To Study Acute Changes in Brain Oxygenation on MRI in Healthcare Workers Using N95 Mask and PPE Kits for Six Hours a Day

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Keywords
► BOLD
► brain oxygenation
► hypoxia
► ASL
► N95 mask and PPE kit

Abstract

Background  Due to long working hours wearing an N95 mask and PPE kit during the COVID-19 pandemic, the healthcare workers (HCWs) complained of headaches, confusion, and exhaustion. This study was therefore performed to study the changes in brain oxygenation.

Aim  To compare brain oxygenation in health care workers wearing an N95 mask with a PPE kit versus a three-ply mask during an intensive care setting for 6 hours.

Materials and Methods  Thirty clinicians and 30 paramedical staff participated in the study. The control (three-ply mask) and subject (N95 mask with PPE) groups included 15 clinicians and 15 paramedical staff. A comparative analysis of brain oxygenation using a 3T magnetic resonance imaging (MRI) machine was performed in these two groups at the beginning and the end of their work shift.

Results  The mean age of the individuals in the control and subject groups was 30.8 and 30.13 years, respectively. The median value of brain oxygenation in the control and subject groups in the pre-shift was between 33 and 31 and post-shift was 30 and 24. The drop in brain oxygenation in subjects was more than the controls (p = 0.004) in the post-shift assessments. The cerebral blood flow (CBF) in the bilateral middle cerebral artery (MCA) using arterial spin labeling (ASL) showed a rise in CBF in both groups post-shift as compared with the pre-shift values. The median values of the right and left MCA in the control and subject groups pre-shift were 82.75/83.45 and 89.75/106.65. The post-shift median values of both MCAs of the control and subject groups were 115.65/115.55 and 109.60/119.49.

Conclusion  MRI-BOLD imaging revealed a significant drop in brain oxygenation in the subject group as compared with the control group. Multiphasic-ASL showed a compensatory rise in CBF in both groups.
Introduction

With rapidly spreading COVID-19 viral infection, healthcare workers (HCWs) around the world were compelled to wear N95 masks and PPE kits for their protection while treating COVID-positive patients. This situation brought a very rapid and sudden change in the way doctors treated patients. Our hospital is a designated tertiary care hospital for treating COVID patients since April 2020. The hospital staff at various levels in the ICU and non-ICU settings have been working with the complete PPE during their working hours in the hospital. Due to fixed working hours daily, for weeks together, the HCW’s encountered symptoms of increasing frequencies of headaches, lethargy, dizziness, blurring of vision, nausea, vomiting, fogging of thoughts with a few experiencing confused state of mind. Thus, there was a need to investigate the underlying cause, and hence the study was undertaken. This is the first observational study of its kind during the pandemic as per our knowledge and one of its kind to demonstrate in vivo changes in human brain oxygenation using MRI.

The human brain even in the resting state is not truly at rest and needs a constant supply of oxygen. The oxygen demand increases significantly during various activities performed throughout the day. Although the total brain volume is ~2 to 3% of the body weight, its oxygen requirement is high and varies from 18 to 20% of the total oxygen available. This makes the brain highly sensitive and susceptible to the alterations in oxygenation levels with resultant vulnerability to hypoxia.\(^1\) It is a known fact that there is a difference in blood supply and oxygenation depending upon the preferred areas in the brain, hence certain parts of the brain that are metabolically active receive more blood supply and therefore more oxygenation than the rest of the brain parenchyma.\(^2\) These high metabolic areas are predominantly cortex and deep gray matter, so we can observe the change in brain oxygenation in these areas during periods of high demand.\(^3\)

Hypoxia

The pathophysiology of hypoxia is extensively documented with various signaling pathways regulating cerebral blood flow to maintain brain tissue oxygenation. Recent studies show the important role of deoxyhemoglobin in regulating CBF and resultant brain tissue oxygenation.\(^4\) As carbon dioxide (CO\(_2\)) is the end product of oxidative metabolism and a principal regulator of the cerebral circulation, its increasing levels during reduced oxygen supply causes vasodilatation and a drop in vascular resistance.\(^5\) This will result in vasodilatation of blood vessels and a resultant increase in the cerebral blood flow for maintaining brain oxygenation. To document these changes, advanced MRI techniques such as resting-state functional MRI BOLD and ASL were used.

BOLD

The BOLD signal depends on the arterial pressure of both oxygen (O\(_2\)) and CO\(_2\) levels and any change in these levels is the basis of fMRI. It indirectly shows the oxygen consumption of brain tissue even in a resting state.\(^6\)

ASL

Multiphasic ASL is a non-contrast perfusion scanning technique of the brain that shows cerebral blood flow through the use of tagged red blood cells.\(^7\)

Materials and Methods

The study was performed at a tertiary care center, approved by the institutional review board. Informed consent was obtained in writing from each participant after explaining the procedure. Participants were also required to fill a form with a list of symptoms that could be associated with brain hypoxia. The symptom of headache was graded on the Wong–Baker pain rating scale.

Participants

Our study consisted of 30 doctors and 30 nurses. The control group consisted of 15 doctors and 15 nurses wearing three-ply masks for 6 hours. Similarly, the subject group also consisted of 15 doctors and 15 nurses wearing N95 masks and PPE for 6 hours. Exclusion criteria were clinically diagnosed cases of hypertension, diabetes mellitus, asthma, and history of cerebrovascular disease.

Study Design

A comparative analysis of brain oxygenation was performed for the HCWs wearing three-ply masks versus those wearing N95 masks with PPE kits enrolled in this study. The study structure was based on a baseline MRI to be performed on the participants at the beginning of their work shifts. The second MRI was performed after working for at least 6 hours. Specific instructions were given to the subjects during the first session of MRI to follow the guidelines for the second MRI protocol, immediately post-doffing without taking anything orally. The mean time taken between post-doffing and the initiation of the MRI image acquisition was 10 minutes.

MRI Acquisition and Post-processing

All MRI sequences were conducted on a 3T MRI scanner (Ingenia; Philips Medical Systems, Netherlands) using a 15-channel digital head coil. A volume three-dimensional (3D) fluid-attenuated inversion recovery (FLAIR) image was acquired and used for the registration of BOLD images. Multiphasic ASL data were obtained and post-processed with a Td of 1.1 seconds.

Acquisition: The imaging protocols used in our study were sagittal 3D FLAIR (TR/TE, 4800/315; flip angle, 1650; FOV, 250; NSA, 2; Voxel size 1.12 x 1.12 x 1.12 and acquisition matrix of 224 x 224), BOLD (TR/TE, 3000/35; FOV, 230; slice thickness, 4 mm; NSA, 1; matrix, 128) and ASL (TE, 16; flip angle, 40; FOV, 240; slice thickness, 5 mm; NSA, 1; matrix, 80).

Images once obtained were post-processed on a Philips workstation using Intellispace Portal (ISP) software, version 10. The images were interpreted by three in-house neuroradiologists with one having over 20 years of experience and the other two with over 4 years of experience.

The 3D FLAIR images were used as the template on which BOLD images were registered and used for localization. The T value of BOLD was adjusted ranging from 3 to 5.
To establish an objective pattern of interpretation for images in the BOLD sequence, a quantitative scoring pattern was formulated. The cortical areas in the frontal, temporal, parietal, occipital lobes, and cingulate gyrus on both sides were quantified. A score of 1 to 4 was allotted to each area on both sides depending on the BOLD signal (<Table 1>). As there is no available software to quantify the BOLD images and scoring system, we used virtual tagging and formulated the following scoring system with an excellent inter-observer agreement with K-value > 9 and p < 0.01.

A total score of 40 was obtained from the four lobes and the cingulate gyrus on each side (<Table 1>). The score reflects the amount of brain oxygenation in the particular region. The scoring was done on both the subjects and the control group at the beginning of the work shift and after 6 hours of work. The control and subject groups consisted of 15 doctors and 15 staff nurses each. Therefore, 120 MRI studies were performed and scored.

On the multiphasic ASL, images with Td of 1.1 seconds were used for quantification, and a region of interest (ROI) of ~15 mm² was placed in the middle cerebral artery (MCA) on both sides in all 120 MRI acquisitions and the quantitative values were documented.

### Statistical Analysis

After data collection, data entry was done in Microsoft Excel. Data analysis was done with the help of SPSS software version 23 (IBM corp. USA). Quantitative data such as the age of study participants, headache on Likert scale, and brain oxygenation levels on MRI of all the lobes pre- and post-work wearing three-ply and N95 masks with PPE and the difference between them are presented using mean, standard deviation, median, and interquartile range (IQR). Qualitative data such as gender, type of symptoms, and occupation are presented using frequency and percentage. K means clustering algorithm was used with R.

The association among study participants who had symptoms and mask groups was assessed using the chi-square (χ²) test. The data not normally distributed were assessed using the Shapiro–Wilk test.

The comparison of MRI values in all the lobes between three-ply and N95 mask users pre- and post-work and the difference between them was assessed using the Mann–Whitney U test.

The level of significance was considered at a 95% confidence interval (p < 0.05).

### Results and Observations

In our study of 60 participants, there were 30 in the control group and 30 in the subject group. In the control group, there were 13 males and 17 females. The minimum age was 20 years, and the maximum age was 50 years with a mean age of 30.8 years. In the subject group, there were 10 males and 20 females. The minimum age was 23 years and the maximum age was 48 years with a mean age of 30.13 years (<Table 2>).

The median value of total brain oxygenation in the control group before the start of their work (pre) was found to be 33 (<Fig. 1A>) and at the end of their work shift (post) was found to be 30 (<Fig. 1B>); (<Table 3>).

![Fig. 1](A) Pre-work resting-state fMRI BOLD image of control showing baseline brain oxygenation. (B) Post-work resting-state fMRI BOLD image of control showing a marginal decrease in brain oxygenation. (C) Pre-work ASL showing baseline CBF. (D) Post-work ASL showing an increase in the CBF. fMRI, functional magnetic resonance imaging.

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### Table 1  Scoring system

<table>
<thead>
<tr>
<th>Percentage of oxygenation (BOLD signal)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25</td>
<td>1</td>
</tr>
<tr>
<td>26–50</td>
<td>2</td>
</tr>
<tr>
<td>51–75</td>
<td>3</td>
</tr>
<tr>
<td>76–100</td>
<td>4</td>
</tr>
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### Table 2  Demographics of study participants

<table>
<thead>
<tr>
<th></th>
<th>3 PLY</th>
<th>N95</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percentage</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>43.3%</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
<td>56.7%</td>
</tr>
<tr>
<td>Mean Age in (y)</td>
<td>30.80</td>
<td>7.76</td>
</tr>
</tbody>
</table>

Abbreviation: SD, standard deviation.
### Table 3 Median comparison of brain $O_2$ and MCA $O_2$ levels before and after using 3 ply mask and N95 mask with PPE kit

<table>
<thead>
<tr>
<th>Variable</th>
<th>3 PLY Pre</th>
<th>IQR</th>
<th>Mean rank</th>
<th>Post</th>
<th>IQR</th>
<th>Mean rank</th>
<th>p-Value</th>
<th>N95 Pre</th>
<th>IQR</th>
<th>Mean rank</th>
<th>Post</th>
<th>IQR</th>
<th>Mean rank</th>
<th>p-Value</th>
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</thead>
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<tr>
<td>Frontal</td>
<td>3.00</td>
<td>1.25</td>
<td>31.52</td>
<td>3.00</td>
<td>1.00</td>
<td>33.53</td>
<td>0.077</td>
<td>3.00</td>
<td>0.25</td>
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<td>1.00</td>
<td>27.47</td>
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<td>Temporal</td>
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<td>1.00</td>
<td>31.82</td>
<td>2.00</td>
<td>1.00</td>
<td>36.10</td>
<td>0.669</td>
<td>2.00</td>
<td>1.25</td>
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<td>2.00</td>
<td>1.00</td>
<td>24.90</td>
<td>0.015</td>
</tr>
<tr>
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<td>1.00</td>
<td>36.00</td>
<td>3.50</td>
<td>1.00</td>
<td>36.65</td>
<td>0.025</td>
<td>3.00</td>
<td>1.00</td>
<td>25.00</td>
<td>2.50</td>
<td>1.00</td>
<td>24.35</td>
<td>0.022</td>
</tr>
<tr>
<td>Occipital</td>
<td>3.50</td>
<td>1.00</td>
<td>37.10</td>
<td>3.00</td>
<td>1.25</td>
<td>37.25</td>
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<td>2.00</td>
<td>2.00</td>
<td>23.75</td>
<td>0.084</td>
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<tr>
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<td>1.00</td>
<td>30.67</td>
<td>4.00</td>
<td>0.25</td>
<td>34.67</td>
<td>0.480</td>
<td>4.00</td>
<td>1.00</td>
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<td>1.25</td>
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<tr>
<td>MCA</td>
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<td>50.95</td>
<td>27.52</td>
<td>115.65</td>
<td>90.36</td>
<td>32.35</td>
<td>&lt;0.001</td>
<td>93.45</td>
<td>49.81</td>
<td>33.48</td>
<td>115.55</td>
<td>68.03</td>
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<td>1.00</td>
<td>33.30</td>
<td>0.018</td>
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<tr>
<td>Temporal</td>
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<td>1.00</td>
<td>31.85</td>
<td>2.00</td>
<td>1.00</td>
<td>36.15</td>
<td>0.405</td>
<td>2.00</td>
<td>1.25</td>
<td>29.15</td>
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<td>1.00</td>
<td>24.85</td>
<td>0.011</td>
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<tr>
<td>Parietal</td>
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<td>1.00</td>
<td>35.58</td>
<td>3.00</td>
<td>1.50</td>
<td>34.95</td>
<td>0.001</td>
<td>3.00</td>
<td>1.25</td>
<td>25.42</td>
<td>2.50</td>
<td>1.00</td>
<td>26.05</td>
<td>0.025</td>
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<tr>
<td>Occipital</td>
<td>3.00</td>
<td>1.00</td>
<td>36.57</td>
<td>3.00</td>
<td>1.25</td>
<td>36.78</td>
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<td>2.00</td>
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<td>24.22</td>
<td>0.097</td>
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<tr>
<td>Cingulate</td>
<td>4.00</td>
<td>1.00</td>
<td>30.20</td>
<td>4.00</td>
<td>1.00</td>
<td>34.28</td>
<td>0.739</td>
<td>4.00</td>
<td>1.00</td>
<td>30.80</td>
<td>3.50</td>
<td>1.25</td>
<td>26.72</td>
<td>0.032</td>
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<tr>
<td>MCA</td>
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<td>87.77</td>
<td>27.52</td>
<td>103.60</td>
<td>71.60</td>
<td>28.75</td>
<td>0.041</td>
<td>106.65</td>
<td>62.76</td>
<td>33.48</td>
<td>119.49</td>
<td>101.18</td>
<td>32.25</td>
<td>0.116</td>
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<tr>
<td>Total</td>
<td>33.00</td>
<td>8.50</td>
<td>34.65</td>
<td>30.00</td>
<td>8.50</td>
<td>37.07</td>
<td>0.032</td>
<td>31.00</td>
<td>10.00</td>
<td>26.35</td>
<td>24.00</td>
<td>10.25</td>
<td>23.93</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Abbreviation: IQR, interquartile range; MCA, middle cerebral artery.
Similarly, the median value of total brain oxygenation in the subject group before the start of their work was found to be 31 (Fig. 2A) and at the end of their work shift was found to be 24 (Fig. 2B). The drop in brain oxygenation in the subject group was found to be more than that in the control group with a $p = 0.004$. Also, it was observed that pre-work shift brain oxygenation of subjects was lower than that in the control group, which dropped significantly further at the end of the work shift (Table 3).

The CBF was measured in bilateral middle cerebral arteries using ASL, which showed a rise in CBF in both the control and subject groups at the end of their work shift as compared with the pre-shift values. The median values of the right and left MCA in the control group pre-shift were 82.75 and 89.75, respectively (Fig. 1C). Similarly, the pre-shift values of the subjects were 93.45 and 106.65 (Fig. 2C). The post-shift median values of both MCAs of the control group were 115.65 (right) and 109.60 (left) (Fig. 1D). The post-shift median values of both MCAs of the subject group were 115.55 (right) and 119.49 (left) (Fig. 2D). The pre-shift ASL values in the subject group were higher than in the control group; however, there was a modest increase in CBF in the subject group as compared with the control in the post-shift study, which suggested possible acclimatization to low levels of O$_2$ in the subject group.

The cingulate gyri in the subjects showed a higher drop in brain oxygenation as compared with that in the control group with $p = 0.018$. It was also observed that the subject population with age less than 25 years and more than 35 years showed a comparatively bigger drop in cingulate gyrus oxygenation (Fig. 3). Also, 80% of the subjects above 35 years of age with a significant drop in oxygenation in the cingulate gyrus had clinical complaints of a confused state.

Headache was reported in 20 subjects and 10 controls. The intensity of headache in the subject group was much higher as compared with that in the control group ($p = 0.002$). It was also observed female subjects had more headaches as compared with the females in the control group. The severity of the headache increased in the subjects that showed a big difference in the pre- and post-ASL than those subjects who showed a relatively smaller difference (Figs. 4 and 5).

**Discussion**

Our study was performed 5 months after the onset of the COVID pandemic in India during which the HCWs in our institute were routinely wearing the N95 masks and PPE kits. We observed in our study that the brain oxygenation significantly dropped in the subjects at the end of their work shifts compared with that in the control group. The subjects showed a drop in brain oxygenation with a median value of 15.5%, while the control group also showed a small drop in brain oxygenation with a median value of 6.7% (Table 4). It

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**Fig. 2** (A) Pre-work resting-state fMRI BOLD image of subject showing baseline brain oxygenation, which is lower than that in the control. (B) Post-work resting-state fMRI BOLD image of subject showing a significant decrease in brain oxygenation. (C) Pre-work ASL showing baseline CBF, which is marginally increased as compared with that in the control. (D) Post-work ASL showing a marginal increase in the CBF. fMRI, functional magnetic resonance imaging.

**Fig. 3** (A and B) The drop in cingulate gyrus oxygenation is relatively more in the age groups less than 25 years and more than 35 years.
was also observed that the baseline brain oxygenation in subjects was at a lower level compared with that in the control group.

The pathophysiology of cerebral hypoxia is complex and multifactorial. It primarily depends upon the severity and duration of hypoxia. Hypoxia can be divided broadly into two categories. One is acute hypoxic hypoxia and the second is chronic hypoxic hypoxia. The duration for acute hypoxia can vary from seconds to hours and days to years for chronic hypoxia. The cerebral vasculature responds to hypoxia and hypercapnia but only after the PaO₂ level drops below 50 mm Hg. As pCO₂ is a strong vasodilator, there is dilatation of the pial vessels and decreased vascular resistance and a resultant increase in CBF. The vasodilatation also occurs in the intracranial and extracranial carotid and vertebral arteries. In chronic hypoxia, the blood is diverted to vital centers such as the brain and heart.

**Fig. 4** (A) Pre-work resting-state fMRI BOLD image of a subject with a severe headache. (B) Post-work resting-state fMRI BOLD image of subject showing no significant change. (C) Pre-work ASL showing baseline CBF. (D) Post-work ASL showing a significant increase in the CBF. fMRI, functional magnetic resonance imaging.

**Fig. 5** Scatter diagram depicting the correlation between headache (Likert scale) and difference in oxygenation at right and left MCA. MCA, middle cerebral artery.

**Table 4** Comparison between median total percentage drop in brain oxygenation on MRI between control and subject groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
</tr>
<tr>
<td>Total drop (%)</td>
<td>6.7</td>
<td>25.9</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; MRI, magnetic resonance imaging.
as the brain stem; hence, the neuronal oxygenation is maintained; however, the non-vital areas will remain relatively hypoxic. This probably explains why the subjects had a lower baseline of brain oxygenation as compared with the control group.

The most affected lobes were the parietal and occipital lobes and to a lesser extent the temporal lobes. There was a redistribution of the brain oxygenation in the frontal lobes and anterior cingulate gyri, as these areas are vital for maintaining cognitive functions as a compensatory mechanism. Seven subjects showed poor compensation in maintaining oxygenation in the anterior cingulate gyri that correlated with clinical symptomatology of a confused state of mind ($p = 0.024$).

**Headache**

Headache was the commonest symptom encountered in both the control and subject groups. Under normal conditions, the brain compensates for hypoxia by increasing the CBF, as CO$_2$ is a potent vasodilator of the arteries. This increase in the CBF is observed on ASL in our study. There are multiple other pathways of CBF autoregulation, which during episodes of prolonged intermittent hypoxia get impaired. This is why the baseline CBF in N95 mask and PPE kit users was higher than in the control group and showed a marginal increase as compared with the control group.

**Confusion**

It was observed that those in the subject group showing more than 50% drop in oxygenation in anterior cingulate gyri showed clinical symptoms of a confused state (-Fig. 6). This is because the cingulate gyrus is an integral part of the cognition circuit.

Although the clinical symptoms of nausea, vomiting, dizziness, blurring of vision, lethargy, somnolence, and fatigability were seen more frequently in subjects than in the control group, there was no statistically significant correlation. No individual from the control and study group reported a loss of consciousness. A large majority of the HCWs in the subject group started showing symptoms especially headache at around 3 hours after wearing an N95 mask and PPE kit.

These findings are significant and predict the future possibility of neurodegenerative diseases. For example, a study by Peers et al revealed how chronic hypoxia increases the likelihood of Alzheimer’s disease.

The shortfall of our study is that there was no software available in the market to quantify brain oxygenation at present, hence we devised this scoring pattern. Also, the sample size of our study was small with only 60 participants and 120 MRI studies in total. However, our proposed method of interpretation provides a template for interpreting and grading BOLD MRI images for any possible future studies.

**Recommendation**

We recommend the following modifications in the work pattern:

1) A 1-hour break after 3 hours of continuous work shifts for HCWs wearing N95 masks with a PPE kit as we have observed in our study that the majority of them showed symptoms at the 3-hour mark.
2) During the break, the HCW should perform deep breathing exercises in the open air.
3) Maintain adequate hydration.
4) Adequate spacing of duties.
5) General population should not wear an N95 mask.
6) Do not exercise/play active sports/strenuous work wearing an N95 mask.

**Summary**

The study showed a significant drop in brain oxygenation with a modest increase in CBF in the subjects, whereas the control group showed a mild drop in brain oxygenation and more increase in CBF.

**Key Results**

1. Comparatively higher drop in brain oxygenation in the HCWs wearing N95 mask and PPE kit ($p = 0.004$).
2. Comparatively higher rise in the cerebral blood flow on ASL imaging in HCWs wearing a three-ply mask.
3. Low baseline brain oxygenation in the HCWs wearing an N95 mask and a PPE kit.
Conflicts of Interest
All authors disclose no relevant relationships.

Acknowledgments
We give our sincere thanks to all the healthcare workers who participated in the study. Special mention to Sister Elizabeth Joseph and Sister Deepthi D. for coordinating the study and technicians Satish Chavan and Atish Shinde for post-processing the images.

References