



## Pulmonary Injury Biomarkers, Oxidative Stress and Systemic Inflammation in Conventional Cigarette, Waterpipe and Electronic Cigarette Users: A Cross-Sectional Comparative Study

NERAN THAAR KLEAB

Department of Pharmacy, Al-Dour Technical Institute, Northern Technical University, Iraq  
neran.tk@ntu.edu.iq

### Abstract

**Background:** Tobacco smoke injures the airway epithelium, imposes an oxidant load and activates innate immunity, but the biological footprint of waterpipe smoking and electronic cigarette (e-cigarette) use is less well characterised, particularly in Middle Eastern populations where all three modalities coexist.

**Objective:** To compare a panel of pulmonary injury biomarkers (CC16, KL-6, YKL-40, galectin-3, TREM-1), oxidative stress indices (malondialdehyde [MDA], superoxide dismutase-1 [SOD-1] protein) and the systemic inflammatory cytokine interleukin-6 (IL-6) among conventional cigarette smokers, waterpipe smokers, exclusive e-cigarette users and non-smokers. **Methods:** In this cross-sectional study, 120 apparently healthy men aged 18–45 years were classified by smoking status into four equal groups (n = 30 each): non-smokers, cigarette smokers, waterpipe smokers and exclusive e-cigarette users. All eight analytes were quantified in serum by enzyme-linked immunosorbent assay. Group differences were tested by one-way ANOVA with Tukey HSD post-hoc comparisons, and associations by Pearson correlation.

**Results:** Serum CC16 was significantly lower, and KL-6, YKL-40, IL-6 and MDA significantly higher, in every smoking group than in non-smokers (all  $p < 0.001$ ;  $\eta^2 = 0.64\text{--}0.74$ ). The largest deviations occurred in the two combustible groups, which did not differ significantly from each other, whereas e-cigarette users showed an intermediate profile lying between non-smokers and combustible smokers. Galectin-3, TREM-1 and SOD-1 protein did not differ among groups ( $p > 0.20$ ). CC16 correlated inversely with the injury, oxidation and inflammation markers ( $r = -0.63$  to  $-0.71$ ), which were positively inter-correlated ( $r = 0.69\text{--}0.73$ ; all  $p < 0.001$ ). **Conclusion:** A coherent signature of airway epithelial injury, lipid peroxidation and IL-6-mediated inflammation separates smokers from non-smokers. The signature is most pronounced with combustible products, including the waterpipe, and is attenuated but clearly present in e-cigarette users. CC16, KL-6, YKL-40, IL-6 and MDA were the most discriminating circulating markers in this population.

**Keywords:** club cell protein 16; Krebs von den Lungen-6; YKL-40; lipid peroxidation; interleukin-6; waterpipe; electronic cigarettes; tobacco harm reduction

### 1. Introduction

Tobacco use remains one of the leading preventable causes of morbidity and mortality worldwide, accounting for more than seven million deaths each year from direct use and over one million further deaths attributable to second-hand smoke [1,2]. Although the age-standardised burden has declined in many high-income countries, the absolute number of tobacco-related deaths continues to rise, and the industry's marketing has shifted towards low- and middle-income countries with weaker regulatory systems [3]. The Eastern Mediterranean region has a distinctive profile: smoking prevalence among men remains high, and while cigarette smoking has fallen by roughly 7% over the past decade, waterpipe smoking has increased by almost 8% over the same period [4]. This shift is clinically relevant

because a single waterpipe session delivers substantially more carbon monoxide, particulate matter and several other toxicants than a single cigarette [5].

Three interconnected mechanisms underlie the respiratory effects of inhaled tobacco toxicants: disruption of the airway epithelial barrier, oxidative injury by reactive oxygen species, and activation of innate and cytokine-mediated immunity [6]. Several circulating proteins index these processes with increasing specificity. Club cell secretory protein-16 (CC16) is an anti-inflammatory pneumoprotein produced by non-ciliated bronchiolar epithelial cells; its serum concentration falls as club cell populations are depleted by chronic smoke exposure, and low CC16 predicts accelerated lung-function decline and incident chronic obstructive pulmonary disease (COPD) [7–10]. CC16 is also implicated in acute epithelial injury and viral lung damage, underscoring its sensitivity as a marker of barrier integrity [11,12]. Krebs von den Lungen-6 (KL-6) is a high-molecular-weight MUC1 glycoprotein released into the circulation when alveolar epithelial cells are damaged [13,14]. YKL-40 (chitinase-3-like protein 1) is secreted by epithelial cells, macrophages and neutrophils, is elevated in smokers, and correlates with airway inflammation, remodelling and neutrophilic disease phenotypes [15–17]. Galectin-3, a  $\beta$ -galactoside-binding lectin involved in tissue remodelling and fibrosis [18,19], and the triggering receptor expressed on myeloid cells-1 (TREM-1), an amplifier of innate immune responses [20,21], have also been proposed as lung injury markers, but their behaviour in otherwise healthy smokers is poorly defined. Large population analyses further indicate that smoking perturbs several systemic immune and inflammatory markers simultaneously, which argues for a multi-marker approach [22].

Oxidative stress is the common biochemical substrate of these processes. Malondialdehyde (MDA), an end-product of membrane lipid peroxidation, reflects the oxidant load imposed by smoke, whereas superoxide dismutase (SOD), a first-line antioxidant enzyme, may increase, decrease or remain unchanged depending on the intensity and duration of oxidant exposure [5,23]. Interleukin-6 (IL-6) links local airway events to low-grade systemic inflammation and is consistently elevated in current smokers [24,25].

Whereas the literature on conventional cigarettes is extensive, few studies have examined such a biomarker panel in cigarette, waterpipe and e-cigarette users drawn from the same demographically matched population. Comparative data are scarcer still for Middle Eastern cohorts, where waterpipe smoking is culturally entrenched and e-cigarettes are gaining popularity among younger adults.

### 1.1 Previous studies and research gap

Regional and international studies provide the context for the present analysis. Al-Tameemi and colleagues, in a cross-sectional study of 108 smokers and 51 non-smokers in Iraq, reported dose-dependent increases in plasma IL-6 (from 1.86 to 2.58 pg/mL) and TNF- $\alpha$  (from 4.93 to 6.29 pg/mL) among smokers [26]. The direction and magnitude of that effect resemble the present findings, although absolute IL-6 concentrations here are higher, a difference most plausibly attributable to the assay platform, sample matrix and population characteristics rather than to a different biological pattern. On the oxidative side, a 2024 pilot study documented a significant rise in MDA together with reduced activities of SOD, catalase and glutathione peroxidase in chronic smokers, confirming the importance of lipid peroxidation and suggesting that antioxidant enzyme activity may decline with heavier or longer exposure [23]. Most directly relevant, a 2025 Saudi study compared respiratory, haematological and inflammatory profiles across cigarette, shisha, e-cigarette and non-smoking groups and found that lung function was more impaired with combustible products than with e-cigarettes, while cigarette and shisha users shared a similar inflammatory profile [27].

This body of work exposes two gaps. First, most regional studies have relied on a conventional oxidative–inflammatory panel (MDA, SOD, catalase, glutathione peroxidase, IL-6, TNF- $\alpha$ , C-reactive protein) rather than on epithelium-specific markers of lung injury. Second, very few have compared cigarette, waterpipe and e-cigarette exposure within a single matched cohort. The present study addresses both gaps by adding CC16, KL-6, YKL-40, galectin-3 and TREM-1 to the panel, thereby focusing on early, subclinical airway injury by smoking modality. Specifically, we aimed to compare these markers of pulmonary injury, oxidative stress and systemic inflammation across four matched male groups, and to identify which circulating markers best discriminate smokers from non-smokers.

## 2. Materials and Methods

### 2.1 Study Design and Setting

This cross-sectional comparative study was conducted at Tikrit Teaching Hospital and its affiliated primary healthcare centres in Salah Al-Din Governorate, Iraq. Recruitment, sampling and laboratory analysis were carried out between January and April 2026.

### 2.2 Participants and Grouping

A total of 120 apparently healthy men aged 18–45 years were enrolled consecutively and classified according to their self-reported smoking status into four equal groups (n = 30 each): non-smokers (controls), conventional cigarette smokers, waterpipe smokers and exclusive e-cigarette users. Participants in the smoking groups were required to have used their respective product for at least one year. Only men were enrolled in order to minimise sex-related variation in IL-6 and oxidative markers [24].

Exclusion criteria were chronic respiratory disease, asthma, tuberculosis, COVID-19 within the preceding three months, autoimmune disease, malignancy, chronic kidney disease, chronic liver disease, and dual or poly-use of tobacco or nicotine products. These criteria were applied to remove the confounding influence of established pulmonary disease, altered renal clearance of the analytes, and the additive effects of multiple exposures.

### 2.3 Sample collection and biomarker assays

Venous blood (5 mL) was drawn from each participant, allowed to clot, and centrifuged at 3000 rpm for 10 min. Serum was separated and stored at –80 °C until analysis. Serum concentrations of the eight analytes (CC16, KL-6, YKL-40/CHI3L1, galectin-3, TREM-1, IL-6, MDA and SOD-1) were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotech Co., Ltd, Hangzhou, China) according to the manufacturer's instructions (Table 1). Protein analytes were quantified by quantitative sandwich ELISA and MDA by competitive ELISA. Samples were diluted five-fold as specified in each kit insert, absorbance was read at 450 nm, and concentrations were interpolated from each kit's standard curve and expressed in the kit's calibrated units. It should be noted that the SOD-1 assay measures the concentration of SOD-1 protein rather than its catalytic activity.

**Table 1.** ELISA kits used for serum biomarker quantification (Sunlong Biotech Co., Ltd, Hangzhou, China).

| Biomarker       | Kit                                                              | Catalogue no. | Assay format |
|-----------------|------------------------------------------------------------------|---------------|--------------|
| CC16            | Human Clara cell protein (CC16) ELISA Kit                        | QS0462Hu      | Sandwich     |
| KL-6            | Human Krebs von den Lungen-6 (KL-6) ELISA Kit                    | SL2082Hu      | Sandwich     |
| YKL-40 (CHI3L1) | Human Chitinase-3-like Protein 1 (YKL-40/CHI3L1) ELISA Kit       | SL2399Hu      | Sandwich     |
| Galectin-3      | Human Galectin-3 ELISA Kit                                       | SL2065Hu      | Sandwich     |
| TREM-1          | Human Triggering Receptor Expressed on Myeloid Cells-1 ELISA Kit | QS1742Hu      | Sandwich     |
| IL-6            | Human Interleukin-6 (IL-6) ELISA Kit                             | SL1001Hu      | Sandwich     |
| MDA             | Human Malondialdehyde (MDA) ELISA Kit                            | SL1135Hu      | Competitive  |
| SOD-1           | Human Superoxide Dismutase 1 (SOD-1) ELISA Kit                   | QS2309Hu      | Sandwich     |

**Note:** All assays were performed according to the manufacturer's protocols, with absorbance read at 450 nm and concentrations interpolated from kit-specific standard curves. The SOD-1 assay quantifies SOD-1 protein concentration rather than enzymatic activity.

### 2.4 Ethical considerations

The study protocol was approved by the Scientific Research Ethics Committee of Northern Technical University (approval no. 127A/26, dated 25 February 2026). All participants provided written informed consent, confidentiality was maintained at every stage, and the study was conducted in accordance with the Declaration of Helsinki.

## 2.5 Statistical analysis

Data were analysed with SPSS version 27 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean  $\pm$  standard deviation (SD). Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test before parametric analysis. Between-group differences were tested by one-way analysis of variance (ANOVA), with eta-squared ( $\eta^2$ ) reported as the effect size and Tukey's honestly significant difference (HSD) test used for post-hoc pairwise comparisons, which controls the family-wise error rate within each marker. Where variances were unequal, results were confirmed with Welch's ANOVA and Games–Howell post-hoc tests. Eight separate ANOVAs were performed (one per biomarker) and no formal correction was applied across markers; however, all significant omnibus tests had  $p < 0.001$  and would remain significant after a Bonferroni correction for eight tests ( $\alpha = 0.00625$ ). Pearson correlation coefficients were calculated among the biomarkers that showed significant group effects. No a priori sample-size calculation was performed; the fixed group size of 30 is acknowledged as a design limitation, and the observed effect sizes are reported descriptively rather than being used to compute retrospective power. A two-sided  $p$  value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1 Demographic comparability

The four groups did not differ significantly in age (one-way ANOVA,  $F = 0.93$ ,  $p = 0.428$ ). Mean ages were  $33.3 \pm 8.6$  years in non-smokers,  $30.8 \pm 8.2$  years in cigarette smokers,  $34.2 \pm 8.3$  years in waterpipe smokers and  $33.5 \pm 8.1$  years in e-cigarette users, supporting the validity of between-group comparison of the biomarker panel.

### 3.2 Pulmonary injury biomarkers

Serum CC16 differed markedly across groups ( $F = 69.85$ ,  $p < 0.001$ ,  $\eta^2 = 0.644$ ; Table 2). The highest concentration was observed in non-smokers ( $15.91 \pm 1.59$  pg/mL). All three smoking groups had significantly lower values: waterpipe smokers showed the lowest concentration ( $10.97 \pm 1.57$  pg/mL), closely followed by cigarette smokers ( $11.17 \pm 1.24$  pg/mL), while e-cigarette users occupied an intermediate position ( $13.09 \pm 1.59$  pg/mL). Each smoking group differed from controls, and e-cigarette users differed from both combustible groups, whereas cigarette and waterpipe smokers did not differ from each other (Tukey  $p = 0.955$ ; Table 3).

The alveolar damage marker KL-6 showed the strongest overall group effect ( $F = 111.28$ ,  $p < 0.001$ ,  $\eta^2 = 0.742$ ), rising from  $220.48 \pm 17.68$  U/mL in non-smokers to  $326.70 \pm 33.45$  U/mL in cigarette smokers and  $346.12 \pm 36.65$  U/mL in waterpipe smokers, with e-cigarette users again intermediate ( $279.35 \pm 24.61$  U/mL). YKL-40 followed a parallel pattern ( $F = 104.69$ ,  $p < 0.001$ ,  $\eta^2 = 0.730$ ), increasing from  $39.94 \pm 4.74$  pg/mL in non-smokers to  $62.76 \pm 6.10$  and  $59.87 \pm 6.47$  pg/mL in the cigarette and waterpipe groups, respectively. The e-cigarette group mean for YKL-40 ( $45.70 \pm 6.08$  pg/mL) lay closer to that of non-smokers than to either combustible group, and the non-smoker versus e-cigarette contrast was the least pronounced of the smoking comparisons ( $p = 0.001$ ). For both KL-6 and YKL-40, the combustible groups did not differ significantly from each other, both exceeded the e-cigarette group, and the e-cigarette group exceeded non-smokers.

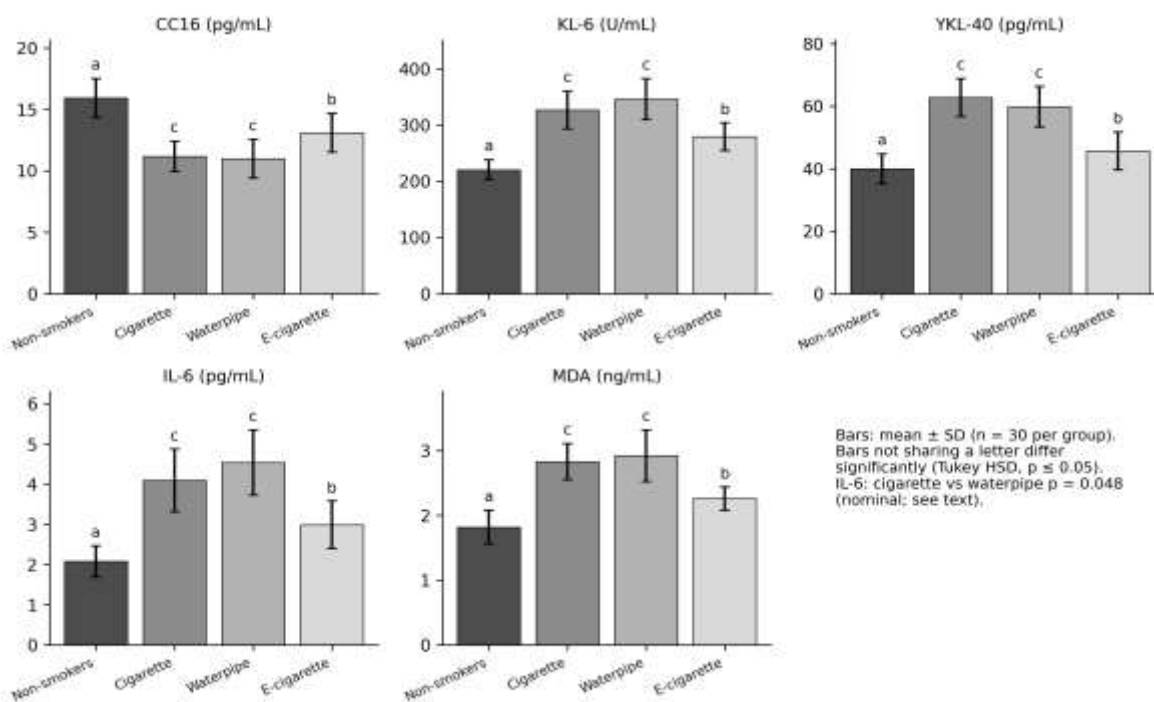
Neither galectin-3 ( $F = 1.48$ ,  $p = 0.223$ ) nor TREM-1 ( $F = 0.15$ ,  $p = 0.927$ ) differed among groups; mean values clustered around 8 ng/mL and 26 pg/mL, respectively, in all four groups. These two markers therefore did not distinguish smokers from non-smokers in this cohort.

**Table 2:** Serum pulmonary injury biomarkers, oxidative stress indices and IL-6 (mean  $\pm$  SD) by study group.

| Biomarker          | Non-smokers        | Cigarette          | Waterpipe          | E-cigarette        | F      | p       | $\eta^2$ |
|--------------------|--------------------|--------------------|--------------------|--------------------|--------|---------|----------|
| CC16 (pg/mL)       | 15.91 $\pm$ 1.59   | 11.17 $\pm$ 1.24   | 10.97 $\pm$ 1.57   | 13.09 $\pm$ 1.59   | 69.85  | < 0.001 | 0.644    |
| KL-6 (U/mL)        | 220.48 $\pm$ 17.68 | 326.70 $\pm$ 33.45 | 346.12 $\pm$ 36.65 | 279.35 $\pm$ 24.61 | 111.28 | < 0.001 | 0.742    |
| YKL-40 (pg/mL)     | 39.94 $\pm$ 4.74   | 62.76 $\pm$ 6.10   | 59.87 $\pm$ 6.47   | 45.70 $\pm$ 6.08   | 104.69 | < 0.001 | 0.730    |
| Galectin-3 (ng/mL) | 7.98 $\pm$ 0.97    | 8.43 $\pm$ 0.95    | 8.49 $\pm$ 1.05    | 8.43 $\pm$ 1.31    | 1.48   | 0.223   | 0.037    |

| Biomarker      | Non-smokers   | Cigarette      | Waterpipe      | E-cigarette   | F     | p       | $\eta^2$ |
|----------------|---------------|----------------|----------------|---------------|-------|---------|----------|
| TREM-1 (pg/mL) | 26.05 ± 2.76  | 26.41 ± 2.94   | 26.48 ± 2.65   | 26.45 ± 2.91  | 0.15  | 0.927   | 0.004    |
| IL-6 (pg/mL)   | 2.08 ± 0.39   | 4.09 ± 0.78    | 4.54 ± 0.81    | 2.99 ± 0.59   | 83.67 | < 0.001 | 0.684    |
| MDA (ng/mL)    | 1.82 ± 0.26   | 2.83 ± 0.28    | 2.92 ± 0.40    | 2.26 ± 0.18   | 94.91 | < 0.001 | 0.711    |
| SOD-1 (pg/mL)  | 121.97 ± 8.16 | 120.72 ± 10.31 | 117.78 ± 12.54 | 118.29 ± 8.37 | 1.18  | 0.319   | 0.030    |

**Note:** Values are mean ± SD; n = 30 per group. F and p from one-way ANOVA ( $df = 3, 116$ );  $\eta^2 = \text{eta-squared}$ . Units are those of each kit's calibrated standard curve with the manufacturer's five-fold dilution applied. CC16, club cell secretory protein-16; KL-6, Krebs von den Lungen-6; TREM-1, triggering receptor expressed on myeloid cells-1; IL-6, interleukin-6; MDA, malondialdehyde; SOD-1, superoxide dismutase-1.



**Figure 1.** Mean (± SD) serum concentrations of the five discriminating biomarkers in the four study groups (n = 30 per group). Bars not sharing a common letter differ significantly by Tukey HSD ( $p \leq 0.05$ ). Cigarette and waterpipe groups share a letter for every marker, indicating comparable measured effects of the two combustible products, with e-cigarette users occupying an intermediate position.

### 3.3 Oxidative stress indices and systemic inflammation

Lipid peroxidation, indexed by MDA, increased significantly across groups ( $F = 94.91$ ,  $p < 0.001$ ,  $\eta^2 = 0.711$ ), from  $1.82 \pm 0.26$  ng/mL in non-smokers to  $2.83 \pm 0.28$  and  $2.92 \pm 0.40$  ng/mL in cigarette and waterpipe smokers, with e-cigarette users intermediate ( $2.26 \pm 0.18$  ng/mL). Systemic IL-6 mirrored this gradient ( $F = 83.67$ ,  $p < 0.001$ ,  $\eta^2 = 0.684$ ), rising from  $2.08 \pm 0.39$  pg/mL in non-smokers to  $4.09 \pm 0.78$  and  $4.54 \pm 0.81$  pg/mL in cigarette and waterpipe smokers, and  $2.99 \pm 0.59$  pg/mL in e-cigarette users. Waterpipe smokers had the numerically highest IL-6 concentration; the contrast with cigarette smokers reached nominal significance (Tukey-adjusted  $p = 0.048$ ) but lies at the margin of the threshold and would not survive correction for the multiple markers tested, so it is interpreted cautiously rather than as evidence of a substantive difference between the two combustible products.

In contrast, SOD-1 protein concentration was closely similar across groups (117.8–122.0 pg/mL) and did not differ significantly ( $F = 1.18$ ,  $p = 0.319$ ). The combination of a clear, graded rise in MDA with unchanged SOD-1 protein indicates a dissociation between the oxidant load imposed by smoking and the expression of this first-line antioxidant protein in these relatively young smokers.

**Table 3:** Tukey HSD post-hoc pairwise comparisons (adjusted p values) for the five biomarkers with significant group effects.

| Comparison                 | CC16    | KL-6    | YKL-40  | IL-6    | MDA     |
|----------------------------|---------|---------|---------|---------|---------|
| Non-smokers vs cigarette   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Non-smokers vs waterpipe   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Non-smokers vs e-cigarette | < 0.001 | < 0.001 | 0.001   | < 0.001 | < 0.001 |
| Cigarette vs waterpipe     | 0.955   | 0.053   | 0.233   | 0.048   | 0.603   |
| Cigarette vs e-cigarette   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Waterpipe vs e-cigarette   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

**Note:** Significance threshold  $p \leq 0.05$ . The recurrent non-significant cigarette-versus-waterpipe contrast indicates a comparable measured effect of the two combustible modalities under the present sampling; in the absence of quantitative exposure data it should not be read as proof of biological equivalence. The IL-6 cigarette-versus-waterpipe contrast ( $p = 0.048$ ) is nominally significant only.

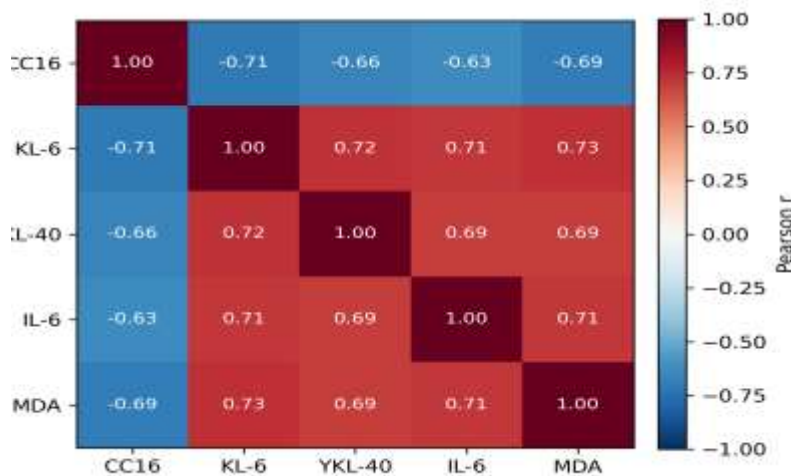
### 3.4 Correlations among biomarkers

Across the whole sample ( $n = 120$ ), the five discriminating biomarkers were strongly inter-correlated in a biologically coherent direction (Table 4, Figure 2). CC16 correlated negatively with every injury, oxidation and inflammation marker ( $r = -0.63$  to  $-0.71$ ; all  $p < 0.001$ ), reflecting loss of the protective epithelial protein as injury, oxidation and inflammation increase. The remaining markers correlated positively with one another ( $r = 0.69$ – $0.73$ ; all  $p < 0.001$ ), the strongest association being between KL-6 and MDA ( $r = 0.73$ ). This pattern is consistent with a single underlying axis linking epithelial injury, lipid peroxidation and IL-6-mediated inflammation.

**Table 4:** Pearson correlation matrix among the five biomarkers with significant group effects (whole sample,  $n = 120$ ).

|        | CC16  | KL-6  | YKL-40 | IL-6  | MDA   |
|--------|-------|-------|--------|-------|-------|
| CC16   | 1.00  | −0.71 | −0.66  | −0.63 | −0.69 |
| KL-6   | −0.71 | 1.00  | 0.72   | 0.71  | 0.73  |
| YKL-40 | −0.66 | 0.72  | 1.00   | 0.69  | 0.69  |
| IL-6   | −0.63 | 0.71  | 0.69   | 1.00  | 0.71  |
| MDA    | −0.69 | 0.73  | 0.69   | 0.71  | 1.00  |

**Note:** All correlations significant at  $p < 0.001$ . Negative coefficients for CC16 reflect its inverse relationship with the injury, oxidation and inflammation markers.



**Figure 2:** Pearson correlation heatmap among the five discriminating biomarkers (whole sample,  $n = 120$ ). The inverse loading of CC16 against the positively co-varying injury, oxidation and inflammation markers is consistent with a single exposure-responsive axis. All correlations were significant at  $p < 0.001$ .

#### 4. Discussion

This study compared a multidimensional biomarker panel among conventional cigarette, waterpipe and e-cigarette users within a single age-matched male cohort. Three findings stand out. First, a consistent and biologically coherent signature (reduced CC16 with elevated KL-6, YKL-40, IL-6 and MDA) discriminated all smokers from non-smokers. Second, the two combustible modalities produced statistically indistinguishable derangements on almost every measure, whereas e-cigarette use produced a consistently intermediate profile. Third, three of the markers examined (galectin-3, TREM-1 and SOD-1 protein) did not discriminate smoking status, a negative finding that carries its own interpretation.

The reduction in CC16 mirrors observations from prospective cohorts in which low circulating CC16 precedes and predicts lung-function decline and incident COPD [9,28]. Because club cells both secrete CC16 and participate in the detoxification of inhaled xenobiotics, depletion of these cells in chronic smokers offers a parsimonious explanation for the observed fall [8,10]; the partial preservation of CC16 in e-cigarette users is consistent with a smaller, though not trivial, epithelial insult. The elevation of KL-6 accords with its established role as a marker of alveolar epithelial damage [13,14,29], and the rise in YKL-40 agrees with earlier reports of increased serum chitinase-like protein in both combustible smokers and e-cigarette users [15,16,30].

It is noteworthy that waterpipe smoking produced derangements statistically similar to, and in the case of IL-6 numerically greater than, those of cigarette smoking, although the IL-6 contrast ( $p = 0.048$ ) is inconclusive. The waterpipe is widely perceived as a benign social alternative to cigarettes, yet toxicant dosimetry shows that users may inhale greater quantities of carbon monoxide and particulate matter than cigarette smokers and are therefore exposed to comparable or greater biological hazard [5,31,32]. Experimental and human studies likewise indicate that waterpipe smoke is at least as pro-inflammatory and pro-oxidant for the lung as cigarette smoke [31,33]. An important caveat applies to this comparison: exposure was not quantified (no pack-years, cotinine or exhaled carbon monoxide data were collected), so the statistical similarity of the two combustible groups may reflect the doses actually delivered rather than the intrinsic toxicity of the products. The finding is therefore best described as a similar measured effect rather than as proof of biological equivalence.

The intermediate position of e-cigarette users is in keeping with an emerging body of evidence. A recent meta-analysis of approximately 24,000 adults found that combustible smokers had higher inflammatory marker concentrations than both non-smokers and e-cigarette users, with the latter group typically falling between the two [34]. The 2025 Saudi comparison of cigarette, shisha, e-cigarette and non-smoking groups reached a convergent conclusion, with combustible products impairing lung function more than e-cigarettes while cigarette and shisha users displayed similar inflammatory profiles [27]. Controlled comparisons of combustible and electronic hookah also show a stronger oxidative and inflammatory signal with the combustible product, yet vaping is far from inert [35]. E-cigarette aerosols induce oxidant generation, epithelial toxicity and IL-6/IL-8 release in vitro and in vivo [36–39], cause vascular oxidative stress and endothelial dysfunction after even short-term exposure [40], and have recognised cardiovascular and systemic effects [41,42]. The present data therefore support a graded interpretation: on these indicators, e-cigarettes are less harmful than combustible products but are not equivalent to non-exposure.

The unchanged galectin-3 and TREM-1 concentrations warrant cautious interpretation. Their stability in a young, clinically healthy cohort suggests that the early, subclinical injury captured by CC16 and KL-6 had not yet progressed to the fibrotic remodelling or acute innate immune amplification that these proteins index [19,21]. The stable SOD-1 value requires even greater care. The present finding is not directly comparable with studies that measured SOD enzymatic activity, such as the 2024 pilot study of chronic smokers that reported reduced activity [23], because the assay used here quantifies the concentration of SOD-1 protein. An unchanged protein concentration does not exclude a loss of function: oxidative inactivation or post-translational modification can reduce catalytic activity without altering the amount of immunoreactive protein. The dissociation between clearly elevated MDA and stable SOD-1 protein should therefore be read as evidence of unchanged antioxidant protein expression, not of preserved antioxidant capacity, and direct activity assays would be needed to distinguish the two. Differences in age and exposure duration

between cohorts may also contribute. Overall, the null result for SOD-1 protein does not contradict the literature on SOD activity but adds a methodologically distinct observation.

The strong inter-correlations among the discriminating markers further support a single exposure-responsive pathway linking epithelial barrier injury, lipid peroxidation and IL-6-mediated inflammation in the systemic circulation. The inverse loading of CC16 on this axis is expected, given its anti-inflammatory and antioxidant properties and its contribution to the resolution of inflammation and repair of the airway epithelial barrier [8,43].

#### 4.1 Strengths and Limitations

The strengths of this study include the balanced group sizes, the age comparability across groups, the exclusion of dual users, and a panel that spans epithelial injury, oxidative stress and inflammation. Several limitations must be acknowledged. The cross-sectional design permits only associative inference, and the biomarker differences represent a single point in time. The most important limitation is the absence of a quantitative exposure measure; smoking was categorised by product only, so the statistical similarity of the combustible groups may reflect delivered dose rather than intrinsic toxicity, and no dose–response relationship could be modelled. Apart from age, potential confounders such as body mass index, socioeconomic status and the duration or intensity of use were not recorded, so no multivariable adjustment (for example by ANCOVA) was possible and residual confounding cannot be excluded. The young, single-sex, single-region sample limits generalisability, and the lack of spirometry prevents the biomarker changes from being linked to functional outcomes. Two analytical caveats also apply. First, the SOD-1 assay measured protein concentration rather than enzymatic activity, so stable SOD-1 does not imply preserved antioxidant activity. Second, all concentrations are expressed in the units of each manufacturer's calibrated standard curve, and the absolute values of some analytes lay near the nominal limits of their kits and depend on the dilution and calibration applied; the results should therefore be interpreted as relative comparisons between groups rather than as absolute reference concentrations, and confirmation on an orthogonal analytical platform would strengthen them.

### 5. Conclusion

In this matched male cohort, conventional cigarette, waterpipe and e-cigarette use were each associated with a clear biomarker profile of airway epithelial injury (reduced CC16, elevated KL-6 and YKL-40), lipid peroxidation (elevated MDA) and systemic inflammation (elevated IL-6). The two combustible products produced statistically similar and most severe derangements, contradicting the common perception of the waterpipe as a benign alternative, whereas e-cigarettes produced a measurable but consistently smaller effect. Because exposure intensity was not quantified, the similarity between the combustible groups should be regarded as a similarity of measured effect rather than of biological equivalence. CC16, KL-6, YKL-40, IL-6 and MDA behaved as sensitive and mutually consistent markers of early, subclinical lung injury, whereas galectin-3, TREM-1 and SOD-1 protein did not discriminate smoking status. These findings do not support the notion that e-cigarettes are a safe alternative to combustible products, while at the same time not overstating their lower biomarker burden. Longitudinal studies incorporating quantitative exposure metrics and lung-function testing are needed to establish causal pathways and clinical relevance.

#### 5.1 Future Directions

Future work should link these biomarkers to spirometric decline, include women and older adults, quantify exposure with cotinine or exhaled carbon monoxide, and follow e-cigarette users over time. Such studies would strengthen the evidence base and help resolve whether the intermediate profile of vaping represents a real reduction in risk or a delayed pathway to the same harm.

#### Declarations

**Ethics approval:** The study was approved by the Scientific Research Ethics Committee of Northern Technical University (no. 127A/26, 25 February 2026) and conducted in accordance with the Declaration of Helsinki.

**Consent to participate:** All participants gave written informed consent.

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**Conflicts of interest:** The author declares no conflict of interest.

**Data availability:** The data analysed in this study are available from the corresponding author upon reasonable request.

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