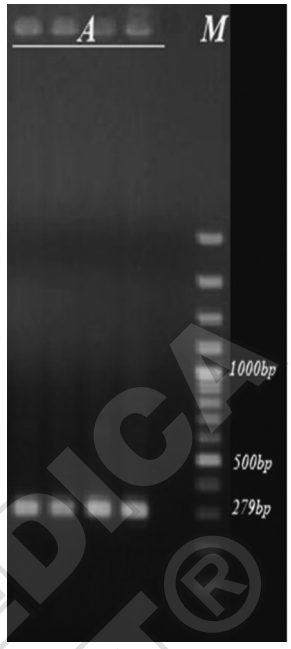

Figure 8.—Electrophoresis was carried out with RT-PCR products. A: five bands of KMP-11 fragment (279bp), M: 100bp ladder as marker.

zi, 0.8 kb and 1.3 kb in *T. brucei* and *L. infantum*.[4] Now, this study confirmed this gene is conserved in Iranian strains of *L. major*. The KMP-11 protein has a high denaturants resistance conditions. For example, it has been determined that the protein is stable at a pH 2-7, its structure is conserved at 50 °C and is completely rolled at 85 °C, progressively loosing its propeller structure.[10] Additionally, comparison of 3D structures of the wild type and double mutant KMP-11 proteins shows that the mutations in position 16 and 41 have led to a change in structure conformation and stability. The prediction indicated that these mutations result in a opener conformation in protein structure. Furthermore, these mutations result in a major stability in protein structure. Studies on KMP-11 showed that this protein has clearly three immunological roles: B-cell immune stimulatory, inducer lymphocyte proliferation and response cytotoxic and immune protective in animal models. Another study by Trujillo showed that the subclasses of immunoglobulin involved in the response to KMP-11 protein are IgG1, IgG3, IgG2 and IgG4.[11, 12] The humeral immune response against KMP-11 protein has been judged not only against the native and recombinant protein but also against synthetic pep-

tides and fractions recombinant protein. But could establish that even these peptides were recognized by serum of visceral leishmaniasis patients (30-58%), by using of lapped three synthetic peptides, this reactivity did not reach the levels recognition of the native protein.[11, 13] The ability of KMP-11 protein to induce proliferation of T lymphocytes was demonstrated. Another study showed that KMP-11 of *L. donovani*, *T.b.rhodesiense*, *T.b.brucei*, *T.congolense* and *T.simiae* is a potent stimulator of $CD4^+$, $CD8^-$ in mice immunized with KMP-11 protein.[14-16] Planelles *et al*. (2002) found that the fusion protein HSP70/KMP-11 is capable of stimulating mouse mature dendritic cells, consequence production of interleukin 12 (IL-12) and tumor necrosis factor (TNFα), and although this effect is mainly due to HSP70, the presence of the KMP-11 increases production levels of cytokines.[17] Ramirez *et al*. showed that immunization of BALB/c mice with an attenuated strain of *Toxoplasma gondii* expressing the *Leishmania* KMP-11 protein induces a specific immune response and is immune protective in such animals.[18] Berberich *et al*. showed that immunization of mice with murine dendritic cells pulsed with a mixture of recombinant Leishmania antigens, including KMP-11 protein, was able to control *L. major* infection.

Evidences in these studies strongly indicate that this gene is a very excellent target for immunotherapy and immunization against leishmaniasis and also trypanosomiasis diseases.[19] Therefore, in this research, KMP-11 gene was cloned into eukaryote vector and we produced a recombinant expression vector (pcKMP-11) that can be used as DNA vaccine against cutaneous and leishmaniasis.

References

1. Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD *et al*. Control of neglected tropical diseases. N Engl J Med 2007;357:1018-27.
2. Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. Lancet 2005;366:1567-77.
3. Maltezou HC. Drug resistance in visceral leishmaniasis. J Biomed Biotechnol 2010;10:1-8.
4. Berberich C, Machado G, Morales G, Carrillo G, Jimenez-Ruiz A, Alonso C. The expression of the *Leishmania infantum* KMP-11 protein is developmentally regulated and stage specific. Biochim Biophys Acta 1998;1442:230-7.
5. Jardim A, Hanson S, Ullman B, McCubbin WD, Kay CM, Olafson RW. Cloning and structure-function analysis of the *Leishmania donovani* kinetoplastid membrane protein-11. Biochem J 1995;305:315-20.
6. Ramirez JR, Berberich C, Jaramillo A, Alonso C, Velez IV. Molecular and Antigenic Characterization of the *Leishmania (Viannia) panamensis* Kinetoplastid Membrane Protein-11. Mem Inst Oswaldo Cruz 1998;93:247-54.
7. Stebeck CE, Beecroft RP, Singh BN, Jardim A, Olafson RW, Tuckey C *et al*. Kinetoplastid membrane protein-11 (KMP-11) is differentially expressed during the life cycle of African trypanosomes and is found in a wide variety of kinetoplastid parasites. Mol Biochem Parasitol 1995;71:1-13.
8. Basu R, Bhaumik S, Mookerjee Basu J, Naskar K, De T, Roy S. Kinetoplastid Membrane Protein-11 DNA Vaccination Induces Complete Protection against Both Pentavalent Antimonial-Sensitive and -Resistant Strains of *Leishmania donovani* That Correlates with Inducible Nitric Oxide Synthase Activity and IL-4 Generation: Evidence for Mixed Th1- and Th2-Like Responses in Visceral Leishmaniasis. J. Immunol 2005;174:7160-71.
9. Stebeck CE, Baron GS, Beecroft RPY, Pearson TW. Molecular characterization of the kinetoplastid membrane protein-11 from African trypanosomes. Mol Biochem Parasitol 1996;81:81-8.
10. Fuertes MA, Pérez JM, Soto M, López MC, Alonso C. Calcium-induced conformational changes in *Leishmania infantum* kinetoplastid membrane protein-11. J Biol Inorg Chem 2001;6:107-17.
11. Jensen AT, Gasim S, Ismail A, Gaafar A, Kurtzhals JA, Kemp M *et al*. Humoral and cellular immune responses to synthetic peptides of the *Leishmania donovani* kinetoplastid membrane protein-11. Scand J Immunol 1998;48:103-9.
12. Trujillo C, Ramirez R, Velez ID, Berberich C. The humoral immune response to the kinetoplastid membrane protein-11 in patients with American leishmaniasis and Chagas disease prevalence of IgG subclasses and mapping of epitopes. Immunol Lett 1999;70:203-9.
13. Thomas MC, Garcia-Perez JL, Alonso C, Lopez MC. Molecular characterization of KMP11 from *Trypanosoma cruzi* a cytoskeleton-associated protein regulated at the translational level. DNA Cell Biol 2000;19:47-57.
14. Maranon C, Tomas MC, Planelles L, Lopez MC. The immunization of A2/K transgenic mice with the KMP-11-HSP70 fusion protein induces CTL response against human cells expressing the *T.cruzi* kmp-11 antigen identification of A2-restricted epitopes. Mol Immunol 2001;38:279-87.
15. Tolson DL, Jardim A, Schnur LF, Stebeck C, Tuckey C, Beecroft RP. The kinetoplastid membrane protein 11 of *Leishmania donovani* and African try panosomes is a potent stimulator of T lymphocyte proliferation. Infect Immun 1994;62:4893-9.
16. Cuellar A, Rojas F, Bolanos N, Diez H, Del Carmen Thomas M, Rosas F *et al*. Natural CD4 (+) T-cell responses against *Trypanosoma cruzi* KMP-11 protein in chronic chagasic patients. Immunol Cell Biol 2009;87:149-53.
17. Planelles L, Thomas MC, Pulgar M, Marañón C, Grabbe S, López MC. *Trypanosoma cruzi* heat-shock protein-70 kD alone or fused to the parasite KMP11 antigen, induces functional maturation of murine dendritic cells. Immunol Cell Biol 2002;80:241-7.
18. Ramirez JR, Gilchrist K, Robledo S, Sepúlveda JC, Moll H, Soldati D *et al*. Attenuated *Toxoplasma gondii* ts-4 mutants engineered to express the leishmania antigen KMP-11 elicit a specific immune response in BALB/c mice. Vaccine 2001;20:455-61.
19. Berberich C, Ramirez JR, Hambrecht C, Alber G, Skeiky AW, Moll H. Dendritic cell (DC)-based protection against an intracellular pathogen is dependent upon DC-derived IL-12 and can be induced by molecularly defined antigen. J Immunol 2003;170:3171-9.

Acknowledgments.—The authors wish to dedicate this article to the memory of Nasibeh Beheshti, our demised colleague who helped us kindly and we now miss.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Received on May 21, 2013.
Accepted for publication on May 29, 2013.