The assessment of leukocyte systemic inflammation index ratios in dyslipidemia patients with dry eye disease

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Abstract

Background

One of the many adverse effects of dyslipidemia (DLP) is dry eye disease (DED), which is brought on by metabolic syndrome and increases inflammation. This research aimed to assess the leukocyte systemic inflammation index ratios in DLP with DED (DED-DLP) as potential biomarkers of systemic inflammation.

Methods

Several sets of blood biomarkers were studied. There were specific leukocyte profile parameters (neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), and neutrophil-to-lymphocyte*platelet ratio (NLPR)); the lipid profile (levels of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein and triglycerides): and an inflammation marker (C-reactive protein (CRP)). These were evaluated in 32 patients with DED-DLP (study group) and 52 subjects without DLP (control group).

Results

The study group had significantly greater levels of monocytes and CRP than did the control group (p = 0.024 and 0.049, respectively). A negative correlation with a statistically significant difference between HDL and NLPR (p = 0.007; r=-0.428) and a negative correlation with a statistically significant difference between albumin levels and PLR values (p = 0.008; r=-0.420) were seen as predictors of DED-DLP.

Conclusion

Individuals with DLP are more likely than those without the condition to experience DED because the levels of their leukocyte systemic inflammation index ratios reflect the seriousness of systemic inflammation. The study found that CRP and NLPR levels might be helpful as predictors of the likelihood of the development of DED by DLP patients. Additional research is needed to determine the additive contribution played by leukocytes and the significance of NLR, PLR, and MLR.

1. Introduction

Dyslipidemia (DLP) is a chronic disease that is related to an imbalanced lipid profile characterised by elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) combined with low levels of high-density lipoprotein cholesterol (HDL-C) (1). There are two classes of DLP: primary (familial hypercholesterolaemia), which is caused by genetic factors, and secondary, which is influenced by many other factors such as obesity and a sedentary lifestyle (2).
DLP is a significant contributor to coronary artery disease. It shows a notable prevalence worldwide. For instance, 19.7% of Korean adolescents, 27% of American adolescents, and 34% of Chinese adults have been reported to have DLP (3). A local study found that a quarter of Saudi Arabian adults, particularly males, experienced DLP (4). Approximately 30% of Saudi adults are classified as overweight or obese, which classification reflects poor dietary habits and an unhealthy lifestyle, and these conditions further increase the risk of developing DLP (5). DLP has been associated with chronic diseases such as diabetes (6), thyroid disease (7) and DED (8).

Numerous investigations have indicated an association between DED and DLP (1, 5, 9–13). The studies propose that lipid composition changes may impact the tear film's stability and contribute to the onset of DED (14). The lipid layer within the tear film is essential to prevent evaporation and to sustain lubrication on the ocular surface (15). DLP, which in many cases is characterised by elevated cholesterol and triglyceride levels, can compromise the quality and quantity of lipids in tears, and this results in high levels of evaporation and discomfort. Recognition of the correlation between DLP and DED is crucial to ensure comprehensive patient care. Multiple investigations, such as those conducted by Dao et al. and Jones et al., have led to awareness of this connection between DLP and DED, and have unveiled potential therapeutic strategies that target lipid irregularities to relieve dry eye symptoms (2, 16).

The inflammatory state that is associated with metabolic syndrome is a risk factor for DLP (17). Many studies have linked elevated levels of inflammatory markers such as erythrocyte sedimentation rate (ESR) and CRP with DLP. One study showed that small LDL-C and LDL scores were positively correlated with high levels of inflammatory indicators, but that mean LDL particle size and significantly high levels of HDL-C were correlated negatively with high levels of these indicators (18). In another study, the association between inflammatory markers and carotid atherosclerosis, which DLP can cause, was investigated. The study showed a significant correlation between levels of inflammatory and atherosclerosis markers, the carotid artery intima-media thickness (IMT), and amounts of carotid plaque. Moreover, ESR and CRP levels were significantly linearly correlated with increased carotid artery IMT (19).

The monocyte-to-HDL-C ratio (MHR) is currently considered a novel marker of systemic inflammation due to the proinflammatory activities of monocytes and the anti-inflammatory effects of HDL. A study in 2018 used the MHR and levels of CRP as markers of inflammation in patients with non-Sjögren syndrome dry eye (NSSDE) (1). A group of 70 NSSDE patients was tested through the application of a questionnaire about DED and the Schirmer 1 test, and the observation of any abnormal ocular surface staining patterns. In NSSDE, the mean MHR value was 12.4 ± 7.7; the mean CRP value was 2.9 ± 1.1mg/dl; both indicators were more significant in the NSSDE group of patients than in the control group (7.7 ± 5.4 and 1.2 ± 0.6mg/dl respectively). As a result, after the establishment of cutoff values, MHR is a potential marker of systemic inflammation and can be used in clinical settings (20).

Neutrophils, lymphocytes, and platelets are cellular indicators of inflammation. However, the NLR and PLR are considered more accurate markers of inflammation than the single measures. They are utilised in various diseases, including cardiovascular diseases, cancer and immune-mediated disorders (21, 22). A
case-control study of proinflammatory markers in DED compared with a healthy control group revealed that individuals with DED exhibited higher levels of NLR than did the healthy group (23). Another inflammation marker, the systemic immune-inflammation index (SII), which is based on platelet counts and the NLR, was examined in a prospective study that involved patients with DED compared with a healthy group. The study concluded that the dry eye group had a higher SII, and this finding suggested that SII could consistently indicate inflammation levels in individuals with DED (24). Additionally, a study in which the association between DED and lipid profile was investigated found that the results for participants with DED were similar to the results for those diagnosed with DLP compared with the healthy group (25). Moreover, a Korean study reported elevated cholesterol levels in individuals with DED, and this finding suggested that there was a need for routine examinations of the eyes of patients diagnosed with DLP due to the strong association between the two conditions (26).

The study explained in this paper aimed to determine the levels of leukocyte count and SII among patients with DLP and DED. The paper describes the involvement of systemic inflammatory markers in the management and prognosis of DLP with DED. These findings will aid in the prognosis and treatment management of patients who have both diseases. The relationships between the levels of lipid profile markers and the inflammatory index predictors were investigated with their implications for public health.

2. Method

2.1. Study design

This study was based on a retrospective cross-sectional research model and convenient sampling methodologies were used. The data was collected from the Ministry of National Guard Health Affairs for all patients who had confirmed DLP and DED between 2016 and 2023 in outpatient communities in Jeddah, Saudi Arabia. The participants' information was obtained from the electronic medical record system, Bestcare.

2.2. Population information

The required sample size was calculated using the clinical sample size calculator on the website http://www.raosoft.com/samplesize.html. Based on previous studies, the prevalence of DLP in DED patients is 40% (27). The required sample size was calculated based on this prevalence and at a 95% confidence level and with a margin of error of ± 5%. The required minimum sample size was determined to be 360. As this sample size was small, we included in our study all the patients with these conditions who attended outpatients during the relevant period.

2.3. Laboratory findings

Laboratory blood tests were performed for parameters related to complete blood counts (CBC), lipid profile (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), and an inflammation biomarker (CRP).
2.4. Blood test calculations

These inflammation index ratios were calculated for all subjects: NLR, PLR, monocyte-to-lymphocyte ratio (MLR) and neutrophil-to-lymphocyte*platelet ratio (NLPR) (28).

2.5. Participant groups and criteria

Patients were divided into the study group (DLP with DED; DLP-DED) and the control group (non-DLP) based on the principal indications of DLP. The number of DLP patients available influenced the sample size.

The inclusion criteria were that patients had to be adults aged ≥ 18 years old. They could be of either gender. Patients with DED were classified based on ocular clinical assessments, and patients with DLP were classified based on lipid profile tests.

The exclusion criteria were patients who were under the age of 18 years; smokers; contact-lens wearers; those who had undergone eye surgery; and people with other chronic diseases such as cancer.

2.6. Data analysis

The data was processed and analysed using PRISM software (GraphPad Inc., San Diego, CA, USA, Release 8.4). Categorical variables were displayed as percentages and integers. Means and standard errors were used to express the parametric data, and significance between groups was determined for normally distributed data (age, lipid profile, CBC and CRP level). The Mann-Whitney test was used for continuously distributed data with anomalous distributions, and Pearson's chi-square test was employed for categorical data (by gender). Spearman's correlation tests were used to examine the lipid profile and inflammation index ratio correlations between the DLP-DED and non-DLP patients. Results with $p < 0.05$ were deemed significant.

2.7. Statistical analysis

PRISM software (GraphPad Inc., San Diego, CA, USA) was used for data processing and analysis. Categorical variables were expressed as percentages and integers. Means and standard deviations were both used to present the parametric data.

A normality test, the Shapiro-Wilk test, was performed for the collected data, which was normally distributed; thus, an unpaired t-test was used for the data to determine the significant differences between the two groups. Correlations were examined for each participant between HDL-C, albumin, and CRP versus inflammatory biomarkers PLR and NLPR.

2.8. Ethical consideration

Ethical approval was obtained by the institutional review board at King Abdullah International Medical Research Centre (IRB RSS23J/007).
3. Results

Two groups of participants were considered from the sample that was used in this study: the DED-DLP study group (n=32) and the non-DLP control group (n=52). The DLP-DED group consisted of 62.5% males and 37.5% females, while the non-DLP group comprised 50.6% males and 49.4% females. There was no statistically significant difference between the groups (p-value=0.259) (Table 1).

Table 1: Gender breakdown of the DED-DLP and non-DLP groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-DLP (%)</th>
<th>DED-DLP (%)</th>
<th>#p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (%)</td>
<td>Male (50.6%) Male (62.5%)</td>
<td>Female (49.4%) Female (37.5%)</td>
<td>0.259</td>
</tr>
</tbody>
</table>

# Mann-Whitney test

The average age for the DLP-DED group was 60.0±11.6 years, and for the non-DLP group was 56.9±5.98, with no statistical significance between the groups (p-value=0.109) (Table 2).

Regarding blood biomarkers, the levels of the systematic inflammatory biomarker CRP in patients with DED-DLP were higher (3.53 ± 4.82mg/L) than were those of the non-DLP group (2.10 ± 1.26mg/L) and monocyte counts were higher (0.54 ± 0.18x10^9/l compared with 0.48 ± 0.14). The differences in these results were statistically significant (p-value=0.024 and 0.049, respectively) (Table 2). The blood data for the non-DLP group was taken from information collected on blood donors.

The remaining blood systematic inflammatory biomarkers showed no statistically significant differences between patients with DED-DLP and the non-DLP group (Table 2). The platelets result for the DED-DLP group was higher (277.2 ± 73.65) compared with that for the non-DLP group (268.1 ± 71.5, p-value=0.288) (Table 2). Lymphocyte and neutrophil counts were higher in patients with DED-DLP compared with non-DLP individuals (lymphocytes 2.67± 0.95 versus 2.60 ± 1.04, p-value=0.383; neutrophils 3.72 ± 1.21 versus 3.29 ± 1.43, p-value=0.081) (Table 2). The NLR value for the DED-DLP group was higher (1.50 ± 0.60) compared with that for the non-DLP group (1.32 ± 0.60; p-value=0.089) (Table 2).

The PLR, MLR, and NLPR values for the DED-DLP and non-DLP groups were: PLR, 113.1 ± 42.77 vs. 0.22 ± 0.11; MLR, 0.0058 ± 0.0026 vs. 114.9 ± 45.69; and NLPR, 0.20 ± 0.063 vs. 0.005 ± 0.0051. These results showed no statistically significant differences between the two groups (p-values: for PLR, 0.428, MLR, 0.099, and NLPR, 0.477) (Table 2).

Regarding the traditional lipid and lipoprotein profiles, the DED-DLP patients had high or borderline high levels in their lipid and lipoprotein profiles. The HDL-C, LDL-C, TC, and TG levels for the study group were
1.282 ± 0.3293, 2.819 ± 0.8488, 4.736 ± 0.965 and 1.344 ± 0.8244, respectively. The albumin level in the DED-DLP patients was 43.6±3.82. Unfortunately, no data on serum lipid or lipoprotein profiles or albumin was available among the non-DLP blood donors (Table 2).

Table 2: Comparison of demographic data and laboratory findings between the DED-DLP and non-DLP groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-DLP (mean ± SD)</th>
<th>DED-DLP (mean ± SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.9 ± 5.98</td>
<td>60.0 ±11.6</td>
<td>0.109</td>
</tr>
<tr>
<td>CRP</td>
<td>2.10 ± 1.26</td>
<td>3.53 ± 4.82</td>
<td>0.024</td>
</tr>
<tr>
<td>Platelet count</td>
<td>268.1 ± 71.5</td>
<td>277.2 ± 73.65</td>
<td>0.288</td>
</tr>
<tr>
<td>Lymphocyte count×10^9</td>
<td>2.60 ± 1.04</td>
<td>2.67 0.95</td>
<td>0.383</td>
</tr>
<tr>
<td>Neutrophil count×10^9</td>
<td>3.29 ± 1.43</td>
<td>3.72 ± 1.21</td>
<td>0.081</td>
</tr>
<tr>
<td>Monocyte count ×10^9</td>
<td>0.48 ± 0.14</td>
<td>0.54 ± 0.18</td>
<td>0.049</td>
</tr>
<tr>
<td>NLR</td>
<td>1.32 ± 0.60</td>
<td>1.50 ± 0.60</td>
<td>0.089</td>
</tr>
<tr>
<td>PLR</td>
<td>114.9 ± 45.69</td>
<td>113.1 ± 42.77</td>
<td>0.428</td>
</tr>
<tr>
<td>MLR</td>
<td>0.20 ± 0.063</td>
<td>0.22 ± 0.11</td>
<td>0.099</td>
</tr>
<tr>
<td>NLPR</td>
<td>0.005 ± 0.0051</td>
<td>0.0058 ± 0.0026</td>
<td>0.477</td>
</tr>
<tr>
<td>HDL-C</td>
<td>NA*</td>
<td>1.282 ± 0.3293</td>
<td>-</td>
</tr>
<tr>
<td>LDL-C</td>
<td>NA*</td>
<td>2.819 ± 0.8488</td>
<td>-</td>
</tr>
<tr>
<td>TC</td>
<td>NA*</td>
<td>4.736 ± 0.965</td>
<td>-</td>
</tr>
<tr>
<td>TG</td>
<td>NA*</td>
<td>1.344 ± 0.8244</td>
<td>-</td>
</tr>
<tr>
<td>Albumin</td>
<td>NA*</td>
<td>43±3.82</td>
<td>-</td>
</tr>
</tbody>
</table>

*NA: not available. Normal range of each parameter: CRP (0-5mg/L), Neutrophils (2-7.50 x 10^9/l), Monocytes (0.2-0.8 x 10^9/l), Platelets (150-450 x 10^9/l), Lymphocytes (1.5-4.0 x 10^9/l), HDL-C (1.55-10mmol/l), LDL-C (<2.59mmol/l), TC (~5.18mmol/l), TG (<1.70mmol/l), and Albumin 35–55 g/L.

# Mann-Whitney test. Statistically significant values (p<0.05) are shown in bold.

In the DED-DLP group, Spearman's correlation analysis illustrated a negative correlation with no statistically significant difference between CRP levels and NLPR (p=0.239; r=-0.155). In the non-DLP
group, a negative correlation showed a statistically significant difference between CRP levels and NLPR (p=0.032; -0.258) (Figure 1).

For the DED-DLP group, Spearman’s correlation analysis demonstrated a negative correlation with no statistically significant difference between HDL-C and the PLR (p=0.231; r=-0.134), Figure 2A, but a negative correlation with a statistically significant difference between HDL-C and the NLPR (p=0.007; r=-0.428), Figure 2B. It also demonstrated a negative correlation with a statistically significant difference between albumin levels and PLR values (p=0.008; r=-0.420), Figure 2C, but a positive correlation with no statistically significant difference between albumin levels and NLPR values (p=0.3735; r=0.0593), Figure 2D.

4. Discussion

Although many studies have been concentrated on DLP, a significant risk factor for cardiovascular disease, few studies have been focused on other conditions associated with DLP, including DED. Since DED is a multifactorial disease, more research on risk factors, such as DLP, is needed to enhance diagnosis, prognosis, and therapy planning. This retrospective study investigated the level of inflammation among people with DLP and DED compared with non-DLP subjects by considering leukocyte biomarker levels.

In our study population, the DLP-DED group comprised more males than females (Table 1). However, there was no statistically significant difference between genders in the DLP-DED group. This finding is aligned with previous research on gender variations in patients with DLP, which demonstrated that DLP was gender-independent and occurred in line with lipid profiles (9, 29–31). In contrast, gender influences the prevalence of DED; studies reveal that the disease shows a 17% greater frequency of occurrence in females than in males, who have 11% (32). Gender also has a role in the symptomatology of the condition, as studies show that females, on average, show higher total symptom scores than males (33). According to a meta-analysis, DLP and DED are statistically significantly correlated, more so among females than males (34). The demographic analysis in this study showed an average age of 60 years in the DLP-DED group, which reflected an older age group (Table 2). This finding is consistent with earlier reports, showing that DLP and DED are age-dependent (1, 13, 35–37).

Blood biomarkers are used to assess the degree of inflammation in people with chronic diseases. Knowledge of the amount of inflammation can assist in limiting the etiology and decrease the severity of the adverse effects, which should maintain or improve the quality of patients’ lives. In this study, the level of the benchmark inflammatory marker, CRP, was higher in the DED-DLP patients than in the non-DLP group to a statistically significant degree, Table 2. This finding is consistent with earlier research on the connections between inflammatory biomarkers and DLP (38, 39). CRP is a well-known indicator of vascular inflammation linked to lipid abnormality (40). Numerous studies have revealed a strong relationship between CRP concentration and the severity of inflammatory diseases such as diabetes and
atherosclerosis, and it has been proven to be the most significant predictor of the risk of future cardiovascular events (41, 42).

White blood cells, including neutrophils (26), monocytes (27), lymphocytes (28), and platelets (43), play a role in inflammation. Monocytes have been shown to trigger an inflammatory response and to increase the risk of atherogenicity as they influence oxidized lipoproteins and phospholipids (44, 45). In our study, monocyte counts were significantly higher in the DLD-DED group compared with the non-DLP group, Table 2. This result has been found by several researchers (46).

Moreover, we reported elevated platelet, lymphocyte, and neutrophil counts in the DED-DLP group, Table 2. This finding is in line with a study by Choi et al., who showed that hypercholesterolemia was substantially common among DED patients, with a higher prevalence in those with severe DED. The only parameter that showed a discernible difference between those with and without DED was HDL-C; those with DED had higher mean HDL-C levels (47).

This study examined novel biomarkers associated with subclinical inflammation levels in patients with DLP. This study showed that the NLR, PLR, MLR, and NLPR levels were higher in the DED-DLP patients than in those who did not have DLP. The PLR and the NLR are more reliable indicators of inflammation in many diseases than other indicators, such as neutrophil, lymphocyte, and platelet counts (48, 49). One study observed that DED patients consistently had greater NLR values than healthy subjects (control) but that PLR values were unaffected (50). Therefore, one possible inflammatory predictor with which to estimate the severity of DED is the NLR (51). These biomarkers were investigated by Ghobady et al. (28) and Hasanefendić et al. (52), who found a correlation between NLR and PLR levels and inflammation. Emerging evidence has discovered a strong connection between DED and the severity of DLP (53).

According to our findings, the lipid profile could be used to predict the risk of DED in people with DLP. The findings indicate that individuals with increased TC levels, particularly women, exhibit DED. Lastly, regarding albumin, the high inflammatory state of the DED-DLP patients did not affect their albumin levels. In addition, the usage of albumin drops has shown no side effects, and lower concentrations of albumin (5%) have had lower healing effects. Meanwhile, a 10% albumin drop showed a healing percentage of 100% (54).

The DED-DLP group demonstrated a negative correlation between CRP levels and the NLPR, which agrees with other reports (55–57). These findings show that the NLPR is an inadequate metric for DED-DLP.

In addition, the DED-DLP group demonstrated a negative correlation between albumin levels and PLR values. This finding was consistent with that reported by Cao et al. (58). Moreover, the negative correlation between the PLR and HDL-C levels and between HDL-C levels and the NLPR are in agreement with those reported by Türkkan et al. (59). Since albumin levels and the NLPR demonstrated a positive correlation, this ratio could thus be a promising inflammatory biomarker in combination with DLP to be used to monitor and predict the incidence and progression of DED in older adults. Future studies should be conducted on the same inflammatory biomarkers in the younger adult DLP population.
5. Conclusion

Findings and observations from this investigation indicate that the NLPR and CRP levels are probable and interesting indicators regarding the development of DED in DLP patients. Individual indices were less predictive of systemic inflammation response than the NLR, PLR, and MLR combinations. These results suggest that further research is required to identify other biomarkers that might be useful to identify and prevent DED in people with DLP.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

Statement of human rights

The study adhered to research ethics and ensured that human rights were protected. The research was a retrospective case-control study that relied on chart reviews and involved no direct contact with the subjects. The research team ensured and maintained the privacy and confidentiality of the data collected throughout the study period.

Consent for publication

Not applicable

Data availability statement

The data presented in this study is available on request from the corresponding author.

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Authorship statement

All authors have read and approved the final version of the manuscript. The corresponding author had full access to all the data collected in this study and accepted complete responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration statement

The supporting source/financial relationships had no involvement in the study.

Transparency statement
Amani Alhalwani affirms that this manuscript is an honest, accurate, and transparent account of the study being reported, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Figures
Figure 1

Scatter plots of CRP vs. NLPR for non-DLP (left) and DED-DLP (right) groups.
Figure 2

Scatter plots of HDL-C vs. the PLR (A), HDL-C vs. the NLPR (B), albumin vs. the PLR (C), and albumin vs. the NLPR (D), all for the DED-DLP group.