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The Meibum Lipid Profile and its Relationship with Clinical Dry Eye Measurement in a Sample of Kuala Lumpur Young Adults

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Tear lipids play a critical role in reducing aqueous evaporation and promoting tear stability, with non-polar lipids (NPoL) being a key subcomponent contributing significantly to this function. Notably, the Asian population exhibit lower tear stability, which may underline the higher prevalence of dry eye disease (DED). This study aimed to characterise the meibum lipid profile and its association with clinical dry eye measurements in a cohort of young adults in Kuala Lumpur, Malaysia. Using reverse-phase high-performance liquid chromatography-electrospray ionising mass spectrometry (RP-HPLC-ESI-MS), a meibum sample from 28 participants was analysed. stratified into dry eye (DE) and non-dry eye (NDE) groups based on prior clinical studies. Fourteen distinct analytes were identified across all samples. The DE group exhibited a significant 12% reduction in NPoL compared to the NDE group ($p<0.05$). While no significant differences were observed in individual lipid species between the two groups ($p>0.05$), phosphatidylserine (PS) was an exception, showing a marked increase in the DE group ($p<0.01$). Cholesterol ester (CE), the sole non-polar lipid (NPoL) identified, demonstrated a moderate yet significant correlation with tear lipid layer (TLL) thickness ($rs= 0.61$, $p<0.05$) and the phenol red thread test (PRT) ($r=0.66$, $p<0.05$). Among polar lipid (PoL) species (PS) exhibited moderate correlation with lid wiper epitheliopathy (LWE) ($rs=0.69$, $p<0.01$), McMonnies dry eye questionnaire score (MDEQ) ($rs=0.57$, $p<0.05$), and tear osmolarity ($rs=0.56$, $p<0.05$). Sphingomyelin (SM) showed the strongest correlation with Efron conjunctiva redness ($rs=0.73$, $p=<0.01$). Notably, NPoL were inversely correlated with LWE ($rs= -0.66$, $p<0.05$) and positively correlated with PRT ($r= 0.56$, $p<0.05$), suggesting that reduced NPoL may contribute to early signs of DED. The elevated presence of PS in DE cases and its strong association with LWE, MDEQ score, and osmolarity highlight its potential as a biomarker for DED. These findings underscore the importance of lipidomic profiling in understanding the pathophysiology of dry eye disease and its clinical manifestations.

Keywords: meibum; tear lipid layer; dry eye; polar lipid; non-polar lipid

I. INTRODUCTION

The terms 'dry eye', 'ocular surface disease', 'ocular surface disorder', or 'tear deficient syndrome' have been widely used to describe symptoms and results of clinical damage to the interpalpebral ocular surface. While these terms collectively enhance our understanding of the condition, they also underscore its multifaced nature, which encompasses abnormalities in tear production or composition, alteration of

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eyelids anatomy or function which includes abnormal or altered tear production or composition, changes in lid anatomy or their function, and related subclinical signs. The definition of dry eye (DE) was updated during the Dry Eye Workshop II (DEWS II) in 2017, characterising it as a multifactorial disease of the ocular surface marked by a loss of tear homeostasis. This condition is accompanied by ocular symptoms, with tear film instability and hyperosmolarity, ocular surface inflammation, damage, and neurosensory abnormalities playing a key etiological role (Craig *et al.*, 2017). Notably, the DEWS II definition highlights tear hyperosmolarity as a central diagnostic indicator of DE. Furthermore, the classification acknowledges two primary subcategories of DE aqueous deficiency dry eye (ADDE) and evaporative dry eye (EDE), which often co-exist without clear demarcation. These subcategories are associated with distinct changes in ocular surface and symptoms profiles, while there are underlying etiological pathways remain an area of active investigation.

The tear film serves as a critical interface between the ocular surface and its immediate external environment, playing a vital role in protecting the eye from environmental stresses. Structurally, the tear film comprises three essential layers: the carbohydrate-rich glycocalyx layer, which anchors the apical microvilli of the superficial corneal epithelial cells; the intermediate aqueous layer, predominantly largely contributed by aqueous secretion from the lacrimal glands that constitute the bulk of the tear film; and the outermost which interfaces directly with the external environment (Joffre *et al.*, 2008). The tear lipid layer (TLL) is particularly crucial for reducing evaporation and maintaining tear film stability between blinks. The TLL consists of two sub-layers: the outer, superficial sub-layer dominantly of non-polar lipids (NPoL), including wax ester (WE), cholesterol ester (CE), triglyceride (TAG); and an inner polar lipid (PoL) layer adjacent to the aqueous interface. Amphiphilic lipids, a unique class of PoL, play a pivotal role in facilitating interaction between these layers. These lipids possess both hydrophilic and hydrophobic moieties, enabling them to act as surfactants that ensure the even distribution of monolayer NPoL with each blink. (McCulley & Shine, 2004; Joffre *et al.*, 2008; Butovich *et al.*, 2009). O-acyl-w-hydroxy fatty acids (OAFHA) have been identified as a novel class of amphiphilic lipids by Butovich *et al.* (2009) and Lam *et al.* (2014), a role previously attributed primarily to phospholipids (PL).

Historically, disruptions in the lipid layer have been implicated in increased tear evaporation, alterations in tear prism and subsequent osmolarity imbalance (Dougherty & McCulley, 1986; Pflugfelder *et al.*, 2000; Shine & McCully, 2003). Meibum, the meibomian gland secretion, constitutes the primary source of lipids for the TLL in humans (McCulley & Shine, 2004). Pathological changes in meibum secretion, volume and composition are often linked to meibomian gland dysfunction (MGD), which can manifest as obstructive gland orifice or hypersecretion secondary to lid inflammations, such as blepharitis.

Additionally, conditions such as seborrhoea, ocular rosacea, overgrowth of normal flora, or infestations by Demodex mites can exacerbate these changes. For instance, *Staphylococcus aureus* and *Staphylococcus epidermidis* produce lipase enzymes that can hydrolyse complex lipids such as triglycerides (TAG), cholesterol ester (CE), wax ester (WE), and diglycerides (DAG) into free fatty acids (FFAs) (Chen & Alonzo, 2019). This lipid degradation diminishes the stability and function of the TLL. Furthermore, Demodex mites (*Demodex folliculorum* and *Demodex brevis*) contribute to ocular surface dysfunction by forming collarettes around eyelash, consuming meibum and obstructing meibomian orifice, thereby exacerbating ocular surface pathology (Cheng *et al.*, 2015).

This study aimed to analyse and compare the content the distinct lipid profiles within the meibum, a critical component of the tear film lipid layer (TLL). Additionally, we sought to investigate the correlations between specific lipids classes and clinical indicators of DE. Understanding this relationship is essential for advancing the management and treatment of DE, as it provides insights into the underlying mechanism driving tear film instability and ocular damage.

II. MATERIALS AND METHOD

This study represents the second phase of two-part investigation, focusing on lipid analysis of the meibum sample. The research employed a cross-sectional study design using a cluster sampling method. The first phase of involved a clinical study of DE, examining parameters such as McMonies dry eye questionnaire (MDEQ) score, tear lipid layer (TLL) thickness, tear break up times (TBUT), phenol red thread test (PRT), tear osmolarity, meiboscore, and lid wiper epitheliopathy (LWE). Subjects were classified into dry eye (DE) and non-dry eye (NDE) groups based on MDEQ score threshold of 14.5 (McMonnies *et al.*, 1998). Inclusion criteria required participants to be aged 19 and 30 years old, with no history of contact lens use, ocular disease, systemic illness, pregnancy, or smoking. The study was approved by the Universiti Kebangsaan Malaysia (UKM) Medical Ethics Committee (NN-115-2013) and adhered to the principles of the Declaration of Helsinki. The clinical investigation was conducted at the Optometry Clinic, Faculty of Health Sciences, UKM. During the initial phase of the clinical study, we systematically evaluated tear film metrics and documented ocular surface alterations associated with DE, as reported in prior publications (Hajar Maidin *et al.*, 2018; Hajar Maidin *et al.*, 2020). The sample size for the current study was calculated using G*Power software (version 3.1.9.3 Heinrich Heine University Düsseldorf, Germany) for a two-tailed t-test with a biserial correlation point. The statistical test power was set at 0.8, significant level at $\alpha = 0.05$, with 0.5 effect size (Faul *et al.*, 2007). A total of 28 samples were randomly selected from both groups for the laboratory analysis. Samples preparation was conducted at the Biomedical Laboratory, Faculty of Health Sciences, UKM, while lipidomic analysis was performed using reverse-phase high-performance liquid chromatography coupled with electrospray ionisation mass spectrometry (RP-HPLC-ESI-MS) at the HPLC Laboratory, Faculty of Food Science Technology, University Putra Malaysia (UPM). This article discusses lipid profile results and their correlation with clinical DE findings.

The following was a summary of sample collection, lipid standard preparation, sample preparation, and RP HPLC-ESI-MS analysis. To prevent contamination from skin sebum, the examiner wore a nitrile-free glove (V-Safe Examination Glove, V-Safe, Malaysia) during sample collection and handling. The meibomian gland was then gently expressed on Schirmer paper and put into a 2 ml HPLC glass vial with a Polypropylene (PTFE)-lined caps were washed with chloroform (Merck, Malaysia), methanol (Merck, Malaysia), and deionised water in a 4:2:1 ratio and vortexed and centrifuged at 5000 rpm at room temperature, forming two distinct layers: an organic layer at the bottom and a supernatant on top. The organic layer was collected and transferred into a fresh vial, while the organic solvent was evaporated under a slow stream of nitrogen gas, and the dried samples were stored at -20°C until analysis.

Lipid standards were prepared according to the manufacturer datasheet, this includes specific instruction for reconstitute with specific solvent, storage and handling the lipid standard. A series of concentrations were prepared to construct a calibration curve. These are the lists of lipid standards used in the study. *1,2 dimyristoyl-sn-glycero-3-phoshocoline*, PC (Avanti Polar Lipids, Alabaster, AL, USA);*1,2 dimyristoyl-sn-glycero-3-phosphoetanolamine*, PE (Avanti Polar Lipids, Alabaster, AL, USA);*1,2 dimyristoyl-sn-glycero-3-phospho-rac-glycerol*, PG (Avanti Polar Lipids, Alabaster, AL, USA), *1,2 dimyristoyl-sn-glycero-3-phospho-L-serine*, PS (Avanti Polar Lipids, Alabaster, AL, USA), *C17-Cermide*, Cer (Avanti Polar Lipids, Alabaster, AL, USA), *C8-Glucosyl-(β)-Cermide*, GluCer (Avanti Polar Lipids, Alabaster, AL, USA), sphingomyelin, SM (Avanti Polar Lipids, Alabaster, AL, USA), dioctanoyl phosphatidylinositol, PI (Echelon Biosciences, Salt Lake City, UT, USA), cholesteryl stearate-26,26,26,27,27,27(d6), CE (CDN Isotopes Inc. Quebec, Canada), *glyceryl-ds-trioctadecanoate 48:0(d5)*, TAG (CDN Isotopes Inc. Quebec, Canada). *O-acyl-w-hydroxy-fatty acids*, OAHFA 18:1/16:0, palmitic acid, and oleic acid were prepared according to Butovich *et al.* (2009). Samples were reconstituted in 800μl chloroform and 400μl methanol prior analysis plastic materials were avoided to prevent contamination from plasticiser (Butovich, 2008, Ewald, 2012).

Lipid profiles were analysed using the reverse phase high-performance liquid colourimetry (RP-HLPC; Alliance 2695 HPLC, Walter Corp., Milford, MA, USA) coupled with mass spectrometry (MS; LCQ Deca Max, Thermo Fisher, USA). The system included an atmospheric pressure unit, and XCalibur software (Thermo Fisher, USA). The method demonstrated excellent linearity, with determination coefficients exceeding 0.99. A 10μl sample was injected into an Xterra MS C18 column (3.5μm, 2.1mm x 100l) (Walter, MA, USA). Separation was achieved based on lipid affinity to stationary phase, with column temperatures were set at 40°C, with the flow rate at 250 μl/min. Mobile phases consisted of water (A) and methanol (B), with the following gradient: 95% A and 5%

B at 0 min; 2% A and 98% B at 5 min, and 98% A 2%B at 10.30 min. Hot nitrogen gas (99% purity) was used as a stealth gas during spray electron ionisation (ESI), while argon gas was for analyte collision in positive ion mode. Mass detection ranged from of 200 to 1000 m/z. Lipids were identified based on retention time and a peak area with concentration calculated from standard curves and reported in parts per million (ppm) or mg/L. The individual lipid content was then compared to the total lipid detected and reported as a percentage.

Data were analysed using the statistical package SPSS version 23.0 for macOS (IBM Corp., Armonk, NY, USA Normality was assessed using the Shapiro-Wilk test. Group comparisons were performed using an independent t-test or Mann–Whitney U rank test, as appropriate. Correlation between individual lipids and clinical measurement or DE classification was evaluated using Pearson's or Spearman's rho correlation coefficients, with a significance level α=0.05.

III. RESULT AND DISCUSSION

The analysis of lipid using HPLC-ESI-MS in positive ionisation mode successful identify chromatographic peaks corresponding to all 14 analytes. The relative abundance of each analyte was calculated as percentage of total concentration of lipids detected. The data for each analyte were summarised as mean ± SD based on group and compared as in Table 1.

Table 1. Mean ± SD percentage Non-Polar Lipid and Polar Lipid detected, Shapiro Wilk test and comparison with independent t-test followed by Levene test

		Mean (μ)	SD (σ)	Shapiro-Wilk Test	Independent t with Levene test
NPoL	NDE	37.19	10.26	p = 0.503	p= 0.028 (t) †
	DE	25.56	6.81	p = 0.171	F=0.513 (p=0.488)
PoL	NDE	62.81	10.26	p = 0.503	p= 0.028 (t) †
	DE	74.44	6.81	p = 0.171	F=0.513 (p=0.488)

† Significant difference at 0.05 (two-sided test)

The study revealed distinct difference in lipid profile composition between local subject groups. Specifically, the ratio of NPoL to PoL was significantly lower in local subjects, with a ratio of 37:63, as detailed in Table 1. Furthermore, the DE group exhibited a 12% reduction in NPoL, compared to the NDE group, a difference that was statistically significant ($p<0.05$), While most individual lipids did not show a significant difference ($p>0.05$), PS was an exception, demonstrating a highly significant difference ($p<0.01$) as outlined in Table 2.

To explore potential relationships between lipid profiles and clinical parameters, Pearson's (r) and Spearman's (r_s) correlation analyses for both individual lipids and lipids as a group. Table 3 revealed strong associations between with the lipid groups and clinical findings, particularly for LWE and PRT. Among individual lipids, SM, PS and CE exhibited significant correlations with clinical data.

Table 2. Mean ± SD percentage of lipid analytes detected, Shapiro Wilk test and Mann Whitney U sum rank test / Independent t followed by Levene test

		Mean (μ)	SD (σ)	Shapiro-Wilk Test	Mann Whitney U/ Independent t with Levene test
CE	NDE	11.61	4.38	p = 0.333	p= 0.08 (t)
	DE	7.70	3.35	p = 0.413	F=1.357 (p=0.267)
WE	NDE	11.74	8.38	p = 0.831	p=0.165 (t)
	DE	6.36	4.72	p = 0.131	F=0.903 (p=0.361)
TAG	NDE	7.24	1.43	p = 0.464	p=0.367 (t)
	DE	5.67	4.19	p =0.323	F=2.509 (p=0.139)
Palmitic Acid	NDE	1.70	0.74	p = 0.431	p=0.832 (t)
	DE	1.59	1.02	p = 0.143	F=0.698 (p=0.420)
Oleic Acid	NDE	4.91	3.24	p =0.061	p=0.712 (t)
	DE	4.23	3.38	p = 0.117	F=0.167 (p=0.690)
OAHFA	NDE	9.60	3.69	p = 0.262	p=0.426 (t)
	DE	11.35	4.27	p = 0.938	F=0.003 (p=.960)
PC	NDE	10.48	3.57	p =0.699	p=0.525 (t)
	DE	9.39	2.62	p =0.587	F=0.88 (p=0.367)
PE	NDE	4.60	2.53	p =0.023	p=0.456 (MW)
	DE	3.52	1.94	p = 0.516	

PS	NDE	12.14	4.62	p =0.757	p=0.007 (t) ††
	DE	21.86	6.48	p =0.398	F=0.564 (p=0.467)
PI	NDE	4.75	3.21	p =0.114	p=0.179 (t)
	DE	7.71	4.45	p =0.381	F=0.325 (p=0.579)
PG	NDE	6.84	4.23	p =0.631	p= 0.253 (t)
	DE	4.74	1.53	p =0.245	F=7.354 (p=0.19)
SM	NDE	2.70	1.45	p = 0.986	p= 0.412 (t)
	DE	3.55	2.19	p = 0.471	F=2.463 (p=0.143)
SER	NDE	1.77	1.18	p = 0.419	p= 0.981 (t)
	DE	1.77	0.85	p = 0.767	F=1.288 (p=0.279)
GluCER	NDE	9.93	6.08	p = 0.794	p= 0.824 (t)
	DE	10.54	3.87	p = 0.387	F=2.076 (p=0.175)

† significant difference at 0.05 (two-sided test)

†† significant difference at 0.01 (two-sided test)

Table 3. Correlation of individual lipid with dry eye clinical measurement and classification

	Correlation	**P**
SM & conjunctival redness	r_s=0.727	p=0.003††
PS & LWE	r_s=0.690	p=0.006††
CE & PRT	r=-0.659	p=0.010†
CE & TLL	r_s= 0.610	P=0.020†
Non-polar lipid & LWE	r_s=-0.656	p=0.011†
Polar lipid & LWE	r_s=0.656	p=0.011†
PS & MDEQ	r=0.573	p=0.032†
Non-polar lipid & PRT	r=0.556	p=0.039†
Polar lipid & PRT	r=-0.556	p=0.039†
PS & Osmolarity	r=0.555	p=0.039†

†significant correlation at 0.05 (two-sided test)

††significant correlation at 0.01 (two-sided test)

Notably, PS showed a moderately positive correlation with LWE (r_s=0.690, p<0.01), as well as a moderately positive and significant correlation with MDEQ (r=0.573, p<0.05) and osmolarity (r=0.555, p<0.05). CE demonstrated a moderately positive and significant correlation with PRT (r=0.659, p<0.05), while SM exhibited has a moderately positive and highly significant correlation with conjunctival redness (r=0.659, p<0.01).

IV. DISCUSSION

The relationship between TLL thickness and EDE has been a subject of long-standing speculation (Korb & Greiner, 1994; Yokai *et al.*, 1996). In the Langmuir model, WE are recognised as the most significant NPoL due to its hydrophobic properties' effectiveness in minimising aqueous evaporation (Rantamaki *et al.*, 2012). Historically, the combination of WE and CE accounts for approximately 60% to 84% of TLL components in Caucasian subjects (Nicholaides *et al.*, 1981; McCulley & Shine, 1997; Mathers et al., 1998; McCulley & Shine, 2003). In contrast, Lam *et al.* (2011) reported significantly higher proportion in Asian subjects, combination of WE and CE, reaching up to 92% However, Lam's findings contrast with the well documented clinical observation that Asians (Chinese, Japanese, Malay, and Korean) have significantly lower tear stability than Caucasians (Cho & Yap, 1993; Toda *et al.*, 1995; Jamaliah & Fathilah, 2002; Mohidin & Amran, 2004; Lee *et al.*, 2017). This discrepancy raises the question, should higher NPoL content theoretically enhances tear film robustness? This highlights potential variation in TLL composition across ethics groups and prompts further inquiry into the implications for clinical DE measurement.

Our result indicated the combined proportion of WE and CE accounted for only 23% of TLL components (with WE and CE each contributing 11%), followed by TAG 7% and FFA, including oleic acid and palmitic acid at 6%. This resulted in a total NPoL composition of 37% in the NDE group of 37%. Notably, TAG content (7%)-slightly exceeded the previously reported range of 2% to 6%. (Cory *et al.*, 1973; McCully & Shine, 1997; Mathers & Lane, 1998; Lam *et al.*, 2011). The NPoL percentage decreased by 12% in the DE group (p<0.05). Among all NPoL, only CE demonstrated a significant correlation with TLL thickness and tear volume.

The production of WE, CE, CHL-OAHFA, free cholesterol, diacylated diols, and a trace of PL and TAG occurs in the meibocytes of the meibomian gland epithelium (Butovich *et al.*, 2017). Fatty acids are converted into alcoholic acids in meibocyte peroxisomes by a series of enzyme reactions, while WE are synthesised in the endoplasmic reticulum membrane via the esterification of fatty acids and fatty acid alcohols (Cheng & Russell, 2004).

The low levels of WE and CE observed in our study are likely attributable to reduced production of these lipids in local population, potentially influenced by dietary factors. Essential fatty acids (EFA), such as monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), which are not synthesised endogenously, play a critical role in meibum production. Omega-3 fatty acids such as alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid are particularly important (Liu & Ji, 2014). However, the dietary intake of omega-3 fatty acids among Malaysians is notably low, comprising only 30% of the recommended nutrient intake (Ng, 2006).

Despite compelling evidence linking low NPoL level to local dietary habits, the underlying reasons for reduced tear stability in Asian remain unclear. Our findings indicate no significant relationship between TBUT with any of the investigated lipids. While the TLL structure, independent of its thickness, can reduce tear evaporation by up to fourfold (Craig & Thomlinson, 1997), we observed no correlation between TLL thickness lipid profiles. This aligns with the finding of Mathers and Lane's (1998), who reported no link between evaporation rate, TLL thickness, and clinical parameters such as tear volume, tear flow rate, Schirmer test, meibum volume, and meibum lubrication. Similarly, clinical study found there were no significant differences in TLL thickness between the DE and NDE groups (Hajar Maidin *et al.*, 2020). The evaporation process occurs at a slow rate, and the TLL primarily serves to decelerate this process (Mathers & Lane, 1998; Bron *et al.*, 2017).

The shortfall of NPoL is compensated by the presence of FFA, particularly palmitic acid (C16) and oleic acid (C18:1). These FFAs help maintain the functionality of the NPOL layer as barrier to aqueous evaporation. However, the impact of FFAs on tear film stability remain debated. Studies by McCully and Shine (2001) and Joffre *et al.* (2008), suggest that FFAs produced by lipolysis of complex lipids, may disrupt the stability of the lipid layer. In contrast, the findings of Mutalib *et al.* (2015) and Nurul Hafizah (2015) in studies on oil-based eyedrops treatment of DE studies indicate that FFAs may not have a detrimental effect and could even contribute positively to tear film stability.

Experimental studies in rabbit models have demonstrated the safety of virgin coconut oil (VCO) as an ocular rewetting agent (Mutalib *et al.*, 2015). VCO, which contains NPoL FFAs such as lauric acid, capric acid, caprylic acid, and TAG, has been shown to improve Schirmer test results and TBUT without altering ocular surface pH (Nurul Hafizah, 2015). Additionally, castor oil has been successfully employed as a lipid emulsion carrier for cyclosporin (Lallemand *et al.*, 2017), and lutein-based phytonutrient eye drops has revealed a potential therapeutic function for DE (Chen *et al.*, 2021). Lipid-based eye drops have been shown to stabilise the tear film lipid layer, reduce evaporation, and alleviate symptoms of MGD and EDE (Rolando & Merayo-Lloves, 2022).

The pathogenesis of DE, as outlined by Bron *et al.* (2017), involves three primary mechanisms: tears hyperosmolarity, tears film instability, and apoptosis of goblet cells. Mucin, the innermost tear component of the tear film, converts the hydrophobic corneal epithelium to a hydrophilic surface. The lacrimal gland produces aqueous layer while the meibomian gland secretes meibum, which forms the outermost TLL. PL functions act as surfactant, facilitating the dispersion of NPoL across the ocular surface with each blink.

Blinking plays a critical role in renewing the tear layer by spreading fresh secretion from the lacrimal gland and meibomian glands across the ocular surface. Lipid and protein contamination of the tear film disrupts tear stability, leading to aqueous evaporation. Hyperosmolarity and LWE further exacerbate tear film dysfunction in DE. Among PL, PS and SM exhibited significant correlation with clinical findings.

PS, a component of PL in cell membrane bilayer showed a moderately positive and highly significant correlates with LWE ($rs=0.690$, $p<0.01$), as well as significant correlation with MDEQ score ($r=0.573$, $p<0.05$) and osmolarity ($r=0.555$, $p<0.05$). LWE, assessed by staining the lid wiper area, serve as early indicator of ocular surface desiccation. Result for our clinical study revealed significant differences in LWE between the DE and NDE groups (Hajar Maidin *et al.*, 2020). The significant association between PS with LWE suggests the accumulation of bilayer membrane components, possibly derived from conjunctival epithelial cells, glandular ductal epithelial cells, or acinar cells. Mechanical forces exerted by the eyelids may also result in immature cell force expression. This may result in a greater detection ratio of PoL to NPoL.

PS also demonstrated a significant relationship with MDEQ symptom scores. While previous study suggested that DE symptoms correlate with disease severity in advance case (Bjerrum *et al.*, 1996; Haga *et al.*, 1999), our finding indicate that the MDEQ score is reliable screening tool for DE, particularly among local subjects (Ahmad *et al.*, 2017; Hajar Maidin *et al.*, 2018; Mohd Ali *et al.*, 2011; Mohammed *et al.*, 2012). MDEQ symptom scores increased as DE severity, reflecting the compensatory tear reflex triggered by dry eye symptoms. This reflex increases aqueous production and blink rate to counteract elevated evaporation rate (Arita *et al.*, 2015; Inaba *et al.*, 2018). Neural adaptations, including changes in sensory thresholds, further complicate the system profile, as highlighted in DEWS II classification (Craig *et al.*, 2017).

PS was also found to significantly correlate with tear hyperosmolarity (r=0.555, p<0.05). The clinical study demonstrated NDE osmolarity value of 286.35 ± 21.31 mOsm/kg (Hajar Maidin *et al.*, 2020), comparable to the control value reported by Ahmad *et al.* (2017) (293.33 ± 13.52 mOsm/L). In contrast, DE osmolarity value exceeded diagnostic thresholds of 316 mOsm/kg (Thomlinson *et al.*, 2006) and 320 mOsm/kg (Thomlinson & Khanal, 2005). Hyperosmolarity is a key diagnostic indicator in both evaporative dry eye (EDE) and aqueous-deficient dry eye (ADDE) (Lemp *et al.*, 2012; Nichols, 2011; Viso *et al.*, 2011). Advances in electrical impedance technology has enabled rapid and accurate measurement of tear osmolarity using nanolitre tear samples, providing a reliable DE diagnostic tool (Masmali *et al.*, 2014).

SM, a sphingolipid located on the inner plasma membrane, functions as a signalling molecule for cell death or autophagy (Young *et al.*, 2013). In our study SM showed a strong correlation with conjunctival redness (rs=0.727, p0.01), particularly in continuous scale measurement. Excessive friction between opposing ocular surfaces may activate conjunctival epithelial cells, triggering inflammatory responses (Swamynathan, 2013). Inflammation, in turn, stimulates the tear reflex and immune activation (Wei & Asbell, 2014). Anti-inflammatories treatments, including lipid emulsions, have been shown to reduce friction, improves tear dispersion, and eventually restores tear layer homeostasis (Herman *et al.*, 2005). Our findings support the use of the LWE as an early clinical indication of dry eye (Korb *et al.*, 2008).

V. CONCLUSION

The lipid profile analysis of young adults in Kuala Lumpur revealed lower NPoL compared to PoL. This disparity was even more pronounced in patient with DE, where NPoL levels were significantly reduced. The presence of PS suggests that the ocular surface has undergone desiccations, likely due to elevated tear osmolarity and LWE. This underscores the importance of PS as a critical parameter in understanding and diagnosing DED.

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