ORIGINAL ARTICLE

IgG Antibodies Levels in Blood Serum of Workers Exposed to Microbial Contaminants of Metal Working Fluids

Mohammed Abdulrazzaq Jabbar[1,2], Zailina Hashim[2], Rukman Awang Hamat[3], Huda Zainuddin[4]

[1] Department of Population Medicine, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, 43000 Kajang, Selangor, Malaysia
[2] Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3] Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4] Department of Community Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Introduction: Water-based metalworking fluid (MWF) provides a suitable environment for microbes to grow. This study aimed at identifying the level and species of microbial contamination of MWF in a metal machining factory and to determine the corresponding Immunoglobulin G (IgG) levels in the workers' blood samples. **Methods:** Total of 298 workers in the production section of a metal machining factory, the production section using MWF as coolant fluid, were involved in this study. The Analytical Profile Index system was used to identify the species of microbes isolated from MWF bulk and air samples. Tryptone soya agar was used to incubate unknown bacteria, and sabouraud dextrose agar was used for unknown fungi. The level of IgG antibodies in workers' blood were measured as an indicator of the exposure to the microbes isolated from MWF, corresponded to the microbial species isolated from MWF. **Results:** The most dominant microbes isolated from the channels were *Candida albicans*, *Klebsiella pneumoniae*, *E. coli* and *Pseudomonas aeruginosa*. A total of 21 (34.4%) workers were positively exposed to E. coli, 30 (42.2%) to Pseudomonas aeruginosa, and 45(75%) exposed to *Candida albicans*. Also, the mean level of optic density of IgG to *Klebsiella pneumoniae* was 0.415 (0.02). **Conclusion:** Water-based metalworking fluid in this metal machining factory was contaminated with bacteria and fungi. The workers in the production section were exposed to MWF as well as the microbes present in MWF. The level of IgG in blood was the biomarkers for occupational exposure to microbial contaminant MWF.

Keywords: Metalworking fluids, Analytical Profile Index, IgG, Biomarkers, Occupational exposure

Corresponding Author:
Zailina Hashim, PhD
Email: zailina@upm.edu.my
Tel: +6017 636 1367

INTRODUCTION

Metalworking fluid (MWF) is a complex mixture used during the machining of metal objects and usually contains substances such as biocides, corrosion inhibitors, metal fines, tramp oils and biological contamination (1-4). Metalworking fluid is also called cutting fluid, machining fluid, or metalworking coolant (5). It can be divided into four major types: Straight oils (mineral and/or fatty oils, insoluble in water), Soluble MWF (water emulsion with high oil content), Semi-synthetic MWF (water emulsion with lower oil content) and Synthetic MWF (chemical solutions of organic compounds and inorganic salts in water without any oils) (6). The functions of MWF are mainly cooling and lubrication at the metal processing point. Generally, a water-based fluid is better for coolants while oil-based fluid is better for lubrication. Currently and for commercial reasons, there is a steady trend towards the use of water-based MWF (7). More than 1,700 companies with approximately 540,000 employees are currently involved in the machinery and equipment sectors in Malaysia, and the number will continuously grow in the future (8). Exposure to MWF aerosols or mists through inhalation can lead to adverse respiratory health effects such as cough, cough with phlegm, wheeze, rhinitis, acute impairment of lung function and hypersensitivity pneumonitis (9). Exposure to MWF aerosols by direct skin contact may lead to adverse skin effects such as contact dermatitis (10-11). According to researchers, water-based MWFs in machining factories bear significant bacterial growth in the fluids and aerosols, which can cause respiratory adverse health effects to exposed workers (12). Hypersensitivity pneumonitis (HP)

is one of the consequences of the respiratory system, occupational exposure to microbial contaminants of water-based MWFs (13-17). Among MWF exposed workers, the prevalence of nasal complaints, cough, chronic bronchitis, and asthma was 25.5%, 22.5%, 4.4%, and 5.7% respectively. On another hand, among MWF unexposed workers the prevalence was 4.1%, 4.1%, 1%, and 1% respectively (1).

The levels of Immunoglobulin G (IgG) in the blood serum of the workers are the indicators of exposure to the microbial contaminants of MWFs. These indicators are a practical tool for occupational health physicians to determine the microbial contaminants of MWFs (10, 18). In a study conducted in Malaysia among 138 metal machinists, there were significant correlations between the total serum IgG levels with the microbial contaminants of MWF in metalworking processes. At the same time, the prevalence of the health symptoms of a cough, skin itching, and inflammation significantly influenced the total serum IgG levels. Therefore, the total serum IgG antibodies may serve as an indicator of occupational exposure to the microbial contaminants in MWF (19). The workers in workplaces using water-based MWF are susceptible for extrinsic allergic alveolitis therefore, these workplaces must be investigated regularly and accurately the quantify the clinical, immunological, and microbiological findings to identify the likely causes and to protect the workers (20). A study was conducted to identify the potential risk between microbial exposure and outbreak of hypersensitivity pneumonitis, and the findings showed there was a spatial relationship between the abundance of a mycobacterium-like organism and the outbreak of MWF-associated hypersensitivity pneumonitis. Further development of sequence-based analytic techniques should assist in the prevention (21). The workers in the metal machining factory were not exceptional as there were high possibilities of being exposed to the microbial contaminants of the water-based MWF. Therefore, this study aimed to assess the species and the levels of contamination of water-based MWF for both bulk and air samples and to determine the level of microorganisms-specific IgG in the workers' blood serum as biomarkers for microbial exposure.

MATERIALS AND METHODS

Methodology

A cross-sectional study was conducted to examine the microbial contamination of MWF bulk and air samples. MWF samples were collected from different locations of the production section. The factory produces three different types of ball bearings, and for each type, there are multiple production sections, and each production section performs a specific metal machining process either turning, grinding or assembly. After determining the microbial species in the MWF, the levels of IgG were also determined against the identified microbes in the workers' blood serum. The management of the factory granted the permission to conduct the study and provided the names of workers in the production section of the factory. The name list was used as the framework for sample selection. The inclusion criteria of workers to be included were a permanent worker. Random sampling was used to select 298 workers from total 400 workers in the production section. Before data collection, ethical clearance was obtained from the Universiti Putra Malaysia (research ethical clearance number UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2 on April 8, 2014). All participants signed the consent form. The female workers did not sign the consent due to some personal reasons and we respected their decision. Therefore, all the respondents were males. The data collection was conducted for a period of 12 months starting from June 2014 to June 2015. The data of this manuscript was part of a study that was conducted among workers in the metal machining factory.

Plant and operation

The study was conducted in a metal machining factory located in Nilai City, about 40 km south of Kuala Lumpur, Malaysia. The factory was chosen because it uses water-based MWF as a coolant in the production section, the number of workers in the production section was adequate for the sample size. The factory management granted us the permission letter to conduct the study in the factory which operates 24 hours a day, for seven days a week and that provided us the time we need to collect the data.

The factory manufactures different types of ball bearings using stainless steel materials. The factory management has requested the factory name to be confidential. Therefore, the three ball bearings were labelled as A, B, and C to preserve the factory anonymity. Product A with three production sections (in the factory named channel) which were labelled as follows: A1, A2 and A3; product B with three channels B1, B2, and B3, and product C with five channels (C1, C2, C3, C4, and C5). The total number of production channels available was 11. The metal machining processes carried out in these channels are turning, grinding, honing and assembling of the ball bearing particles. The ball bearings production process starts with the turning process of a ball bearing which provides the inner and outer soft rings (soft ring before heat treatment) of the ball bearing their rough shapes. After the heating treatment, the grinding process is carried out to provide the inner and outer hard rings with precise shapes and diameters. The honing process is to provide the inner and outer hard rings with the shiny and bright looks. At the end of the production process, the ball bearing parts (the inner rings, outer rings, balls/rollers, with the nets and cages) were put or arranged on assembly stations to assemble and construct the ball bearings. Although auto-assembly machines were available to carry out the process, workers were required to do the manual assembly to reach the production target. The workers in those three units were presumed

to have been exposed to MWF.

The workers were distributed into three teams; all were working in three different shifts on a rotational basis per week - morning, afternoon and night shifts - which cover the 24 hour-working periods. On Saturdays, the workers only work an 8 hour-morning shift. Their schedules are regularly rotated to ensure that the three shifts are equally spread amongst them. In total, 298 workers from the production section were involved in the study.

Assessment of Microbial Contamination of Metalworking Fluids

The assessment process was started by collecting bulk and air MWF samples and inoculated using tryptone soya agar (TSA) that formulated from Tryptone 15 g/l, soy peptone 5 g/l, sodium chloride 5 g/l. The agar TSA used for the cultivation of a wide variety of bacteria as well as sabouraud dextrose agar (SDA) that formulated from peptone 10 g/l and dextrose 40 g/l for fungi cultivation (22). The MWF bulk samples were collected from the tanks in each of the production section (channel) on the morning by using a sterile pipette with a maximum sampling volume of 5000µl. The samples were then transferred into 5 ml autoclaved glass containers. The air samples, on the other hand, were collected by using a dual-head portable microbiological air sampler (DUO SAS 360) which was installed in the middle of a production channel of 1.5-meter height and was operated on a standard mode for 10 minutes for each channel. The samplers contained two chambers for 2 Petri dishes - one with TSA and the other with SDA agars. The samples were then transferred directly from the air into the agar in the production channel. The bulk and air samples were transported from the factory immediately after the collection process was completed to the microbiology media laboratory in Universiti Putra Malaysia (UPM) in a cooling container filled with ice. The bulk samples were inoculated into agars on the same day inside a safety cabinet and were incubated with the air samples at 37°C (22). The microbial analysis has been started after 24 hours of incubation to identify the microbes in the bulk and air MWF samples by using the Analytical Profile Index (API). API 20E was used to identify gram-negative rods with 18-24 hours' incubation, API 20 NE to identify non-enteric gram-negative rods with 24-48 hours incubation, API Staph to identify staphylococci with 18-24 hours incubation, API strep to identify streptococci with 4-24 hours incubation and API candida to identify yeasts. Because catalase, oxidase, and coagulase tests are not available in the identification systems, they were conducted before using API. The interpretation of the final results was carried out using the numerical coding of results of the API systems to identify the microorganisms' species (23). The microbes which were isolated from the MWF bulk samples and air samples were *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Candida albicans*. In order to achieve the quality control of microbial species determination and to prevent the cross contamination, the microbial detection procedures were conducted by following the Standard Operational Procedure (SOP) available in the API kits.

Assessment of Microorganisms-specific IgG Levels in Workers' Blood Serum

The indicator of exposure to microbial contaminants of MWF was the microbes' specific IgG levels in the blood serum of the metal machinists (18). After the isolation and identification of microorganism species in the bulk and air samples of MWF that was obtained from each production channel, specific enzyme-linked immunosorbent assays (ELISA) on workers' blood serum samples were used to determine the level of microbes-specific IgG. The amount of blood were obtained from workers was 6 CC. The blood sampling was carried out by the main researcher who is a medical doctor and registered in Malaysian Medical Council with valid annual practicing certificate. *Candida albicans* IgG ELISA kit, Human Anti-*Escherichia coli* IgG ELISA kit and *Pseudomonas aeruginosa* FC IgG ELISA kit were used to determine the levels of microbes-specific IgG. The procedures to detect the levels of IgG antibodies were carried out by the Standard Operational Procedure (SOP) available in the kits. With regards to *Klebsiella pneumoniae*, there was no standardized kit to identify the microbe-specific IgG; thus, a method was adopted (24) to analyze the *Klebsiella pneumonia*e specific IgG in the workers' blood serum.

Assessment of Workers' Respiratory and Skin Health

Beside the microbial contamination assessment, the respiratory health of the workers was assessed using Medical Research Council questionnaire (MRCQ) (25), and skin health was assessed using the Nordic Occupational Skin Questionnaire NOSD 2002 (26). The questionnaires content and face validity were assessed during the pilot study before the commencement of the original study. The questionnaires were translated from the original English language into Malaysian language with the help of the linguistics. The health assessment was conducted throughout face to face interview. The response rate to the study was 99%.

RESULTS

As summarized, Table I, the level of microbial colonies in different production channels was obtained by measuring the bacterial and fungal concentration in the bulk and air samples of each of the production channels. The highest air bacterial colony was found in channel A2 (66.5 x 101 CFU/m^3), air fungal colony in channel C1 (37.0 x 101 CFU/m^3), MWF bulk bacterial colony in channel B2 (45 x 103 CFU/m^3) and MWF bulk fungal colony in channel C2 (39 x 103 CFU/m^3).

The most dominant microbial species isolated from MWF bulk and air samples were *Candida albicans*, *Klebsiella*

Table I: Level of dominant microbes found in MWF in different production sections

Channels	Level of Dominant Microbial Contaminants			
	MWF Air Samples Bacteria (cfu/m^3)	**MWF Air Samples Fungal** (cfu/m^3)	**MWF Bulk Samples Bacteria** (cfu/m^3)	**MWF Bulk Samples Fungal** (cfu/m^3)
A1	24.5×10^1		42×10^3	
A2	66.5×10^1		32×10^3	
A3	37.0×10^1		43×10^3	
B1	24.5×10^1		45×10^3	
B2	19.0×10^1		36×10^3	
B3	20.0×10^1		37×10^3	
C1		37.0×10^1		34×10^3
C2		14.0×10^1		39×10^3
C3	25.0×10^1		43×10^3	
C4	21.5×10^1		43×10^3	
C5	21.0×10^1		32×10^3	
Mean (S.D)	28.58×10^1 (13.13×10^1)	23.12×10^1 (8.05×10^1)	42.92×10^3 (4.64×10^3)	36.50×10^3 (4.13×10^3)

pneumoniae, E. coli and *Pseudomonas aeruginosa*. Table II summarises the characteristics of these microbes and from different channels where they were isolated.

The following descriptive analysis of the microbes-specific IgG antibodies in the workers' blood serum showed the total number of workers exposed to each of the microbial species; to *Klebsiella pneumoniae* 106, to *E. coli* 61, *Pseudomonas aeruginosa* 71 and *Candida albicans* 60. A total of 21 (34.4%) workers were positively exposed to *E. coli*, 30 (42.2%) positively exposed to *Pseudomonas aeruginosa* and 45 (75%) exposed to *Candida albicans*. With regards to *Klebsiella pneumoniae*, the mean level of optic density of *Klebsiella pneumoniae* specific IgG was 0.415 (0.02).

The workers in the production section had shown signs and symptoms of respiratory and skin disorders. The most common respiratory disorders found were nasal complaints (a runny nose, stuffy nose, or nasal itching),

Table III: Descriptive analysis, IgG antibodies to the specific microorganism in the serum of the respondents

Microbe-specific IgG	Number of exposed workers	Variables	IgG total Results	
			Mean(SD)	n (%)
Klebsiella pneumoniae	106	Optic Density	0.415 (0.02)	
Escherichia coli	61	Positive Negative		21 (34.4) 40 (65.6)
Pseudomonas aeruginosa	71	Positive Negative		30 (42.2) 41 (57.8)
Candida albicans	60	Positive Negative		45 (75) 15 (25)

The IgG status for workers with potential exposure to the microbe that was isolated from their production sections.

Table II: Characteristics of the most dominant microbes isolated from the environment and MWF at operation channels

Microbes	Taxonomy	Agar Materials	Oxidase Test	API system	Production Sections (where the microbe isolated)	Number of workers
Candida albicans	Kingdom: Fungi Division: Ascomycota Class: Saccharomycetes Order: Saccharomycetales Family: Saccharomycetaceae Genus: Candida Species: *Candida albicans*	Sabouraud Dextrose Agar (SDA)	Negative (because grown in SDA)	API Candida	C1, C2	60
Klebsiella pneumoniae	Kingdom: Bacteria Phylum: Proteobacteria Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae Genus: Klebsiella Species: *Klebseilla pneumoniae*	Tryptone Soya Agar (TSA)	Positive	API 20 E	A2, B2, C4	106
E. coli	Domain: Bacteria Kingdom: Eubacteria Phylum: Proteobacteria Class: Gammaproteobacteria Order: Enterobacteriales Family: Enterobacteriaceae Genus: Escherichia Species: *E. coli*	Tryptone Soya Agar (TSA)	Positive	API 20 E	B1, B3,	61
Pseudomonas aeruginosa	Domain: Bacteria Phylum: Proteobacteria Class: Gammaproteobacteria Order: Pseudomonadales Family: Pseudomonadaceae Genus: Pseudomonas Species: *Pseudomonas aeruginosa*	Tryptone Soya Agar (TSA)	Positive	API 20 E	A1, A3, C3, C5	71

cough and cough with phlegm. Skin disorders such as itchiness, redness, scaling, rash or dryness were most commonly found on the workers' hands, forearms and front torso. Table IV shows that exposure to *Klebsiella pneumoniae* was significantly associated with cough (p=0.031) and cough with phlegm (p=0.029). Exposure to *Pseudomonas aeruginosa* was associated with cough (p=0.041) and cough with phlegm (p=0.03). Exposure to *Candida albicans* was associated with nasal complaints (p=0.032) and cough with phlegm (p=0.07).

Furthermore, exposure to *Candida albicans* was significantly associated with hand skin disorders (p=0.02), forearm skin disorders (p=0.018) and front torso skin disorders (p=0.02) as shown in Table V.

Table IV: Association of microbial exposure with respiratory complaints among workers with potential exposure to microbes

Microbes		Respiratory Complaints					
		Nasal Complaints		Cough		Phlegm	
		Yes	No	Yes	No	Yes	No
Klebsiella pneumoniae [A] (106 workers)	Optic Density	0.44 (0.01)	0.42 (0.02)	0.47 (0.01)	0.36 (0.02)	0.48 (0.01)	0.35 (0.02)
p value		0.82		0.031*		0.029*	
Pseudomonas aeruginosa [B] (71 workers)	Positive (30)	18 (60%)	12 (40%)	18 (60%)	12 (40.0%)	10 (33.3%)	20 (66.6%)
	Negative (41)	22 (53.6%)	19 (46.4%)	17 (41.4%)	24 (58.6%)	26 (63.5%)	15 (36.5%)
p value		0.052		0.041*		0.03*	
Candida albicans [B] (60 workers)	Positive (45)	29 (64.4%)	16 (35.6%)	23 (51.1%)	22 (49.9%)	19 (42.25%)	26 (57.75%)
	Negative (15)	6 (40%)	9 (60%)	8 (53.3%)	7 (46.7%)	6 (40%)	9 (60%)
p value		0.032*		0.65		0.07	

N= 298, A continuous variables independent t test was conducted, B categorical variables chi square test was conducted. *significant at <0.05

Table V: Association of microbial exposure with skin disorders in most common body sites (hands, forearms, and chest/abdomen) among workers with potential exposure to microbes

IgG Specific to		**Respiratory Complaints**					
		Hands Skin Disorders		**Forearms Skin Disorders**		**Front torso Skin Disorders**	
		Yes	**No**	**Yes**	**No**	**Yes**	**No**
Klebsiella pneumoniae A (106 workers)	Optic Density	0.38 (0.01)	0.41 (0.02)	0.41 (0.01)	0.42 (0.02)	0.39 (0.02)	0.43 (0.015)
p value		0.72		0.94		0.76	
E. coli (61 workers)	Positive (21)	6 (28.5%)	15 (71.5%)	10 (47.6%)	11 (52.4%)	14 (66.7%)	7 (33.3%)
	Negative (40)	20 (50%)	20 (50%)	22 (55%)	18 (45%)	23 (57.5%)	17 (42.5%)
p value		0.08		0.64		0.52	
Pseudomonas aeruginosa (71 workers)	Positive (30)	13 (43.3%)	17 (56.7%)	15 (50%)	15 (50%)	14 (46.6%)	16 (43.4%)
	Negative (41)	18 (43.9%)	23 (56.1%)	17 (41.4%)	24 (58.6%)	20 (48.7%)	21 (51.3%)
p value		0.58		0.31		0.81	
Candida albicans (60 workers)	Positive (45)	32 (71.1%)	13 (28.9%)	33 (73.3%)	12 (26.7%)	28 (62.2%)	17 (37.8%)
	Negative (15)	5 (33.3%)	10 (66.6%)	6 (40%)	9 (60%)	5 (33.3%)	9 (66.6%)
p value		0.02*		0.018*		0.02*	

N= 298, [A] continuous variables independent t test was conducted, chi square test was conducted for categorical variables, *significant at <0.05

DISCUSSION

The water-based metalworking fluid is the most common coolant used in metal machining processes (1, 4). Water-based MWF consists of approximately 90% water and 10% petroleum oil with emulsifier materials, anticorrosion and biocides. This fluid is considered a good environment for microbes to live and grow (27). Researchers have stated that the most common microbes which can survive in the fluid were gram-negative bacteria and fungus. These findings were similar to what was reported in this study whereby the machinists in the metal machining factory who were exposed to the fluid had subsequently been exposed to the microbes (28).

It was possible that the water-based metalworking fluid was contaminated with microbes as *Pseudomonas aeruginosa* was isolated in B1, B3, C3 and C5 channels, *Klebsiella pneumoniae* was isolated in B2, A2, and C4, *Escherichia coli* was isolated in A1 and A3, and *Candida albicans* was isolated in C1 and C2. These findings are consistent with the results of other studies (10). In the following article (10) the researchers have reviewed some references on microbial exposure from MWF and found the most common microbes which can live and exist on MWF are gram-negative bacteria. The existing literature substantially confirms that respiratory problems are associated with exposure to microbial contaminated MWF (29). These microorganisms were isolated from the air environment of different channels in the production section of the factory, and have the potential to produce serious health effects on the exposed workers (10, 12, 13).

A study conducted in Malaysia on water-based MWF and the level of contamination with microbes among metal machinists in a metal machining factory has found that the coolant was contaminated with different species of bacteria from gram-negative such as *Pseudomonas aeruginosa, E. coli*, and *Klebsiella pneumoniae*. The findings are consistent with that of this study (10). Other researchers have isolated *Pseudomonas aeruginosa* from MWF (30) and found that the MWF was contaminated with *Pseudomonas aeruginosa, E. coli*, and *Staphylococcus aureus* (31). Microbes that survive on MWF will become highly resistant to biocides, and this means that adding in biocides will not resolve the problem of germs in MWF (30). Microbial contaminated MWF can lead to severe skin disorders especially among workers with poor hand hygiene (20, 32, 33).

The MWF workers showed significantly higher IgG antibody responses to bacterial antigens than did the controls. In occupational hygiene measurements, the IgG antibodies against antigens identified from MWF samples could be a practical tool for occupational health physicians to confirm the exposure (18). Table III shows that the concentration of IgG to *Klebsiella pneumoniae* was 0.415 (0.02) ng/ml. About 34.4% of workers were considered positively exposed to *E. coli*, 42.2% were exposed to *Pseudomonas aeruginosa*, and 75% were exposed to *Candida albicans*. These results provide the evidence that workers in the production section of the metal machining industry who are exposed to MWF are probably exposed to its microbial contaminants too. These findings are consistent with that of other studies which concluded that water-based MWF is most commonly used in Malaysian metal machining industries and that the fluid provides a good environment for microbial growth (10). The limitation of this study was the MWF bulk and air samples were collected in one day. Therefore, we were unable to find out the trend of the microbial colonies and species exist in MWF bulk and air samples.

Workers must be aware of the existing hazards in the workplace such as MWF and its microbial contaminants, namely *Klebsiella pneumoniae, E. coli, Candida albicans* and *Pseudomonas aeruginosa*. The findings of this study must be communicated to the workers and employers so that necessary measures can be taken to reduce worker's exposure to microbial contaminated MWF. For future study, we recommend researchers to collect the MWF bulk and air samples every week for six months to order to identify the trend of microbial colonies and species exist in MWF and this will help to understand more details which microbe is more dominant in the fluid and what are the differences in the level of contamination in different times.

CONCLUSION

Water-based MWF is used as a coolant in metal machining factories. MWF bulk and air samples were contaminated with microbes like bacteria and fungus, and the dominant species isolated were *Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa* and *Candida albicans*. The analysis on the workers' blood serum showed that the level of biomarkers (IgG) was significantly linked to the types of the microbes isolated from the MWF. Thus, the production section workers are at risk of developing serious respiratory and skin disorders due to the exposure to the MWF contaminated with microbes if preventive measures to further exposure is not taken.

ACKNOWLEDGEMENT

The completion of this research would not have been possible without the support of Universiti Putra Malaysia which provided us with the research ethical clearance number of UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2 as well as the Ministry of Science, Technology & Innovation for funding the study with the Research Grant number of 5524410. Thanks to all the staff of the microbiology laboratory at the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for their help and cooperation during the study.

REFERENCES

1. Jabbar MA, Hashim Z, Zainuddin H, Munn-Sann L. Respiratory health effects of Metalworking fluid among metal machining workers: Review article. Asia Pacific Environmental and Occupational Health Journal. 2017;3(2):15-19.
2. Burton CM, Crook B, Scaife H, Evans GS, Barber CM. Systematic Review of Respiratory Outbreaks Associated with Exposure to Water-Based Metalworking Fluids. Annals of Occupational Hygiene. 2012;56:374–88.
3. Cherrie JW, Semple S. Dermal exposure to metalworking fluids and medium-chain chlorinated paraffin (MCCP). Annals of Occupational Hygiene. 2010;54:228–35.
4. Jabbar MA, Hashim Z, Zainuddin H. Metalworking fluid exposure and consequences on skin health in a metal working factory: Review article. International Journal for Empirical Education and Research. 2018;1(4):27-35.
5. Woskie S R, Virji M A, Hallock M, Smith T J and Hammond S K. Summary of the findings from the exposure assessments for metalworking fluid mortality and morbidity studies Applied Occupational Environmental Hygiene. 2003;18:855–64.
6. Lillienberg L, Burdorf a., Mathiasson L, Thurneby L. Exposure to metalworking fluid aerosols and determinants of exposure. Annals of Occupational Hygiene. 2008;52(7):597–605.
7. Simpson a. T, Stear M, Groves J a., Piney M, Bradley SD, Stagg S, Crook B. Occupational exposure to metalworking fluid mist and sump fluid contaminants. Annals of Occupational Hygiene. 2003;47:17–30.
8. Malaysian Investment and Development Authority. Machinery & Equipments and Advanced Engineering. [Internet]. MIDA Malaysia; 2014 [cited 2014 January 28]. Available from: http://www.mida.gov.my/home/administrator/system_files/ modules/photo/uploads/20141111033003_Machinery%20Equipment%20&%20Engineering _Nov2014_.pdf
9. Lillienberg, L., Andersson, E., Jarvholm, B. and Toren, K. Respiratory symptoms and exposure-response relations in workers exposed to metalworking fluid aerosols. Annals of Occupational Hygiene. 2010;54:403-11.
10. Maizura H, Hashim Z, Rukman A. A review: Health implication of microbial exposure of metalworking fluids (MWFs) to chemical and industrial machinists. Advance in Environmental Biology. 2015;9:220-5.
11. Brown, T. Strategies for prevention: occupational contact dermatitis. Occupational Medicine. 2004;54:450-7.
12. Perkins, S. and Angenent, L. Potential pathogenic bacteria in metalworking fluids and aerosols from a machining facility. FEMS Microbiology Ecology. 2010;74:643-54.
13. Burge P. Hypersensitivity pneumonitis due to metalworking fluid aerosols. Current Allergy Asthma Reports. 2016;16:59.
14. Rayan, K., Cesta, M., Herbert, R., Brix, A., Cora, M., Witt, K., Kissling G, Morgan, DL. Comparative pulmonary toxicity of inhaled metalworking fluids in rats and mice. Toxicology and Industrial Health. 2016;1:1-21.
15. Barber, C., Burton, C., Hendrick, D., Pickering, A., Robertson, A., Robertson, W. Burge, S. Hypersensitivity Pneumonitis in workers exposed to Metalworking fluids. American Journal of Industrial Medicine. 2014;57:872-80.
16. Lewis, D., Janotka, E., Whitmer, M. and Bledsoe, T. Detection of microbial antigens in metalworking fluids. International Biodeterioration & Biodegradation. 2001;47:89-94.
17. Passman, F. Metalworking Fluids Microbes-What we need to know to understand cause-and-effect relationships successfully. Tribology and Lubrication Technology. 2008;3:2-12
18. Laitinen S1, Linnainmaa M, Laitinen J, Kiviranta H, Reiman M, Liesivuori J.Endotoxins and IgG antibodies as indicators of occupational exposure to the microbial contaminants of metal-working fluids. Int Arch Occup Environ Health. 1999 Oct;72(7):443-50.
19. Hashim NM, Hashim Z, Hamat RA. Total serum IgG and respiratory symptoms as determinants of occupational exposure to the microbial contaminants in metal working fluids among machining industry workers Ann Trop Med Public Health [serial online] 2017 [cited 2018 Aug 19];10:82-9. Available from: http://www.atmph.org/text.asp?2017/10/1/82/205545.
20. David Fishwick, Paul Tate, Joanne Elms, Edward Robinson, Brian Crook, Frank Gallagher, Roderick Lennox and Andrew Curran Respiratory symptoms, immunology and organism identification in contaminated metalworking fluid workers. What you see is not what you get. Occupational Medicine 2005;55:238–241 doi:10.1093/occmed/kqi049
21. Phillip L James, Julie Cannon, Christopher M Barber, Laura Crawford, Heather Hughes, Meinir Jones, Joanna Szram, Steven Cowman, William O C Cookson, Miriam F Moffatt, Paul Cullinan Metal worker's lung: spatial association with Mycobacterium avium Occupational lung disease doi:10.1136/thoraxjnl-2017-210226
22. Atlas, R. Handbook of Microbiological media for the examination of food. 2nd ed. Florida: Taylor & Francis; 2006.
23. Luis M, Marie T, Baron E. Color Atlas of diagnostic microbiology. 1st ed. St. Louis Missouri: Mosby;1997.
24. Ahmadi, K., Wilson, C., Tiwana, H., Binder, A. and

Embringer, A. Antibodies to Klebsiella pneumoniae lipopolysaccharide in patients with ankylosing spondylitis. British Journal of Rheumatology. 1998;37:1330-3.
25. Medical Research Council's Committee (MRC). Environmental and Occupational Health Questionnaire on respiratory symptoms [Internet]. Medical Research Council's Committee UK; 1986; [cited 2018 August 13]. Available from https://mrc.ukri.org/documents/pdf/questionnaire-on-respiratory-symptoms-1986/.
26. Kurpiewska J, Liwkowicz J, Benczek K. A survey of work-related skin diseases in different occupations in Poland. International Journal of Occupational Safety and Ergonomics. 2011;17(2):207-214.
27. Health and Safety Executive Cross contamination of metalworking fluid system [Internet]. London HSE UK; 2006; [cited 2013 September 19]. Available from: www.hse.gov.uk/research/rrpdf/rr441.pdf.
28. Marchand, G., Lavoie, J., Racine, L., Lacombe, N., Belanger, Y. and Lemelin, C. Evaluation of bacterial contamination and control methods insoluble metalworking fluids. Journal of Occupational and Environmental Hygiene. 2003;7:358-66.
29. Bracker, A., Storey, E., Yang, C. and Hodgson M. An outbreak of hypersensitivity pneumonitis at a metalworking plant: A longitudinal assessment of intervention effectiveness. Applied Occupational and Environmental Hygiene. 2003;18:96-108.
30. Trafny, E., Lewandowski, R., Kozlowska, K., Zawistowska-Marciniak, I. Stepinska, M. Microbial contamination and biofilms on machines of metal industry using metalworking fluids with or without biocides. International Biodeterioration and Biodegradation. 2015;99:31-8.
31. Veillitte, M., Thorne, P., Gordon, T. Duchaine, C. Six months tracking of microbial growth in a metalworking fluid after system cleaning and recharging. Annals of Occupational Hygiene.2004;48:541-6.
32. Dilger, S., Fluri, A. and Sonntag, H. Bacterial Contamination of Preserved and Non-Preserved Metalworking Fluids. International Journal of Environmental Health. 2005;208:467-76.
33. Awosika-Olumo A., Trangle, K. and Fallon, L. Microorganism-induced skin disease in workers exposed to metalworking fluids. Occupational Medicine. 2003;53:35-40.