



## • Perspective

# Mitochondria disease due to humidifier disinfectants: diagnostic criteria and its evidences

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Humidifier disinfectant damages caused by the misuse of humidifier disinfectants, such as polyhexamethylene guanidine (PHMG), resulted in chemical disasters in South Korea in 2011. About four million people were exposed to humidifier disinfectants (HDs) in the 17 years between 1994 and 2011. Although fatal lung damage was initially reported, investigations into the victims' injuries revealed that the damage was not limited to the lungs, but that systemic damage was also confirmed. Considering the spread of HD from the lungs to the whole body, the toxic effects of PHMG from reactive oxygen species (ROS), NOTCH signaling pathways, and mitochondrial dysfunction resulted in endothelial damage in the lungs, blood vessels, liver, kidneys, bone marrow, nerves, and muscles. The main toxic mechanisms involved in HD damage may be the NOTCH pathway and mitochondrial damage. There are many case reports which include neurologic disorders (ADHD, depression, posttraumatic stress disorder), muscular disorder (exercise intolerance, myalgia), energy metabolism disorder (chronic fatigue syndrome), and immunologic disorder (rheumatoid arthritis) in HDs victims. These case reports involve multi-system involvement in HDs victims. Further well-designed study is needed to clarify whether mitochondrial dysfunction is associated with multi-organs involvement in HDs victims.

**Keywords:** Humidifier disinfectants, PHMG, mitochondria diseases, reactive oxygen species, NOTCH pathway, Humidifier disinfectants syndrome

## Introduction: Humidifier disinfectants (HDs) study results so far achieved

From 2002 through 2020, about 1,600 people were reported to die of fatal injuries associated with the use of humidifier disinfectants (HDs) in South Korea. Several chemical disinfectants used for household humidifiers were later clinically confirmed to cause HD-associated lung injury (HDLI). Polyhexamethylene guanidine phosphate (PHMG-P) is the main ingredient of the HDs associated with lung disease. Epidemiological studies, animal studies, and dose-response analysis demonstrated a strong association between HDs use and lung injuries. The diagnostic criteria for HDLI was defined on the basis of the clinical, pathological, and radiological attributes of the patients [1]. Because of the fatal lung injuries in South Korea, investigations into the adverse health effects of HDs have

focused on the lung toxicity of PHMG-P [2]. Therefore, the following toxicologic studies focused on the association between HDs and pulmonary fibrosis. PHMG-P aerosol particles induce pulmonary inflammatory and fibrotic responses [3]. As PHMG-P-induced lung fibrosis is different from that induced by known fibrogenic agents, such as bleomycin, it is important to understand the molecular mechanisms underlying this effect. Guanidine-based disinfectants, PHMG-P, polyhexamethylene biguanide (PHMB), and oligo (2- (2-ethoxy) ethoxyethyl guanidinium chloride (PGH) induce epithelial-mesenchymal transition in A549 alveolar epithelial cells [4]. PHMG-P promotes reactive oxygen species (ROS) generation and consequently increases the expression of DNA damage markers, such as ATM and H2AX phosphorylation [5]. NOTCH1 transcripts significantly increased in lung tissues from HDLI cases compared to unexposed controls ( $p=0.05$ ), so NOTCH1 may play an important role in the pulmonary fibrosis of HDLI [6]. PHMG-P induced epithelial-mesenchymal transition (EMT) through Akt/NOTCH signaling pathways and ZEB2 played an important role in PHMG-P-induced lung toxicity [7].

Age-period-cohort (APC) analysis using health insurance data showed that many diseases, such as asthma, bronchiec-

Received: April 16, 2020 Accepted: May 13, 2020

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tasis, interstitial lung disease (ILD), pneumonia, and toxic hepatitis, are associated with exposure to HDs [8]. According to a recent survey [9], the high prevalence of symptoms or chronic diseases, such as ophthalmologic diseases, nasal diseases, cardiovascular disease, chronic fatigue, attention-deficit/hyperactivity disorder (ADHD), and developmental disorders, may be related to HDs. Are there common underlying toxic mechanisms for all these diseases, including HDLI? NOTCH signaling on mitochondrial proteome, which in turn affects the functioning of key metabolic pathways, connects an important signaling pathway to the regulation of cellular metabolism [10]. The NOTCH-activated signaling cascade interacts with mitochondrial remodeling proteins to regulate cell survival [11]. A previous study reported that HDLI is caused by the NOTCH-activated signaling cascade. Interestingly, interstitial fibrosis can be caused by the NOTCH-activated signaling cascade. Many researchers have identified and described the underlying processes that result in metabolic dysregulation, metabolic reprogramming, and mitochondrial dysfunction observed in the cells of idiopathic pulmonary fibrosis (IPF) lungs [12]. Mitochondrial dysfunction is the molecular treatment target of IPF [13]. To identify the mechanism of PHMG hepatotoxicity, HepG2 cells were exposed to PHMG-P for 72 hr. The cell viability was significantly decreased by PHMG-P in time- and concentration-dependent manners. The mitochondrial membrane potential was markedly reduced by PHMG-P and the apoptotic signaling cascade was activated [14].

### New hypothesis about mitochondrial diseases

Many studies already showed that biocides can cause various non-communicable diseases (NCD) by inducing mitochondria dysfunction. Typical examples are organophosphate, herbicide, and pyrethroids [15-17].

PHMG overexposure leads to metabolic overload of the mitochondria and the damaged mitochondria are likely to result in muscle insulin resistance and  $\beta$ -cell dysfunction [18]. Unexplained cardiovascular disease, such as angina pectoris and myocardial infarction at young ages, could be associated with exposure to PHMG. Cardiovascular toxicity caused by PHMG was observed in an animal study [19]. Mitochondria in airway epithelium, smooth muscle, and fibroblasts play differential roles, being consistent with their contributions to disease biology, such as asthma and chronic obstructive pulmonary disease (COPD) [20]. Mitochondrial dysfunction could trigger developing asthma and COPD. ROS and mitochondrial dysfunction could be the cause of bronchiectasis. Mitochondria

dysfunction could play a core role in developing immune disorders such as rheumatoid arthritis [21].

Mitochondrial defects were detected in ADHD cybrids created by patients' platelets, implying that a bioenergetic crisis in the mitochondria could be a contributory factor in ADHD pathology and/or phenotypes [22]. Mitochondrial dysfunction could play a critical role in cancer progression, cognitive decline, and repeated stroke at young ages [23-25].

Toxicological studies to date have tried to show an association between HDs exposure and HDLI. Researchers have tried to elucidate the mechanism of pulmonary fibrosis but did not investigate the toxicological mechanisms of asthma, interstitial pneumonia, pneumonia, toxic hepatitis, cancer, chronic fatigue syndrome or ADHD.

Considering the movement of HD in the lungs, which spreads from the lungs to the whole body [26], the toxic effects of PHMG on pulmonary epithelial cells through ROS and the NOTCH signaling pathways, and mitochondrial dysfunction have also been found in blood vessels, liver, kidneys, bone marrow, nerves, and muscles. Bone marrow damage can be associated with idiopathic pulmonary fibrosis by abnormal immune modulation of immune cells [27].

HDs were shown to exert typical toxicologic effects on various target organs, such as the skin, conjunctiva, nasal mucosa, bronchial mucosa, and alveoli, which shared common toxicological responses. Diverse diseases can occur at the same time with a common toxicologic mechanism and cause humidifier disinfectant syndrome (HDS) [28].

Mitochondria disease criteria was suggested by E. Morava et al. [29] Mitochondria diseases due to HDs can be diagnosed as follows. First, exposure to HDs should be confirmed. Second, upper or lower respiratory tract diseases, such as asthma, interstitial lung disease, bronchiectasis, pneumonia, and COPD should be confirmed. Third, mitochondria diseases criteria should be met. Fourth, Other causes, such as genetic factor, radiation exposure, and other occupational and environmental exposures should be ruled out.

HD victims are at increased risk of developing many HD-related diseases. However, HD exposure no longer occurs because the use of HD was banned in 2011. Thus, why do HD-related diseases develop and continue? Damaged lymphocyte are still observed in many HDs victims. This can be due to HD-induced genetic mutations [30] and epigenetic changes [31] in the mitochondria.

Mitochondrial diseases are very important because most NCDs are associated with these diseases. So, to investigate into the possible environmental causes of mitochondrial diseases has tremendous importance. To clarify the association

between HDs and mitochondrial disease can be good step to prevent NCDs.

To treat and prevent these kinds of diseases, the concept of HDS should be considered. Many therapies to target mitochondrial dysfunction are being developed [32,33] and data has accumulated on the toxicologic mechanism of HDs. Therefore, it is necessary to update the diagnostic criteria for HD-related diseases and establish strategies to treat and prevent HD-related diseases to care for HDs victims.

## Conclusion

There are many case reports which include neurologic disorders (ADHD, depression, posttraumatic stress disorder), muscular disorder (exercise intolerance, myalgia), energy metabolism disorder (chronic fatigue syndrome), and immunologic disorder (rheumatoid arthritis) in HDs victims. These case reports involve multi-system involvement in HDs victims. Further well-designed study is needed to clarify whether mitochondrial dysfunction is associated with multi-organs involvement in HD victims.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRedit author statement

JHL: Conceptualization; Writing - Original Draft; HCK: Writing - Reviewing & Editing

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