ORIGINAL ARTICLE

Exposure to Microbial Contaminants in Metalworking Fluids (MWF) and the Fractional Exhaled Nitric Oxide (FeNO) Levels among Machining Industry Workers

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ABSTRACT

Introduction: Water based Metalworking fluids (MWF) are commonly used in machining industries and are excellent media for microorganism growth. The study aimed at determining the relationship between the airway inflammation as indicated by fractional exhaled nitric oxide (FeNO) with the microbial contaminants of MWF in aerosol and bulk sample as well as the workers' reported respiratory health symptoms. **Methods:** This cross sectional study was carried out on 138 machining workers. Their FeNO were measured using NIOX-MINO instrumentation. The microbial assessments of bacteria and fungus were carried out on the MWF bulk samples and the aerosol using a sampler DUO SAS SUPER 360TM. **Results:** Findings showed significant difference in the FeNO levels in workers from various job sections (p=0.01). Significant relationships found between high FeNO levels with their closeness to the machines (p=0.03), high number of machines in the workplaces (p=0.02), high environmental bacteria colonies (p=0.04), longer employment years (p<0.001) and more frequent cough reported (p=0.03). **Conclusion:** Risk factors in the workplace which contributed to higher airway inflammation include their short distance and high number of machines, high environmental bacteria colonies in aerosol and bulk samples as well as longer employment years. Exposures to MWF had also resulted in significantly increased coughing among the respondents.

Keywords: Airway inflammation, Fractional exhaled nitric oxide, Machining workers, Respiratory health symptoms

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INTRODUCTION

Metalworking fluids (MWF) are used as lubricant, coolant, cutting fluid, machining fluid including hydraulic oil (1). MWF can helps in preventing metal corrosion and act as a metal scrap remover while undergoing machining process (2). It comprises four types; straight oil, soluble oil, synthetic and semi-synthetic MWF (3). The use of MWF may potentially produce contaminants such as bacteria and fungi, other than soluble metal ions such as chromium, nickel as well as cobalt in alloyed steel and hard metal (4). These fluids are extensively used as industrial lubricants to facilitate the lubricating and the cooling of metalworking operations by reducing friction at tool-work piece interfaces, thus reducing heat. MWF are also used to wash away waste metal debris, thus improving the machining performance and as a result, prolonging the life of the cutting tool.

The National Occupational Exposure Survey conducted in 1981-1982 by the National Institute for Occupational Safety and Health (NIOSH) in the USA reported that the potential of workers exposed to MWF was estimated around 1.2 million (5). Previous study stated water-containing MWF which widely replaced oil coolants was associated with an increased incidence of occupational respiratory diseases (6). In addition, exposure to MWF was associated to various health diseases such as cancer, respiratory outcome and skin diseases (7). Respiratory health effects such as upper respiratory irritation, asthma, and hypersensitive pneumonitis (HP) are caused from exposure to diluted MWF, microbial and chemical contaminants of the fluids (7). Dermatitis is the most common complaint as the side effects associated with MWF. Used MWF containing water had high risk of microbial contamination. Workers were exposed to the MWF either through inhalation of the diluted fluids mist (aerosol) or via skin absorption through contact while handling the bearings coated with MWF (3).

A non-invasive tool called the fractional exhaled nitric oxide (FeNO) has been increasingly used to measure inflammation of the airway (8). The aim of this study was to determine the relationship between the airway inflammation with the microbial contaminants in MWF aerosols and the health symptoms as reported by the machining workers.

MATERIALS AND METHODS

Study population

This cross-sectional study was conducted in one of the factories located in Negeri Sembilan, Malaysia. This studied factory was a bearing manufacturing factory, where MWF were extensively used in the metal working process. The name list of all workers (200 workes) whose work tasks involved with MWF were obtained from the Human Resource Department. Permission forms were distributed to the listed workers and about one hundred and sixty nine workers (169 workers) volunteered to participate in the study. From these, only one hundred and thirty eight workers (138 workers) had written consent and fulfilled the inclusion criteria of the study which were, healthy adults with no chronic diseases, between the age range of 20 to 58 years, and have been employed for more than 6 months at the time of the study. Approval from the factory management were also obtained. This study obtained ethical approved from the Universiti Putra Malaysia, Medical Research Ethics Committee of (FPSK (FR14) P002).

Workplace and metalworking fluids (MWF)

The production of bearings within the factory consist of four main sections namely the self-aligning ball bearing (SABB), deep groove ball bearing (DGBB), spherical roller bearing (SRB), and the large size roller (LSR). From these sections, the factory is further divided into 12 sub-sections namely SABB 1, SABB 2, SABB 3, DGBB 1, DGBB 2, DGBB 3, SRB 1, SRB 2, SRB 3, SRB 4, SRB 5, and finally LSR. All of these sections used almost similar types of MWF, namely Hysol x, Castrol Solvent D100, H 460, Biogard 25 and Koolgard 16.

Environmental sampling

The microbial air samplings were performed using a DUO SAS SUPER 360™ for a duration of 15 minutes at each of the 4 locations in the work sections, to identify the number of colonies of bacteria and fungus in the environment. There were two different types of agar plates that were used; tryptic soy agar (TSA) for bacteria and sabouraud dextrose agar (SDA) for fungus. The air flow speed was set at 200 L/min. After incubating, both the agar plates at 25°C for 2 to 7 days, colonies formed were counted to estimate the bacteria and fungus concentration by using the colony forming unit (CFU).

Bulk sampling of metalworking fluids (MWF)

About 10 ml of bulk samples were collected in sterile containers from 12 MWF tanks in various work sections. These were serially diluted to tenfold dilution in 0.85% NaCl and inoculated into 2% SDA for fungus and into TSA for bacteria. All plates were incubated at 25°C for 2 to 7 days and colonies were counted to estimate the bacteria as well as fungi CFU in various work sections.

Measurement of fractional exhaled nitric oxide (FeNO)

FeNO was used to determine the degree of airway inflammation. Respondents' FeNO levels were measured using portable electrochemical detection device called NIOX-MINO®; Aerocrine AB (9) with reference to the standard procedure for FeNO measurement (10). Those respondents with cold on the particular day were excluded and later measured when recovered. Prior to the FeNO test, respondents were advised to avoid nitrate rich food at least 3 hours before and from strenuous activities at least 1 hour before. During the FeNO test, nose clips were avoided to ensure that exhaled nitric oxide did not accumulate in the nose which may potentially leak into the exhaled air stream through the posterior nasopharynx (10). During the inhalation phase, respondents were required to breathe through a disposable nitric oxide scrubbing filter and exhale twice before the FeNO measurement. On the third breath, respondents were required to inhale through the disposable filter and exhale into the FeNO analyzer. The exhaled FeNO measurements, with a flow rate of 50 mL/s for 10 seconds were guided by a light and sound signal to assure steady flow (11).

Data Analysis

Data collected were analysed using descriptive and multivariate statistics with the application of the Statistical Package for Social Sciences (SPSS) Version 22. Kolmogorov-Smirnov statistics was used to test the normality of the continuous variables. If the data is normal, parametric statistical test was used while if not normal, nonparametric statistical test was used Descriptive analysis was used to explore and analyse socio-demographic data and other related variables in terms of mean value, standard deviation and frequency. Pearson correlation was used to determine the correlation between FeNO levels and environmental aerosol as well as MWF bulk sample microbial contaminants. Multiple linear regression and multiple logistic regression were used to determine the relationship between the selected independent variables with the dependent variables.

RESULTS

Socio-demography, work information, and smoking habits of 138 respondents were obtained through an interview and a self-constructed questionnaire as shown in Table I. The respondents were within the range of 20 to 29 years old were 40.6% while 42% of the respondents were found to be overweight, 43.5% were smokers and only 5.8% reported to consume alcohol on a daily basis. Nearly half of the respondents have been working in the factory for 6 months to 5 years (47.1%).

Table I: Socio-demographic Information of Respondents

Variables		Frequency n (%)
Age (Years)		
	20 to 29	56 (40.6)
	30 to 39	18 (13.0)
	40 to 49	45 (32.6)
	≥50	19 (13.8)
BMI		
	Underweight (<18.5)	5 (3.6)
	Normal (18.5 - 24.9)	54 (39.1)
	Overweight (25 - 29.9)	58 (42.0)
	Obese (≥ 30)	21 (15.2)
Smoking Habit		
	Yes	60 (43.5)
Alcohol Intake		
	Yes	8 (5.8)
Employments Years		
	6 month - 5years	65 (47.1)
	5 years – 10 years	13 (9.4)
	10 years – 20 years	32 (23.2)
	>20 years	28 (20.3)
Working Duration		
	8 hours	80 (58.0)
	12 hours	58 (42.0)
Past Working Experience		
	0 month	29 (21.0)
	<6 months	22 (15.9)
	6 months – 5 years	72 (52.2)
	5 years – 10 years	11 (8.0)
	10 years – 20 years	4 (2.9)

(N=138)

The distribution of respondents, number of MWF machines and their distances in the 12 work sections are shown in Table II. Most respondents came from Section DGBB 1 and SABB 1. The sections with the most machines were SABB 1 and SABB 2 where the MWF machines were also placed closer together with the distances of not more than 3 metres.

Table II: Workplace Information at Different Job Sections

Job Sections	No. of Respondents (N=138) n (%)	No. of Machines (N= 84) n (%)	Distance between machines (m)
SABB 1	17(12.3)	10(11.9)	<3m
SABB 2	5(3.6)	10(11.9)	<3m
SABB 3	10(7.2)	8(9.52)	<3m
DGBB 1	18(13.0)	6(7.14)	>3m
DGBB 2	6(4.3)	8(9.52)	<3m
DGBB 3	13(9.4)	7(8.33)	<3m
SRB 1	10(7.2)	7(8.33)	>3m
SRB 2	15(10.9)	8(9.52)	>3m
SRB 3	6(4.3)	5(5.95)	>3m
SRB 4	7(5.1)	6(7.14)	<3m
SRB 5	16(11.6)	5(5.95)	<3m
LSR	8(5.8)	4(4.76)	>3m
All plant	7(5.0)	84(100.0)	

The microbial contaminants levels were measured in various job sections. The microbial levels were obtained by measuring the bacterial and fungal concentrations in the environment as well as from the bulk MWFs sampled from the machines. Table III shows the microbial contaminant levels and the distribution of FeNO levels in various job sections. Sections SABB reported the highest bacterial and fungal levels within the working environment. Total mean value for the environmental aerosol bacteria and fungus in all the job sections were 285.83 cfu/m^3 and 231.2 cfu/m3 respectively. Whereas, the microbial contamination means in the MWF bulk samples were 37,916.7 cfu/m3 for bacteria and 38,833.3 cfu/m^3 for fungus respectively.

The SRB Section respondents had the highest FeNO level which was recorded at 34.46 ppb. Workers in Section SRB 1 reported the highest lung inflammation of 43.00 ppb. The normal FeNO range is between 20 and 25 ppb. Table III shows a comparative one way ANOVA test which determined the difference in the FeNO levels between each job section, which showed significant

Table III: Microbial Levels in Various Job Sections and FeNO Levels among Respondents

Job Sections	Level of Microbial Contaminants				N	Level of FeNO in each Sub-section Mean (S.D) (ppb)	Level of FeNO in Combined Sections Mean (ppb)	F(df)	p
	Environmental Bacteria (cfu/m^3)	Environmental Fungal (cfu/m^3)	Bulk MWF Bacteria (cfu/m^3)	Bulk MWF Fungal (cfu/m^3)					
SABB 1	24.5 x 10^1	23.0 x 10^1	42 x 10^3	42 x 10^3	19	30.68 (12.86)			
SABB 2	66.5 x 10^1	37.0 x 10^1	32 x 10^3	32 x 10^3	5	32.40 (15.57)	28.96		
SABB 3	37.0 x 10^1	35.0 x 10^1	43 x 10^3	44 x 10^3	10	23.80 (13.04)			
DGBB 1	24.5 x 10^1	22.0 x 10^1	42 x 10^3	43x 10^3	6	32.17 (17.23)			
DGBB 2	19.0 x 10^1	19.0 x 10^1	36 x 10^3	44 x 10^3	13	27.31 (12.78)	29.22		
DGBB 3	20.0 x 10^1	21.0 x 10^1	37 x 10^3	37 x 10^3	22	28.17 (12.28)			
SRB 1	28.5 x 10^1	35.0 x 10^1	42 x 10^3	34 x 10^3	5	43.00 (10.56)			
SRB 2	33.5 x 10^1	14.0 x 10^1	36 x 10^3	39 x 10^3	15	35.67 (13.61)			
SRB 3	25.0 x 10^1	21.5 x 10^1	41 x 10^3	42 x 10^3	5	39.60 (10.16)	34.43		
SRB 4	21.5 x 10^1	18.5 x 10^1	41 x 10^3	40 x 10^3	5	33.40 (11.68)			
SRB 5	21.0 x 10^1	16.0 x 10^1	32 x 10^3	43 x 10^3	15	20.47 (9.02)		2.24 (12)	0.01*
LSR	22.0 x 10^1	15.5 x 10^1	32 x 10^3	43 x 10^3	4	29.75 (16.07)	29.75		
All plant					14	27.71 (12.55)	27.71		
Total Mean (S.D)	28.58 x 10^1 (13.13 x 10^1)	23.12 x 10^1 (8.05 x 10^1)	37.92 x 10^3 (4.64 x 10^3)	38.83 x 10^3 (4.13 x 10^3)					
Total					138	29.58 (12.55)	29.58		

One way ANOVA *Significant at p<0.05

differences among the sections (p <0.01).

From the total of 138 respondents, the highest reported respiratory symptoms were wheezing (29%), followed by phlegm (18.1%), cough (17.4%). Reported skin symptoms, was skin itchiness (39.9) as the highest, followed by rashes (39.1%) and skin inflammation (23.9) which were the most common skin symptoms.

From Table IV, there was a significant relationship between FeNO levels and cough. Airway symptoms were reported in 39% of the respondents exposed to MWF although the measured MWF levels in the work place were low. Moreover, respondents who operated machines using MWF had significantly higher prevalence of work-related nasal irritation and coughing than the unexposed workers at the same factory (14). Incidence rate in airway inflammation accompanied by asthma like symptoms such as cough and chest tightness were more than twice as high among the machinists compared to other apprentices (13).

From the statistical test in determining the relationship between FeNO levels with the microbial contaminants environmental aerosol and MWF bulk samples, a significant relationship between the FeNO levels with environmental aerosol bacteria was found (p=0.05).

Table IV: Relationship between Airway Inflammation Levels (FeNO) with Health Symptoms

Variables	FeNO Levels[a] (N=138)	
	OR (95%CI)	p-value
Respiratory Symptoms		
Cough[a]	2.06 (3.64-6.58)	0.03*
Phlegm[a]	1.01 (0. 98-1.04)	0.59
Chest Tightness[a]	1.01 (0.98-1.04)	0.67
Wheezing[a]	1.01 (0.96-1.03)	0.93
Skin Symptoms		
Rashes[a]	1.02(0.99-1.04)	0.25
Itching[a]	1.02(0.99-1.04)	0.22
Inflammation[a]	1.02(0.99-1.05)	0.25

Multiple Logistic Regression Test
[a]Dependant variables (Reported health symptoms for the past 6 months during the interview)
*Significant at p<0.05

Table V shows the relationship between FeNO levels with the socio-demographic, lifestyle, and workplace characteristics. There were significant relationships between employment years (OR=8.93, p <0.001), distance of machine OR=0.79, p=0.03) and numbers of machines (OR=2.55, p=0.02) with FeNO levels.

DISCUSSION

All of the respondents were males within the age range of 20 to 29 years, high percentage were obese and smokers. A minority consume alcohol since almost all respondents were of Malay ethnic and Muslims. The biggest group were those employed less than 5 years as they were young energetic segment of the population. Majority worked 8 hours daily while the rest had additional over time work hours. In term of work experience, most of the respondents had at least 6 months to 5 years work experience. The employer also prefer young workers with experience.

Table V: Relationship between FeNO levels with Socio-demographic, Lifestyle and Workplace Information

Variables	FeNo Level (N=138)	
	OR (95%CI)	p-value
Age	0.06 (0.14 - 0.25)	0.56
Body Mass Index	0.19 (0.02 – 0.40)	0.08
Employments Years	8.93 (7.33 – 10.54)	<0.001**
Work Duration	0.80 (3.00 – 1.40)	0.47
Past Working Experience	0.19 (0.82 – 0.45)	0.56
Alcohol Intake	0.32 (7.37 – 0.99)	0.13
Smoking	0.66 (1.46 – 2.77)	0.54
Distance machine	1.79 (1.64 - 1.96)	0.03*
No machines	2.55 (1.11 - 5.82)	0.02*

Multiple Linear Regressions Test (method enter)
*Significant at p< 0.05
**Significant at p< 0.001

Information on their work areas / sections showed that those workers from SABB 1 and 2, with high number of machines and close distance between them were the most likely to be exposed to the MWF due to the crowdedness.

The high microorganism levels in the atmospheric environment and the MWF machine in Section SABB could be because of the proximity between the each of the MWF machines were less than 3 meters. It is known that the closer the machines are to one another, the higher the bacteria and fungus levels in the atmosphere. These findings suggest that the total number of bacteria collected in the air surrounding the machines was inversely related to the distance measured between the machines (12). Job Section SABB 2 had the highest number of water-based MWF machines in the factory, suggesting the microorganisms could be from the MWF aerosols emitted from the machine which had accumulated over time.

The high FeNO levels in Section SRB were due to the high total number of sub-sections. Besides that, SRB job section had the highest number of machines and workers. Previous study has shown that workers exposed to aerosol microbes were associated with specific occupational asthma, increased respiratory symptoms and hypersensitivity pneumonitis (13). Most of the respondents in the Section SRB 2 had longer employment years which contributed to the lung inflammation due to the prolonged exposure.

According to an in vitro test performed in a study (14), samples of used MWF taken directly from a cutting machine at a factory had considerably higher inflammatory promoting effect than an unused MWF. The fact that if this was caused by higher amounts of antibacterial formaldehyde, microbial growth or the generation of pro-inflammatory products such as endotoxin or enrichment of particulate matter remained unknown. However, it can reasonably argued that the use of MWF over a period of time is most likely to increase airway inflammation (14). In our study, findings showed correlation between FeNO levels with the atmospheric environmental bacteria. Thus, suggesting that most of the microorganism present in the environment were emitted from MWF machine in a form of aerosol which most likely inhaled and resulted in airway inflammation among the respondents. Previous findings suggested that endotoxin contamination of MWF in the form of aerosol is the causative agent for inducing inflammatory response in airways (15, 16).

Based on a study, the increase in bronchial responsiveness was associated with duration of exposure to water-based MWF (13). In accordance to our study, high employments years (higher duration of exposure) were related to high FeNO level (airway inflammation). Our findings also were consistent with other studies which reported the distance between MWF machine were inversely related to the FeNO level, had resulted in high microbial contaminant levels. Findings showed that the total number of bacteria collected with a Reuter centrifugal sampler in the air surrounding machines was inversely related to the distances from the machines (12). Bacterial contamination of the MWF might have contributed to the airway inflammation (14). In this study, job section was also one of the risk factors for high FeNO levels. High FeNO levels indicated high lung inflammation were found in respondents who had worked in job sections with high environmental bacteria levels, high number of machines and close proximity between the machines. In addition, the effect of cigarette smoking on FeNO levels in this study should also be taken into consideration. In a previous study, the FeNO levels of steroid-naive asthmatics smokers were found to be lower than asthmatics non-smokers. In general, smoking reduces the FeNO levels (17). On the contrary, our results showed that smoking did not influence the FeNO levels. (Table VI).

Airway symptoms were reported in 39% of the workers exposed to MWF although the measured MWF levels in the work place were low. Moreover, workers who operated machines using MWF had significantly higher prevalence of work-related nasal irritation and coughing than the unexposed workers at the same factory (14). Incidence rate in airway inflammation accompanied by asthma like symptoms such as cough and chest tightness were more than twice as high among the machinists compared to other apprentices (13).

Based on a study, elevation of FeNO level helped to make a final diagnosis of chronic cough (15). Another study (17) also concluded that FeNO measurements could be used as a diagnostic marker for prolonged cough, especially in identifying bronchial asthma and cough variant asthma from eosinophilic bronchitis without asthma.

CONCLUSION

In conclusion, this study showed that respiratory symptoms among respondents exposed to MWF were common occupational hazard. Exposure to MWF also resulted in significantly frequent cough symptoms. Risk factors from the workplace such as the number of machines and their low proximity, high environmental aerosol bacteria colonies and duration of employment years had positive significant relationships with the airway inflammation (FeNO). These findings warranted that FeNO test is useful to diagnose respiratory diseases from exposure to atmospheric pollutants within a workplace.

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