TÜBİTAK

LETTER TO THE EDITOR

Turk J Med Sci
2007; 37 (4): 251-253

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K26 Antigen from *L. infantum* Mon1: Sequence Based Function-Localization Analysis

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Received: March 06, 2007
Accepted: July 20, 2007

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To the Editor,

Visceral leishmaniasis (VL), a vector-borne disease caused by the *Leishmania donovani* complex, is a zoonotic disease. Annual incidence is estimated at 500,000 cases. Ninety percent of VL cases occur in just five countries: Bangladesh, India, Nepal, Sudan and Brazil. Overall prevalence is 12 million people and 350 million people are at risk for the disease. *Leishmania* K26 protein, which has a 14 amino acid repeat region, has been evaluated as an immunoresponsive diagnostic marker that might be more reliable than K36 or other candidates. In order to develop serological diagnosis, some investigations were conducted to evaluate the immunogenic properties of K26, but there are no data about its function or cellular localization (1-5). Therefore, secondary structure prediction, protein motifs based comparison as well as conserved regions, molecular electrostatic potential and possible location of the protein were used to predict a closely related function and cellular location for K26 protein.

Analysis of *L. infantum* K26 protein by Gene Runner software (ver 3.08) predicted that this protein is hydrophilic and very acidic with isoelectric point (pHi) of 4.59 with 247 amino acids with molecular weight of 26.1 kD. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) indicated the recombinant K26 protein was 41 kD. This result depends on SDS-protein interactions, in which the mobilities of proteins with the same molecular weight are different in the gel because of the hydrophilic or hydrophobic property as well as isoelectric point of the protein. Results of pairwise alignment revealed 99% similarity between *L. chagasi* and *L. infantum* Mon1 for k26 gene and K26 protein (under accession number of DQ192034 and ABA55016 for *L. infantum* Mon1 in the National Center for Biotechnology Information-NCBI) using Gene Doc software (ver 2.6.002) under the PAM 20 scoring matrix and Blosum 100 scoring matrix, respectively.

Alignment of protein sequences detected only one substitution at the 67th position of the *L. infantum* K26 protein in which the arginine of *L. chagasi* K26 was replaced with histidine in *L. infantum* K26. Multiple alignment by ClustalX (ver 1.8) on eight different protein sequences with highest similarity in NCBI indicated a high similarity for *L. infantum* and *L. chagasi* K26 with *L. donovani* HASPB1. The 56% similarity between *L. infantum* K26 and *L. donovani* HASPB1 proteins is notable because it could be a high similarity among different proteins. Cluster analysis of protein sequences via the neighbor joining (NJ) method of Saitou and Nei (6) classified them into two groups (Figure 1), which was confirmed via stepwise discriminant analysis (7) on groups with 0.062 for significant Wilks lambda ($F_{1,6}$=90.938, p<0.000). Therefore, the genetic distances between *L. infantum* and *L. chagasi* K26 with *L. donovani* HASPB1 were 0.081 and 0.085, respectively. Prediction of protein secondary structure using online programs of DSC, DPM, GOR I, GOR III, GOR IV, HNN, SIMPA96, PHD, PREDATOR and SOPM in PBIL (8) and motif search coincided with the alignment results in which a

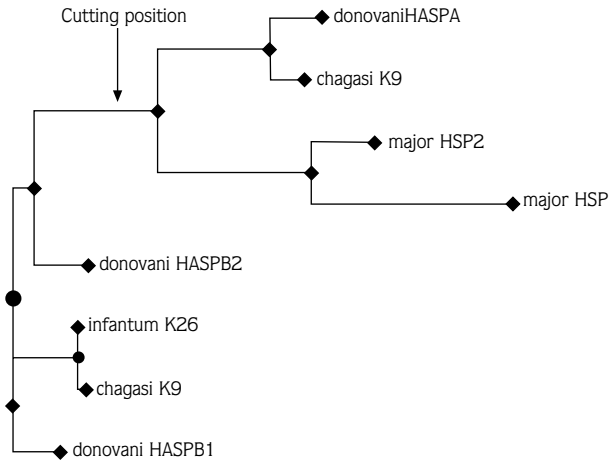

Figure 1. Phylogenetic tree construction via the NJ (Neighbor Joining) method of Saitou and Nei. Bootstrapping of sequences (reported sequences are available in the GenBank databases under the accession numbers of: Infantum K26: ABA55016, Chagasi K26: AAD55244, Donovani HASPB1: CAA09789, Donovani HASPB2: CAA09788, Donovani HASPA: CAA09787, Chagasi K9: AAD55243, Major hydrophilic surface protein: AAA66474, Major hydrophilic surface protein 2: CAB05670) was done under 2000 for number of bootstrap trials and 500 for random number generator seed.

similar second structure was predicted for K26 and HASPB1 (Table 1). Using BioEdit (ver 5.0.9), two conserved regions, CTKDSAKE and NEDGND, were detected for all of the aligned sequences at positions 5 to 12 and 386 to 391, respectively, with average entropy of 0.0000. The first position included casein kinase II phosphorylation site and protein kinase C phosphorylation site, but no motif was defined on second conserved region. However, it is possible the positions from 5 to 12 and from 386 to 391 have a crucial role from the point of view of molecular function for all of the subjected proteins. In addition, positions 5 to 28, 42 to 66, 75 to 94, 101 to 116, 166 to 192 and 369 to 396 were found as conserved regions for protein sequences of *L. infantum* K26, *L. chagasi* K26 and *L. donovani* HASPB1. All of these conserved regions were hydrophilic and acidic with the exception of the first region that was basic with pHi ~ 8. These results indicated K26 and HASPB1 are closely related with possible similar functions as well as subcellular localizations. These results are congruent with estimations of electrostatic potential for investigated proteins by DeepView/Swiss-pdbViewer (ver 3.7) using the coulomb method, which approximately computed the same charge distribution for HASPB1 and K26 (Figure 2). All of the sequences were positively charged (acidic) and only their N-terminal regions had negative charge (basic). It is notable that the high similarity in N-terminal and C-terminal for the protein sequences corresponded to similar electrostatic potential in these regions (Figure 2), which could be evidence for possible important cellular functions. SOSUIsignal, a program for classification and secondary structure

Table 1. Prediction of protein secondary structures with different tools.

	Prediction Tools										
Sequences	DPM	DSC	GOR1	GOR3	GOR4	HNNC	PHD	Predator	SIMPA96	SOPM	Sec.Cons
Infantum K26	c	c	c	c	c	c	c	c	c	c	c
Chagasi K26	c	c	c	c	c	c	c	c	c	c	c
Donovani HASPB1	c	c	c	c	c	c	c	c	c	c	c
Donovani HASPB2	c	?	h	c	c	c	c	c	c	c	c
Donovani HASPA	c	h	h	c	c	c	c	c	c	c	c
Chagasi K9	c	?	c	c	c	c	c	c	c	c	c
Major HSP*	c	c	h	c	c	c	c	c	c	c	c
Major HSP2*	c	c	h	c	c	c	c	c	c	c	c

h: Alpha helix. c: Random coil. ?: Ambiguous states.

*Hydrophilic Surface Protein

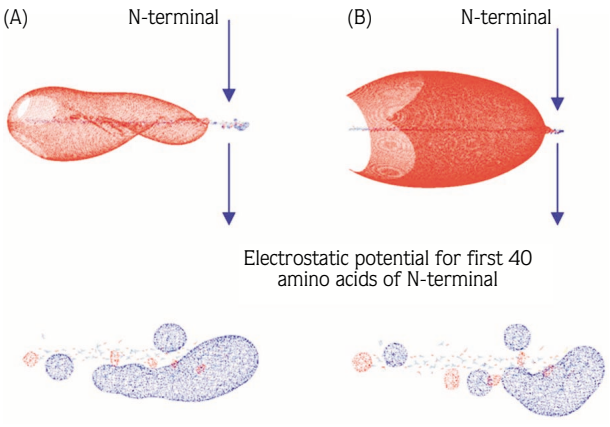

Figure 2. Computation of electrostatic potential for K26 (A) and HASPB1 (B) proteins.

prediction of membrane proteins, did not indicate the presence of signal peptides on K26, and the protein was not predicted to be a transmembrane or mitochondrial protein by the TMpred and MitoProt programs, respectively. Furthermore, analysis with use of iPSORT program did not indicate any signal or mitochondrial targeting peptide sequences. However, WoLFPSORT suggested 32 nearest neighbors, including 14 mitochondrial, 11 cytoplasmic, 5 secreted and 2 nuclear protein neighbors. Therefore, sequence similarity, secondary structure and electrostatic based evaluation indicate that K26 is an extracellular or secreted protein which corresponds to the predicted five secreted protein neighbors.

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