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ANALYSISOF HYDROPHOBICITYANDANTIGENIC EPITOPE PREDICTION FROM D. MEDINENSIS

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ABSTRACT

The long-established human infection which needs to be eradicated after the small pox is guinea worm disease. The causative agent is Dracunculusmedinensis, the only human infecting species. The Cyclops ingeststhe larvae of parasite (Dracunculusmedinensis) which is further ingested by the human from the contaminated stagnant water sources. The Cyclops digested by stomach digestive juices and ledto release of the larvae. These larvae travel and penetrate the digestive wall of the human and get entry into the abdominal cavity and retroperitoneal space. These larvae mature in adults and soon after the copulation the female mature and grows in size up to 60 cm to 3 m in size whereas the male dies. A year after the incubation period the mature female worm come towards the skin subcutaneous tissue and start formation of a small round bulge on the skin , generally on the distal lower extremity and start secreting an irritating chemical. ATP synthetase subunit 6 (mitochondrion) proteins of the Dracunculusmedinensis is a 191 aa protein which is used for the identification of the antigenicity through B- cell epitopes prediction methods .The result obtained shows that the region of maximal hydrophilicity is likely to be antigenic site having the hydrophobic characteristics and contain the segments of low complexity and high-predicted flexibility. This predicted antigenic protein from D. medinensis could be the new paradigm of synthetic vaccine development and target validation.

Keywords: Antigen, Dracunculusmedinensis, Epitope, Protein, Dracunculiasis, ATP Synthetase Subunit 6 (Mitochondrion)

I. INTRODUCTION

Guinea worm or *Dracunculusmedinensis*is considered as the neglected tropical disease. Like other filarial nematode guinea worm has got six developmental stages life cycle with incubation period of the approximately more than a year. This disease has its own clinical importance and needs to be eradicated after small pox [1]. Mature adult female soon after the copulation produces millions of eggs in its uterus, whereas male dies. After an incubation period the female worm release the larvae which induces a painful blister (1 to 6cm diameter) on the skin of lower limbs(in 80-90% reported cases); the symptoms comprised of slight fever , local skin redness , swelling and severe pruritus around the blister . Other symptoms include diarrhea, nausea, vomiting and dizziness. The blister burst within 1 to 3 days and female worms one or more slowly comes out from the wounds which causes an excessive burning sensation and pain [2].Immersing or pouring water over the blister provide pain reliever. But this the moment that adult female is exposed to the external environment [3]. Duringemergence of the limbs in open water sources it recognizes the temperature difference and releases the milky white liquid in the water which contains millions of immature larvae, when larvae released in water are

ingested by copepods where they mount twice and become infective larvae within two weeks [4]. The *D.medinensis* antigen peptides can be most desirable segment for the subunit vaccine development because with the single epitope, the immune response can be generated in large population. This approach is usually based on the phenomenon of cross-protection, whereby infected with the mild strain and is protected against a more severe strain of the same. The phenotype of the resistant transgenic hosts includes fewer centers of initial infection, a delay in symptom development and low accumulation. In this study ATP synthase is used for the investigation of the antigenicity.ATP synthase is an important enzyme which dispenses energy for the cell to use through the synthesis of adenosine triphosphate (ATP). ATP is well known as "energy currency" of cells.

The overall biochemical reaction sequence is: ADP + Pi → ATP, where ADP and Pi are joined together by ATP synthase

ATP synthase is located with the thylakoid membrane and inner mitochondrial membrane.It is comprise of the 2 regions i.e., the F_O portion is within the membrane and the F_1 portion of the ATP synthase is above the membrane, inside the matrix of the mitochondria. Mitochondrial encoded ATP synthase 6 gene is also called as MT-ATP6 commonly which encodes for the ATP synthetase subunit 6 (mitochondrion) proteins. The MT-ATP6 protein is a subunit of a large enzyme ATP synthase which is also known as complex V. This found to be responsible for the terminal step of oxidative phosphorylation. The MT-ATP6 gene associated to a mitochondrial respiratory chain complex gene family. Any kind of alteration in the MT-ATP6 gene has got adverse affect on health. The anomaly MT-ATP6 gene is found in the Leigh syndrome. Leigh syndrome is a progressive brain disorder that usually appears in infancy or early childhood. Children with the deficiency/mutation of this gene experience delayed development, muscle weakness, problems with movement, or breathing difficulty. The common genetics changes which has seen in the Leigh syndrome is due to the replacement of the thymine nucleotide with the guanine nucleotide at position 8993(written as T8993G). This mutation led impact on the function or stability of the ATP synthase complex, which inhibit ATP production and oxidative phosphorylation impairing. The enigma of impaired oxidative phosphorylation has not yet been explained clearly but researcher believes that this king of impaired oxidative phosphorylation usher the cell death because of decline energy availability in the cell. The tissue which requires immense quantity of energy for their functionality , such as the brain, muscles, and heart, are seem very sensitive to decreases in cellular energy. The brain cell death and other sensitive tissue death is likely causes the characteristic changes in the brain which commonly seen in Leigh syndrome. Due to mutation in the MT-ATP6 gene there are less severe disorder has been detected, like neuropathy, ataxia, and retinitis pigmentosa. The new haplotypes of the ATP synthase subunit 6 of mtDNA is generally associated with acute lymphoblastic leukemia [5]. In the other case from a family with a very rare, maternally inherited missense m.8851T>C mutation in the mitochondrial MTATP6 gene has been reported [6]. It has been identified that the mtDNA mutation m.9185T>C in MT-ATP6Genetic dysfunction of MT-ATP6 causes axonal Charcot-Marie-Tooth disease [7].In an isolated case of mental retardation and ataxia without retinitis pigmentosa, de Coo et al. (1996) found an 8993T-G transversion (516060.0001)[8].Tatuch et al. (1992) and Shoffner et al. (1992) demonstrated that the nucleotide 8993 mutation can cause Leigh syndrome and they also found the heteroplasmicmtDNA mutation in a female infant showing lactic acidemia, hypotonia, and neurodegenerative disease leading to death at the age of 7 months[9]. Santorelli et al. (1993) found the T-to-G point mutation at nucleotide 8993 in 12 patients with Leigh syndrome from 10

unrelated families[10]. Antigen protein prediction from *D. medinensis* is necessary for few paradigms of synthetic vaccine development and target validation [11-12].

II. METHODOLOGY

B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system. Knowledge of B-cell epitopes may be used in the design of vaccines and diagnostics tests. It is therefore of interest to develop improved methods for predicting B-cell epitopes [13]. In this investigation we have used methods for predicting continuous antibody epitope from ATP synthetase subunit 6 sequences with the consideration of the parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of polypeptides chains, hydrophobicity and have been correlated with the location of continuous epitopes. The methods used for the determination of the antigenic epitopes of the antigen protein using the Gomase in 2007,Welling,Eisenberg, Parker , Rao& Argos, Malanvalan,Bepipred Linear Epitope Prediction, Emini Surface Accessibility Prediction, Karplus& Schulz Flexibility Prediction, Kolaskar&Tongaonkar Antigenicity, Parker Hydrophilicity Prediction[13-19].

III. RESULT AND INTERPRETATIONS

The ATP synthetase subunit 6 protein sequences (191 aa protein) is analysed through different types B- cell epitope prediction methods. Through Chou & Fasman Beta-Turn Prediction the higher peak with highest score is with start and end point is between 55-60(Fig.1),inEmini Surface Accessibility Prediction method highest peak is between 80-85(Fig.2), Karplus & Schulz Flexibility Prediction method it was observed between 160-165 (Fig.3),in Kolaskar & Tongaonkar Antigenicity method between 70-75 (Fig.4) and through Parker Hydrophilicity Prediction method its between 55-60(Fig.5). All prediction calculations are based on propensity scales for each of the 20 amino acids. Each scale consists of 20 values assigned to each of the amino acid residues on the basis of their relative propensity to possess the property depicted by the scale. On the graphs (Figs. 1-5), the Y-axes depict for each residue the correspondent score whereas X-axes depict the residue positions in the sequence. The tables (Tables. 1) provide values of calculated highest scores for each residue. The larger score for the residues might be interpreted as that the residue might have a higher probability to be part of epitope (those residues are colored in yellow on the graphs)(Figs. 1-5). This in turns indicates that there might be probability of residue to be a part of the epitope. We have also found that the region of maximal Hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because the terminal regions of antigen protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein (Fig. 6-7). It was shown that an antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Figs. 8-10). The predicted antigenic protein segments of ATP synthetase subunit 6 proteins can take active part in the host immune reactions. In future study the predicted antigenic protein ATP synthetase subunit 6 protein fragments can be used in the investigation of MHC molecules binding and it can be the first bottlenecks in vaccine design.

IV. FIGURES AND TABLES

Fig 1- Chou &Fasman Beta-Turn Prediction Graph

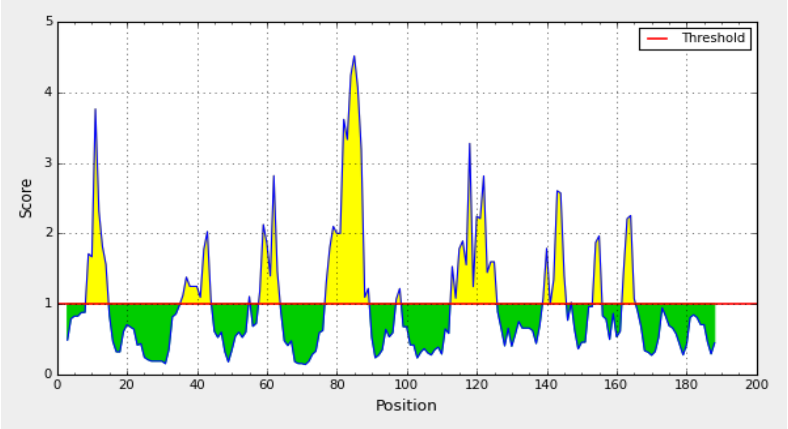

Fig 2- Emini Surface Accessibility Prediction Graph

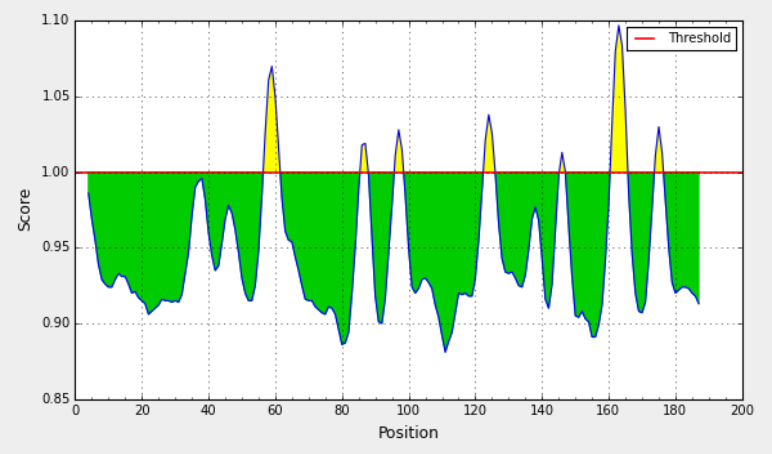

Fig 3- Karplus& Schulz Flexibility Prediction Graph

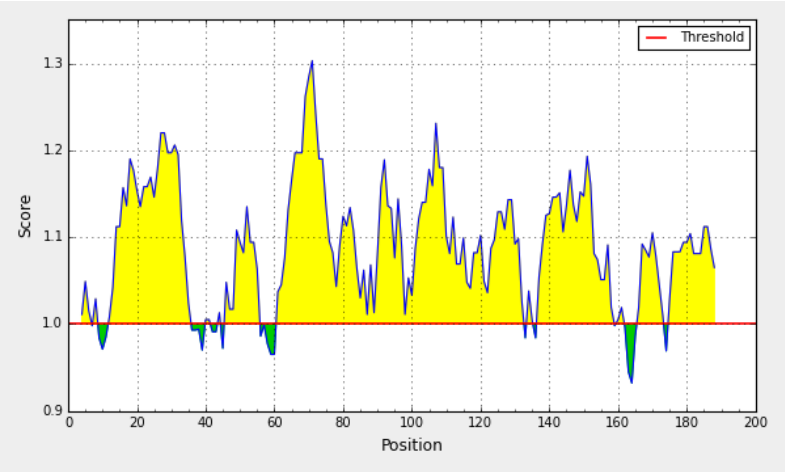

Fig 4- Kolaskar &Tongaonkar Antigenicity Graph

Fig 5- Parker Hydrophilicity Prediction Graph

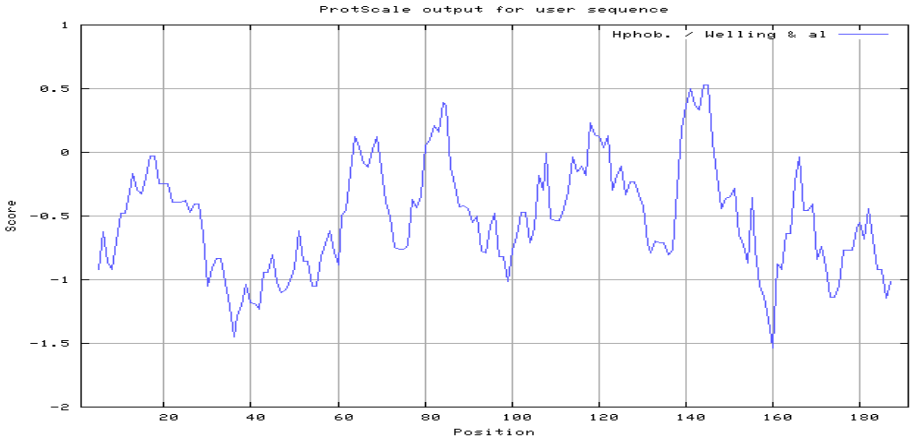

Fig.6. Hydrophobicity plot of antigen by Hphob/Welling & al., scale

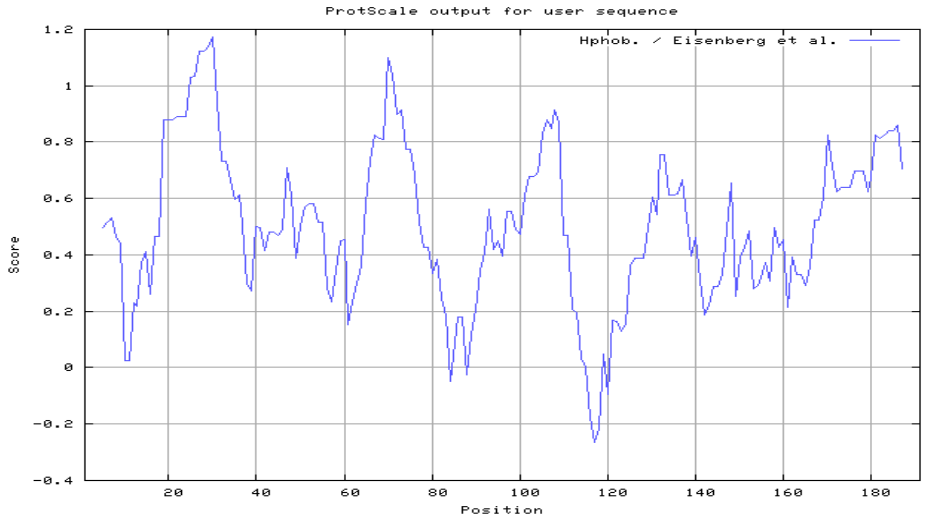

Fig.7. Hydrophobicity plot of antigen by Hphob/Eisenberg, et al., scale

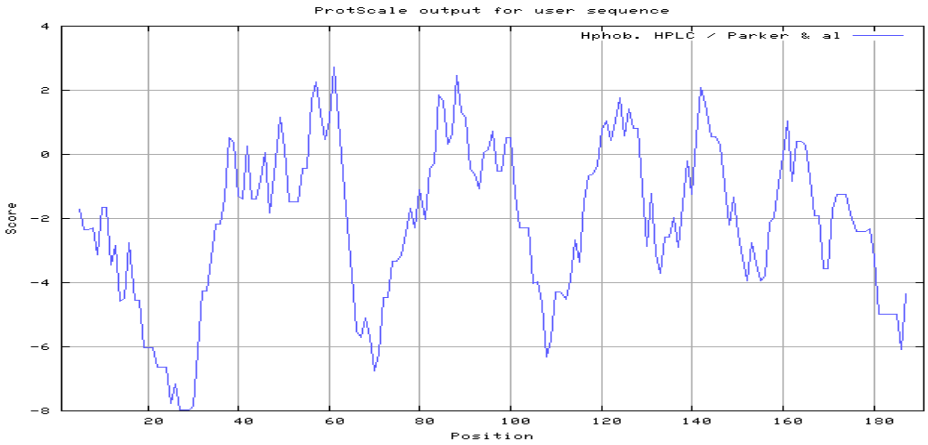

Fig.8. Hydrophobicity plot of antigen by Hphob. HPLC/Parker & et al., scale

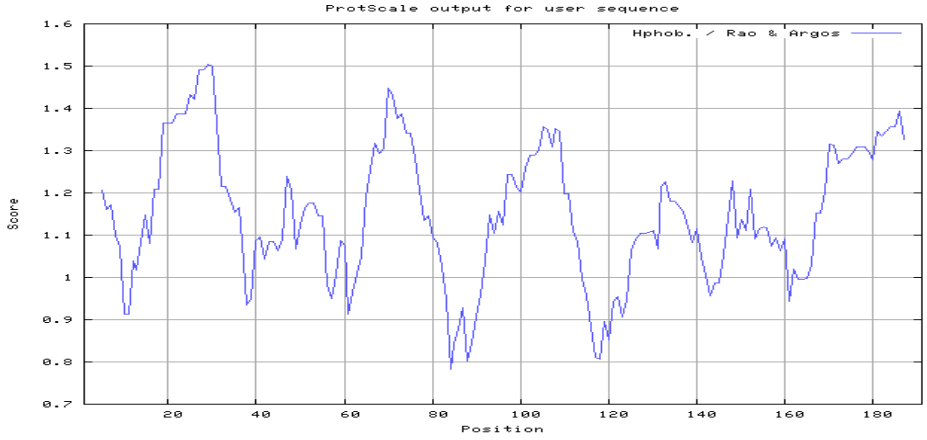

Fig.9. Antigenicity plot of antigen protein by Hphob. / Rao& Argos, scale

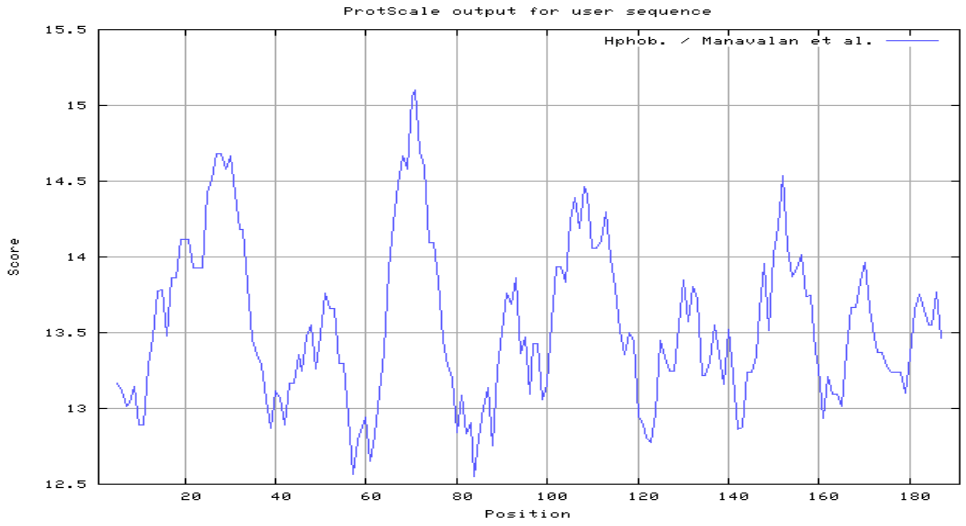

Fig.10. Antigenicity plot of antigen protein by Hphob. / Manavalan et al., scale

Table 1:Chou &Fasman Beta-Turn Prediction and Parker Hydrophilicity Prediction - Predicted Residue Scores

		Position	Residue	Start	End	Peptide	Score
1	Chou & Fasman Beta-Turn Prediction- Predicted residue scores	55	L	52	58	CNYLFGG	1.171
		56	F	53	59	NYLFGGG	1.224
		57	G	54	60	YLFGGGD	1.21
		58	G	55	61	LFGGGDS	1.251
		59	G	56	62	FGGGDSY	1.33
		60	D	57	63	GGGDSYF	1.33
		Position	**Residue**	**Start**	**End**	**Peptide**	**Score**
2	Parker Hydrophilicity Prediction Predicted residue scores	55	L	52	58	CNYLFGG	-0.071
		56	F	53	59	NYLFGGG	0.543
		57	G	54	60	YLFGGGD	0.971
		58	G	55	61	LFGGGDS	2.171
		59	G	56	62	FGGGDSY	3.214
		60	D	57	63	GGGDSYF	3.214

V. CONCLUSION

An antigenic protein ATP synthetase subunit 6 from *D. medinensis*can plays an important role in vaccine development. The peptide fragments of antigen protein can be used to select nonamer for use in rational vaccine design and can develop the understanding of roles in the immune system in infectious disease.

5.1 Conflicts of Interest

The authors declare no conflict of interest.

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