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Enolase in vector-borne pathogens: A Potential Therapeutic Target

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Abstract
Enolase is one of the most abundantly expressed cytosolic proteins in many organisms. It is a key glycolytic metalloenzyme found from archaebacteria to mammals and its sequence is highly conserved. Enolase, a multifunctional protein, exhibits diversity in its location as well as in function. Besides its function in glycolysis and gluconeogenesis, it also serves as a surface protein and surface receptor for the binding of plasminogen on the surface of the variety of haematopoetic, epithelial and endothelial cells for the invasion of pathogens. Enolase is transported to the cell surface through an unknown mechanism. It efficiently binds with plasminogen and converts it to a serine protease, plasmin that helps to degrade the fibrin matrix together with other extracellular matrix surrounding the targeted host cell for the invasion of pathogen. Presence of enolase and its key role in the metabolism and in the pathogen invasion process in many of the vector-borne pathogens from arboviral to nematode pathogens are being recognised recently. The location and possible function of enolase and its importance as therapeutic target in vector-borne pathogens are discussed.

Keywords: Enolase; vector borne pathogens; therapeutic target

Introduction
Vector-borne diseases (VBD) are those diseases that are transmitted by infected arthropod vectors from one person to another or from animal hosts to humans. There are a large number of viral, rickettsial, bacterial and parasitic diseases that account for about 17% of the estimated global burden of all infectious diseases (Table 1). Among these diseases malaria is the most prevalent VBD with over 2.4 billion people around the world at risk and > 275 million cases reported every year with > 1 million deaths in children [1]. Besides mortality, morbidity resulted from infection with such diseases led to major economic losses and increased burden in terms of disability adjusted life years (DALY). Within two decades global trade, rapid international travel, and environmental changes such as climate change and urbanization result in vectors and vector-borne diseases to re-emerge or spread beyond borders [2]. Vectors that transmit different diseases may share similar habitats. Single vector may transmit more than one disease as in the case with malaria and anopheline transmitted filariasis. Strategies to control these diseases include use of insecticides against vectors, immunization by live attenuated cell line vaccines, chemotherapy and anti-parasitic drugs. However, increasing reports on the development of resistance to anti-parasitic drugs prompted a situation where designing and developing new alternative anti-parasitic drugs or molecular vaccines for parasites/other pathogens are very essential in the forthcoming years.

Pathogens and invasion
Pathogens, from bacteria to helminthes, are equipped with a variety of potent mechanisms to invade several host tissue barriers to reach their final destination. Within their target cells the pathogens are safe to proliferate and equip for transmission to the next host. To survive within the host, the pathogens suppress the host immune system through sophisticated mechanisms of antigenic variation through specialized enzymatic and biochemical means including adhesion molecules and cellular receptors [3]. Basic metabolic pathways and biochemical features of vector-borne parasites are potential chemotherapeutic targets for developing antiparasitic drugs for the treatment of diseases caused by these parasites. A number of pathogenic organisms display specialized proteins on their cell surface to help in the invasion, one of the best characterized is the glycolytic enzyme enolase.

General aspects of enolase

Enolase (EC No. 4.2.1.11) is one of the most abundantly expressed cytosolic proteins in many organisms. It is a key glycolytic metalloenzyme that catalyses the dehydration of 2-phosphoglycerate (2-PGA) to phosphoenol pyruvate (PEP), in the last steps of the catabolic glycolytic pathway (Fig. 1). Enolase has an absolute requirement for magnesium as the natural cofactor, in the presence of which it imparts highest activity in the metabolic pathway of fermentation in general and the glycolytic pathway, in particular, and hence is ubiquitously present in abundance in the biological world [4]. Enolase is found from archaebacteria to mammals and its sequence is highly conserved. It has been also localized on the surface of several pathogens like bacteria, fungi, protozoa and helminthes. Indeed, in many organisms (vertebrates, *Saccharomyces cerevisiae*, *Toxoplasma gondii*) the presence of different enolase isoforms has been reported, often with kinetic properties favouring a flux in the glycolytic or gluconeogenic direction resulting in multifaceted functions [5-7].

Importantly, in pathogenic organisms, enolase is mainly responsible for the invasion of pathogen into its host cells (Fig. 2). Its surface expression depends on the pathophysiological, metabolic or developmental conditions of cells. The enzyme has been found in the cell wall of *S. cerevisiae* and constitutes an immunodominant antigen at the surface of the pathogenic yeast *Candida albicans* during invasive candidiasis[6]. Surface enolase has been highlighted as an important virulence factor in *Bacillus anthracis, Streptococcus pneumonia* and *S. mutans* [8-10]. Additionally, in *Entamoeba invadens*, enolase expression is induced by environmental signals, and it shows association with cytoplasmic vesicle-like structures that transport the protein to the cyst wall where it plays an essential but so far unknown function [11]. Enolase may also be excreted into the extracellular environment where it mediates degradation of host tissues and immune evasion, which has been observed in the human pathogenic *Streptococcus. pyogenes* and the arthropod parasite, *Aphidius ervi* [12, 13].

Diversity in location and function of enolase

Enolase, a multifunctional protein, exhibits diversity in its location as well as function. It is reported as a surface protein [14], surface receptor for the binding of plasminogen [15] and a Myc-binding protein localized in the nucleus as a DNA binding protein [16]. Based on its functional diversity enolase is identified as an eye crystalline protein [17], a heat shock protein [18], a neurotropic factor [19] and a cytoskeletal and chromatin binding protein [20].

Enolase is present in many vector-borne pathogens including *Leishmania mexicana* and *Plasmodium falciparum*. The cell surface enolases of pathogens efficiently capture plasminogen (proenzyme) from the surrounding environment which is subsequently converted into active plasmin, a strong serine protease that facilitates the invasion process [21, 22]. Activation of plasminogen into active plasmin involves cleavage of the Arg_{561}-Val_{562} bond yielding an N-terminal A (heavy) chain which remains linked by two disulphide bonds to the smaller C-terminal B (light) chain. The B-chain contains the protease-active residues [23]. The plasminogen N-terminal region contains five 80-amino-acids-long kringle domains. Plasminogen binds strongly and specifically to surface enolase via interaction of its Kringle domain with the C-terminal or internal lysine motifs of enolase [24].

Through binding to plasminogen and nucleic acid, enolase plays an important role as a cell surface receptor in host pathogen interactions and pathogenic diseases [25, 26]. This enzyme was also identified as a major component of excretory-secretory products (ESP) of many parasites[27] which play key role in controlling the parasite growth [28]. In this review, the role of surface enolase in various vector-borne pathogens and its therapeutic importance with regard to control of vector-borne diseases are discussed.

Role of surface enolase and vector-borne diseases:

Viral diseases

Dengue

Dengue virus, a mosquito-borne single stranded positive RNA virus, includes five serotypes from 1 to 5. It is transmitted to humans mainly by mosquito vectors *Aedes aegypti and Ae. albopictus* [29, 30] by infecting primary human cells such as peripheral blood leukocytes, blood monocytes/macrophages, dendritic cells, and B lymphocytes [31]. Dengue virus (DENV) attaches to the host epithelial cell receptors through envelop (E) protein [32, 33] and enters the cell mainly via this receptor by clathrin-dependent endocytosis[34, 35]. Several DENV receptors have been described in mammalian cells [36] as well as in mosquito cells [37]. Enolase has been identified as a 67 kDa DENV binding protein from protein extracts of C6/36 cell and in the brush border of midgut of *Ae. aegypti* mosquitoes from DS3 and DMEB strains [38, 39] and also been shown to be bound to DENV-2 presumably through the envelope protein on the virus surface [40]. This reinforces the idea that enolase may be a DENV receptor of *Ae. aegypti* midgut[41]. Recently, Higa *et al*. [42] also showed that DENV infection alters the secretion pattern of hepatic HepG2 cells, with α-enolase appearing as one of the major proteins secreted in higher levels by infected cells and suggested an association between plasma levels of α-enolase and disease severity. Since α-enolase binds plasminogen and modulates its activation, it is plausible to speculate the association of the increase in α-enolase secretion by infected hepatic cells with the haemostatic dysfunction observed in dengue patients including the promotion of fibrinolysis and vascular permeability alterations. Further investigation on enolase is essential for targeting the protein as a suitable therapeutic candidate against all serotypes of dengue viruses.

West Nile

West Nile virus (WNV) is a positive sense, single-stranded RNA arbovirus of the Flavivirus family with potential to cause meningoencephalitis [43]. Humans and other mammals are incidental hosts with transmission through bites of infected mosquitoes and the virus is maintained in nature in a mosquito-bird cycle with *Culex* species being the primary vector. West Nile virus can cause serious illness in man, resulting in encephalitis and death, and is soon expected to be endemic in most of the United States, South America, Greece and Italy [44-46]. Neuron is basically affected by WNV leading to meningoencephalitis. Enolase is a glycolytic enzyme found in neurons and neuroendocrine cells of the human host and is currently used to identify neurons [47]. Antibodies against neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP) and WNV were used to develop the method of double-label immunohistochemical staining, which allowed independent assessment of neuron status and WNV distribution in the brain cells of monkey [48]. Recently, 18 mosquito proteins including enolase (gi|157121051) that interact with WNV were identified using tandem affinity purification (TAP) assay. Enolase has been shown to be

required for virus transcription via its interaction with tubulin [49] and there may be a similar requirement for this enolase protein in other flavivirus infection. Not much information is available on the mechanism of interaction of viral enolase with the vector. More research on this protein and its association with flaviviruses may provide some clues to design novel targets [50] to inhibit infection of the vector or block transmission to humans [40].

Tick-borne Encephalitis

Tick-borne encephalitis (TBE) single-stranded RNA virus causes severe encephalitis with serious sequelae in humans. The disease is characterized by fever and debilitating encephalitis that can progress to chronic illness or fatal infection [51]. TBE virus (TBEV) has 3 subtypes: European, Siberian, and Far Eastern. Enolase is secreted in the saliva of the tick that provides convincing evidence for a role of this salivary protein as a plasminogen receptor, most likely stimulating host fibrinolysis and maintaining blood fluidity during tick feeding [52]. TBEV infection, in addition to causing fatal encephalitis in mice, induces considerable breakdown of the blood-brain barrier (BBB) due to drastic changes in permeability of BBB in animal models. The permeability of the BBB increased in later stages of TBE infection when high virus load was present in the brain with neurological signs associated with sharp declines in body weight and temperature [53]. In human patients with a severe form of TBE, neurospecific proteins such as neuron-specific enolase or α-1 brain globulin were elevated in serum, indicating BBB breakdown [54-57]. However, despite this finding, dysfunction of the BBB has not been extensively studied in TBE so far. The RNAi experiments and immunization trials indicated that enolase could be involved in the regulation of tick reproduction, suggesting new potential control strategies [52].

Japanese Encephalitis

The mosquito-borne Japanese encephalitis virus (JEV) is an enveloped, positive-sense single-stranded RNA virus and a member of the genus *Flavivirus* under the family Flaviviridae[57]. JE has a high fatality rate of 30% and around half of the JE survivors show severe neurological sequellae [58]. JEV is the sole etiologic agent of Japanese Encephalitis (JE), a neurotropic killer disease being one of the major causes of acute viral encephalitis in human in wide areas of southern and eastern Asia. Following entry into the host system through mosquito bite JE virus (JEV) may replicate in various organs such as liver and spleen and reaches the CNS resulting in rapid inflammatory response. Recent study on the microarray analysis showed that 437 genes in spleen and 1119 genes in brain were differentially expressed in response to JEV infection with obviously upregulated genes like chemokines and cytokines and down regulated genes such as enolase 2 (Eno2). These genes may play a critical role as antiviral response of host against JEV and also as biomarkers [59]. The mRNA profile obtained may provide foundation for further investigations of JEV pathogenesis and therapeutic methods [60, 61] (Yang *et al*., 2011; Peng and Jiang, 2015).

Bacterial disease

Lyme Disease

Borrelia burgdorferi, the Lyme disease spirochete, causes the most common arthropod-borne disease in the United States and many other temperate regions of the world. This tick-borne bacterial pathogen causes a disseminated infection involving multiple organs known as Lyme disease. It is significant cause of morbidity and continues to be a serious public health concern [62, 63]. *Borrelia burgdorferi* is a successful extracellular pathogen that co-opts host proteins for its own advantage. The enolase of *B. burgdorferi* is both cytoplasmic and surface exposed [64] and may directly participate in microbial virulence by facilitating pathogen dissemination via interaction with host factors. A fraction of the *B. burgdorferi* chromosomal gene product BB0337, annotated as enolase is associated with spirochete outer membrane and is surface exposed. Recent work also revealed that the enolase of *B. burgdorferi* moonlights as a surface-exposed plasminogen-binding protein [65]. *B. burgdorferi* enolase, either in a recombinant form or as a membrane-bound native antigen, displays enzymatic activities intrinsic to the glycolytic pathway. The protein interacts with host plasminogen, potentially leading to its activation and resulting in *B. burgdorferi*-induced fibrinolysis [66, 67]. Further, it penetrates the endothelium and activates matrix metalloprotease-9 and matrix metalloprotease-1 [68]. Spirochete enolase immunization of murine hosts significantly reduced the acquisition of spirochetes by feeding ticks, suggesting that the protein could have a stage-specific role in *B. burgdorferi* survival in the feeding vector [69, 70]. Strategies to interfere with the function of surface enolase could contribute to the development of novel preventive measures to interrupt the spirochete infection cycle and reduce the incidences of Lyme disease. Surface-exposed regions of enolase could potentially serve as vaccine targets or antigenic regions that could alter the course of natural Lyme disease.

Protozoan diseases

Malaria

Malaria, caused by protozoan parasite *Plasmodium* species, remains one of the most infectious diseases in the third world causing about 198 million infections and over 500,000 deaths per year. It is estimated that 443,000 children die each year mostly in sub-saharan Africa due to severe complications of *Plasmodium falciparum* malaria [71]. The most severe form of human malaria parasite is caused by *P. falciparum*. It encodes an enolase of about 50 kDa. The *Plasmodium falciparum* enolase protein is homo dimers similar to the enolases from several other sources [72]. *P. falciparum* enolase (Pfen) the *Plasmodium falciparum* enolase is a homo dimer similar to the enolases can be a potential target for antimalarial drugs [73]. The deduced sequence of *P. falciparum* enolase exhibits high homology with mammalian enolases (68-69%), but differs in containing a plant-like pentapeptide, a dipeptide insertion and some different residue [74]. Patients infected with *P. falciparum* and *P. vivax* had high anti-enolase antibody titres and hence it was concluded that enolase could be used for the Immunodiagnostics of malaria [75]. Immunofluorescence as well as electron microscopic examinations revealed localization of the enolase protein on the merozoite cell surface. These observations establish malaria enolase to be a potential protective antigen [76]. Another study supports the hypothesis that enolase on the surface of Plasmodium ookinetes plays a dual role in midgut invasion: by acting as a ligand that interacts with the midgut epithelium and, further, by capturing plasminogen via enolase's internal lysine motif (DKSLVK), whose conversion to active plasmin promotes the invasion process [77]. Using a novel approach, Marcelo Jacobs-Lorena and colleagues originally identified a structural mimic of the ookinete surface protein enolase by screening a phage display library and

named this peptide SM1. It was shown to inhibit the invasion of rodent malaria and was hypothesized to interact with a mosquito midgut receptor and thereby block the natural receptor / ligand interaction [78]. Thus, it may be worthwhile to evaluate enolase, a protein critical to the survival of the parasite [79], for the immunoprophylactic and immunotherapeutic control of malaria.

Leishmaniasis

Leishmaniasis can occur as three different forms: i) cutaneous leishmaniasis is the most common and mild form of the disease characterized by the presence of ulcers that appear at the places of parasite inoculation ii) mucocutaneous leishmaniasis is manifested by destruction of the mucosa membranes of mouth, nose, and throat after parasite spreading iii) visceral leishmaniasis is the most dangerous form of the disease in which organs such as liver and spleen are compromised. Over 90% of new cases occur in 6 countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan [80]. Leishmania parasites are transmitted by sand flies (*Phlebotomus* sp.*) Leishmania mexicana* belong to the family Trypanosomatidae are the causative agents of a variety of diseases collectively called Leishmaniasis [81]. Pathogenic stages of *Leishmania*, the amastigotes living intracellularly in phagolysosomes of macrophages, utilize amino sugars as a source of carbon and energy thereby indicating that glycolysis is important [82]. Even during hexose uptake, gluconeogenesis has been shown to be an essential pathway for the synthesis of glycoconjugates and *β*-mannan, both required for the virulence of the parasite [83, 84]. Pathogenic stage of *Leishmania* expresses a protein of about 50 kDa called *L. mexicana* enolase on its surface. The plasma membrane localization of enolase and its association with external surface of *L. mexicana* was demonstrated by Immuno-blotting analysis and Immuno-fluorescence microscopy [85]. Cell surface enolase has been reported as a protein capable of binding plasminogen[5] and laminin [86]. Enolase in *L. mexicana* is able to bind plasminogen [87] and this binding might contribute to the virulence of this parasite[88]. This location and possible function of enolase offer additional perspectives for both drug discovery and vaccination.

Trypanosomiasis

Trypanosoma brucei is the causative agent of human sleeping sickness, a disease that threatens millions of mainly impoverished people in 36 countries of the African continent [89]. This parasite is transmitted by several species of the genus *Glossina*, insects commonly known as tsetse flies, which are endemic in the affected sub-Saharan countries. Without treatment, human sleeping sickness is considered fatal. *Trypanosoma cruzi* is responsible for Chagas' disease in South and Central America, a form of trypanosomiasis that threatens 15 million people on the American continent. This parasite is mainly transmitted to humans via the faeces of infected triatomine bugs. Detailed studies specifically devoted to parasite's enolase have confirmed that its activity is exclusively present in the cytosol of *T. brucei* bloodstream forms [90], *T. cruzi* epimastigotes and *L. mexicana* promastigotes [85]. In bloodstream-form *T. brucei*, enolase has been genetically validated as a drug target by RNA interference (RNAi). Upon induction of RNAi, enolase activity gradually decreased during the first 24 h to 16%, leading to growth arrest followed by trypanosome death after two days [91]. In *T. cruzi*, the enzyme was detected by peptide mass fingerprinting in the different developmental stages with a higher expression in trypomastigotes and amastigotes compared to epimastigotes [92]. *T. cruzi* glycolytic enzymes including enolase have been shown to be inhibited by inorganic pyrophosphate (PPi) [93, 94] whereas no such inhibition was found for *T. brucei* enzymes. Glycolysis and glyconeogenesis play crucial roles in the ATP supply and synthesis of glycoconjugates, important for the viability and virulence, respectively, of the human-pathogenic stages of *T. brucei, T. cruzi*, and *Leishmania* spp. These pathways are, therefore, candidate targets for drugs/vaccines against these parasites [95]. Nonetheless, potentially important differences exist between the trypanosomatid and host enzymes, with three unique, reactive residues close to the active site of the former that might be exploited for the development of new drugs. Parasites's enolase is one among several proteins showed strong potential for diminishing or even disrupting fly vector competence, and their application holds great promise for improving the control of sleeping sickness [96].

Helminthic diseases: Filariasis

Nematodes are responsible for this most common parasitic infection of humans. In particular, the tissue-dwelling filarial nematodes—including *Onchocerca volvulus, Loa loa, Wuchereria bancrofti, Brugia timori* and *B. malayi* cause the most severe pathologies associated with these infections, including blindness, extensive skin lesions (in long-standing disease) and elephantiasis [97]. Relatively little is known about the filarial proteins that interact with the human host. Although the filarial genome has recently been completed [98], protein profiles have been limited to only a few recombinants or purified proteins of interest.

5.4.1. Onchocerciasis

Human onchocerciasis, the fifth common cause of blindness in the world, is caused by the filarial parasite *Onchocerca volvulus*. Circulating microfilariae of *O. volvulus* cause persistent debilitating itching, severe dermatitis and ocular lesions resulting in blindness (river blindness). *O. volvulus* affects nearly 37million people in 34 countries and is most abundant in Africa, with small foci in Southern and Central America [99]. Over 25 million people are infected and nearly 130 million persons are at risk of infection mainly in tropical Africa; 6.5 million suffer from severe itching or dermatitis and 270,000 are blind DALYs estimated at nearly 600,000 worldwide. The disease also occurs in some parts of Latin America and Yemen [100-103]. The parasite is transmitted to humans through exposure to repeated bites of infected female blackflies of the genus *Simulium*. The severity of illness is directly proportional to the number of infected microfilariae and the power of the resultant inflammatory response and incubation period in humans is about one year. In skin involvement, the infected larvae remain localized in the skin and grow into adults producing large numbers of microfilariae resulting in disease symptoms. Through genomics approach and the leads from the model nematode *Caenorhabditis elegans,* proteins/enzymes involved in the invasion of the parasite or pathogenesis of the diseases was studied. One of the enzymes, α-enolase, involved in the parasite invasion was targeted. Complete cDNA of 1615 bp of from *O. volvulus* (Ov-ENO) was isolated which encodes a polypeptide of 435 aminoacids with calculated molecular mass of about 38 kDa. Immunolocalization of Ov-ENO in adult *Onchocerca* worms and microfilariae were observed in the afibrillar portion of the muscles of the body wall and of the uterus [104]. The plasminogen-binding property of Ov-ENO may support

plasmin-mediated proteolysis including degradation of host's extracellular matrix thereby promoting the migration of larval stages through tissues. The recognition by antibodies in sera of *O. volvulus*-infected persons indicates an involvement of the protein in the interaction between the parasite and the human host. A recent study on the occurrence of filarial proteins in onchocerciasis patients indicated that alpha-enolase and other compounds of *O. volvulus* were expressed in the cells of the neoplasms [105]. The mechanism by which the protein is transported to the outer cell surface needs to be determined to explore the possibilities of Ov ENO as a drug target/vaccine candidate.

Loasis

Loa loa is a blood dwelling nematode that is parasitic in humans and about 13 million people infected [106] and nearly 30 million are at risk [107]. The adult worm wanders through the subcutaneous tissue but is most obvious as it crosses the conjunctiva of the eye hence leading to its common name, the African eye worm. The worms migrate through the skin causing local inflammatory reactions called Calabar swellings. Infection with *L. loa* is spread by biting deer fly *Chrysops* which lives in swampy areas of the forest, principally in the Congo River region, Sudan, and Ethiopia. The American deer fly, *Chrysops atlanticus*, had been reported to be a competent intermediate host of *L. loa* and able to spread the worm to monkeys. This is of some public health concern but so far *L. loa* has remained isolated to Africa. *L. loa* does not contain the obligate intracellular *Wolbachia* endosymbiont. Desjardins *et al*. described the 91.4 Mb genome of *L. loa* and predicted 14,907 *L. loa* genes on the basis of microfilaria RNA sequencing. Under the genome sequencing project of *L. loa,* enolase gene (203 bp long and 22 kDa) has been identified (NCBI-GeneID: 9938290) [108]. However, more work is needed to uncover the role of enolase in the pathology of this debilitating filarial infection and for developing new treatment or diagnostic strategies.

Brugian Filariasis

Of all lymphatic filarial (LF) infections, 90% are caused by *Wuchereria bancrofti* and the remaining is caused by *Brugia malayi* and *Brugia timori*. The human filarial parasite *Brugia malayi* harbours an endosymbiotic bacterium of the genus *Wolbachia. Wolbachia* represent an attractive target for the control of filarial induced disease as elimination of the bacteria affects moulting, reproduction and survival of the worms. Two *Wolbachia* surface proteins (WSPs) WSP-like proteins (wBm0152 and wBm0432) were localized to various host tissues of the *B. malayi* female adult worms and are present in the excretory/secretory products of the worms. Evidences show that both of these proteins bind specifically to *B. malayi* crude protein extracts and to individual filarial proteins to create functional complexes. The wBm0432 interacts with several key enzymes involved in the host glycolytic pathway, including enolase and aldolase. It may also function as an anchor between *Wolbachia* and the *B. malayi* cytoskeleton using the ATP produced at the surface as an energy source to engage the actin cytoskeletal network to support its motility and distribution within the host [109, 110]. The other glycolytic enzymes detected are enolase (Bm1_24115) and transaldolase (Bm1_04195). While transaldolase was maximally observed in the secretions of microfilariae and sparingly in adults, enolase was detectable only in the secretions of the adult male parasites. Recently, in our laboratory complete cDNA of 1314 bp of enolase from *B. malayi* was isolated using PCR based approach with an open reading frame encoded for 436 amino acids (GenBank Acc. No: KF830990). Further characterization of the protein as a therapeutic candidate is underway.

Bancroftian filariasis

Bancroftian Filariasis, caused by *Wuchereria bancrofti,* is a vector-borne, parasitic filarial nematode disease that has been targeted for elimination. In 1995, WHO ranked LF as the second leading cause of disability worldwide after mental illness. Over 120 million infected individuals are infected with the causative agents of lymphatic filarial parasites *W. bancrofti* and *B. malayi***,** 40 million disfigured or incapacitated with >25 million men with genital disease; > 15 million people with lymphoedema or elephantiasis of the leg; >1.4 billion people at risk [99] and DALYs estimated at 2.8 million worldwide[103]. Currently, one-third of the people infected with LF live in India; one-third is in Africa and one-third is in South Asia, the Pacific, and the Americas.

The Global Programme for the Elimination of Lymphatic Filariasis (GPELF), launched in 1998, aims to eliminate LF by 2020 [111]. Diagnostic tools based on the detection of parasite DNA/antigen/antibody are essential for monitoring and certification of GPELF. To achieve the goal, development of potential diagnostic/drug/vaccine targets for lymphatic filariasis is given utmost priority. Recently several enzymes including surface enolase have been shown to perform important role in different biological and pathophysiological processes of parasitic nematode [112]. The development of molecular methods and techniques has lead to the advancement in isolation, purification and characterization of nucleic acids and proteins from filarial parasites. In our laboratory, partial cDNA (621 bp) has been isolated from L3 cDNA library of *W. bancrofti*, and sequences deposited in the Genbank (EU370162). Based on these coding sequences a phylogenetic tree of selected nucleotide sequences of enolases of some parasites/pathogens was constructed (Fig. 3). The tree formed two major clusters of which the upper cluster included most of the parasites within which the filarial nematodes are grouped as a separate sub cluster. The ubiquitous presence of the enzyme and the sequence homology between enolases from extant pathogens belonging to different phyla indicate that an enolase gene has already been present in the common ancestor and diversified by speciation of organisms and gene duplication within organisms [113]. Further knowledge on the molecular and functional characterization of this parasite enzyme and its genetic basis may help to understand parasite evolution and also to design new chemotherapeutic tools.

Therapeutic importance of enolase

Enolase may have one or more of three important functions in different vector-borne pathogens, in glycolysis, in gluconeogenesis and/or as plasminogen receptor. The presence of surface enolases in vector-borne pathogens and their potential role as a cell surface receptor in host pathogen interactions, make them potential therapeutic and diagnostic targets. Since enolase is abundantly expressed in most of the cells, it has been proved useful as a model for studying basic mechanisms of enzyme action as well as structural analysis. The gene that expresses enolase is not a house keeping gene since its expression varies according to the pathophysiological, metabolic or developmental conditions of the cells in the pathogens. Experiments carried out earlier on binding of plasminogen to surface exposed enolase [114] epitope mapping of anti-enolase mAb, immunizing mice with

anti-recombinant enolase have indicated the therapeutic prospects of targeting enolase to combat bacterial pathogens of human.

The available information on the presence of enolase in many arboviral (dengue virus, tick-borne encephalitis virus, and several other viruses which may cause encephalitis) particles as a possible requirement for the transcription of the virus and its secretion in higher levels in infected cells suggest that this protein may be explored as a potential vaccine target for arboviral infections. In tick-borne spirochetes, *Borrelia* spp., antigenic regions of enolase which is associated with spirochete outer membrane and surface exposed are aimed to be therapeutic candidates. High anti-enolase antibody titre in *P. falciparum* and *P. vivax infected* patients and increased amount of enolase in parasitized RBCs led to a conclusion that enolase can be a potential protective antigen for the immunoprophylactic and immunotherapeutic control of malari [75]. During hexose uptake gluconeogenesis has been shown to be an essential pathway for synthesis of glycoconjugates and *β-manna,* both required for the virulence of the Leishmania parasite [115]. Therefore, enolase could be considered as vital, and thus a drug target in this organism. Glycolysis represents the only process through which ATP is synthesized by the *T. brucei* and *T. Cruzi* parasites and inhibition of glycolysis, therefore, leads to the death of these parasites and hence enolase might be a candidate drug target. In blood stream-form *T. bruci*, enolase has been genetically validated as a drug target by RNA interference (RNAi), leading to decrease in enolase activity, growth arrest and followed by trypanosome death.

In helminthes particularly in parasites causing filarial diseases, enolase could be detected in most tissues of microfilariae, infective larvae and adults of *Onchocerca volvulus* as a surface protein involved in the interaction between parasite and human host which do not possess a classical machinery for surface transport in spite of that they are transported to the surface and anchored to cells by an unknown mechanism. Though partial and full length cDNA encoding enolase in *W. bancrofti* and *B. malayi* respectively have been reported, the real function of this enzyme in the interaction with the plasminogen is to be investigated further. The advent of completed genomes and the development of crucial experimental tools such as RNA interferons, provide the basis for further investigations into enzymology of enolase in the parasite nematodes. Molecular basis of filarial parasite modulation of host immunity increases the possibility of identifying vaccine candidates and new drug targets, and may also aid in the development of protein probes for selective and sensitive diagnosis of filariasis.

The wide variety of vector-borne pathogens discussed above need to penetrate the physical barriers such as extracellular material and tissue epithelium for their development in their mammalian or respective host. These pathogens from arbovirus to nematode prefer to recruit plasminogen- plasmin system for their survival. Since pathogen is devoid of fibrin to capture plasminogen on the surface of the pathogen, enolase a metabolic enzyme, is expected at the cell surface where the lysine motif of the enzyme physically interacts with kringle domain of the plasminogen. The genome of the pathogen mostly has a single enolase gene encoding a protein with multiple functions related to its subcellular location. Generally enolase enzyme lacks signal sequence and hence what mechanism operates to assign different functions to this enzyme and also its surface location is not yet known. No information is available as to how the mechanism of plasminogen activation and binding to enolase on the pathogen surface occur except the evidence that tPA binds to the surface of the mosquito midgut epithelium in *Plasmodium* for the activation of plasminogen on the ookinete surface at the time of ookinete-midgut epithelium contact.

Further in depth research on the mechanism of plasminogen activation and binding to enolase on the pathogen surface will unravel the complexity involved in tissue invasion. Knowledge on the nature of the protein substrates of surface plasmin and the mode of cleavage by plasmin will pave way for the innovative approaches for the development of therapeutic candidates towards vector-borne diseases control.

Prospects

The fact that enolase plays a crucial role in the metabolism of perhaps all vector-borne pathogens and is most likely an important virulence factor in some intracellular parasites provide two prospects for using this protein to combat or prevent an infection. In the first possibility, the presence of the plasminogen-receptor form of enolase could be used in two different ways to interfere with the virulence of the pathogens. Enolase of any vector-borne pathogen or unique part of its sequence, may be used either as an antigen for an infected person to enhance his immune system in combating the pathogen or it may be administered as a potential vaccine as in the case of *Clonorchis sinensis* prevention [116] to protect people at risk of being infected. On the other hand, compounds may be developed as drugs that prevent or disrupt the receptor-plasminogen interaction. The availability of crystal structure of parasite enolase has revealed the second possibility to design parasite-enzyme selective inhibitors whose selectivity and irreversible inhibition can be increased by including suitable substitutions. Easy production of purified bacterially expressed enolases (of different vector-borne pathogens) makes high-throughput screening of large libraries of drug like compounds possible. Further steps of optimization for any successful hits obtained by such screening through successive cycles of structure-activity relationship analysis may lead to development of drug candidates against vector-borne diseases.

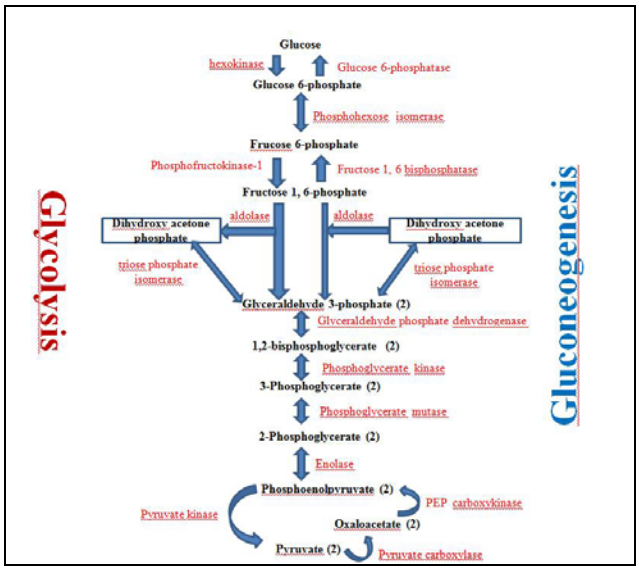

Fig. 1. Glycolytic pathway

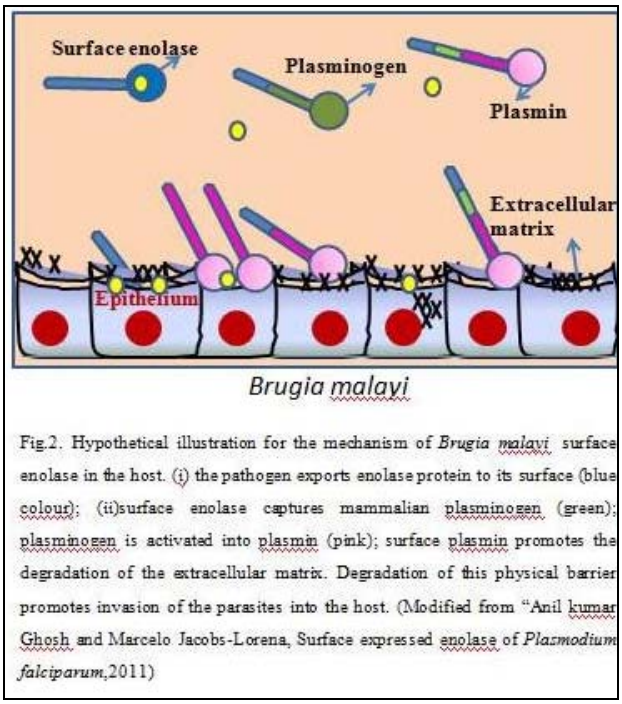

Fig.2. Hypothetical illustration for the mechanism of *Brugia malayi* surface enolase in the host. (i) the pathogen exports enolase protein to its surface (blue colour); (ii)surface enolase captures mammalian plasminogen (green); plasminogen is activated into plasmin (pink); surface plasmin promotes the degradation of the extracellular matrix. Degradation of this physical barrier promotes invasion of the parasites into the host. (Modified from "Anil kumar Ghosh and Marcelo Jacobs-Lorena, Surface expressed enolase of *Plasmodium falciparum*,2011)

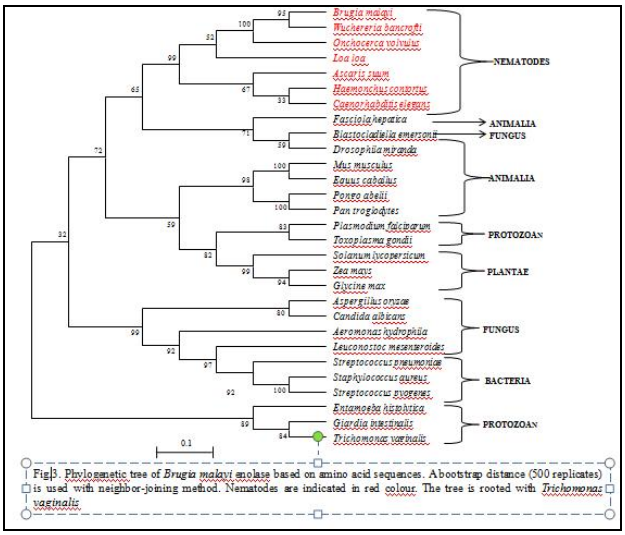

Fig3. Phylogenetic tree of *Brugia malayi* enolase based on amino acid sequences. Abootstrap distance (500 replicates) is used with neighbor-joining method. Nematodes are indicated in red colour. The tree is rooted with *Trichomonas vaginalis*

Table 1: Selected Vector-borne diseases of public health importance@.

Disease	Pathogens	Vector	Geographical distribution	Clinical manifestations
Viruses:				
Dengue	Flavivirus	Mosquito Aedes sp	Africa, Caribbean, Pacific, FarEast, India	Hemorrhagic fever
Japanese Encephalitis	Flavivirus	Mosquito Culex vishnui	Japan, Far East, India	Encephalitis
West Nile	Flavivirus	Complex Mosquito	Africa, India, Europe, North America	Encephalitis
Tick borne Encephalitis	Flavivirus	Ixodic tick	Former USSR, Europe, Africa	Encephalitis
Bacteria:				
Lyme disease	*Borrelia burgdorferi*	Ticks	Europe, North America	Arthritis
Protozoan parasites:				
Malaria	*Plasmodium* spp.	Mosquitoes	Widespread in tropics	Febrile illness with high mortality
African trypanosomiasis	*Trypanosoma brucei*	Tsetseflies	Africa	African sleeping Sickness
American Trypanosomiasis	*T. Cruzi*	Triatomine bugs	Central & South America	Chagas disease
Leishmaniasis	*Leishmania* spp	Sand flies	Africa, Africa & South America	Visceral Cutaneous & Mucocutaneous
Helminth parasites:				
Lymphatic Filariasis	*Wuchereria bancrofti*	Mosquito	Tropics	Elephantiasis
Onchocerciasis	*Brugia spp. Onchocerca*	Mosquito Blackflies	SouthEast Asian countries Africa, Central & South America	Dermatitis, Blindness
Loaiasis	*Volvulus Loaloa*	Tabanid flies	West &Central Africa	Calabar swelling

@ - Modified from Cook, G. C. (1996) Manson's Tropical Diseases, 20th Edn. London: WB Saunders.

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References

1. Cook GC. Manson's Tropical Diseases, 20th edn. 1996. London: WB saunders.
2. Hunter PR. Climate change and waterborne and vector-borne disease. J Appl. Microbiol. 2003; 94:37s-46s.
3. Pizarro-Cerdá J, Cossart P. Bacterial adhesion and entry into host cells. Cell. 2006; 124:715-727.
4. Wold F. Enolase. In: The enzyme, pp. Boyer P.D. Boyer P.D (ed), Academic press, New York. 1971; 499-538.
5. Pancholi V. Multifunctional alpha-enolase: its role in diseases. Cell. Mol. Life Sci. 2001; 58:902-920.
6. Lopez-Villar E, Monteoliva L, Larsen MR. Genetic and proteomic evidences support the localization of yeast

enolase in the cell surface. Proteomics. 2006; 6:S107-118.
7. Ruan J, Mouveaux T, Light SH, Minasov G, Anderson WF, Tomavo S *et al*. The structure of bradyzoite-specific enolase from *Toxoplasma gondii* reveals insights into its dual cytoplasmic and nuclear functions. Acta. Crystallogr. D. Biol. Crystallogr. 2015; 71(Pt 3):417-426.
8. Bergmann S, Wild D, Diekmann O. Identification of novel plasmin (ogen)- binding motif in surface displayed α-enolase of *Streptococcus pneumonia*. Mol. Microbiol.2003; 49:411-423.
9. Jones MN, Holt RG. Cloning and Characterization of α-enolase of the oral pathogen *Streptococcus mutans* that binds human plasminogen. Biochem. Biophy. Res. Comm. 2007; 364:924-929.
10. Agarwal S, Kulshreshtha P, Bambah Mukku D, Bhatnagar R. α-enolase binds to human plasminogen on the surface of *Bacillus anthrasis.* Biochim. Biophy. Acta. 2008; 1784:986-994.
11. Segovia-Gamboa NC, Chavez-Munguia B, Medina-Flores Y. *Entamoeba invadens*, encystations process and enolase. Exp. Parasitol. 2010; 125:63-69.
12. Walker MJ, McArthur JD, McKay F, Ranson M. Is plasminogen deployed as a *Streptococcus pyogenes* virulence factor? Trends Microbiol. 2005; 13:308-313.
13. Fallabella P, Riviello L, Stradis ML. *Aphidius ervi* teratocytes release an extra cellular enolase. Insect. Biochem. Mol. Biol. 2009; 39:801-813.
14. Pancholi V, Fischetti VA. α-Enolase, a novel strong plasmin(ogen) binding protein of the surface of the pathogenic streptococci. J. Biol. Chem. 1998; 272:14503-14515.
15. Miles LA, Dahlberg CM, Plescia J, Felez J, Kato K, Plow EF. Role of cell-surface lysines in plasminogen binding to cells: identification of alpha-enolases as a candidate plasminogen receptor. Biochemistry. 1991; 30:1682-1691.
16. Subramanian A, Miller DM. Structural analysis of alpha-enolase. Mapping the functional domains involved in down regulation of the c-myc protooncogene. J Biol. Chem. 2000; 275:5958-5965.
17. Wistow GJ, Leitman T, Williams LA, Stapel SO, deJong WW, Horwitz J. Taucrystaline /alpha-enolase: one gene encodes both an enzyme and a lens structural protein. J. Cell. Biol. 1988; 107:2729-2736.
18. Aaronson RM, Graven KK, Tucci M, McDonald RJ, Farber HW. Non neuronal enolase is an endothelial hypoxic stress protein J Bilo. Chem. 1995, 27752-27757.
19. Takei N, Kondo J, Nagaike K, Oshawa K, Kato K, Kohsaka S. Neuronal survival factor from bovine brain is identical to neuron-specific enolase. J. Neurochem. 1991; 57:1178-1184.
20. Al-Giery AG, Brewer JM. Characterization of the interaction of yeast enolase with polynucleotides. Biochim. Biophys. Acta. 1992; 1159:134-140.
21. Lottenberg R, Minning-Wenz D, Boyle MDP, Kain SJ, Schroeder BL, Curtiss III R. Capturing host plasmin (ogen): a common mechanism for invasive pathogens. Trends Microbiol. 1994; l 2:20-24.
22. Ehinger S, Schubert WD, Bergmann S, Hammerschmidt S, Heinz DW. Plasmin (ogen) binding alpha-enolase from *Streptococcus pneumonia*: Crystal structure and evolution of plasmin (ogen)-binding sites. J. Mol. Biol. 2004; 343:997-1005.
23. Pontig CP, Marshall JM, Cederholm-Williams SA. Plsminogen: a structural review. Blood coagul Fibrinolysis.1992; 3:605-614.
24. Derbise A, Song YP, Parikh S, Fischetti VA, Pancholi V. Role of the C-terminal lysine residue of streptococcal surface enolase in Glu- and lys-plasminogen binding activities of group A *Stretococci.* Infect. Immun. 2004; 72:94-105.
25. Mundodi V, Kucknoor AS, Alderete JF. Immunogenic and plasminogen binding surface associated alpha enolase of *Trichomonas vaginalis*. Infect. Immun. 2008; 26:523-531.
26. Feo S, Arcuri D, Piddin E, Passanthino R, Giallongo. Eno I gene product binds to the c-myc promoter and acts as a transcriptial repressor: relationship with myc promoter – binding protein I (MBP-1). FEB Lett. 2000; 473:47-52.
27. Bernal D, De la Rubia JE, Carasco-Abad AM, Mas-Coma S, Marcilla A. Identification of enolase as a plasminogen binding protein in excretory-secretory products of *Fasciola hepatica.* FEBS Lett. 2004; 563:203-206.
28. Wang L, Liu J, Li X, Shi J, Hu J, Cui R *et al*. Growth propagation of yeast in linear arrays of microfluidic chambers over many generations. Bioinformatics. 2011; 5(4):44118-441189.
29. Chow VT, Chan YC, Yong R, Lee KM, Lim LK, Chung YK *et al*. Monitoring of dengue viruses in field-caught *Aedes aegypti* and *Aedes albopictus* mosquitoes by a type-specific polymerase chain reaction and cycle sequencing. Am. J. Trop. Med. Hyg. 1998; 58:578-586.
30. Joshi V, Mourya DT, Sharma RC. Persistence of dengue-3 virus through transovarial transmission passage in successive generations of *Aedes aegypti* mosquitoes. Am. J. Trop. Med. 2002; 7:158-161.
31. Guzman MG, Halstead SB, Artsob H. Dengue: a continuing global threat. Nature Reviews. 2010; 8:S7-S16.
32. De Lourdes Mu˜noz M, Cisneros A, Cruz J, Das P, Tovar R, Ortega A. Putative dengue virus receptors from mosquito cells. FEMS Microbiol. Lett. 1998; 168:251-258.
33. Mercado-Curiel RF, Black IV WC, M̃uoz MDL. A dengue receptor as possible genetic marker of vector competence in *Aedes aegypti*. BMC Microbiol. 2008; 8:118.
34. Mosso C, Gal'van IJ, Ludert JE, Del Angel RM. Endocytic pathway followed by dengue virus to infect the mosquito cell line C6/36 HT. Virology. 2008; 378:193-199.
35. Van der Schaar HM, Rust MJ, Chen C. Dissecting the cell entry pathway of dengue virus by single-particle tracking in living cells. PLoS Pathogens Article ID e1000244. 2008, 4.
36. Castaneda E, Brummer E, Pappagianis D, Stevens DA. Chronic pulmonary and disseminated paracocidioidomycosis in mice: quantitation of progression and chronicity. J. Med. Vet. 2000; 23:377-387.
37. Mercado-Curiel RF, Black WC. 4th, Muñoz Mde L. A dengue receptor as possible genetic marker of vector competence in *Aedes aegypti.* BMC Microbiol 2008; 8:118.
38. Chee HY, Bakar AS. Identification of a 48 kDa tubulin or tubulin-like C6/36 mosquito cells protein that binds dengue virus 2 using mass spectrometry. Biochem. Biophys. Res. Commun. 2004; 320:11-1.
39. Sumanochitrapon W, Strickman D, Sithiprasasna R,

Kittayapong P, Innis BL. Effect of size and geographic origin of *Ae. aegypti* on oral infection with dengue-2 virus. Am. J. Trop. Med. Hyg. 1998; 58:283-286.
40. Colpitts TM, Cox J, Nguyen A, Feitosa F, Krishnan MN, Fikrig E. Use of a tandem affinity purification assay to detect interactions between West Nile and dengue viral proteins and proteins of the mosquito vector. Virology. 2011; 417:179-187.
41. Popova-Butler A, Dean DH. Proteomic analysis of the mosquito *Aedes aegypti* midgut brush border membrane vesicles. J. Insect. Physiol. 2009; 55:264-272.
42. Higa LM, Curi BM, Aguiar RS, Cardoso CC, De Lorenzi AG *et al*. Modulation of α-enolase post-translational modifications by dengue virus: increased secretion of the basic isoforms in infected hepatic cells. PLoS. 2014; 1(29):9:e88314.doi: 10.1371/journal.pone.0088314.
43. Petersen LR, Marfin AA. West Nile virus: A primer for the clinician. Ann. Intern. Med. 2002; 137:173-179.
44. Mackenzie JS, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of *Japanese encephalitis*, West Nile and dengue viruses. Nat. Med. 2004; 10(Suppl.):S98-S10910: 1038/nm1144.
45. Rappole JH, Derrickson SR, Hubálek Z. Migratory birds and spread of West Nile virus in the Western Hemisphere. Emerg. Infect. Dis.2000; 6:319-328. doi: 10.3201/eid0604.000401.
46. Bangarelli P, Marinelli K, Trotta D, Monachetti A, Tavio M, Del Gobbo R *et al*. Nicoletti. Human case of autochthonous West Nile virus lineage 2 infection in Italy. Euro. Surveill. 2011; 16:20002.
47. Hatfield RH, McKernan RM. CSF neuron-specific enolase as a quantitative marker of neuronal damage in a rat stroke model**.** Brain Res. 1992; 577:249-52.
48. He X, Ren J, Xu F, Ferguson MR, Li G. Localization of West Nile Virus in monkey brain: double staining antigens immunohistochemically of neurons, neuroglia cells and West Nile Virus. Int. J. Clin. Exp. Pathol. 2010; 3:56-161.
49. Ogino T, Yamadera T, Nonaka T, Imajoh-Ohmi S, Mizumoto K. Enolase a cellular glycolytic enzyme, is required for efficient transcription of Sendai virus genome. Biochem. Biophys. Res. Commun. 2001; 285:447-455.
50. Mackenzie JS. Emerging flaviviruses: the spread and resurgence of *Japanese encephalitis*, West Nile, and dengue viruses. Nat. Med. 2004; 10:S98-S109.
51. CDC. Tick-borne encephalitis among US travelers to Europe and Asia-2000-2009. MMWR Morb. Mortal. Wkly. Rep. 2010; 59(11):335-8.
52. Díaz-Martín V, Manzano-Román R, Oleaga A, Encinas-Grandes A, Pérez-Sánchez R. Cloning and characterization of a plasminogen-binding enolase from the saliva of the argasid tick *Ornithodoros moubata*. Vet. Parasitol. 2013; 191:301-314.
53. Ruzek D, Jiri S, Singh SK, Kopecky J. Breakdown of the Blood-Brain Barrier during Tick-Borne Encephalitis in Mice Is Not Dependent on $CD8^+$ T-Cells. PLoS 2011; 1(6):e20472.x.
54. Beliaeva IA, Antonova OM, Anin AN, Chekhonin VP. Resistance of the hemato-encephalic barrier in tick-borne neural infection (Lyme disease and tick-borne encephalitis). Zh. Nevrol. Psikhiatr. Im. S. S. Korsakova. 1995; 95:25-29.
55. Chekhonin VP, Zhirkov YA, Belyaeva IA, Ryabukhin IA, Gurina OI. Serum time course of two brain-specific proteins, alpha (1) brain globulin and neuron-specific enolase, in tick-born encephalitis and Lyme disease. Clin. Chim. Acta. 2002; 320:117-125.
56. Davidson MM, Williams H, Macleod JA. Louping ill in man: a forgotten disease. Journal of Infection. 1991; 23(3):241-49.Ebel GD. Update on Powassan virus: emergence of North American tick-borne flavivirus. Ann. Rev. Entomol. 2010; 55:95-110.
57. World Health Organization. *Japanese encephalitis* vaccines. Wkly. Epidemiol. Rec. 2006; 25:81(34/35):331-40.
58. Solomon T, Thao LT, Dung NM, Kneen R, Hung NT, Nisalak A *et al*. Rapid diagnosis of Japanese encephalitis by using an immunoglobulin M dot enzyme immunoassay. J. Clin. Microbiol. 2000; 36(7):2030-2034.
59. Burke DS, Nisalak A, Ussery MA, Laorakpongse T, Chantavibul S. Kinetics of IgM and IgG responses to *Japanese encephalitis* virus in human serum and cerebrospinal fluid. J. Infect. Dis. 1985; 151(6):1093-1099.
60. Yang Y, Ye J, Yang X, Jiang R, Chen H, Cao S. *Japanese encephalitis* virus infection induces changes of mRNA profile of mouse spleenand brain. Virol. J 2011; 8:80-91.
61. Peng Jiang, Matthew L, Freedman, Jun S, Xiaole Shirley Liu. IgG responses to *Japoanese encephalitis* virus in human serum and crerbrospinal fluid. J Infect Dis. 2015; 154:1089-1099.
62. Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease. J. Clin. Invest. 2004; 113:1093-1101.
63. CDC Lyme disease data and statistics. http://www.cdc.gov/lyme/stats/index.html. 2011.
64. Nowalk AJ, Nolder C, Clifton DR, Carroll JA. Comparative proteome analysis of subcellular fractions from *Borrelia burgdorferi* by NEPHGE and IPG. Proteomics. 2006; 6:2121-2134.
65. Floden AM, Watt JA, Brissette CA. *Borrelia burgdorferi* enolase is a surface-exposed plasminogen binding protein. PLoS 2011; 1(6):e27502.
66. Coleman JL, Gebbia JA, Piesman J, Degen JL, Bugge TH. Plasminogen is required for efficient dissemination of *Borrelia burgdorferi* in ticks and for enhancement of spirochetemia in mice. Cell, 1997; 89:1111-1119.
67. De Silva AM, Telford SR, Brunet LR, Barthold SW, Fikrig E. *Borrelia burgdorferi* ospA is an arthropod-specific transmission-blocking Lyme disease vaccine. J. Exp. Methods. 1996; 183:271-275.
68. Gebbia JA, Coleman JL, Benach JL. *Borrelia* spirochetes upregulate release and activation of matrix metalloproteinase gelatinase B (MMP-9) and collagenase 1 (MMP-1) in human cells. Infect. Man.
69. Coleman JL, Gebbia JA, Piesman J, Degen JL, Bugge TH. Plasminogen is required for efficient dissemination of *Borrelia burgdorferi* in ticks and for enhancement of spirochetemia in mice. Cell, 1997; 89:1111-1119.
70. Barbour AG, Hayes SF, Heiland RA, Schrumpf ME, Tessier SL. A Borrelia-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. 1986; 52(2):549-554.
71. World Malaria Report. World Global Malaria Programme. 2013, 255.
72. Wold F, Ballou CE. Studies on the Enzyme Enolase.2. Kinetic Studies. J. Biol. Chem. 1957; 227:313-328.
73. Roth EF, Raventos-Suarez C, Perkins M, Nagel RL. Glutathione stability and oxidative stress in Plasmodium

falciparum infection *in vitro*: responses of normal and G6PD deficient cells. Biochem. Biophys. *Res.* Commun. 1982; 109:355-362 10.1016/0006-291X (82)91728-4.
74. Read M, Hicks KE, Sims PF, Hyde JE. Molecular characterisation of the enolase gene from the human malaria parasite *Plasmodium falciparum*. Evidence for ancestry within a photosynthetic lineage. Eur. J Biochem. 1994; 220:513-20.
75. Sato K, Kano S, Matsumoto Y, Glanarongran R, Krudsood S, Looa-reesuwan S *et al*. Application of yeast enolase as antigen for immunodiagnosis of malaria. Southeast Asian J. Trop. Med. Pub. Hlth. 2000; 31(Suppl 1):79-84.
76. Pal-Bhowmick I, Mehta M, Coppens I, Sharma S, Jarori GK. Protective properties and surface localization of *Plasmodium falciparum* enolase: Inf. immunity. 2007; 75:5500-5508.
77. Ghosh AK, Isabelle Coppens I, Henrik Gårdsvoll H, Michael Ploug M, Jacobs-Lorena M. Plasmodium ookinetes coopt mammalian plasminogen to invade the mosquito midgut PNAS. 2011; 108:17153-17158.
78. Vega-Rodriguez J, Ghosh AK, Kanzok SM, Dinglasan RR, Wang S, *et al*. Multiple pathways for *Plasmodium* ookinete invasion of the mosquito midgut. Proc. Natl. Acad. Sci. USA. 2014; 111(4): E492-E500.
79. Witkowski B, Amaratunga C, Khim N. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect. Dis. 2013; 13:1043-9.
80. Jeronimo SMB, de Queiroz Sousa A, Pearson RD. Leishmaniasis. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases. 2nd ed. Philadelphia: Churchill Livingston Inc.; 2006, 1095-107.
81. Croft SL, Coombs GH. Leishmaniasis-current chemotherapy and recent advances in the search for novel drugs. Trends. Parasitol. 2003; 19:502-508.
82. Naderer T, Heng J, McConville MJ. Evidence that intracellular stages of *Leishmania* major utilize amino sugars as a major carbon source. PLoS Pathog. 2010; 6:e1001245.
83. Naderer T, Ellis MA, Sernee MF. Virulence of *Leishmania* major in macrophages and mice requires the gluconeogenic enzyme fructose-1, 6-bisphosphatase. Proc. Natl. Acad. Sci. USA. 2006; 103:5502-5507.
84. Rodríguez-Contreras D, Landfear S. Metabolic changes in glucose transporter-deficient *Leishmania mexicana* and parasite virulence. Biol. Chem. 2006; 281:20068-76.
85. Quinones W, Pena P, Domingo-Sananes M, Caceres A, Michels PA, Avilan L *et al*. Exp. Parasitol. 2007; 116:241-25.
86. Carneiro D, Bavia ME, Rocha W, Lobão J, Filho CM, Oliveira JB *et al*. Identificação de áreas de risco para a leishmaniose visceral americana através de estudos epidemiológicos esensoriamento remoto orbital em Feira de Santana, Bahia, Brasil (2000-2002). Rev. Baiana. Saude. Publica. 2004; 28:19-32.
87. Avilan L, Calcagno M, Figuera M, Lemus L, Puig J, Rodríguez AM. Interaction of *Leishmania mexicana* promastigotes with the plasminogen-plasmin system. Mol. Biochem. Parasitol. 2000; 110:183-193.
88. Maldonado J, Calcagno M, Puig J, Maizo Z, Avilan L. A study of cutaneous lesions caused by *Leishmania mexicana* in plasminogen-deficient mice. Exp. Mol. Pathol. 2006; 80:289-294.
89. Simarro P, Jannin J, Cattand P. Eliminating human African trypanosomiasis: where do we stand and what comes next? Plos Medicine 2008; 5:e55.
90. Hannaert V, Alber MA, Rigden DJ. Kinetic characterization, structure modelling studies and crystallization of *Trypanosoma brucei* enolase. Euro. J. Biochem. 2003; 270:3205-3213.
91. Weitz B. The properties of some antigens of *Trypanosoma brucei*. J. Gen. Microbiol. 1960; 23:589-600.
92. Paba J, Santana JM, Teixeira ARL, Fontes W, Sousa MV, Ricart CAO. Proteomic analysis of the human pathogen *Trypanosoma cruzi*. Proteomics. 2004; 4:1052-1059.
93. Caceres AJ, Portillo R, Acosta H. Molecular and biochemical characterization of hexokinase from *Trypanosoma cruci*. Mol. Biochem. Parasitol. 2003; 126:251-262.
94. Pabon MA, Caceres AJ, Gualdron M, Quinones W, Avilan L, Concepcion JL. Purification and characterization of hexokinase from *Leishmania Mexicana.* Parasitol. Res. 2007; 100:803-810.
95. Thurston JP. The effect of immune sera on the respiration of *Trypanosoma brucei in vitro*. Parasitology. 1958; 48(3-4):463-467.
96. Smith IM. The protection against trypanosomiasis conferred on cattle by repeated boses of antrycide, alone or with *Trypanosoma congolense.* Ann. Trop. Med. Parasitol. 1958; 52(4):391-401.
97. World Health Organization. Global programme to eliminate lymphatic filariasis: progress report for 2012. WER 13 September 2013; 88(37):389-400. http://www.who.int/lymphatic_filariasis/epidemiology/en
98. Ghedin E, Wang S, Spiro D, Caler E, Crabtree J, Allen JE. Draft genome of the filarial nematode parasite, *Brugia malayi*. Science. 2007; 317:1756-60.
99. World Health Organization. Global Burden of Disease, 2012. http://www.who.int/entity/healthinfo/global burden disease/GHE DALY WHOReg6 2012.xls (DALY).
100. World Health Organization. Japanese encephalitis vaccines. Wkly Epidemiol Rec. 1995; 25:81(34/35):331-40.
101. World Health Organization. First WHO report on neglected tropical diseases: working to overcome the global impact of neglected tropical diseases (Data 2008) (*Onch).*
102. World Health Organization. Global programme to eliminate lymphatic filariasis: progress report for 2012. WER 2013; 88:37:389-400. http://www.who.int/lymphatic_filariasis/epidemiology/en
103. World Health Organization. Japanese encephalitis vaccines. Wkly Epidemiol Rec. 1996; 29:81(34/35):331-406.
104. Jolodar A, Fischer P, Bergmann S, Büttner DW, Hammerschmidt S, Brattig NW. Molecular cloning of an alpha-enolase from the human filarial parasite *Onchocerca volvulus* that binds human plasminogen. Biochim. Biophy. Acta. 2003; 1627:111-120.RAPLOA). PLoS Negl. Trop. Dis. 2011; 5(6):e1210.
105. Brattig NW, Hoerauf A, Fischer PU, Liebau E, Bandi C, Debrah A *et al*. Immunohistological studies on neoplasms of female and male *Onchocerca volvulus*: filarial origin and absence of *Wolbachia* from tumor cells. Parasitol. 2010; 137:841-854.
106. Hotez PJ, Kmath A. Neglected tropical disease in sub-

saharan Africa: review of their prevalence, distribution, and disease burden. PLos Negl. Trop. Dis. 2009; 3(8).
107. Zoure HGM, Wanji S, Noma M, Amazigo UV, Diggle PJ. The Geographic Distribution of *Loa Loa* in Africa: Results of large-scale Implementation of the Rapid Assessment procedure for Loiais (RAPLOA). PLoS Negl. Trop. Dis. 2011; 5(6):e1210.
108. Nutman TB, Fink DL, Russ C, Young S, Zeng Q, Koehrsen M *et al* The genome sequence of *Loa loa***.** The Broad Institute Genome Sequencing Platform. Submitted to the, EMBL/GenBank/DDBJ databases. Cited for**:** NUCLEOTIDE SEQUENCE [LARGESCALE GENOMIC DNA], 2010. http://www.uniprot.org/uniprot/E1GL52.
109. Melnikow E, Xu Liu J, Bell AJ, Ghedin E, Unnasch TR, Lustigman S. A Potential Role for the Interaction of *Wolbachia* Surface Proteins with the *Brugia malayi* Glycolytic Enzymes and Cytoskeleton in Maintenance of Endosymbiosis. PLOS Neg. Trop. Dis. 2013; 7:e2151 www.plosn;7: e2151.
110. Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, *et al*. The *Wolbachia* genome of *Brugia malayi*: Endosymbiont evolution within a human pathogenic nematode. PLoS Biol. 2005; 3:0599-0614. doi: 10.1371/journal.pbio.0030121.
111. Zagaria N, Savioli L. Elimination of lymphatic filariasis: a public-health challenge. Ann. Trop. Med. Parasitol. 2002; 96(Suppl. 2):S3-S13.
112. Rana AK, Misra-Bhattacharya S. Current drug targets for helminthic diseases. Parasitol. Res. 2013; 112:1819-31. Doi: 10.1007/s00436-013-3383-6.
113. Day INM, Peshavaria M, Quinn GB. A differential molecular clock in enolase isoprotein evolution. J. Mol. Evol. 1993; 36:599-601.
114. Bergmann S, Rohde M, Preissner KT, Hammerschmidt S. The nine residue plasminogen-binding motif of the pneumococcal enolase is the major cofactor of plasmin-mediated degradation of extracellular matrix, dissolution of fibrin and transmigration. Thromb. Haemost. 2005; 94:304-311.
115. Brewer JM, Claiborne VC, Glover, Michael J Holland MJ, Lebioda L. Effect of site-directed mutagenesis of His373 of yeast enolase on some of its physical and enzymatic properties. Biochim. Biophys. Acta. 1997; 1340:88-96.
116. Wang X, Shang M, Li X, Yu X, Muang Y. Surface display of *Clonorchis sinensis* enolase on *Bacillus subtilis* spores potentializes an oral vaccine candidate. Vaccine, 2014; 132:1335-1345.
117. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.protein I (MBP-I) FEB Lett 2000; 473:47-52.
118. Fitch WM, Margollash E. Construction of phylogenetic trees. Science. 1967; 155:279-284.