*Research article*

# Altered ocular surface health status and tear film immune profile due to prolonged daily mask wear in health care workers

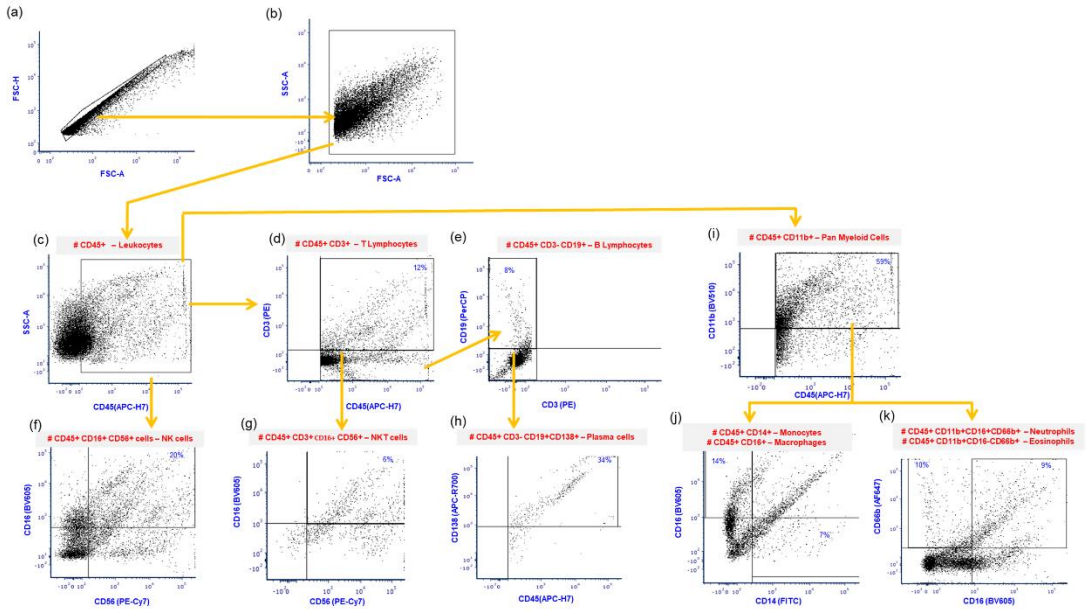


**Figure S1:** Gating strategy to phenotype ocular surface immune cell subsets: Representative images shows gating strategy used to determine the immune cell populations (specifically Leukocytes, T cells, B cells, Natural Killer cells, NKT cells, Pan Myeloid cells, Monocytes, Macrophages, Neutrophils, Eosinophils) in the ocular surface wash samples. The respective samples were stained for immune cell type-specific fluorochrome-conjugated antibodies and manual gating strategy (marked with arrows) were performed. (a) The scatter plot marked Forward scatter-A (FSC-A) versus Forward scatter-H (FSC-H) represents singlet population and (b) marked region in Forward scatter (FSC-A) versus Side Scatter (SSC-A) plot represents the total cell population. (c) The marked region CD45 (APC-H7) versus Side Scatter (SSC) represents the leukocytes population. (d) The upper right quadrant in this panel indicates T cells positively stained for both CD3 (PE) and CD45 (APC-H7). Bottom right quadrant which indicates leukocytes devoid of T cells were used to determine B cell population in the next panel. (e) The upper left quadrant in the panel represents B cell population - CD19 (PerCP) positive but CD3 (PE) negative cells. (f) The upper right quadrant in this panel indicates CD45 (APC-H7) positive cells stained for both CD16 (BV605) and CD56 (PE-Cy7) and they represent NK cells while (g) NKT cells represented in the plot are T cells shown in panel d that are positively stained for CD16 (BV605) and CD56 (PE-Cy7) as can be seen in the upper right quadrant. (h) plasma cells represented in the plot are B cells shown in panel e that are positively stained for CD138 (APC-R700) as can be seen in the upper right quadrant. (i) The upper right quadrant represents pan-myeloid cells stained positive for both CD45 (APC-H7) and CD11b (BV510). (j) The scatter plot represents pan-myeloid cells from panel h that are positive cells stained for CD14 (FITC) or CD16(BV605). The lower right quadrant shows monocytes that are exclusively positive for CD14 (FITC) while upper left quadrant shows macrophages that are exclusively stained positive for CD16 (BV605). (k) CD16 (BV605) versus CD66b (AF647) plot is used to identify neutrophils and eosinophils within in the pan-myeloid cells as seen in panel i. Neutrophils are cells that are positive for both CD16 (BV605) and CD66b (AF647) – upper right quadrant. Eosinophils are represented in the upper left quadrant are stained only for CD66b (AF647). The percentage of each immune cell subsets for each study subject was computed using number of positively stained cells (cell specific markers) to number of CD45 positive cells (leukocytes) obtained from FCS express 6.

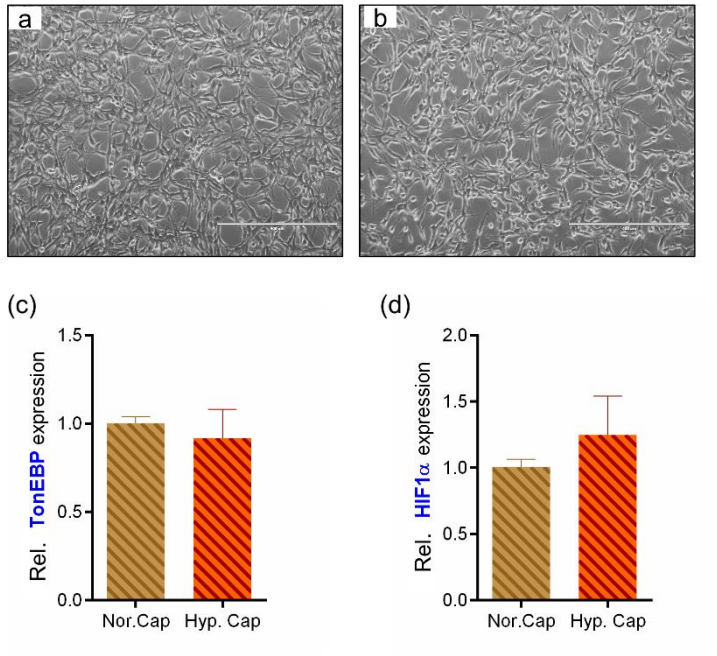


**Figure S2:** Effect of hypercapnia on cellular morphology and osmotic stress response genes in human corneal epithelial cells. Panels shows the morphology of SV40 immortalized human corneal epithelial cells (HCE2) following exposure to either (a) 5% CO2 (Normocapnia – Nor. Cap) or (b) 20% CO2 (Hypercapnia – Hyp. Cap), in vitro for a period of 24 hours in vitro using a bright field microscope at 10x magnification. The microscopic images shown are representative images of three different fields from three independent experiments. Graphs indicate mean relative mRNA expression of TonEBP (c) and HIF1α (b) in SV40 immortalized human corneal epithelial cells (HCE2) exposed to either 5% CO2 (Normocapnia – Nor. Cap) or 20% CO2 (Hypercapnia – Hyp. Cap), in vitro for a period of 24 hours. The expression of TonEBP and HIF1α were normalized to expression of β-Actin (housekeeping gene). Bar graph indicates Mean±SEM from two technical replicates for each of the three biological replicate experiments. TonEBP – Tonicity-responsive enhancer-binding protein; HIF1α – Hypoxia-inducible factor-1α.

**Table S1:** The levels of cytokines in the tear fluid of study subjects.

| Analytes (pg/ml) | Pre-FM | | | Post-FM | | | P value |
|---|---|---|---|---|---|---|---|
| | Mean | Stdev | SEM | Mean | Stdev | SEM | |
| IL-1α | 56 | 61 | 10 | 81 | 58 | 10 | 0.037 |
| IL-1β | 166 | 171 | 29 | 296 | 196 | 34 | 0.005 |
| IL-2 | 15 | 32 | 5 | 44 | 85 | 15 | 0.033 |
| IL-6 | 4 | 8 | 1 | 2 | 8 | 1 | 0.045 |
| IL-8 | 1189 | 3962 | 679 | 50 | 45 | 8 | < 0.0001 |
| IL-12/IL-23p40 | 1297 | 1853 | 318 | 542 | 725 | 124 | 0.095 |
| IL-13 | 14 | 27 | 5 | 3 | 10 | 2 | 0.030 |
| IL-17A | 1 | 3 | 1 | 1 | 4 | 1 | 0.882 |
| IL-18 | 95 | 102 | 18 | 41 | 60 | 10 | 0.001 |
| IL-21 | 602 | 933 | 160 | 1099 | 1761 | 302 | 0.249 |
| IL-33 | 522 | 960 | 165 | 1322 | 1053 | 181 | 0.001 |
| IFNα | 15 | 24 | 4 | 26 | 66 | 11 | 0.723 |
| IFNβ | 525 | 602 | 103 | 902 | 720 | 123 | 0.010 |
| IFNγ | 2 | 11 | 2 | 8 | 33 | 6 | 0.465 |
| TNFα | 5 | 10 | 2 | 1 | 2 | 0 | 0.101 |

**Table S2:** The levels of chemokines and growth factors in the tear fluid of study subjects.

| Analytes (pg/ml) | Pre-FM | | | Post-FM | | | P value |
|---|---|---|---|---|---|---|---|
| | Mean | Stdev | SEM | Mean | Stdev | SEM | |
| Fractalkine | 1228 | 1146 | 197 | 937 | 962 | 165 | 0.488 |
| GRO$\alpha$ | 1950 | 1988 | 341 | 1489 | 2099 | 360 | 0.661 |
| IP-10 | 688731 | 3967968 | 680501 | 9091 | 18000 | 3087 | 0.278 |
| I-TAC | 997 | 2426 | 416 | 153 | 145 | 25 | 0.002 |
| MCP-1 | 182 | 164 | 28 | 187 | 172 | 30 | 0.919 |
| MIG | 768 | 2026 | 353 | 564 | 1543 | 265 | 0.129 |
| RANTES | 57 | 105 | 18 | 16 | 19 | 3 | 0.001 |
| BDNF | 169 | 197 | 34 | 350 | 246 | 42 | 0.001 |
| NGF | 10 | 7 | 1 | 15 | 15 | 3 | 0.062 |
| HGF | 1708 | 1247 | 214 | 1157 | 1252 | 215 | 0.005 |
| VEGF | 721 | 529 | 91 | 358 | 251 | 43 | < 0.0001 |

**Table S3:** The levels of soluble cell adhesion molecules, soluble receptors and enzymes in the tear fluid of study subjects.

| Analytes (pg/ml) | Pre-FM | | | Post-FM | | | P value |
|---|---|---|---|---|---|---|---|
| | Mean | Stdev | SEM | Mean | Stdev | SEM | |
| sICAM1 | 6244 | 10808 | 1854 | 2864 | 5146 | 882 | 0.062 |
| sVCAM | 440 | 781 | 134 | 42 | 126 | 22 | 0.001 |
| sL-selectin | 2249 | 4114 | 706 | 130 | 449 | 77 | 0.001 |
| sP-selectin | 710 | 1635 | 280 | 255 | 517 | 89 | 0.184 |
| sIL-1R1 | 324 | 264 | 45 | 164 | 270 | 46 | 0.001 |
| sIL-1R2 | 337 | 620 | 106 | 48 | 39 | 7 | 0.024 |
| sIL-2Ra | 124 | 107 | 18 | 166 | 136 | 23 | 0.048 |
| sTNFRI | 126 | 153 | 26 | 257 | 311 | 53 | 0.001 |
| sTNFRII | 44 | 56 | 10 | 14 | 19 | 3 | 0.008 |
| LIF | 2268 | 2146 | 368 | 3973 | 2454 | 421 | 0.001 |
| Angiogenin | 51572 | 65803 | 11285 | 46033 | 37368 | 6409 | 0.723 |
| NGAL | 2319 | 1936 | 332 | 633 | 549 | 94 | < 0.0001 |
| Granzyme | 2811 | 1718 | 295 | 1398 | 1145 | 196 | 0.001 |
| Perforins | 409 | 540 | 93 | 618 | 606 | 104 | 0.027 |
| TSLP | 102 | 152 | 26 | 264 | 191 | 33 | 0.001 |