Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/aedc3c86

IDENTIFICATION OF UNCHARACTERIZED WUCHERARIA BANCROFTI PROTEINS USING PHYRE 2.0 AND INTERPRO SOFTWARE

Swathi varri[1] *, Dr. D Narendra[1], Navya[1], Sowjanya[2], Rupa Sri[3], Deepika[4], Johaparvaz[5]

[1*]Assistant Professor, Department of Pharmaceutics, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh- India.
[1]Principal &Professor, Department of Pharmaceutical analysis, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh – India.
[1]Student, Department of Pharmacy, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh – India.
[2]Student, Department of Pharmacy, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh – India.
[3]Student, Department of Pharmacy, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh – India.
[4]Student, Department of Pharmacy, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh – India
[5]Student, Department of Pharmacy, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh – India

***Corresponding Author:** Swathi varri
[*]Assistant Professor, Department of Pharmaceutics, VJ's college of Pharmacy, Rajahmundry, Andhra Pradesh- India.

ABSTRACT:
METHOD: Globally, filariasis is one of the most common diseases effected by wucheraria bancrofti parasite. The two uncharacterized wucheraria bancrofti were inserted into phyre 2 for knowing protein homology and 3D structure of proteins and interpro used for understanding domain annotation, visualisation and family of protein. RESULTS: The 1st protein showed the highest confidence and coverage level of 100% followed by second protein, first protein is having 11% disorderness and majority of beta-helix and alpha-helix and 23% and 23% and 21% respectively. Whereas second protein has no disorderness with 26% alpha-helix. The two proteins visualization exhibited secondary structure and more than 50 amino acid residues for each protein. CONCLUSION: The second protein would be (o44786 ID) best target lead for wcheraria bancrofti. This research aims to understand the structure and function of two uncharacterized wcheraria bancrofti proteins (P35666 ID, o44786 ID) using bioinformatic methods in an aim to learn more about the parasite methods. The two uncharacterized proteins were given input into phyre 2 for determining the active site, domains 3D structures and sequence function. Interpro is also used to determine the SUPER FAMILY of given proteins.

INTRODUCTION:

Habit and Habitat:

Wuchereria bancrofti is a filaroid nematode, causing a very tragic, horrifying and debilitating disease known as filariasis. This disease has been known from antiquity and was the first discovery of insect (culex mosquito) transmission of a human disease. In general, infection with any of the filarial nematode may be called as filariasis but traditionally, the term filariasis refers to lymphatic filariasis caused by Wuchereria or Burgie species. Ap-prox-i-mately 106 mil-lion peo-ple in 76 coun-tries are in-fected, with one bil-lion peo-ple thought to be at risk, Wuchereria bancrofti is a dreadful endoparasite of man; adults harbouring the lymphatic vessels and lymph nodes.[1] The genus Wuchereria was named after Wucherer, a Brazilian physician who reported the presence of larvae in chylous urine in 1866. The adult female was described by Bancroft in 1876. Wuchereria bancrofti is distributed widely in tropics and subtropics of Asia (India, Bangladesh, Myanmar, China, Japan), Africa and South America[2]

The adult worms are whitish, translucent, threadlike worms with smooth cuticle and tapering ends. Adult worms These are long, hair-like, transparent, translucent, thread-like worms with smooth cuticle and tapering ends, The head is slightly swollen and bears two circles of well-defined papillae. The mouth is small and lack buccal capsule.[3] The female worm is larger (80-100 mm long and 0.24-0.3 mm wide) than the male (40 mm long and 0.1 mm wide) The vulva is near the level of the middle of the oesophagus. Males and females remain coiled together usually in the abdominal and inguinal lymphatics and in the testicular tissue of human. Sexes are separate with distinct sexual dimorphism. The adult worms live for many years, probably 10-15 years or more.[4]

Life Cycle Of Wuchereria Bancrofti:

Wuchereria Bancrofti requires two hosts for comple-tion of its life cycle. Man is the only definitive host and no animal host or reservoir is known for W. bancrofti. The intermediate host is the female mosquito of the genus Culex. The major vector in India and most other parts of Asia is Culex fatigues. The disease is endemic in 83 countries with more than 1.2 billion at risk. They are found in India, West-Indies, Puerto Rico, Southern China, Japan, Pacific Island, West and central Africa, South America[5].

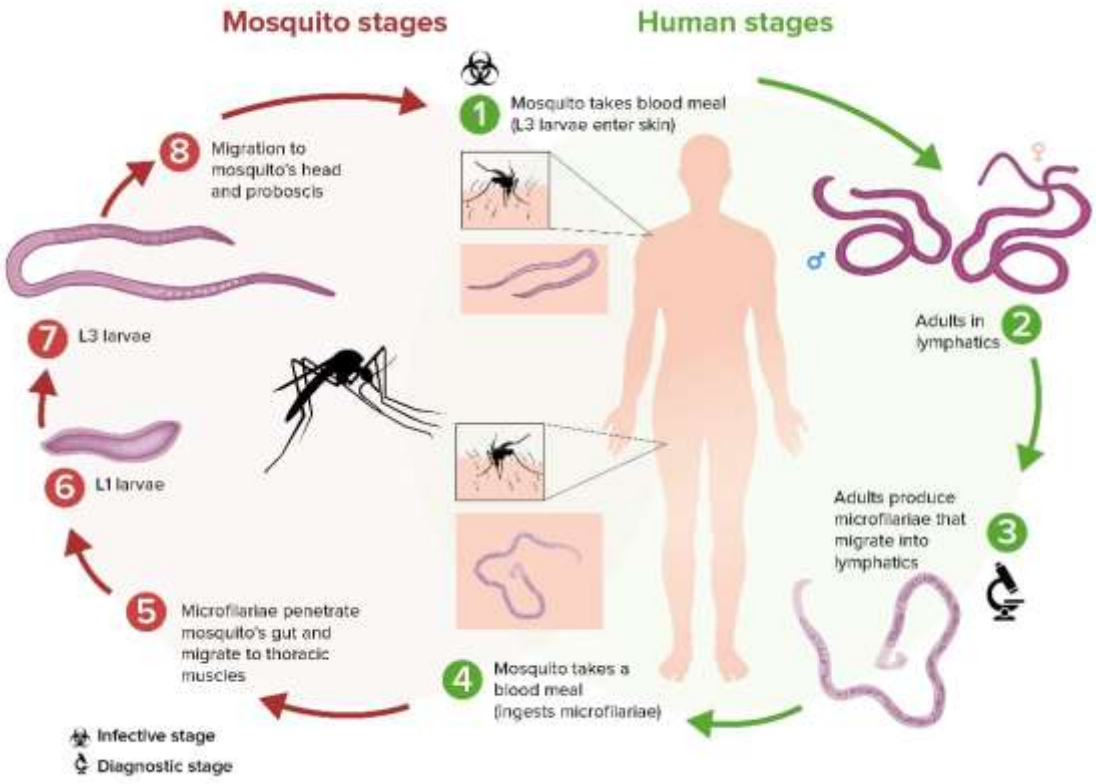

Fig:1 life cycle of Wuchereria Bancrofti gives the complete description as a flow chart [26]

STAGES IN MOSQUITO AND HUMAN:

A. IN MOSQUITO:

When a vector mosquito feeds on a carrier the microfilariae are taken in with the blood meal and reach the stomach of the mosquito. Within 2 to 6 hours, they cast off their sheath (ex-sheathing), penetrate the stomach wall and within 4 to 17 hours migrate to the thoracic muscles where they undergo further development. is a digenetic parasite and requires two hosts to complete its life cycle. Infection is acquired by the bite of infected mosquito during which L3 larva are deposited on the. Metamorphosis completes by 10-11days with distinct features such as the tail atrophies to a mere stump and the digestive system, body cavity and genital organs are now fully developed. This is the third stage larva L3. These L3 larva are the infective form which enters the proboscis sheath of the mosquito on or about the 14th day. Development in mosquito takes place within 10-20 days. In another week, it develops internal structures and becomes the elongated third-stage filariform larva (J3), measuring 1,500-2,000 x 15-25 µm. It is actively motile and infective. It enters the proboscis sheath of the mosquito, awaiting opportunity for infecting human, the defi-nitive host. The larvae possess a rudimentary digestive tract.[6]

B. In Human:

Wuchereria bancrofti is a filarial nematode that causes Wuchereriasis or filariasis (commonly called elephantiasis) in human beings. When a mosquito with infective larvae in its proboscis feeds on a person, the larvae get deposited, usually in pairs, on the skin near the puncture site. The larvae then enter through the puncture wound or penetrate the skin by themselves. When this infected mosquito pierces its proboscis in the warm and moist skin of man, the larvae creep out of labium to human skin.[7] The infective dose for man is not exactly known, but many larvae fail to penetrate the skin and many more are destroyed in the host tissues by immunological and other defence mechanisms. However, it is found that a very large number of infected mosquito bites are required to ensure transmission to man – perhaps as many as 15,000 infective bites per person.[8] Then, it penetrates the skin and finally come to settle down into lymphatics. After penetrating the skin, the third-stage larvae enter the lymphatic vessels and are carried usually to abdominal or inguinal lymph nodes, where they develop into adult forms. There is no multiplication at this stage, and only one adult develops from one larva, male or female. The female worm is viviparous or ovoviviparous, it releases numerous larvae called microfilariae into the bloodstream. During the daytime, the microfilariae tend to stay in the deep blood vessels of man.[9]In the peripheral blood, the microfilariae do not undergo any further development. It they are not taken up by a female vector mosquito they die. Their life-span is believed to be about 2 to 3 months. It is estimated that a microfilaria density of at least 15 per drop of blood is necessary for infecting mosquitoes[10.] Densities of 20,000 microfilariae or more per ml. of blood may be seen in some carriers. Metamorphosis finally completes by 10-11 days into third-stage filariform larvae (L3) which measure about 1500 to 2000 µm in length and 18-23 µm in diameter.[11]

MAJOR PROTEINS IN WUCHERERIA BANCROFTI:

1. Macro phase migration inhibitory factor homolog
2. Cuticular glutathione peroxidase

1.**MACRO PHASE MIGRATION INHIBITORY FACTOR HOMOLOG**

Introduction of Macro phase migration inhibitory factor homolog

Lymphatic filariasis, a neglected tropical disease caused primarily by Wuchereria bancrofti, persists as a major public health problem in tropical and subtropical regions. The success of this parasite in establishing long-term infections is largely attributed to its ability to manipulate the host immune system through secreted effector molecules. Among these, the macrophage migration inhibitory factor (MIF) homologs represent a unique class of parasite-derived immunomodulators. MIF is a conserved cytokine in mammals that regulates inflammation, T-cell activation, and macrophage recruitment. Interestingly, filarial nematodes including W. bancrofti encode two MIF homologs (Wb-MIF-1 and Wb-MIF-2), which display structural similarity to the human MIF protein. Experimental studies have

demonstrated that these homologs are secreted during infection and interact with host immune cells to alter their function. [12]

Functions of macro phase migration inhibitory factor homolog:

1. Modulation of Cytokine Profiles

Wb-MIF homologs influence the balance of host cytokines. They suppress the expression of pro-inflammatory mediators such as tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), both of which are central to parasite killing. At the same time, they enhance the secretion of IL-4, IL-5, and IL-10, which are associated with Th2-biased responses. This immune profile Favors long-term parasite persistence.[13]

2. Effects on Antigen-Presenting Cells. These homologs interfere with the normal function of dendritic cells and other antigen-presenting cells. By dampening their ability to stimulate effector T-cell responses, Wb-MIF proteins help establish an immune environment that is less hostile to the parasite.[14]

3. Skewing Toward Th2 Immunity

The immune manipulation caused by Wb-MIF promotes a Th2-dominated immune environment. While Th2 responses encourage antibody production, they are less effective at clearing intracellular or tissue-dwelling parasites. This shift reduces the effectiveness of protective Th1 responses and prolongs infection.[15]

TABLE: 1 Properties of macro phase migration inhibitory factor homolog

S .NO	PROTERTIES	SIGNIFICANCE
1.	Structural and Molecular Features	The parasite's MIF homolog is a small soluble protein (around 12–15 kDa) that usually forms a trimeric structure. It resembles mammalian MIF in its overall fold and enzymatic domain but contains unique parasite-specific sequence regions. The N-terminal proline residue, characteristic of MIF proteins, is also present and contributes to its enzymatic site.[22]
2.	Production and Secretion	This protein is expressed across different stages of the parasite's life cycle. Its presence has been detected in the blood of infected individual, indicating its role in ongoing infections.
3.	Immunological Functions	Acts as a functional mimic of host MIF, capable of binding to the CD74 receptor on immune cells. Restricts macrophage migration, preventing effective recruitment of immune cells to infection sites.[23]
4.	Effects on the Host Immune System	The homolog functions as a parasite cytokine mimic. By binding to the CD74 receptor on host immune cells, it alters normal signalling. It can block macrophage movement, preventing effective immune surveillance. In addition, it promotes a Th2-dominated or regulatory immune profile instead of a pro-inflammatory Th1 response. It also enhances production of IL-10, a cytokine that helps suppress inflammation, thereby favouring parasite survival.[24]

Classification of macro phase migration inhibitory factor homolog

1. By Origin

The protein is parasite-derived, encoded by the genome of W. bancrofti, a filarial nematode responsible for lymphatic filariasis. It is part of the excretory–secretory (ES) protein pool, meaning it is actively released into the host during infection.

2. By Molecular Characteristics

A small soluble protein, approximately 12–15 kDa in size. Typically forms a trimeric structure, a hallmark feature of MIF proteins. Contains a conserved N-terminal proline, which contributes to its enzymatic tautomerize activity.[16]

2.Cuticular glutathione peroxidase

INTRODUCTION OF CUTICULAR GLUTATHION PEROXIDASE:

Wuchereria bancrofti is a filarial nematode parasite responsible for lymphatic filariasis, a debilitating disease affecting millions of people worldwide, particularly in tropical and subtropical regions. The parasite resides within the human lymphatic system for extended periods, often surviving for years despite constant exposure to the host's immune defences. One of the major challenges faced by W. bancrofti within the host is oxidative stress, generated by reactive oxygen species (ROS) released during the host immune response. To counteract this, the parasite expresses a range of antioxidant enzymes, among which cuticular glutathione peroxidase (GPx) plays a critical role. Like other helminth parasites, W. bancrofti has developed specialized mechanisms to survive within the hostile environment of the human host, where it is continuously exposed to oxidative stress generated by host immune cells. One of the key defence strategies of the parasite involves antioxidant enzymes that neutralize reactive oxygen species (ROS). Cuticular glutathione peroxidase (GPx) represents an important component of this protective system. This enzyme is expressed on the cuticle, the outer protective layer of the parasite, and functions to detoxify peroxides, particularly hydrogen peroxide and lipid peroxides, which are produced as part of the host's oxidative immune response. [17]

FUNCTIONS OF CUTICULAR GLUTATION PEROXIDASE:

Cuticular glutathione peroxidase (GPx) in Wuchereria bancrofti plays an important role in protecting the parasite from the host's immune defence mechanisms. This enzyme is primarily located on the cuticle, which is the outer protective surface of the worm, and it helps neutralize harmful molecules such as hydrogen peroxide and lipid hydroperoxides that are generated by the host during oxidative stress. These molecules, including hydrogen peroxide and lipid peroxides, can damage cellular membranes, proteins, and nucleic acids of the parasite. The localization of GPx on the cuticle provides a first line of Défense, as it catalyses the reduction of peroxides into harmless products using glutathione as a cofactor. This action protects the cuticular surface, which is critical for maintaining the structural integrity and viability of the worm.[18]

TABLE: 2 PROPERTIES OF CUTICULAR GLUTATHION PEROXIDASE:[25]

S.NO	PROPERTIES	SIGNIFICANCE
1.	Antioxidant Function	Antioxidant Function
2.	Surface Localization	Unlike intracellular GPx in host cells, this enzyme is located on the cuticle (outer surface) of the filarial worm. This strategic positioning allows it to immediately neutralize oxidative attacks from host immune cells.
3.	Glutathione Dependency	Requires reduced glutathione (GSH) as an electron donor to carry out its catalytic activity. The GSH → oxidized glutathione (GSSG) conversion cycle is essential for its enzymatic function.

RESEARCH PROCEDURE

Phyre2.2:

Sequences, often including those of entire proteomes, are being determined at an ever-increasing rate. Consequently, many researchers in the bioscience and biomedical communities are looking for models of proteins to provide insight into structure/function relationships and to guide further experiments. For many years template-based modelling, also known as homology modelling, has been the most accurate approach for structure prediction and has been made accessible to the community via web servers including Phyre21 and SWISS-Model.2 The result of a sequence search

identifies a sequence similarity between the query to be predicted and a known structure from which one infers that the unknown structure will adopt a similar conformation to the known coordinates which accordingly acts as a template for modelling. But often these are not of the same quality as the models obtained by template-based modelling.[19]

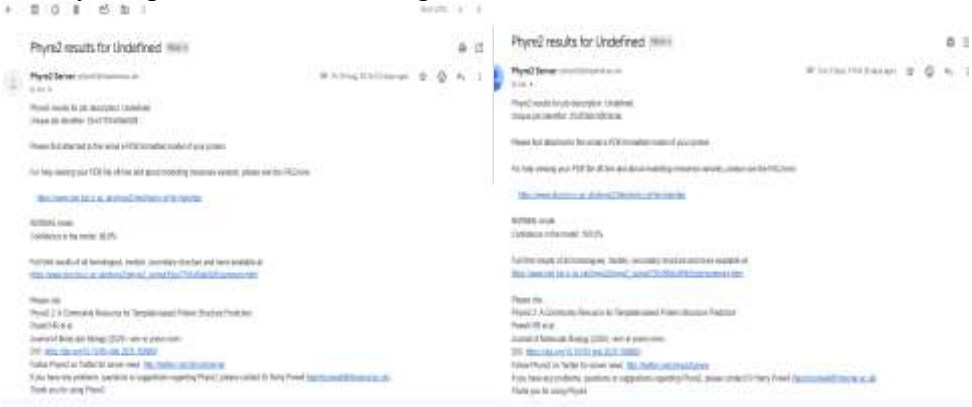

Fig:2 and fig :3 describes about the running of the software in the phyre 2.0 by generating the resukt as follows

InterPro

It is a prominent bioinformatics resource created and maintained by EMBL-EBI. It provides a comprehensive platform for the classification of protein families, domains, and functional motifs by integrating predictive models from numerous partner databases.

- **Database integration**: Executes predictive models (signatures) from more than 15 partner resources, such as Pfam, PROSITE, PANTHER, SMART, TIGRFAMs, CDD, and Gene3D.
- **Consistent annotation**: Links sequence matches to InterPro entries that provide standardized names, descriptions, and associated Gene Ontology (GO) terms.
- **Flexible input**: Supports protein FASTA files, translated nucleotide sequences, and bulk submissions.[20]

fig 4 and fig 5 describes about the result generating in the soft ware interpro

RESULT:

Cuticular Glutathione Peroxidase (cGPx) and Macrophage Migration Inhibitory Factor (MIF) homologs exhibit distinct structural and functional properties as revealed by Phyre2 and InterPro analyses. Structural modeling through Phyre2 indicates that cGPx possesses a thioredoxin-like fold consisting of α-helices and β-strands, forming a compact globular architecture with conserved cysteine or selenocysteine residues that are critical for its peroxidase activity. From a functional perspective, cGPx primarily contributes to parasite survival by detoxifying host-derived reactive oxygen species, whereas MIF homologs modulate host immune responses by mimicking cytokine activity and suppressing macrophage activation. Collectively, these findings suggest that cGPx and MIF homologs operate through complementary mechanisms—antioxidant defense and immune evasion—that together enhance parasite persistence within the host.[21]

3D IMAGES:

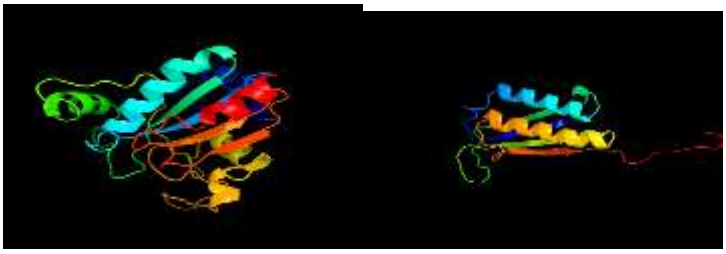

fig 6: describes about the Cuticular Glutathione Peroxidase
fig 8: describes about the Macro phase migration inhibitory factor homolog

SECONDARY STRUCTURE AND DISORDER PROTECTION

Fig:8 SECONDARY STRUCTURE AND DISORDER PROTECTION of the Cuticular Glutathione Peroxi

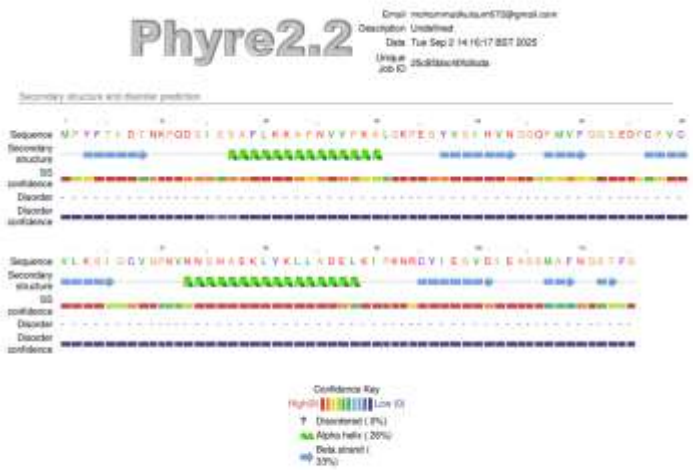

Fig: 9 SECONDARY STRUCTURE AND DISORDER PROTECTION of the Macro phase migration inhibitory factor homolog

CONCLUSION:

Cuticular Glutathione Peroxidase (cGPx) and Macrophage Migration Inhibitory Factor (MIF) homologs embody distinct yet complementary mechanisms that support parasite survival. cGPx, with its thioredoxin-like fold and conserved catalytic residues, is specialized for the detoxification of reactive oxygen species, thereby mitigating oxidative stress encountered within the host. Conversely, MIF homologs, characterized by a tautomerase fold and frequent oligomerization, function as cytokine mimics that suppress host immune responses, particularly macrophage activation. Collectively, cGPx provides antioxidant protection, whereas MIF homologs enable immune

modulation, and together they enhance the parasite's capacity for persistence and pathogenic adaptation in the host environment.

REFERENCES:

1.2Guy K. Wuchereria bancrofti. Animal Diversity Web. University of Michigan Museum of Zoology; [cited 20 Sep 2025]. Available from: https://animaldiversity.org/accounts /Wuchereria_bancrofti/

2.World Health Organization. Lymphatic filariasis fact sheet. Geneva: WHO; [cited 2025 Sep 19]. Available from: https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis

3.History, Epidemiology and Control of Filariasis. PLoS Neglected Tropical Diseases. 2011;5(4):e1185.

4.Canada.ca. Infectious Substances – Wuchereria bancrofti: Pathogen Safety Data Sheet. Ottawa: Government of Canada; [cited 2025 Sep 19]. Available from: https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/wuchereria-bancrofti.html

5.World Health Organization. Lymphatic filariasis. Geneva: WHO; 2023 [cited 2025 Sep 19]. Available from: https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasi.s

6.Taylor MJ, Hoerauf A, Bockarie M. Lymphatic filariasis and onchocerciasis. Lancet. 2010;376(9747):1175-85. doi:10.1016/S0140-6736(10)60586-7.

7.Chandy C, Thanikachalam S, Ramalingam S. Basic science of lymphatic filariasis. Indian J Urol. 2005;21(1):10-16. doi:10.4103/0970-1591.19552.

8.Nutman TB. Insights into the pathogenesis of disease in human lymphatic filariasis. Lymphat Res Biol. 2013;11(3):144-8. doi:10.1089/lrb.2013.0015.

9.Simonsen PE, Mwakitalu ME. Urban lymphatic filariasis. Parasitol Res. 2013;112(1):35-44. doi:10.1007/s00436-012-3226-x.

10.Michael E, Bundy DA, Grenfell BT. Re-assessing the global prevalence and distribution of lymphatic filariasis. Parasitology. 1996;112(4):409-28. doi:10.1017/S0031182000066646.

11Centers for Disease Control and Prevention. Parasites – Lymphatic Filariasis. Atlanta: CDC; 2022 [cited 2025 Sep 19]. Available from: https://www.cdc.gov/parasites/lymphaticfilariasis.

12. Chauhan N, Sharma R, Hoti SL. Identification and biochemical characterization of macrophage migration inhibitory factor-2 (MIF-2) homologue of human lymphatic filarial parasite, Wuchereria bancrofti. Acta Tropica. 2015;142:71-78. doi:10.1016/j.actatropica.2014.10.009

13.Sharma R, Hoti SL, Meena RL, Vasuki V, Sankari T, Kaliraj P. Molecular and functional characterization of macrophage migration inhibitory factor (MIF) homolog of human from lymphatic filarial parasite Wuchereria bancrofti. Parasitology Research. 2012;111(5):2035-2047. doi:10.1007/s00436-012-3051-2.

14. Non-mammalian MIF superfamily review. Immunology & Cell Biology. 2017;95(5):372-381. doi:10.1111/imcb.12003 (or similar – see "The non-mammalian MIF superfamily" PMC article).

15.Singh I, Kanichery A, Kotimoole CN, Modi PK, Prasad TSK, Hoti SL. Unpacking immune modulation as a site of therapeutics innovation for nematode parasite Wuchereria bancrofti: a temporal quantitative phosphoproteomics profiling of macrophage migration inhibitory factor 2. OMICS. 2024;28(3):125-137. doi:10.1089/omi.2024.0002.

16.Sharma R, Hoti SL, Meena RL, Vasuki V, Sankari T, Kaliraj P. Molecular and functional characterization of macrophage migration inhibitory factor (MIF) homolog of human from lymphatic filarial parasite Wuchereria bancrofti. Parasitology Research. 2012;111(5):2035-2047. doi:10.1007/s00436-012-3051-2.

17.Nematode Orthologs of Macrophage Migration Inhibitory Factor (MIF) Homologs. Pathogens. 2022;11(2):258. doi:10.3390/pathogens11020258.

18.Sureshan M, Prabhu D, Saraboji K, et al. Structure-based designing of anti-filarial molecules targeting the key antioxidant enzyme glutathione peroxidase (GPx) of Wuchereria bancrofti: an integrated in silico and in vitro approach. Computers in Biology and Medicine. 2025 Sep;195:110669. doi:10.1016/j.compbiomed.2025.110669.

19.Filarial selenium glutathione peroxidase: a probable immunodiagnostic marker for lymphatic filariasis. [Conference/Abstract or Journal]. [year]; [volume(issue)]:[page numbers]. (Exact details may need verifying).
20. Powell HR, Islam SA, David A, Sternberg MJE, et al. Phyre2.2: A community resource for template-based protein structure prediction. Journal of Molecular Biology. 2025;437(15):168960. doi:10.1016/j.jmb.2025.168960.
21. Cho Y, Jones BF, Vermeire JJ, Leng L, DiFedele L, Harrison LM, et al. *Structural and functional characterisation of a secreted hookworm macrophage migration inhibitory factor (MIF) that interacts with the human MIF receptor CD74.* J Biol Chem. 2007;282(34):23447-56.
22. Pastrana DV, Raghavan N, Fitzgerald P, Eisinger SW, Metz C, Bucala R, et al. *Filarial nematode parasites secrete a homologue of the human cytokine macrophage migration inhibitory factor.* Infect Immun. 1998;66(12):5955-63.
23. Swagata Ghosh, Nona Jiang, Laura Farr, Renay Ngobeni, Shannon Moonah. *Parasite-Produced MIF Cytokine: Role in Immune Evasion, Invasion and Pathogenesis.* Front Immunol. 2019;10:1995.56
24. Muthusamy S, Prabhu D, Saraboji K, et al. Structure-based designing of anti-filarial molecules targeting the antioxidant enzyme glutathione peroxidase of *Wuchereria bancrofti*: an integrated in silico and in vitro approach. Comput Biol Med. 2025;195:110669.
25. Veerapathran A, Dakshinamoorthy G, Gnanasekar M, Reddy MVR, Kalyanasundaram R. Evaluation of *Wuchereria bancrofti* glutathione S-transferase as a vaccine candidate for lymphatic filariasis. PLoS Negl Trop Dis. 2009;3(6):e457.