Original article:

Adenosine deaminase(ADA) activity in relation to oxidative stress in lymphatic filariasis in an endemic area

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Abstract:

Lymphatic filariasis is a major public health problem affecting about 119 million people all over the world. India has a significant share in it with 48 million people harbouring microfilariae and suffering from disease manifestations. Being a characteristic debilitating disease, it causes little direct mortality but provokes a spectrum of clinical symptoms. Hence the present study was undertaken to evaluate oxidative stress and antioxidant status along with adenosine deaminase activity to know the cell mediate immunity in the lymphatic filarial cases in an endemic area.

1. The Malondialdehyde values in lymphatic filariasis were found to be significantly high compared to the levels of endemic normal. This observation indicated the existence of oxidative stress in lymphatic filariasis.
2. The enzyme Superoxide Dismutase (SOD) activity was found to be significantly low in lymphatic filarasis compared to endemic normals.
3. The antioxidant vitamin C concentration was significantly low in lymphatic filariasis compared to endemic normals. This imbalance between pro and antioxidant processes observed, this might pla a role in the pathology associated with lymphatic filariasis.
4. Adenosine Deaminase activity in lymphatic filariasis was significantly high compared to endemic normal, attributed to more differentiated lymphocytes in the cell mediated immune response occurring in lymphatic filariasis.

Introduction:

Lymphatic Filariasis resulting from Wucheriria bancrofit and brugia malayi infections continues to be a major cause of clinical morbidily in developing countries. Lymphatic filariasis is a disabling and disfiguring disease affecting mankind since antiquity. It is a debilitating disease attaching social stigma to afflicted people living in most of the tropical countries in the world. This infection is prevalent in both urban and rural areas. Lymphoedema is a common clinical problem. In india, commonest cause of lymphoedema of the limbs is due to Filariasis a disease that is endemic in many parts of our country. As lymphoedema is the commonest clinical manifestation of Filariasis, there exist a vast number of patients afflicted by this crippling disease.

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Repeated attacks of fever, lymphangitis and lymphadenitis are accompanied by progressive oedema of the limbs. This is associated with fibrosis and thickening of the skin resulting in the hideous looking limbs of ten called Elephantiasiss.[1,21]

Globally about 120 million people are affected with the disease of which one third live in India alone. This disease is caused by one of the parasites Wuchereria bancrofti, Brugia malayi, or Brugia timori, It is a disease of the poor and is prevalent in urban, peri urban and rural areas. The transmission is from man to man by mosquitoes of the genus Culex. In human beings the adults parasite live in the lymphatic system, producing millions of microfilarae which propagage the infection. The infection is usually acquired in childhood. Infection by B.timori does not occur in India.[11]

The disease caused by Wucheria Bancroft is often called Bancroftian Filariasis. Brugia malayi causes Malayan filariasis. The similar infection caused by Brugia malayi and Brugia timori are grouped together as brugia filariasis. Bancroftian filariasis occurs around the world in the latitudes between 41^0 N and 30^0 S, especially in Srilanka, India, Bangladesh, Burma, Thailand, Malasia, China, The Philippines, Indonesia and less in the Middle East, North and Central Africa, the Caribbean, central and south America, the Pacific islands. Malayan filariasis occurs in Burma, Thailand, Vietnam, Korea, Japan, Borneo, New Guinea. Brugai timori only causes disease only in Indonesia as shown in Table-2.

In india, infection of filariais have been recorded as early as 6^{th} century B.C., by the famous Hindu Physician "Susruta" in his treatise, "Susruta Samhita". Madhavakar (700 A.D) described the signs and symptoms of the disease in his treatise "Madhava Nidhana", which holds good even today. As early as 1500BC, the laws of Manu stated that a priest should not marry a woman with a family history of tuberculosis, epilepsy, leprosy of elephantiasis. The famous sun temple at Konark near Puri Orissa (1300 AD) depicts a couple with hydrocele and elephantiasis. Thus this disease has been with us through the centuries.

Lymphoedema was also known in the Biblical times so much so that it was stated in the Deuteronomy: it was an act of providence that for all the wanderings of the Hebrews in the desert, not a single case of swollen dough like feet was noticed (Deut (8 :4)

Hippocrates (460-355 BC) used to practice scarification for all kinds of oedema including lymph edema. He term elephantiasis however originated as a Roman soldier slang for the natives of Libya. This was describing the huge elephant hide type legs and scrotums that they saw during a campaign there. With on set of Christianity, medicine underwent decline. No significant progress was made in all diseases including lymph edema. The next description of this disease was rcorded in persia through the two great physicians Rhages (865 – 925 AD) and Avicenna (980 -1037 AD) naming it as elephantiasis arabicum through some of the lesions were confused with leprosy.

Wucher (1820 -1873) was a German Physician in Brazil, Bancroft (1836 – 1894) an English physician from Australia, and Brug (1879 -1947) a Dutch physician who worked in Indonesia have demonstrated various facts about the Filariasis. Demarquay (1863) first demonstrated microfilaria from hydrocele fluid while Wucherer (1866) found it in chylous urine. Lerois (1872) found it in peripheral blood where as Bancroft (1876) demonstrated microfilaria in the blood and in urine of a patient with

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lymphoedema and established Culex Fatigens as the vector.

In 1789, Clark described elephantoid legs in cochin, south india, as Malabar legs. Manson and Bahr (1959) have reviewed various aspects of Filariasis caused by Wuchereria. Demarquey (1863) first noted microfilariae in hydrocele fluid of a resident of Hawana, Cuba. After three years wucherer in 1866 in Bahia, Brazil, discovered microfilarae in the urine of a patient while investigating a case of suspected urinary bilharziasis. The discovery of microfilariae in the peripheral blood of a person wa reported by Lewis in Calcutta and the parasite was described by the term "filarial sanguis hominis", later it was named after Bancroft who described the adult female worms in a lymph node abscess from the arm of a Chinese patient in Brisbane, Ausralia.

Codbold in 1877, in London, coined the term "Filaria Bancrofti" to the parasite (Filar means thread like), by its morphology. The generic name, wuchereria was given byDe Silva Aranjo, when he described microfilariae in the peripheral blood of a patient in Brazil in 1878. Manson demonstrated thatculex quanquifaciatus mosquito acts as the intermediate host in 1887. Filariasis is the first disease proved to be transmitted by insectss.[18]

Although lymphatic Filariasis has been identified as eradicable or potentially eradicable among the six infectious diseases by the international Task force for disease eradication, it is still a major public healh problem in many parts of the world including India[14].

In India a total of 553 million people are estimated to be harboring there are approximately 30 muillion people are estimated to be harboring Wuchereria bancrofti microfilariae. 21 million people with symptomatic filariasis and about 20 million suffer with chronic manifestations such as hydrocele (13 million) and lymphoedema(7 millionh). W.Bancrofti is the predominant species accounting for about 98% of the national burden, widely distributed in 17 states and 6 union territories. B.malayi around river basins, and eastern and wesern costal parts of India (map)[12,20].

The term Lymphatic Filariasis covers infection with three closely related nematode worms – W. Bancrofti, B.malayi and B.temori. all three infections are transmitted to man by the bites of Infective mosquitoes. Filariasis is a global problem. It is a major social and economic sourage in the tropics and subtropics in Africa, Asia, Western Pacific and parts of the Americas, affecting over 120 million people in 80 countries. More than 1.1 billion people live in areas where there is a risk of infection. Infected areas are found in UP, Bihar, AP, Orissa, Tamilnadu, Kerala and Gujarat. Though not fatal, the disease is responsible for considerable suffering, deformity and disability. In East Godavari District of Andhra Pradesh, specially Kakinada and surrounding areas are highly infective. The aim of the present study is to assess the oxidative stress due to filarial infection and estimate the antioxidant response by the infected individual there was no proper information in this regard.

- To assess the oxidative stress in lymphatic filariasis.
- To assess the antioxidant response or status of the infected individuals
- Estimating the enzyme superoxide Dismutase and antioxidant Vitamin C
- To know the cell mediate immunity in the lymphatic filariasis by estimating
- the Adenosine Deaminase activity.

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Materials & methods

1.Estimation of adenosine deaminase by Galantio Glusti Colorimetric Method.

2.SOD activity in human blood is measured by the method of Murlelumb & Murklund (1974). This method utilizes the inhibition of anto oxidation of pyrogallol by SOD enzyme. The final essay mixture for the autooxidation of pyrogallol contained in a volume of 3ml.

3.Estimation of Malondialdehyde (MDA) by Thiobarbituric acid (TBA) reaction.- Mahalouz et al 1978.

4.Estimation of Ascorbic acid by Photometric Method.

Observations and results:

In the present study a total number of 100 individual suffering from lymphatic filariasis were included as summarized in the table 5. The patients were from the O.P of Regional Filarial control Unit in Kakinada, East Godavari dt, Andhra Pradesh.

Table-1

Disease burden of lymphatic filariasis in the world and in India:

Categories of cases	NUMBER OF CASES IN MILLIONS					
	W.Bancrofti		B.Malayi		Total	
	World	India	World	India	World	India
Microfilaria Carriers	73.27	29.46	10.36	1.80	83.63	31.26
Lymphoedema	13.27	6.58	2.81	0.86	16.02	7.44
Hydrocele	26.79	12.88	--	--	26.79	12.88
Total Cases	106.19	45.52	12.91	2.58	119.10	48.11

Excludes cases which have both infection and disease.

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Table-2

Filarial disease in Global Scenario:

S.NO	CONTINENT/REGION	NO. of COUNTRIES	Population in CRORES	Bancroftian Filaria cases In Crores
1.	Central America	07	44.10	0.04
2.	Africa	38	51.20	4.00
3.	Asia(Madhyadhara Sea Region)	01	5.20	0.03
4.	Asia-china	01	85.00	4.56
5.	Asia – south and west Pacificregion	20	113.10	1.44
6.	Asia – south and west pacific region	20	113.10	1.44
	Total	68	412.10	10.62

Table-3

Filarial disease in Indian Scenario:

S.NO	POPULATION	INDIA In Crores	ANDHRA PRADESH	EAST GODAVARI	KAKINADA
1.	Population at Risk	41.0	5.24 Crores	0.30 Crores	0.02 Crores
2.	In Urban Ara	10.8	1.27 Crores	0.16 Crores	0.02 Crores
3.	In Rural Area	30.2	3.97 Crores	0.22 Crores	---
4.	Filarial Cases	4.73	0.53 Crores	0.05 Crores	35,000

Table-4

Distribution of study cases of Filariasis:

Study Group	Number	Male	Female
Endemic Normal	`45	20	25
Lymphatic Filariasis	100	33	67

Age and sex wise distribution of all the study cases is shown in table-6 And maximum number of individuals representing 50% of the total 100 were in the age group of 31-50 years. While male and female ratio was almost 1:2 in all the filarial cases.

Showing values of serum MDA, Adenosine Deaminase, plasma vit.C Superoxide dismutase (SOD) in endemic normals and Lymphatic filarial cases SD + mean.

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Table-5

Showing values of serum MDA, Adenosine Deaminase, plasma vit.C Superoxide dismutase (SOD) in endemic normals and Lymphatic filarial cases SD + mean:

Parameter	Endemic normals N=45	Filarial cases n=100	Lymphedema left leg n=57	Lymphedema right leg n=43
Serum MDA nmol/dl	229 + 69	418 + 83	416+88	425 + 76
Serum ADA U/L	44 + 6.4	152 + 18.9	151 + 19	154 + 19
Plasma Vit.C mg%	0.94+0.3	0.4+0.08	0.41+0.09	0.42+0.07
Serum SOD U/ml	2.4+0.46	1.21+0.39	1.2+0.34	1.22+0.4

Table-6

Showing values of serum MDA, Adenosine Deaminase, plasma Vit.C Superoxide Dismutase (SOD) in endemic normal and Lymphatic Filarial cases SD + mean and 'p' values:

Serum MDA nmol/dl		Serum ADA U/L		Plasma Vit.C mg%		SOD U/ml	
Endemic normals	Lymphotic Filariasis	Endemic normals	Lymphatic filariasis	Endemic normals	Lymphatic Filariasis	Endemic normals	Lymphatic Filariasis
229+69	418+83	44+6.4	152+18.9	0.94+0.3	0.4+0.08	2.4+0.46	1.21+0.39
P=>0.001		P=>0.001		P=>0.001		P=>0.001	

Table -7

Showing the values of serum ADA, MDA,SOD and plasma Vit.C in Endemic normal and lymphatic Filarial cases 't' and 'p' values:

Parameter	Endemic normals n=45	Lymphatic Filariasis n=100
	't'	'p'
Serum Adenosine Deaminase U/L	37.37	>0.001
Serum Malondialdehyde nmol/dl	13.338	>0.001
Plasma Vit.C mg%	16.875	>0.001
Serum Superoxide Dismutase U/ml	16.06	>0.001

Discussion:

Lymphatic filariasis caused by W. bancrofti and B.malayi has remained as a major cause of clinical morbidity in the affected population. It is associated with a remarkably wide range of clinical signs, symptoms and sequelae[16,22].

The clinical manifestations of lymphatic filariasis are influenced by a variety of factors including the patient's age, gender, the species and perhaps the strain of the parasite, the anatomic location of the adult warms, previous exposure to the parasite, the immune response and secondary bacterial infections. In the area where lymphatic filariasis is endemic,

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most infected persons are asymptomatic despite the presence of up to a million of mf circulating in the peripheral blood, known as microfilaraemic/mf carriers. Although they may be asymptomatic, there is a growing body of evidence indicating that they do have some degree of sub clinical disease. Approximately 40% of microfilaria positive cases have microscopic or occasionally macroscopic haematuria and proteinuria[3,5].

The transmission is from man to man by mosquitoes. In human beings the adult parasites live in the lymphatic system, producing millions of microfilariae which propagate the infection. The infection is usually acquired in childhood. The adult worms cause dilatation of lymphatic vessels resulting in their damage and dysfunction. This causing obstruction to the lymph flow resulting in lymphoedema. Initially there is a n acute inflammatory response characterized by infiltration of polymorphs, histiocytes, eosinophils and lymphocytes around the luymphatic. This is followed by formation of epitheloid granuloma and foreign body giant cells after the death of the adult worms. Which may calcify, become lysed and surrounded by fibrosis. Lymphatic abscesses may form at the site of dead and degenerating worms. Finally lymphatics are obliterated by fibrosis with the disappearance of microfilariae from the blood[13].

Lymph statis results in increased susceptibility to secondary bacterial infection particularly with group a streptococci resulting in episodic attacks of adenolymphangitis (ADL). Each attack of ADL results in the progression of chronicity of disease and increased lymph stasis, there by again increased susceptibility to ADL attacks. This culminates in fibrosis with the formation fo solid edema asnd probable decline in ADL attacks[17].

In human beings the adult parasites live in the lymphatic system, producing millions of microfilariae which propagate the infection. The infection is usually acquired inb childhood. The adult worms cause dilatation of lymphatic vesels resulting in their damange and dysfunction. This leads to slow flow of lymph which may cause lymphoedema, kidney damage and chyluria-from rupture of dilate lymphatics in to urinary system. Typical acue inflammatory attacks of Lymphatic Filariasis occur due to the entry of bacteria through breaks in the lymph edematous skin. Stasis of lymph provides conditions for rapid growth of these bacteria. Damage to small lymphatic vessels results in fibrosis and progression of elephantiasis.

In areas where lymphatic filariasis is endemic, most infected persons are asymptomatic despite the presence of up to a million of mf circulating in the peripheral blood, known as microfilaraemics/mf carriers. Although they may be asymptomatic, there is a growing body of evidence indicating that they do have some degree of sub clinical disease. Approximately 40% of microfilaria positive cases havemicroscopic or occasionally macroscopic haematuria and proteinuria[5,3].

The normal function of the lymphatics is to return proteins, lipids and water from the interstitium to the intravascular space. Forty to fifty percent of serum proteins are transported by this route each day. Interstitial fluid normally contributes to the nourishment of tissues. About 90% of the fluid returns to the circulation via entry into venous capillaries. The remaining 10% is composed of high molecular weight proteins and their oncotically associated water, which are too large to readily pass through venous capillary walls. This leads to flow into the lymphatic capillaries where pressures are

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typically sub atmospheric and can accommodate the large size of the proteins and their accompanying water. The proteins then travel as lymph through numerous filtering lymph nodes on their way to join the venous circulation. In a diseased state, the lymphatic transporot capacity is reduced. This causes the normal volume of interstitial fluid formation to exceed the rate of lymphatic return, resulting in the stagnation of high molecular weight proteins in the interstitium.

Free radicals are atoms or molecules carrying odd number of electroms at outer atomic or molecular orbits. These compounds formed in organisms via metabolic pathways and by the external effects. Free radicals such as hydroxyl, alcoxyl, peroxyl, superoxide, nitric oxide and nitrogen dioxide are basically derived from oxygen[9]. Free radicals when accumulated cause extensive damage to lipids, proteins and even DNA molecules leading to cell death[8]. Under pathological conditions, much larger amounts of oxygen free radical are formed than normal and these can overwhelm the defence of the cells and lead to lipid peroxidation and even death of the cell. To overcome the harmful effects of free radicals, al aerobic cells are endowed with externsive antioxidant defence mechanisms that include enzymes viz., superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione S-transferase and molecules like carotenoids, vitamin E and C, glutathione etc[10].

There has been accumulating evidence to imply the reactive species produced by polymorph nuclear leukocytes and possibly other sources may contribute to the complex pathogenesis of the diseases associated with inflammation [6].

There are no reports available on the status of oxidative stress and antioxidant levels in lymphatic filariasis. Hence the present study was under taken to estimate levels of malondialdehyde as indicator of oxidative stress, enzyme superoxide dismutase activity and Vit.C concentration to reflect antioxidant status of enzymatic and non enzymatic source. Adenosine Deaminase enzyme has been implicated in T lymphocyte proliferation and differentiation in order to prevent accumulation of toxic metabolites[2].

ADA has been considered as a marker of cells mediated immunity so the Adenosine Deaminase levels were estimated to assess the ccell mediated immunity.

A total of 100 individuals suffering from lymphatic filarial cases were included in the present study. The lymphatic filariasis cases showed significantly high MDA levels (p>001) compared to he levels of endemic normals. (Figs-0). This suggests the association of oxidative stress in filariasis and its possible involvement in the pathogenesis of disease process. Increased amounts of reactive oxygen species and reactive nitrogen intermediates are known to be produced as a consequence to phagocyte burst.

In the present study the activity of enzyme superoxide dismutase (SOD) was also found to be significantly low in filarial cases in filarial cases were significantly decreased compared to endemic normals (p>0.001) (Fig9). Vit C levels in filarial cases were significantly decreased compared to endemic normals (p>0.001) (Fig-0) indicated the non enzymatic defence also efficient in combating oxidative stress. The imbalance between pro and antioxidant processes observed in all the stages of filarial infection suggests tghat such an imbalance might possibly play a role in the pathology associated with lymphatic filariasis.

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In the present study the activity of enzyme Adenosine Deaminase activity was significantly increased compared to endemic normals (p>0.001) (Fig-11). Adenosine Deaminase enzymes has been considered as a marker of cell mediated immune because raised level of ADA under antigenic stimulation in order to deal with the accumulation of toxic metabolities. Therefore, significantly high serum ADA levels in patients with lymphatic filarisis as compared to those of endemic normals is in contrast to the proposed hypothesis of filarial specific immune suppression antigen mimicry and immune paralysis[4,15,16].

Conclusion:

Lymphatic filariasis is a major public health problem affecting about 119 million people all over the world. India has a significant share in it with 48 million people harbouring microfilariae and suffering from disease manifestations. Being a characteristic debilitating disease, it causes little direct mortality but provokes a spectrum of clinical symptoms. Disease morbidily may range from transient fevers or mild itching to extensive and virtually, immobilizing oedema of the limbs and hydrocele or occult infections such as TPE, arthritis etc the acute attacks causes disfigurement resulting psychological problems and reduced economic output. Consequently, this disease has become a major impediment to the socioeconomic progress in the endemic regions.

Hence the present study was undertaken to evaluate oxidative stress and antioxidant status along with adenosine deaminase activity to know the cell mediate immunity in the lymphatic filarial cases in an endemic area.

The Malondialdehyde (MDA) levels, enzyme superoxide Dismutase (SOD) and Adenosine Deaminase (ADA) activities and antioxidant Vitamin Vit.C levels were estimated in 100 cases belonging to Lymphatic Filariasis and compared the values with the values 45 endemic Controls.

1. The Malondialdehyde values in lymphatic filariasis were found to be significantly high (p>0.001) compared to the levels of endemic normal. This observation indicated the existence of oxidative stress in lymphatic filariasis.
2. The enzyme Superoxide Dismutase (SOD) activity was found to be significantly low in lymphatic filarasis compared to endemic normals (p>0.001).
3. The antioxidant vitamin C concentration was significantly low (p>0.001) in lymphatic filariasis compared to endemic normals. This imbalance between pro and antioxidant processes observed, this might pla a role in the pathology associated with lymphatic filariasis.
4. Adenosine Deaminase activity in lymphatic filariasis was significantly high (p>0.001) compared to endemic normal, attributed to more differentiated lymphocytes in the cell mediated immune response occurring in lymphatic filariasis.

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