TABLE OF CONTENTS

S.No	CONTENTS	Page No.
		140.
1	Review of literature	
1	Introduction	1
1	The history of homocysteine	1
1.0	Measurement of plasma Homocysteine	2
1.1	Homocysteine metabolism	3
1.2	Homocysteinylation of proteins	5
1.3	Homocysteine and Iron	6
1.4	Cystathionine β – synthase and Iron	6
1.5	Homocysteine and Ferritin	7
1.6	Clinical Studies on the association of Homocysteine and Iron	8
1.7		8
1.8	Glutathione synthesis	10
1.9	Homocysteine and Growth factors	11
1.10	Homocysteine and Vascular diseases	12
1.11	Homocysteine and other diseases	15
1.12	Other diseases	17
1.13	Homocysteine and Eye The effect of vitamin supplementation in hyperhomocysteinemia	18
1.14	The effect of vitamin supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet and the supplementation in hyperments yet and the supplements yet are supplements yet and the supplements	19
1.15	Factors influencing Homocysteine levels	19
1.15.1	Age and gender	20
1.15.2	Dietary factors	21
1.16	Retinal Vascular diseases such as Eales' Disease and Age related	
	macular degeneration	21
1.16.1	Eales' disease	21
1.16.2	Age related macular degeneration	22
1.17	Gap in existing literature	
i		24
2	Outline of work	24
3	Methodology	
3.1	Selection criteria for patients and control subjects	28

3.2	Blood tests	30
3.3	Separation of Peripheral blood mononuclear cells	30
3.4	Determination of Homocysteine and its related amino acids by High	30
	Performance Liquid Chromatography	
3.4.1	Principle of Precolumn derivatization of Homocysteine	30
3.4.2	Processing of standards	31
3.4.3	Processing of standard DL-Homocysteine and its related amino acids	32
3.4.4	Processing of plasma samples	33
3.4.5	Method Standardization	33
3.5	Determination of Plasma Homocysteine-Thiolactone	37
3.6	Determination of protein-cys bound Homocysteine and protein-lys	39
	bound Homocysteine	
3.7	Determination of Iron, Iron binding capacity and haemoglobin levels	39
	by spectrophotometer	
3.8	Determination of aminolevulinic acid synthase in serum and peripheral	40
	blood mononuclear cells	
3.9	Determination of heme in serum and peripheral blood mononuclear cells	42
3.10	Determination of ferritin, transferrin and serum transferrin receptor in	43
	serum and peripheral blood mononuclear cells	
3.10.1	ferritin	43
3.10.2	Transferrin	44
3.10.3	Sorum transferrin receptor	45
3.11	Determination of heme oxygenase in serum and peripheral blood	46
3.11	mononuclear cells	
3.12	Determination of Vascular Endothelial Growth Factor	47
3.13	RNA analysis	49
3.13.1	Total RNA extraction	49
3.13.2	Conversion of RNA to cDNA	49
3.13.3	Real time PCR	50
3.14	Western blot	50
3.14.1	Sample Preparation	50
3.14.1	Polyacrylamide gel electrophoresis	50
3.14.2	Lamuno detection	51
	instign of Glutathione by spectrophotometer	51
3.15	Determination of reduced and oxidized glutathione by High Performance	51
ا، د	Liquid Chromatography using Electrochemical Detector	
3.16	Determination of reduced and oxidized grands	

33

The state of the s

3.17	Determination of activity of -glutamate-cysteine ligase by	55
	spectrofluorometer	
3.18	Modelling for GCLC	56
3.18.1	Homology modeling	56
3.18.2	Molecular Dynamic Studies	56
3.19	Cell Culture experiment	57
	Retinal pigment epithelial cells	57
	Human Umbilical vein endothelial cells	58
3.19.1	Maintenance of cells	58
3.19.2	Subculture	59
3.19.3	Cell Viability	59
3.19.4	Cytotoxicity assays	59
3.20	Statistical analysis	59
4	Results	60
4.1	LIBLO analysis of amino acids involved in Homocysteine pathway	60 60
4.1.1	Levels of homocysteine and its related amino acids in plasma samples of	60
	ED	62
4.1.2	HPLC analysis of homocysteine and its related amino acids in plasma	02
	samples of ARMD	65
4.2	Structural damage to proteins due to Hcy and its metabolite HcyTL in	0.5
	LADAD	65
4.2.1	Analysis of protein modification, namely protein-cys bound Hcy and	
	Hey by HPLC using FLD in ED	67
4.2.2	of protein modification such as protein-cys bound frey and	
	to hound Hey by HPLC using FLD III ARMID	67
4.3	the mocysteine associated with Iron metabolism	68
4.3.1	a facility haemoglobin, Iron and Total Iron briding capacity	70
4.3.2	c Corriting transferrin and serum transferrin receptor	72
4.3.3	Levels of aminolevulinic acid synthase (ALAS), Heme and Heme	
	oxygenase	75
4.3.4	Levels of VEGF in ED	76
4.3.5	mRNA expression of Iron regulators industrial factors (HIF1 and HIF2)	76
4.3.6	Over expression of Hypoxia inducible factors (IIII I and IIII =)	77
4.3.7	Typression of Iron regulators	79
4.4	Analysis of glutathione by spectrofluorometer	13

6	CONCLUSION	***************************************
5	DISCUSSION	109
4.7.3		100
	modifier unit of GSH in HUVECs Homocysteine lowers the mRNA expression of Nrf2 in HUVECs	98
4.7.2	Homocysteine suppressed the mRNA expression of catalytic unit and	5 10
4.7.1	Cytotoxicity Assay	97
	model system for vascular diseases (In Vitro).	96
4.7	Human umbilical vein endothelial cells (HUVECs) as a cell culture	
4.6.5	Homocysteine stimulates mRNA expression of Nrf2 in ARPE -19 cells	95
	modifier unit of GSH in ARPE -19 cells	05
4.6.4	Homocysteine induces mRNA expression of catalytic unit and the	94
4.6.3	Homocysteine alters the amino acids level in ARPE-19 cells	93
4.6.2	Intracellular GSH levels	92
4.6.1	Cytotoxicity assay	91
4.6	Cell culture model system for ARMD – ARPE 19 cells (<i>In Vitro</i>).	91
4.5.5	Binding pose analysis	89
4.5.4	Distance between Cso/Cys and Glu	87
4.5.3	Ligand Positional RMSD	87
	Protein backbone RMSD	
4.5.2	Molecular Dynamics Simulations studies of GCLC-Cys and GCLC-Cso	86
4.5.1	Structural comparison of GCLC-Cys and GCLC-Cso complex	85
	Molecular Dynamics stimulation study.	
4.5	To understand the effect of homocysteine in glutamate-cysteine ligase by	84
4.4.4	Expression of Nuclear erythroid related factor 2 (Nrf2)	83
4.4.3	Expression of -glutamate-cysteine ligase	83
4.4.2	Analysis of -glutamate-cysteine ligase by spectrofluorometer	81
4.4.1	Analysis of reduced and oxidized glutathione by HPLC using ECD	80