Evaluation of Interleukin-6 in Tears and Serum and Its Associated Factors in Age Related Macular Degeneration Patients

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10th Conjoint Ophthalmic Scientific Conference

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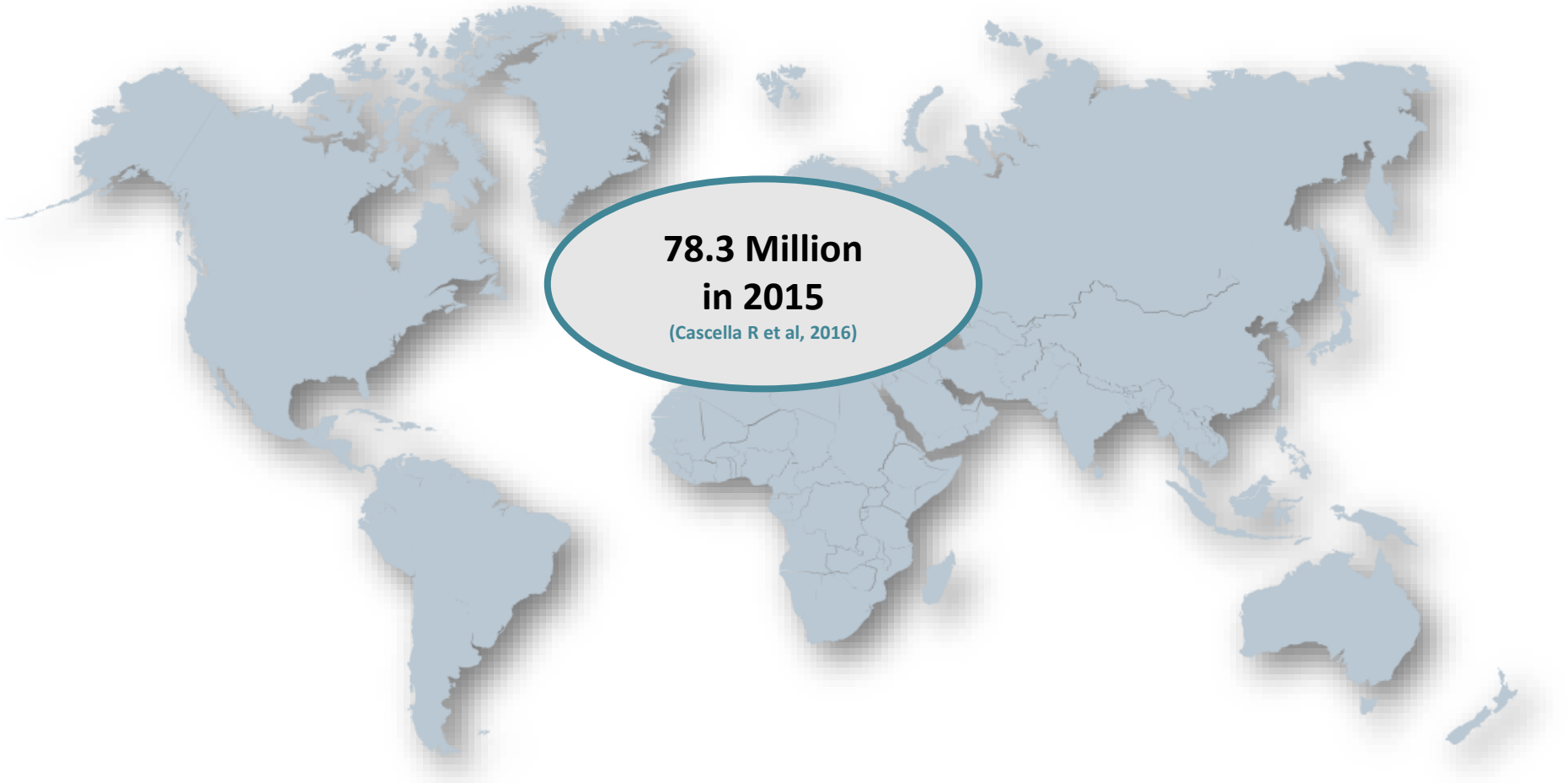
Age Related Macular Degeneration (AMD)

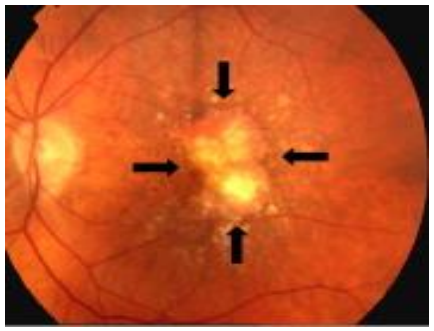


AMD Classification

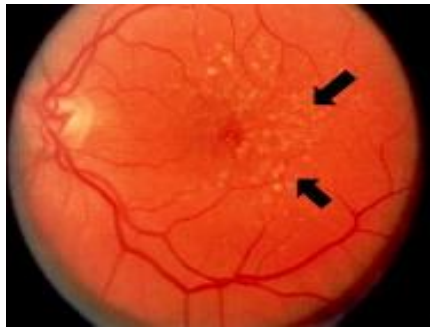
Wisconsin Age-related Maculopathy Grading System (WARMGS)

AMD

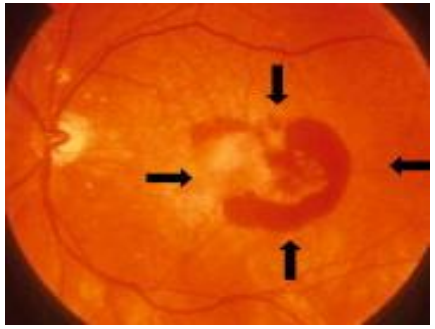
Early AMD



Late AMD



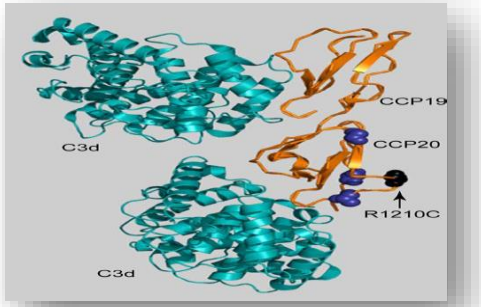
Geographical Atrophy



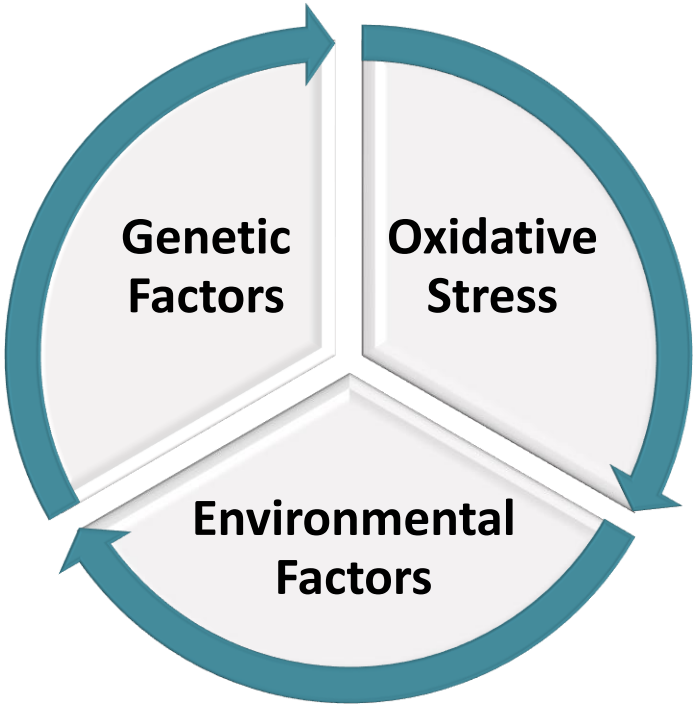
Neovascular AMD

The Royal College of Ophthalmology, 2013

Risk Factors of AMD



Complement Factor-H



Smoking

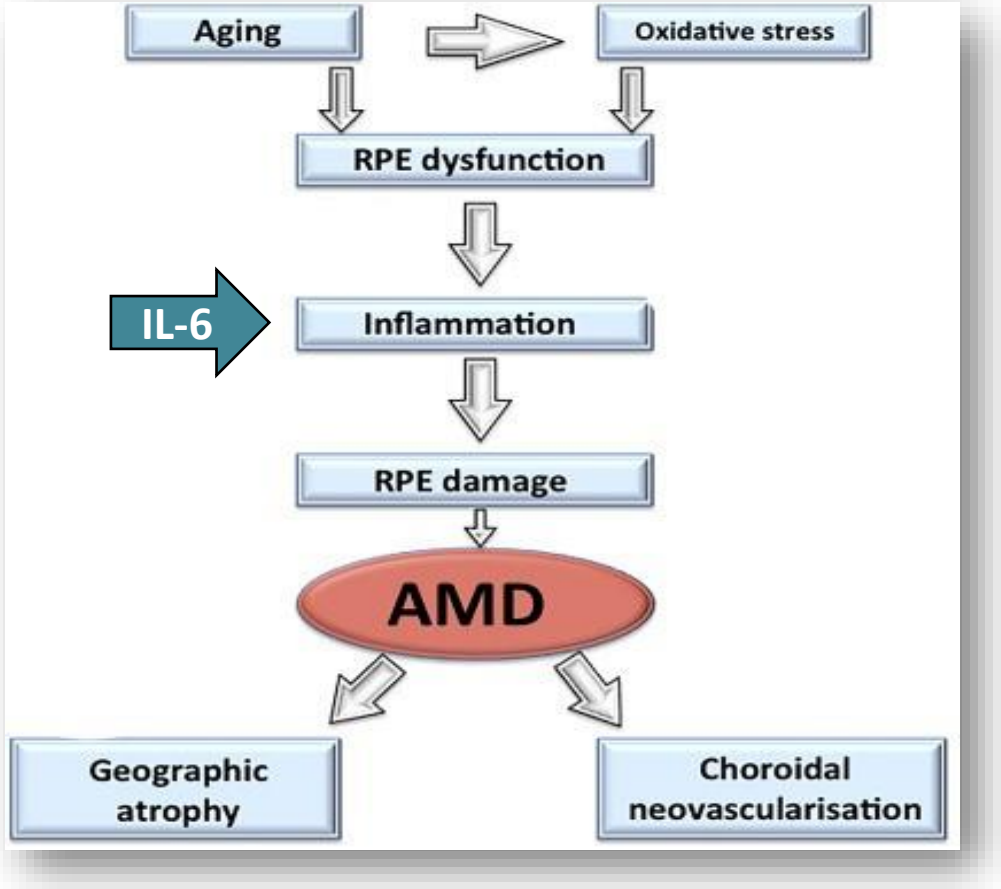
Age

Coleman HR et al, 2008; Chen M et al, 2015; Cascella R et al, 2014; Knickelbein JE et al, 2015

Pathogenesis of AMD

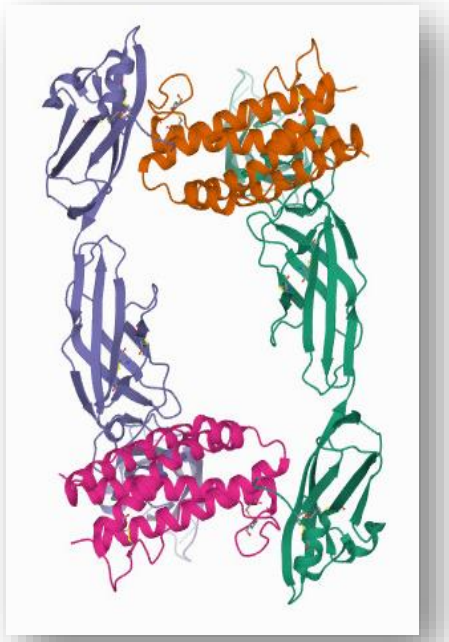
Activation of endothelial cells, lead to increase in vascular permeability and leakage of fluid and protein

Up-regulation of VEGF secretion



Kauppinen A et al, 2016; Naldini A et al, 2005; Tracey KJ et al, 1994; Yildirim Z et al, 2012

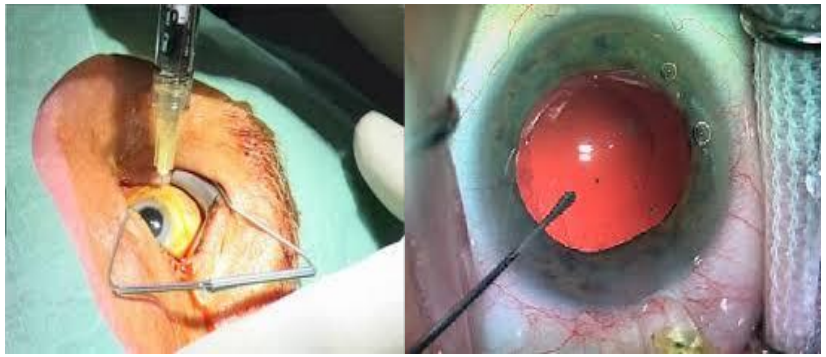
Interleukin-6



Crystal structure of IL-6

- Pro-Inflammatory cytokine
- AMD - elevated in
  - Serum (Yildirim Z et al, 2012)
  - Aqueous & Vitreous (Sato K et al, 2018; Abcouwe SF et al, 2013)
- Dry eye disease – elevated in
  - Tears (Yoon et al, 2007)

IL-6 Quantification



Aqueous & Vitreous

- Invasive
- Surgical risks
- Complications

Tears

- Less invasive
- Safe
- Comfortable

Rationale of Study

- Significant higher level of IL-6 found in serum, aqueous and vitreous in AMD patient
- Significant correlation between IL-6 and VEGF which is the angiogenic factor in neovascular AMD
- IL-6 level in tears can be use as a **non-invasive biomarker** for AMD screening

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General Objective

To evaluate the level of IL-6 in tears and serum and its associated factors in AMD patients

Specific Objectives

1. To compare the level of IL-6 in tears between AMD and Control

2. To compare the level of IL-6 in tears between Early and Late AMD

3. To compare the level of IL-6 in serum between AMD and Control

4. To compare the level of IL-6 in serum between Early and Late AMD

5. To identify the associated factors (AMD status, duration of AMD, serum level of IL-6 and smoking status) of tears IL-6 in AMD patients

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Methodology

Design	Comparative Cross-Sectional Study
Population	Newly diagnosed patients with AMD and Control
Location	Ophthalmology Clinic, Hospital USM
Duration	June 2018 to May 2021
Ethical Approval	Human Research Ethics Committee, USM [USM/JEPeM/ 18100488]
Funding	Malaysian Society of Ophthalmology (MSO) Small Research Grant

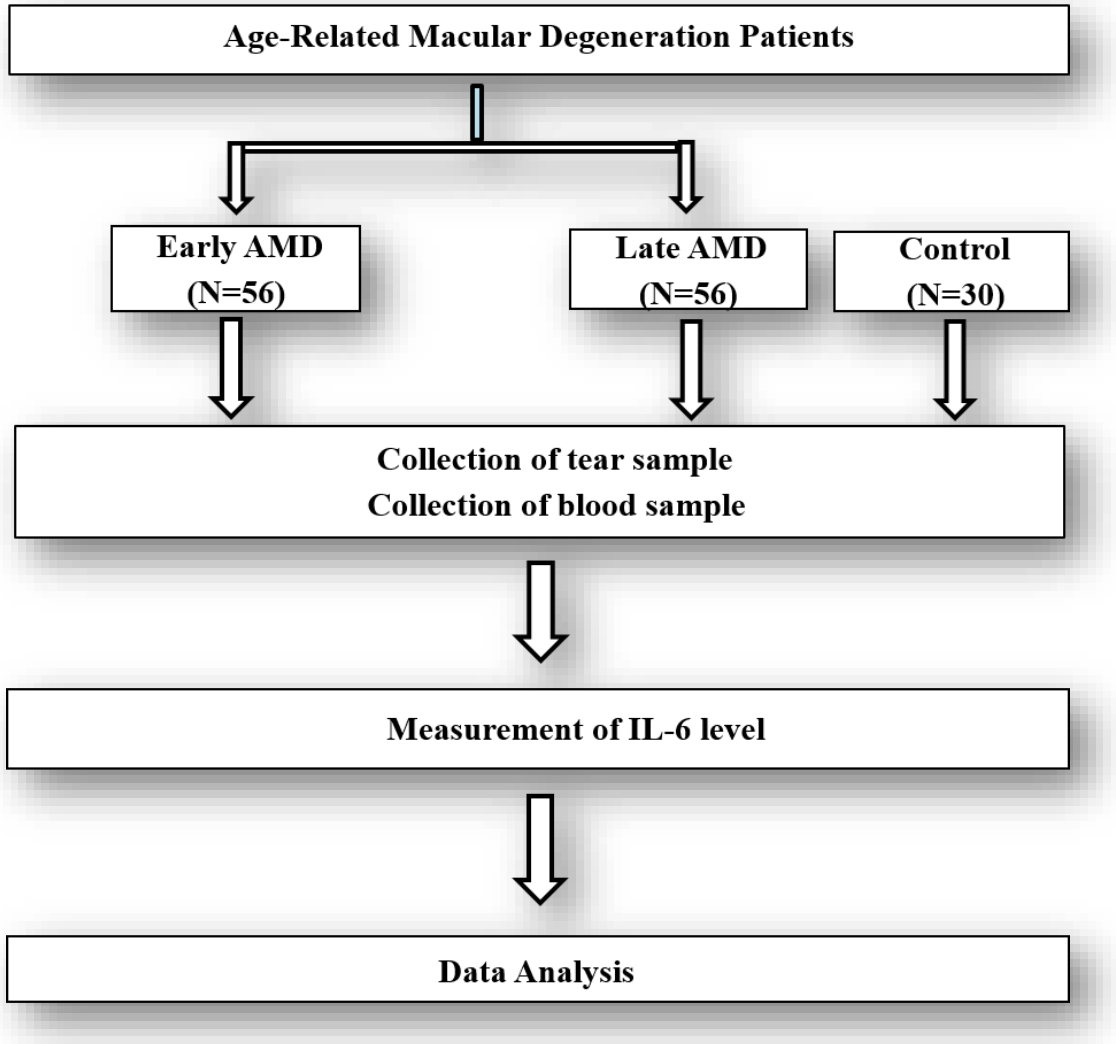
Flow Chart

Inclusion Criteria

- AMD patients
- Age: 45 - 70 years old

Exclusion Criteria

- Vitreoretinal pathology (DR,CRVO)
- Refractive error > -5.00DS
- Other macular pathology
- Ocular surgery within last 6 months
- History of ocular surface disease
- History of cornea pathology
- Ocular/systemic inflammation
- Chronic systemic disease

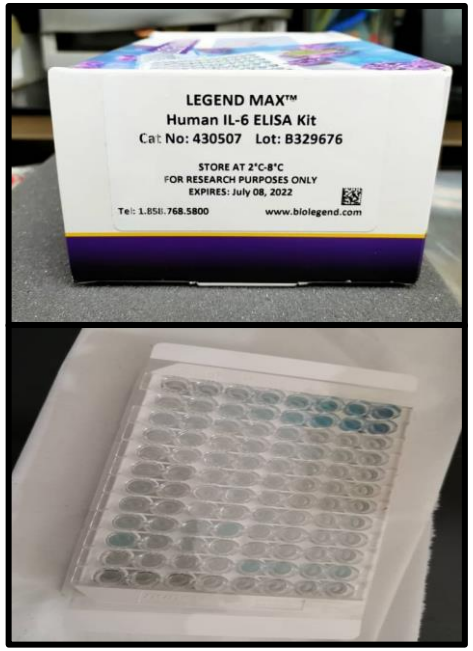


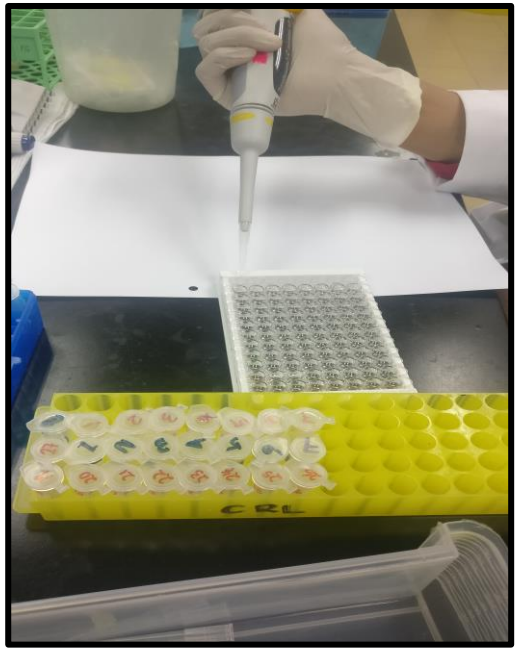
Tears and Serum Collection

Blood taking

EDTA Tube

IL-6 Measurement





Human IL-6 ELISA Kit

Sample Processing

ELISA Machine

Laboratory Analysis

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Demographic Data

Variable	Early AMD n=56	Late nAMD n=56	Control n=30	stat (df)	p-value
Age (years)	66.73 (5.43)*	66.20 (4.13)*	64.30 (5.65)*	2.37 (2)	0.097[a]
Ethnicity					
Malay	40	45	23	1.23 (2)	0.540[b]
Chinese	16	11	7		
Gender					
Female	33	32	18	0.07 (2)	0.964[b]
Male	23	24	12		
Duration of AMD (Months)	40.45 (23.74)*	38.04 (28.78)*	NA	0.23 (1)	0.630[c]

*Mean (SD); [a]ANOVA test; [b]Chi-square test; [c]Independent t-test;
Abbreviation : AMD: Age-Related Macular Degeneration; nAMD: neovascular Age-Related Macular

Clinical Profile

Variable	Early AMD n=56	Late nAMD n=56	Control n=30	stat (df)	p-value
Comorbidities					
Yes	51	43	24	4.33 (2)	0.115[b]
No	5	13	6		
Types of Comorbid					
Diabetes Mellitus	30	26	17	0.99 (2)	0.609[b]
Hypertension	40	38	21	0.17 (2)	0.918[b]
Dyslipidaemia	23	22	11	0.16 (2)	0.923[b]
Smoking Status					
Non-smoker	43	44	25	0.51 (2)	0.776[b]
Active Smoker	13	12	5		

*Mean (SD); [a]ANOVA test; [b]Chi-square test; [c]Independent t-test;
Abbreviation : AMD: Age-Related Macular Degeneration; nAMD: neovascular Age-Related Macular

IL-6 in Tears & Serum: AMD vs Control

Variable	AMD	Control	Mean Different (95% CI)	stat (df)	p-value
Tears IL-6 level (pg/ml)					
Crude Mean (SD)	21.97 (10.95)	16.06 (10.00)	-5.91 (-10.29, -1.54)	-2.67 (140)	**0.008[a]**
Adj. Mean (95% CI)	21.91 (19.89, 23.93)	16.27 (12.33, 20.22)	-5.64 (-10.10, -1.18)[c]	6.25 (1,136)	**0.014[b]**
Serum IL-6 level (pg/ml)					
Crude Mean (SD)	12.00 (6.04)	8.53 (4.13)	-3.47 (-5.79, -1.16)	8.99 (140)	**0.004[a]**
Adj. Mean (95% CI)	12.01 (10.93, 13.08)	8.51 (6.41, 10.62)	-3.49 (-5.87, -1.11)[c]	8.42 (1,136)	**0.004[b]**

[a]Independent t-test; [b]ANCOVA test adjusted with covariates: Age, Gender, Presence of Comorbidities & Smoking Status, $p<0.05$, significant; [c]Adjusted with Bonferroni Correction

IL-6 in Tears & Serum: Early vs Late nAMD

Variable	Early AMD	Late nAMD	Mean Different (95% CI)	stat (df)	p-value
Tears IL-6 level (pg/ml)					
Crude Mean (SD)	22.33 (9.62)	21.60 (12.21)	0.73 (-3.39, 4.85)	3.13 (110)	0.726[a]
Adj. Mean(95% CI)	21.76 (18.89, 24.64)	22.17 (19.30, 25.05)	-0.41 (-4.53, 3.71)[c]	0.04 (1, 106)	0.844[b]
Serum IL-6 level (pg/ml)					
Crude Mean (SD)	10.11 (5.41)	13.89 (6.08)	-3.78 (-5.94, -1.63)	0.72 (110)	**0.001[a]**
Adj. Mean(95% CI)	10.03 (8.49, 11.58)	13.97 (12.43, 15.52)	-3.94 (-6.15, -1.73)[c]	12.48 (1,106)	**0.001[b]**

[a]Independent t-test; [b]ANCOVA test adjusted with covariates: Age, Gender, Presence of Comorbidities, Smoking Status & Duration of AMD, $p<0.05$, significant; [c]Adjusted with Bonferroni Correction

Associated Factors for Tears IL-6

Variable	Simple Linear Regression[a]				Multiple Linear Regression[b]			
	Crude β	95% CI	t-stat	p-value	Adj. β	95% CI	t-stat	p-value
Duration of AMD (Months)	-0.05	-0.13, 0.03	-1.27	**0.207**	-0.05	-0.13, 0.03	-1.20	0.235
Serum IL-6 level (pg/ml)	0.24	-0.07, 0.55	1.51	**0.133**	0.17	-0.19, 0.53	0.92	0.359
Smoking Status	1.17	-3.31, 5.65	0.52	0.606	1.50	-3.46, 6.46	0.60	0.550
AMD Status	0.73	-4.85, 3.39	-0.35	0.726	-1.46	-5.80, 2.88	-0.67	0.508

[a]Simple Linear Regression test, $p<0.25$, significant, [b]Multiple Linear Regression test, $p<0.05$, significant

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IL-6 in Tears

The mean level of IL-6 in tears significantly higher in AMD group compare to Control group

Indicating active role of IL-6 in pathogenesis of AMD

- Increased level of vascular permeability and angiogenesis by stimulating the expression of VEGF **(*Tzafra C et al, 1996*)**
- Increase endothelial permeability through its induction of gap formation between adjacent cells **(*Naoko M et al,1992*)**

IL-6 in Tears

No significance difference in the mean level of IL-6 in tears between Early AMD and Late nAMD

Study done by ***Ulhaq et al, (2020)*** found no significant difference of IL-6 in vitreous and aqueous between dry and wet AMD

IL-6 in Serum

The mean level of serum IL-6 was significantly higher in AMD group compare to control group. Further analysis also showed significant higher level of serum IL-6 in late nAMD group

- Serum IL-6 increased in AMD patients and recognized as an important factor in prognosis of AMD progression ***(Yildrim et al, 2012)***
- Positive correlation between systemic levels of IL-6 with progression of AMD ***(Klein R et al, 2014; Seddon JM et al, 2005)***

Associated Factors for Tears IL-6

1. No significant association with duration of AMD

The duration of AMD in our study was standardized from the day of diagnosis. This may not correlate with the onset of disease.

Associated Factors for Tears IL-6

2. No significant association with serum IL-6 level

- Mean level of IL-6 was much higher in tears.
- May explained by the site of production of these inflammatory mediators
- Within the eye, structures like RPE, iris, ciliary body and muller cells were able to secret IL-6 ***(Ahmed HM et al, 2014)***

Associated Factors for Tears IL-6

3. No significant association with smoking

- Different biochemical pathways in pathogenesis of AMD ***(Velilla S et al, 2013)***
- Pro-oxidant compounds, cause oxidative damage to the RPE, contributes to the development and progression of AMD, and the alterations in the metabolic support of the RPE cause apoptosis of the photoreceptors ***(Beatty S et al, 2000; Jiyang C et al, 2000)***

Associated Factors for Tears IL-6

4. No significant association with AMD status

- Possibility of involvement of other factors that determine AMD progression
- Future controlled studies are needed to explore the association of IL-6 with other factors in AMD

Limitations and Recommendations

Limitations

- Single centre study
- Lack of racial data variation
- Lack of number of advance dry AMD with GA
- Limited budget

Recommendations

- Multicenter study
- More patients with advance dry AMD with GA
- Comprehensive data collection method
- Industrial involvement in future study

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Conclusion

- There was significantly higher mean IL-6 in both tears and serum in AMD compared to Control group
- Therefore, tears sample can be used as non-invasive biomarker for AMD screening
- There was no significant association between IL-6 in tears with duration of AMD, serum IL-6, smoking status and AMD status

References

-Abcouwe SF. (2013). Angiogenic Factors and Cytokines In Diabetic Retinopathy. *J Clin Cell Immunol*. 1-12 doi: 10.4172/2155-9899

-Ahmed HM et al. (2014). Serum level of Interleukin-6, C-Reactive Protein in Diabetic Patients and their Relation to Progression of Diabetic Retinopathy. *Med J Babylon.* 11:1-3

-Beatty S et al. (2000). The Role of Oxidative Stress in the Pathogenesis of Age-Related Macular Degeneration. *Surv. Ophthalmol.* Vol. 45(2): 115-134 doi: 10.1016/s0039-6257(00)00140-5.

-Cascella R et al. (2014). Age-related macular degeneration: Insights into Inflammatory Genes. *J Ophthalmol*. ID 582842, 1-9 doi: 10.1155/2014/582842

-Coleman HR et al. (2008). Age-related Macular Degeneration. *Lancet*. Vol: 372: 1835-1845 doi: 10.1016/s0140-6736(08)61759-6

-Chen M et al. (2015). Parainflammation, Chronic Inflammation, And Age-related Macular Degeneration. *J Leukoc Biol.* Vol: 98: 713-725 doi: 10.1189/jlb.3RI0615-239R

-Jiyang C et al. (2000). Oxidative Damage and Protection of the RPE. *Prog. Retin. Eye Res.* Vol. 19(2): 205-221 doi: 10.1016/s1350-9462(99)00009-9

-Klein R et al. (2014). Markers of Inflammation, Oxidative Stress, And Endothelial Dysfunction And The 20-year Cumulative Incidence Of Early Age-related Macular Degeneration: The Beaver Dam Eye Study. *JAMA Ophthalmol*. 132:446–55 doi: 10.1001/jamaophthalmol.2013.7671.

-Knickelbein JE et al. (2015). Inflammatory Mechanisms Of Age-related Macular Degeneration. *Int Opthalmol Clin*. 55(3): 63-78 doi: 10.1097/IIO.0000000000000073

-Kauppinen A et al. (2016). Inflammation And Its Role In Age-related Macular Degeneration. *Cell. Mol. Life Sci.* 73:1765–1786 doi: 10.1007/s00018-016-2147-8

-Naldini A et al. (2005). Role of Inflammatory Mediators in Angiogenesis. *Curr Drug Targets -Inflamm Allergy* Vol. 4(1): 3-8 doi: 10.2174/1568010053622830

-Naoko M et al. (1992). IL-6 Increases Endothelial Permeability in Vitro. *Endocrinology.* 131: 710-714 doi: 10.1210/endo.131.2.1639018

-Sato K et al. (2018). Interleukin-6 Plays a Crucial Role in the Development of Subretinal Fibrosis in a Mouse Model. *Immunol Med*. 41(1): 23-29 https://doi.org/10.1080/09114308.1451609

-Seddon JM et al. (2005). Progression Of Age-related Macular Degeneration: Prospective Assessment of C-reactive Protein, Interleukin 6, and Other Cardiovascular Biomarkers. *Arch Ophthalmol*. 123: 774–82 doi:10.1001/archopht.123.6.774

-The Royal College of Ophthalmologists, Guidelines for Management AMD 2013

-Tzafra C et al. (1996). Interleukin 6 Induces the Expression of Vascular Endothelial Growth Factor. *J. Biol. Chem.* 271: 736-741 doi: 10.1074/jbc.271.2.736

-Tracey KJ et al. (1994). Tumor necrosis factor: A pleiotropic Cytokine and Therapeutic Target. *Annu. Rev. Med*. 45: 491-503 doi: 10.1146/annurev.med.45.1.491

-Velilla S et al, (2013). Smoking and Age-Related Macular Degeneration: Review and Update. *J Ophthalmol. ID* 895147 http://dx.doi.org/10.1155/2013/895147

-Wakefield D et al. (1992). The Role of cytokines in the pathogenesis of Inflammatory Eye Disease. *Cytokine*. Vol. 4(1): 1-5 doi: 10.1016/1043-4666(92)90028-p.

-Yildirim Z et al. (2012). Choroidal Neovascular Membrane in Age- Related Macular Degeneration is Associated with Increased Interleukin-6. *J Gerontol Geriatr Res.* 101-104

-Yoon et al. (2007). Interleukin-6 and Tumor Necrosis Factor-α Levels in Tears Patient With Dry Eye Syndrome. *Clin Sci Cornea*. 26: 431-437 doi: 10.1097/ICO.0b013e31803dcda2.

Acknowledgement

Supervisor

Professor Datin Dr Zunaina Embong

Co-supervisors

Asst. Prof Dr Aidila Jesmin bt Jabbari

Assoc. Prof Dr Khairidzan Mohd Kamal

Assoc. Prof Dr Che Badariah Ab Aziz

THANK YOU