

APPENDIX I

Chemicals:

All the fine chemicals used in the study were purchased from Sigma Chemical Company (St Louis, MO, USA) unless otherwise specified. All other chemicals were analytical grade obtained from Merck Chemicals (Mumbai, India).

Buffer preparation for High performance liquid chromatography

Buffer A:

Sodium acetate trihydrate - 3.04 g

Triethyl amine - 45 μ l

Milli Q water - 500 ml

pH - 7.0

Adjust the pH with 1N acetic acid.

Buffer B:

Sodium acetate trihydrate - 3.04 g

Triethyl amine - 45 μ l

Methanol – 450 ml

Milli Q water - 50 ml

pH - 7.0

Adjust the pH with 1N acetic acid. All the chemicals and solvents are HPLC grade one.

Borate buffer 0.1M pH 10.5:

1.9069 g of disodium tetraborate made upto 50 ml with milliwater; adjust the pH to 10.5 by using 1N sodium hydroxide.

Borate buffer 0.1M pH 11.5:

1.9069 g of disodium tetraborate made upto 50 ml with milliwater; adjust the pH to 11.5 by using 1N sodium hydroxide.

Perchloric acid 0.05M:

15 μ l stock perchloric acid made upto 5 ml with milliwater.

Iodoacetic acid 0.8M:

148.8 g of iodoacetic acid in 1 ml of 0.1M borate buffer pH10.5.

Ortho-phthaldialdehyde (OPA) Stock 0.4M:

5.3 mg of OPA / 100 µl of methanol.

Working OPA:

20 µl of OPA + 40 µl of β-mercaptoethanol + 140 µl of 0.1M borate buffer pH 11.5

Buffer preparation for Glutathione estimated by spectrofluorometer:

Sodium phosphate buffer pH 8.0 (1M)

Monosodium phosphate 1M – 1.37 g

EDTA – 146 mg

Milli Q water – 100 ml.

Ortho-phthaldialdehyde: 1 mg/ml of methanol.

Buffer preparation for High performance liquid chromatography using Electrochemical detector estimation of reduced and oxidized glutathione**Buffer A**

Trifluoroacetic acid - 0.1%

Acetonitrile - 2 %

MilliQ water - 98 ml

Dithiothreitol 50 mM

134 mg DTT/1000 µl of water.

Reagent preparation for the estimation of gammaglutamylcysteine ligase by spectrofluorometer

GCL reaction cocktail: pH 7.4

Tris 400 mM (4.8 g)

ATP 40 mM (2.02 g)

L- glutamic acid 20 mM (294 mg)
EDTA 2.0 mM (74 mg)
Sodium borate 20 mM (76 mg)
Serine 2 mM (20 mg)
MgCl₂ 40 mM (800 mg)
Water – 100 ml

TES/SB: pH 7.4

Tris 20 mM (121 mg)
EDTA 1 mM (18 mg)
Sucrose 250 mM (4 g)
Serine 2 mM (10 mg)
Sodium borate 20 mM (381 mg)
Cysteine 2 mM (12 mg)
Water – 100 ml.

Sulphosalicylic acid 200 mM

2.5 g SSA dissolved in 50 ml of water

Naphthalene-2,3-dicarboxaldehyde (NDA) preparation 10 mM

Stock: 18 mg (NDA) dissolved in 10 ml of dimethyl sulfoxide (DMSO)

Working NDA preparation

Tris 50 mM pH 10.0 (30 mg) dissolved in 50 ml of water.

NaOH 0.5 N (200 mg) dissolved in 100 ml of water.

NDA derivatization solution, 50 mM Tris and 0.5 N NaOH was prepared and cyclomixed in the ratio of (1.4 ml / 0.2 ml /0.2 ml).

From this, 180 µl of NDA was added to the wells.

Reagent Preparation for Aminolevulinic acid Synthase by spectrophotometer

1. Sodium acetate buffer 1.0 M pH = 4.7 adjust with 20% HCl.
2. Tris-HCl buffer 1.0 M pH = 7.5 adjust with 20 % NaOH
3. Potassium phosphate buffer 50 mM pH =7.0
Prepared by mixing 8 mL of 50 mM KH_2PO_4 and 20 mL of 50 mM K_2HPO_4
4. Glycine 1.0 M – 10 mL
5. Succinate 1.0 M – 20 mL
6. MgCl_2 0.1 M – 20 mL
7. ATP 0.2 M – 10 mL
8. Pyridoxal phosphate 0.01 M – 6.75 mL
9. Coenzyme A 0.01 M – 8.75 mL
10. Ehrlich's reagent
1.0 g of para-dimethyl-aminobenzaldehyde to 92 ml of glacial acetic acid, adding 8 mL of 60 % perchloric acid. Prepared this reagent every day.
11. ALA standard solutions
12. Trichloroacetic acid 10 %.

Preparation of Solutions

Enzyme substrate cocktail

- Glycine 1.0 M (10 mL)
Succinate 1.0 M (10 mL)
 MgCl_2 0.1 M (10 mL)
Tris-HCl buffer 1.0 M (5 mL)

Stored in the refrigerator

Enzyme control cocktail

- Water (10 mL)
Succinate 1.0 M (10 mL)
 MgCl_2 0.1 M (10 mL)
Tris-HCl buffer 1.0 M (5 mL)

Stored in the refrigerator

Cofactor cocktail

- ATP 0.2 M (10.0 mL)
CoA 0.01 M (8.75 mL)
Pyridoxal phosphate 0.01 M (6.75 mL)
Water (10.0 mL)

Reagent Preparation for Heme oxygenase

1. Phosphate buffer pH 7.4 100 mM
 - A. Sodium dihydrogen phosphate = 3.561 g / 100 ml
 - B. Disodium hydrogen phosphate = 7.164 g / 100 mlA (81 ml) + B (19 ml) = 100 ml pH 7.4
2. Cytosol (2 mg)

Commercially available 20 mg /ml vial per protein. Stored at - 80°C.
20 mg/1000 µl, i.e., **2 mg/100 µl** (used for assay)
3. NADPH 0.8 mM
weighed 0.64 mg / 1000 µl. From this **100 µl** taken for the assay corresponds to 0.064 mg /100 µl leads to 0.8mM.
4. Glucose-6-phosphate 1mM
Weigh 3 mg / 1000 µl. from this **100 µl** (0.3 mg) corresponds to 1 mM for the assay.
5. Glucose-6-phosphate dehydrogenase 0.2 U
Commercially available 250 U / 0.061 ml.
i.e., 250 U / 61 µl
20 U / 4.88 µl
Stock:
4.88 µl /1000 µl = 20 U
0.488 µl / 100 µl = 2 U
0.0488 µl / 10 µl = 0.2 U.
6. Hemin 10 µg / ml.
1mg /ml
1000 µg / 1000 µl.
10 µg / 10 µl.
7. Chloroform 1 ml to stop the reaction.
8. Bilirubin std
1 mg / 1 ml chloroform.

APPENDIX II

Table I: Demographic details of the Eales' patients Group I

Age/Sex	Clinical Diagnosis	Medications at time of collection
24/M	active vasculitis, cotton wool spot (OD,OS)	Nil
33/M	active vasculitis, vitreous hemorrhage	Nil
36/M	active vasculitis, vitreous hemorrhage	Nil
26/M	OD-perivasculitis,choroiditis	Nil
30/M	active vasculitis,vitreous hemorrhage,ED	Nil
35/M	Vasculitis	Nil
24/M	Active vasculitis,dispersed vitreous hemorrhage	Nil
25/M	active vasculitis	Nil
30/M	Vitreous hemorrhage, healed vasculitis	Nil
34/M	Vasculitis	Nil
39/M	active vasculitis, neovascularization,	Nil
29/M	active vasculitis, retinal detachment	Nil
18/M	peripheralvasculitis,vitreous hemorrhage,	Nil
38/M	active vasculitis, periphlebitis	Nil
26/M	active vasculitis,vitreous exudates, ED	Nil
23/M	active vasculitis,vitreous hemorrhage,	Nil
53/M	active vasculitis,hemorrhage	Nil, prednisone
44/M	active vasculitis	Nil
45/M	ED	Nil
17/M	Active vasculitis	Nil

Table II : Demographic details of the Eales’ patients. Group II

Age/Sex	Clinical Diagnosis	Medications at time of collection
25/M	active vasculitis, cotton wool spot (OD,OS)	Nil
20/M	active vasculitis, vitreous hemorrhage	Nil
21/M	active vasculitis, vitreous hemorrhage	Nil
26/M	OD-perivasculitis,choroiditis	Nil
20/M	active vasculitis,vitreous hemorrhage,ED	Nil
43/M	Vasculitis	Nil
18/M	activevasculitis,dispersed vitreous	Nil
32/M	hemorrhage	Nil
42/M	active vasculitis	Nil
33/M	Vitreous hemorrhage, healed vasculitis	Nil
23/M	Vasculitis, mild vitreous hemorrhage	Nil
30/M	active vasculitis, neovascularization	Nil
	active vasculitis, vitreous hemorrhage, Bronchial tuberculosis	
42/M	active vasculitis, vitreous hemorrhage	Nil
28/M	peripheralvasculitis,vitreous	Nil
	hemorrhage,phlebitis, healed vasculitis	
15/M	active vasculitis, subhyaloid hemorrhage	Nil
23/M	active vasculitis,vitreous exudates, ED	Nil
53/M	active vasculitis,vitreous hemorrhage,	Nil, prednisone
17/M	active vasculitis,hemorrhage	Nil
30/M	active vasculitis,vitreous hemorrhage	Nil
17/M	activevasculitis,vitreous hemorrhage, periphlebitis	Nil

Table III. Demographic details of the ARMD patients. Group III

Age / Sex	Clinical Diagnosis	Medications at time of collection
69 / M	CNVM	Nil
89 / M	CNVM	Nil
63 / M	CNVM	Nil
64 / M	CNVM	Anti VEGF treatment
65 / M	Dry ARMD	Nil
70 / M	CNVM	Nil
70 / M	Dry ARMD	Nil
79 / M	CNVM	Nil
72 / M	CNVM	Nil
78 / M	CNVM	Nil
75 / F	CNVM	Nil
73 / M	CNVM	Nil
65 / F	CNVM	Nil
57 / F	Disciform scar	Anti VEGF treatment
73 / M	CNVM	Nil
59 / F	Dry ARMD	Nil



**Characterization of a novel 88-kDa protein found in patients with
Eales' Disease.**

CONSENT LETTER

The Research worker has explained the purpose of this study to me. I understand that Blood sample shall be taken for the research purpose that may help in understanding the disease mechanism of Eales' Disease.

I consent to participate in the study, having understood its objectives and outcome. I was informed about the strict maintenance and confidentiality of the results obtained. I voluntarily give my consent to participate and fully cooperate in this study.

Signature: _____

Date: _____

Name : _____

Parent/Guardian: _____



“Studies on thiolation and homocysteinylation of serum proteins in the two retinal diseases - Age related macular degeneration and Eales’ disease”.

CONSENT LETTER

The Research worker has explained the purpose of this study to me. I understand that Blood sample shall be taken for the research purpose that may help in understanding the disease mechanism of Age Related Macular Degeneration and Eales’ Disease.

I consent to participate in the study, having understood its objectives and outcome. I was informed about the strict maintenance and confidentiality of the results obtained. I voluntarily give my consent to participate and fully cooperate in this study.

Signature: _____

Date: _____

Name : _____

Parent/Guardian: _____

INSTITUTIONAL APPROVAL LETTER



S NARAYAN
VRF MANAGER

Dr SR

October 26, 2006

Your projects titled

- ❖ Studies on thiolation and homocysteinylation of serum proteins in Age related macular degeneration – **40-2006-P**
- ❖ Studies on thiolation and homocysteinylation of serum proteins in Eales' disease – **41-2006-P**

has been approved in the Research/Ethics Subcommittee meeting held on 16th October 2006. Please mention the following project numbers for your future correspondence.

PROJECT NO: 40-2006-P, 41-2006-P


S NARAYAN
VRF MANAGER

INSTITUTIONAL APPROVAL LETTER



• Molecular cloning, sequencing, overexpression and characterization of a novel 88KDA protein found in patients with Eales' disease

Dr. KNS presented the project. Members felt that this as a very useful project and possibly will help the institute to hold newer techniques. The project has been approved by the Research Sub-Committee.



CLINICAL PROFORMA

Eales' Disease and Age related macular degeneration

1. S. No.

2. Name

3. Age

4. MRD NO.

Sex 1. M
2. F

5. Onset of symptoms (days)

6. Diagnosis of disease (anywhere)

7. Symptoms

1. Floaters 2. Black spots 3. Dimness of vision
4. Pain and redness 5. Others

8. Systemic history

1. Tuberculosis 5. Sarcoidosis
2. Diabetes mellitus 6. Hemoglobinopathies
3. Hypertension 7. Sickle cell disease
4. Collagen vascular disorder 8. Any others (specify)
9. Nil

9. Prior treatment taken

- 1. Topical steroid
- 2. Systemic steroid
- 3. Subtenon injection
- 4. Combination
- 5. Anti TB medication
- 6. Laser
- 7. Vitrectomy.
- 8. Vitamin supplements/Antioxidants
- 9. Aspirin or dipyridazole with antiplatelet
- 10. Others (specify)

10. Best corrected visual acuity **1. 6/5, 2. 6/6, 3. 6/9, 4. 6/12, 5. 6/18, 6. 6/24, 7. 6/36, 8. 6/60, 9. 3/36, 10. 3/60, 11. 2/60, 12. 1/60, 13. CF CF, 14. HM, 15. PL, 16. NPL.**

OD
OS

11. Near Vision **1. N6, 2. N8, 3. N10, 4. N12, 5. N18, 6. N36 7. <N36**

OD
OS **1. Lids 2. Conjunctiva 3. Cornea 4. Sclera 5. Iris
6. Anterior chamber 7. Pupil 8. lens 9. WNL, 10. Others (specify)**

12. Anterior segment

13. Intra ocular pressure OD OS

14. Fundus:

a. Periphlebitis OD
OS **1. Active 2. Inactive 3. Normal 4. others**

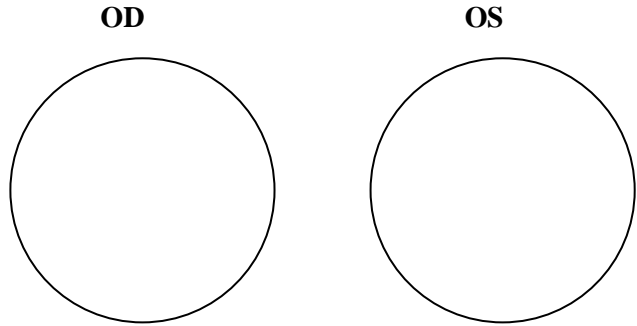
Extent of involvement OD
OS **0. No QD, 1. 1QD 2. 2QD 3. 3QD, 4. 4QD**

b. Neovascularization OD
OS **1. Y**

NVD **1. Y** NVE **1. Y** Extent **0. No QD, 1. 1QD 2. 2QD 3. 3QD, 4. 4QD**

c. Retinitis proliferans

1. Vitreal **1. Y**
 2. ERM **2. N**
 3. Subretinal membrane **OD OS**



- d. Vitreous h'ge**

- e. Tractional RD** **1. Y**

- f. Combined RD** **2. N**

- 15. Diagnos** 1. Central
 2. Peripheral 3. Others (specify)

- 16. Treatment**
 1. Laser 2. Systemic steroids 3. RD surgery 4. Vitamin supplementation 5. Others

APPENDIX III

S.No	Product	Company
1.	Vented flask and culture dishes	Nunc
2.	Fibronectin	Sigma
3.	Collagenase	Sigma
4.	Antibiotic solution	Gibco
5.	Endothelial growth medium 2	Lonza
6.	Gelatin	Sigma
7.	Dimethyl sulfoxide	Merck
8.	MTT	Sigma
9.	Paraformaldehyde	Merck
10.	Transwell inserts	Millipore
11.	ECM matrix	Millipore
12.	Phen green FL	Invitrogen
13.	Cell death ELISA kit	Roche
14.	TRIzol	Sigma
15.	Chloroform	Merck
16.	Iso propanol	Merck
17.	Ethanol	Merck
18.	iScript RT-PCR Kit	Biorad
19.	SYBR Green	Eurogentech
20.	Bradford	Thermoscientific
21.	Nitrocellulose membrane	Millipore
22.	Horse radish peroxidase tagged antibody	Santacruz
23.	Oasis column	Waters
24.	DTT	Sigma
25.	Iodaoacetic acid	Sigma
26.	SDS	Sigma
27.	Acrylamide	Sigma
28.	Bisacrylamide	Sigma

29.	TEMED	Biorad
30.	Ammonium per sulphate	Biorad
31.	EDTA	Merck
32.	Tris	Sigma
33.	Glycine	Sigma
34.	Trypsin for cell culture	Hi media
35.	Foetal bovine serum	Hi media
36.	Primers	Eurogentech
37.	Skimmed milk powder	Nestle
38.	DMEM / F12	Biocorporals
39.	Diethylpyrocarbonate	Sigma
40.	DL - Homocysteine	Sigma
41.	DL - Cysteine	Sigma
42.	L- Glutamic acid	Sigma
43.	L - Glycine	Sigma
44.	L- Methionine	Sigma
45.	L- Taurine	Sigma
46.	ODS Column C18	Phenomenex
47.	Gluathione	Sigma
48.	Oxidized Glutathione	Sigma
49.	ODS Column C18	Phenomenex
50.	Homocysteine-thiolactone hydrochloride	Sigma
51.	Poly Sulfoethyl Asparatamide column	Agilent
52.	γ - Glutamate – Cysteine	Sigma
53.	Napthalenedicarboxaldehyde	Sigma
54.	Aminolevulinic acid	Sigma
55.	Acetyl acetone	Sigma
56.	Hemin	Sigma
57.	Pyridoxal-L-phosphate	Sigma

58.	C0A enzyme	Sigma
59.	Para dimethyl amino benzaldehyde	Sigma
60.	Succinate	Sigma
61.	Adenosine di phosphate	Sigma
62.	Cytosol	Sigma
63.	NADPH	Sigma
64.	Glucose-6-phosphate	Sigma
65.	orthophthaldehyde	Sigma
66.	Methanol – HPLC grade	Merck
67.	Ferritin KIT	Best Surgicals
68.	Transferrin KIT	Best Surgicals
69.	Serum Transferrin Receptor KIT	Bio Vendor
70.	Vascular Endothelial Growth Factor KIT	R & D Systems
71.	Heme KIT	Quantichrome

LIST OF PUBLICATIONS:

1. Bharathselvi.Muthuvel, Vidhya Srinivasan, Pukhraj Rishi, Sulochana N. Konerirajapuram. Increased Vitreous Heme Oxygenase Activity is Associated with Proliferative Diabetic Retinopathy. Ind J Clin Biochem. 2015
2. Muthuvel.Bharathselvi, Sayantan Biswas,Rajiv Raman, Radhakrishnan Selvi,Karunakaran Coral,Angayarkanni Narayanansamy,Sivaramakrishnan Ramakrishnan, and Konerirajapuram.N.Sulochana. Homocysteine and its metabolite Homocysteine-Thiolactone and Deficiency of Copper in patients with Age Related Macular Degeneration. IJMR . 2015
3. Muthuvel.Bharathselvi, Jyothirmay. Biswas, Radhakrishnan. Selvi,Karunakaran.Coral, Angayarkanni. Narayanansamy, Sivaramakrishnan. Ramakrishnan, and Konerirajapuram.N.Sulochana. Increased Homocysteine, Homocysteine-Thiolactone, Protein Homocysteinylolation, Oxidative Stress in circulation of patients with Eales' Disease. Annals of clinical Biochemistry. 2013.
4. Narayanan Gomathy; S.R Bharathidevi; Vuyyuru Harish; Muthuvel Bharathselvi; Natrajan Sulochana. CTR1 silencing inhibits angiogenesis by limiting copper entry in endothelial cells. PLOS ONE.2013.
5. S. Ramakrishnan, R.Selvi,A.V.Saijyothi, Jyotirmoy Biswas, M.BharatSelvi and N.Angayarkanni Clinical and Biochemical benefits of administration of vitamins E & C in patients with Eale's Disease. BIOMEDICINE.2012
6. Coral K, Angayarkanni N, Gomathy N, Bharathselvi M, Pukhraj R, Rupak R. Homocysteine levels in the vitreous of proliferative diabetic retinopathy and rhegmatogenous retinal detachment: its modulating role on lysyl oxidase.Invest Ophthalmol Vis Sci. 2009.

7. Coral K, Angayarkanni N, Madhavan J, Bharathselvi M, Ramakrishnan S, Nandi K, Rishi P, Kasinathan N, Krishnakumar S. Lysyl oxidase activity in the ocular tissues and the role of LOX in proliferative diabetic retinopathy and rhegmatogenous retinal detachment. Invest Ophthalmol Vis Sci. 2008 .
8. Selvi R, Angayarkanni N, Bharathselvi M, Sivaramakrishna R, Anisha T, Jyotirmoy B, Vasanthi B. Increase in Fe³⁺/Fe²⁺ ratio and iron-induced oxidative stress in Eales disease and presence of ferrous iron in circulating transferrin. Curr Eye Res. 2007.

MANUSCRIPT UNDER PREPARATION:

1. Dysregulation of Iron homeostasis in Eales' Disease.
2. Exposure of Homocysteine negatively influences glutathione synthesis in human Retinal pigment epithelial (ARPE-19) cells.
3. Simultaneous determination of homocysteine and its related amino acids by High performance liquid chromatography with FLD detector.

POSTER PRESENTED:

1. Poster presented in BITS Pilani -2011 – Increased Thiolation and Homocysteinylation of proteins are responsible for protein damage in Eales' Disease.
2. Poster presented in IERG 2011- LVPEI Hyderabad – Increased Thiolation and Homocysteinylation of proteins are responsible for protein damage in Eales' Disease and Age related macular degeneration.
3. Poster presented in IERG 2010 - LVPEI Hyderabad - Exposure to Homocysteine negatively influences Glutathione Synthesis in Human Retinal Pigment Epithelial (RPE) Cells.
4. Poster presented in SBCI 2009 – NCCS Pune - First report on the elevated levels of free amino acids Homocysteine, Methionine, Cysteine, Taurine, Serine and Glycine in Aqueous humour of patient with Pseudoexfoliation syndrome Syndrome.

AWARD:

1. Received Travel grant, for Indian eye research group meeting in LVPEI, Hyderabad, India, 2010.

BRIEF BIODATA OF CANDIDATE

Ms. M.Bharath selvi obtained her B.Sc. Microbiology degree from Madras University in 2002. She did her MS in medical laboratory technology in 2002 from Birla institute of Technology & Science – Pilani in collaboration with Medical research foundation. She did MSMLT internship in Microbiology Dept on the topic of Standardization of western blotting technique for detecting of IgG and IgM anti Toxoplasma antibodies against antigens of Toxoplasma gondii tachyzoites in ocular fluids. She later joined the department of Biochemistry and cell biology as a junior scientist in 2005. she registered for PhD in 2009 under the guidance of Dr. K.N. Sulochana. She was recruited as a Junior research fellow in ICMR funded grant. During which she made 4 poster presentation, 1 workshop. She has published 3 paper in her PhD topic and other 3 manuscripts are under preparation. She handled several cell lines like ARPE 19, HUVECs and cells isolated from peripheral blood mononuclear cells. Her keen interest is on Homocysteine and vascular diseases. She is also involved in patient care and teaching classes for optometry students.

BRIEF BIODATA OF SUPERVISOR

Prof. K.N. Sulochana is currently Sr. Professor & Director, R.S. Mehta Jain Department of Biochemistry and Cell Biology, Vision Research Foundation, Chennai. She has been Reader in Biochemistry Research Dept, Sankara Nethralaya for nearly 10 yrs, Sr. Research Fellow, National University Singapore, for 2 yrs and as a Sr. Lecturer at Bradford University Singapore campus and Sr. Research Scientist, in pharma industry for 3 yrs. She did her B.Sc.(Chemistry); M.Sc. (Biochemistry)–University of Madras and Ph.D. (Biochemistry-Enzymology) SV. University, India.

She has keen interest in research, teaching and patient care. Her teaching area includes Basic Biochemistry, Biomolecules, Instrumentation, Clinical Biochemistry and Ocular biochemistry. Patient care includes optimization and Development of clinical investigations for oxidative stress, analysis of vitamin, amino acids, and enzymes.

Her current interests are Ocular Angiogenesis, Protein- Protein interactions, Molecular mechanisms of metabolic diseases, Drug targets, Design and development of inhibitors of angiogenesis, Pharmacokinetics and Preclinical studies. She has more than 80 Research article published, 5 reviews and 4 Book Chapters.

She has participated in many National and International conferences. She has received Dr. BC. Roy Award, Silver Jubilee Research Award of Medical Council of India, 1996, Swarn Lata Punshi award as "Best Research Worker" Medical Research Foundation, 1997, Women of the Year American Biographical Institute, Inc (2000), The biographical sketch inclusion, 19th edn of Who's Who in the World by Marquis (2001) and Outstanding Woman of the 21st Century", American Biographical Institute, NC, USA, 2001.

She's also a life member of professional bodies in Society of Biological Chemistry (I) (Life Member), Association for the promotion DNA fingerprint DNA technology (Life Member), Active participant in Annual Meeting of IERG for the past 7 yrs, Member in ARVO, Member in Association for clinical biochemist Singapore since 2002 (SAC), Member of the Management development institute of Singapore (MDIS) since Jan-2008.