Which are the best in protecting the upper respiratory tract, KN95® or Acchadana®-herbal masks on IL-6 perspective?


ABSTRACT

Background: In the new normal era, face masks are very important for everyday use. Masks began to be developed involving herbal plants with anti-inflammatory and antibacterial potential. This study aims to prove the potential of KN95 masks and Acchadana® Herbal Masks.

Methods: This randomized control trial has 50 laboratory workers as subjects. Subjects were divided into two groups. The control group wore KN95® masks (n=25), and the treatment group wore Acchadana® herbal masks (n=25, 1 dropout). Each group wore a mask for 60 days. Then a nasal wash sample was taken for a laboratory test for IL-6 levels using the ELISA method.

Result: There was no significant comparison of IL-6 levels between the KN95® mask group and the herbal mask group in the Mann Whitney test (p=0.242). When viewed from the high and low levels of IL-6 in the respiratory tract of laboratory workers after 60 days of wearing masks, IL-6 levels in the Acchadana® mask group experienced a greater decrease compared to the KN95 mask group. However, the protection of the two masks was not significantly different (KN95® = -0.604 pg/ml and Herbal = -1.026 pg/ml; p>0.05).

Conclusion: KN95® masks and Acchadana® herbal masks have equal ability to protect the respiratory tract.

Keywords: Acchadana herbal mask, IL-6, KN95, upper respiratory tract.

INTRODUCTION

During the Covid-19 pandemic, face masks have become very important for everyday use. Masks are part of comprehensive prevention and control to limit the spread of viruses that attack the respiratory tract. Masks have been recommended as potential personal protective equipment (PPE) in preventing the transmission and spread of Covid-19, and the high cases of Covid-19 have caused a global shortage of N95®/KN95® respirators and surgical masks used by health workers, where N95 masks */KN95® is considered to have the best potential. As PPE, masks protect the respiratory tract from harmful particles in the air that can initiate infection or inflammation, especially in the respiratory tract. Air that is contaminated badly causes the respiratory tract to catch a lot of foreign particles excessively and trigger inflammation. Inflammation of the respiratory tract will easily occur in inhaling reactive chemicals continuously and repeatedly.

Face mask modifications continue to be developed to maximize respiratory protection. There are many modifications to face masks, such as the materials used, sizes, and types of filters. Several previous studies have shown that there are innovations in facial masks containing a combination of Nephrolepis exaltata herbal plant extracts and Hibiscus rosa sinensis as an antibacterial or anti-inflammatory, which is patented under the Acchadana® brand (Brand Patent No. IDM000921225). Acchadana® herbal masks can improve the nasal mucociliary transport rate, increase sinonasal IgA antibody levels, and improve lung function in industrial textile workers. Acchadana® herbal masks were also reported to significantly reduce Reactive Oxygen Species (ROS) levels and inhibit the pro-inflammatory cytokine interleukin-6 (IL-6) initiation in the respiratory tract.

In general, IL-6 is involved in innate and adaptive immune response mechanisms to defend against bacteria, viruses, or pathogens. Interleukin-6 is a pleiotropic cytokine produced by monocytes and macrophages in response to tissue damage and infection. IL-6 will activate Toll-Like Receptors (TLRs) as an inflammatory mediator in further chronic inflammation. Although the Acchadana® herbal mask has been proven to have this medical potential, it is not yet known whether it is comparable to KN95®, and this study proves its protective potential by measuring the levels of the cytokine IL-6 formed in the sinonasal.
Table 1. Demographics and subject research data based on mask groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mask</th>
<th>KN95</th>
<th>Acchadana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Age</td>
<td>33.36 ± 8.93</td>
<td>33.96±9.47</td>
<td>33.96±9.47</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>20</td>
<td>80.0</td>
<td>19</td>
</tr>
<tr>
<td>Woman</td>
<td>5</td>
<td>20.0</td>
<td>5</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>130.20±24.45</td>
<td>1.38±21.76</td>
<td>1.38±21.76</td>
</tr>
<tr>
<td>Diastolic</td>
<td>82.88±10.35</td>
<td>79.33±12.87</td>
<td>79.33±12.87</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>8.00</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>92.00</td>
<td>20</td>
</tr>
<tr>
<td>Respiratory disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>8.00</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>23</td>
<td>92.00</td>
<td>24</td>
</tr>
</tbody>
</table>

METHODS

Study Design
This research is a randomized control trial with a purposive sampling technique. The subjects were 50 laboratory workers from different laboratories (blood service, blood component, serology-hematology and infection). Each laboratory is taken half randomly and divided into two groups. The control group wore KN95® masks (n=25), and the treatment group wore Acchadana® herbal masks (n=25, 1 dropout). Each subject received a mask, according to the group, which was given periodically every two weeks. The inclusion criteria in this study were subjects who were in good health based on the results of a doctor's examination and willing to be involved in research by signing informed consent. Exclusion criteria included pregnant subjects, heavy smokers who had consumed alcohol and had a history of respiratory problems, allergic rhinitis and asthma. Masks produced by CV. Beauty Kasatama Surabaya. The research subjects wore masks for 60 days, then were examined for IL-6 levels from nasal wash liquid. The cytokine IL-6 is used as a marker of the severity of respiratory tract inflammation in laboratory workers.

Acchadana® Herbal Mask
Herbal mask extract Acchadana® Nephrolepis exalata – Hibiscus rosa sinensis was made at the Diponegoro University Applied Sciences Laboratory. Acchadana® masks have received brand patents with No. IDM000921225 and KN95® Masks with coded GB2626-2006.

Sample Collection
Samples were taken by washing the nose using 10cc of 0.9% NaCl. Sampling was done by asking the subject to sit with the head tilted forward 45°, according to the Naclerio technique. Subjects were instructed to take a deep breath and hold their breath. A needless syringe filled with 20 ml of saline solution is inserted into one nostril. Subjects were asked to hold this position for a few seconds, bend over, and slowly squirt the liquid into the container. Nasal wash samples were collected in a container tube for laboratory testing for IL-6 levels. The IL-6 level of the sample was measured using the ELISA method. Tests were carried out using the Human IL-6 ELISA kit ABDonal RK00004. This research has received approval from the Ethics Commission of the Faculty of Medicine UNDIP with number 152/EC/KEPK/FK-UNDIP/VI/2022.

Statistical analysis
The data was processed and analyzed using IBM SPSS version 24 software for windows. Data normality test from each group was analyzed using the Shapiro-Wilk test. The statistical analysis showed that the data were not normally distributed, a significant difference test if p <0.05.

Table 2. The difference in IL-6 levels after using the mask for 60 days.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mask Type</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>KN95</td>
<td>-0.604</td>
</tr>
<tr>
<td></td>
<td>Acchadana</td>
<td>-1.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.242†</td>
</tr>
</tbody>
</table>

*Significant (p < 0.05); †Mann Whitney

RESULT

Subject characteristics
Participants in this study were 79.60% male and 20.40% female. The research subjects were divided into 2 groups of masks, namely KN95 masks consisting of 80.00% men and 20.00% women. In the Acchadana® herbal mask group, 79.20% were men, and 20.80% were women. In the KN95® mask group, 8.00% of subjects had a smoking history, while in the Acchadana® herbal mask group, 16.7% of subjects. Only 2 (8.00%) subjects had respiratory problems in the KN95® mask group. In the Acchadana® herbal mask group, all subjects had no history of respiratory problems, as stated in Table 1.

Comparison of IL-6 levels between the KN95® mask group and the herbal mask group in the Mann-Whitney test had a p-value = 0.242 (p <0.05), as stated in Table 2. The protection of KN95® masks and herbal masks did not differ significantly from the high or low levels of IL-6 in the respiratory tract of laboratory workers after 60 days of wearing masks (KN95® = -0.604 pg/ml and Herbal = -1.026 pg/ml).
Differences in IL-6 levels in the KN95® mask and Acchadana® herbal mask groups are also presented in a box plot diagram as depicted in Figure 1.

**DISCUSSION**

This study tested two types of masks, namely KN95® masks and Acchadana® herbal masks. KN95® masks and Acchadana® herbal masks have equal protection capabilities. This was proven by the results of ELISA readings for IL-6 levels which showed no significant difference after 60 days of wearing masks. KN95® masks are widely recommended during a pandemic because they claim up to 90% protection level.

The Acchadana® herbal masks used in this study contain Nephrolepis exaltata (Ne) and Hibiscus rosa sinensis (Hrs) extracts. Ne plants are reported to have the ability to absorb and neutralize Volatile Organic Compounds (VOC) and absorb pollutant compounds such as formaldehyde, benzene, toluene, and xylene irritating substances. The content of flavonoids, saponins and steroids in Hrs plants is thought to be responsible for significant inflammation inhibition, involving inhibition of prostaglandins, as well as blocking oxidative stress.

In another study, it was stated that Hibiscus has diverse potential and synergistically plays many roles in relieving inflammation and anti-microbial. Still, it is also involved in skin protective functions. As a pro-inflammatory cytokine, the low levels of IL-6 in this study indicated that the respiratory tract was in good condition. IL-6 cytokines have a crucial functional role, especially as a marker of immune status in many ways. Research on patients with Chronic Obstructive Pulmonary Disease (COPD) showed overexpression of the parameters IL-6, TNF-α and MCP-1. This study also served as an explanation for our study that the respiratory tract conditions of laboratory workers who used KN95® masks or the Acchadana® herbal mask did not experience significant inflammation (KN95® = -0.604 pg/ml and the Acchadana® herbal mask = -1.026 pg/ml). The high or low expression of IL-6 as a marker of inflammation or infection in the respiratory tract is closely related to the presence of macrophages. Macrophages regulate the inflammatory response through the release of chemoattractants and proteases, which then take control of inflammatory responses.

Differences in IL-6 levels in the KN95® mask and Acchadana® herbal mask groups are also presented in a box plot diagram as depicted in Figure 1.

**Figure 1.** Differences in IL-6 levels after wearing a mask for 60 days. IL-6 levels in the Acchadana® mask group experienced a greater decrease than in the KN95 mask group.

The design of the Acchadana® herbal mask consists of 3 layers of filters, with a density of 0.7-1.0 µm, better than medical masks (3µm). Although not studied in depth, the herbal components of the Acchadana® mask are likely to affect the balance of oxidants and antioxidants due to repeated inhalation. The development of the Acchadana® herbal mask has been tested in several previous studies regarding its ability to protect the respiratory tract by looking at levels of ROS and SOD-3, as well as the rate of nasal mucociliary transport.

As a pro-inflammatory cytokine, the low levels of IL-6 in this study indicated that the respiratory tract was in good condition. IL-6 cytokines have a crucial functional role, especially as a marker of immune status in many ways. Research on patients with Chronic Obstructive Pulmonary Disease (COPD) showed overexpression of the parameters IL-6, TNF-α and MCP-1. This study also served as an explanation for our study that the respiratory tract conditions of laboratory workers who used KN95® masks or the Acchadana® herbal mask did not experience significant inflammation (KN95® = -0.604 pg/ml and the Acchadana® herbal mask = -1.026 pg/ml). The high or low expression of IL-6 as a marker of inflammation or infection in the respiratory tract is closely related to the presence of macrophages. Macrophages regulate the inflammatory response through the release of chemoattractants and proteases, which then take control of inflammatory responses.
signaling by recruiting inflammatory cells to areas of inflammation by activating them via TLR-3. Thus if research subjects are continuously exposed to reactive substances, the cells Inflammatory cells will immediately give a signal as a form of notification of inflammation and command the release of pro-inflammatory cytokines such as IL-6.

In the process, the mechanism of action begins with IL-6 binding to IL-6R, forming the IL-6–IL-6R complex. This complex then associates with the IL-6R subunit signal transduction β (gp130), initiating intracellular signaling. Signal transduction within cells involves activation of the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) and RAS-dependent mitogen-activated kinase (MAPK). Activation of intracellular signaling via gp130 also initiates a negative feedback loop via the signaling suppressor cytokine 3 (SOCS3), ultimately leading to signaling termination. IL-6 is released by neutrophils and enters the circulation to be transported to areas of inflammation.

Furthermore, IL-6 forms trans-signals in smooth muscle cells, endothelial cells, mesothelioma, epithelium, and fibroblasts, producing various chemokines and cytokines. In this study, we utilized the secretion of cytokines by cells in the nasal area as an image projection—the condition of the subject's respiratory tract.

IL-6 cytokines play an important role because IL-6 is a strong predictor of many incidences of respiratory tract disease, including COPD exacerbations, allergic rhinitis, sinusitis, and rhinosinusitis. In many respiratory disorders studies, cytokines are used as a reliable marker. IL-6 cytokine belongs to the Th2 type and is predominant in the nasal mucosa. Previous studies on in vitro observations of the nasal mucosa showed that the expression of several cytokines, such as IL-6 and TNF-α, was widely released from nasal epithelial cells. This study still has limitations. The magnitude of the decrease in IL-6 cytokines in each mask group cannot be compared because the ELISA examination was only carried out at the end of the study. Future studies need to do a pre-post examination of the immunological parameters involved in the study. It is necessary to check cytokine levels at the beginning of the study to clarify the high or low IL-6 difference detected at the end of using the mask. The addition of comparison parameters will probably give more satisfactory results.

CONCLUSION

KN95* masks and Acchadana* herbal masks as respiratory protection have equal protective abilities in protecting the respiratory tract from exposure to pollutants, viruses, pathogens, and free reactive compounds.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

ETHICAL CONSIDERATION

This research has received approval from the Ethics Commission of the Faculty of Medicine UNDIP with number 152/EC/KEPK/FK-UNDIP/V1/2022.

FUNDING

The authors are responsible for all the study funding without a grant or external funding source.

AUTHOR CONTRIBUTION

All authors have contributed to this research process.

REFERENCES


