RELATIONSHIP BETWEEN SERUM IgG LEVEL, FRACTIONAL EXHALED NITRIC OXIDE AND MICROBIAL CONTAMINATION IN METALWORKING FLUIDS AMONG MACHINISTS IN NILAI, NEGERI SEMBILAN, MALAYSIA

NURUL MAIZURA BINTI HASHIM

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By

NURUL MAIZURA BINTI HASHIM

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RELATIONSHIP BETWEEN SERUM IgG LEVEL, FRACTIONAL EXHALED NITRIC OXIDE AND MICROBIAL CONTAMINATION IN METALWORKING FLUIDS AMONG MACHINISTS IN NILAI, NEGERI SEMBILAN, MALAYSIA

By

NURUL MAIZURA BINTI HASHIM

December 2016

Chairman : Professor Zailina Hashim, PhD
Faculty : Medicine and Health Sciences

Introduction: Metalworking fluids (MWFs) are commonly used in machining industry during metal working process such as cutting, turning, milling, drilling and grinding. Water based metalworking fluids (MWFs) are commonly used in machining industries and are excellent media for microorganism growth. Machining’s workers are most exposed to the contaminant of metalworking. Problem Statement: Machining industry is one of the industries in Malaysia that are rapidly growth. Soluble metalworking fluids (MWFs) are commonly used by machining industry. These fluids represent a high potential for health risk due to high water content present in the emulsion which is excellent for micro-organism growth. Respiratory problem and skin problem may be implicated by the microbial contaminants exposure of metalworking fluids (MWFs). Upper airway inflammation was a common problem among machinists and it is reported that, about 39% of the workers exposed to MWFs had airway symptoms. MWFs exposure containing a mixture of reactive and pro-inflammatory agents may induce a toxic response in the upper cell-linien, resulting in decreased formation of normally expressed immune defence protein. Objective: To study the relationships between serum IgG levels and airway inflammation with the microbial exposure among MWFs workers. Methodology: This cross sectional study was carried out on 138 machining workers exposed to MWF. Background, working, health and resedential information of respodents were collected by a set of questionnaire adapted from American Thoracic Society (ATS-DLD-78). Two ml blood were drawn from the cubital to be tested for the level of total and specific IgG antibody in blood. The workers’ FeNO were measured using NIOX-MINO instrumentation. The microbial assessments were carried out on the MWF bulk samples and the aerosol in the work environment. DUO SAS SUPER 360TM sampler was used to sample the bacteria and fungi in the air. The data were analysed using the SPSS Version 22.0. Result and Discussion: This study found that, the mean value for the environmental bacteria and fungal in all work sections were 285.83cfu/m³ and 231.2cfu/m³ respectively. Whereas, the means for microbial contamination in bulk samples were 37916.7 cfu/ml³ (bacteria)
and 38833.3 cfu/ml³ (fungal) respectively. The mean of the IgG in all work sections was 13.79 g/litre. The environmental bacteria levels has a significant correlation with the total IgG antibodies levels (p=0.003). There were also a significant relationship between BMI (p=0.044), work duration (p=0.014), smoking (p=0.014) and environmental contaminants (0.049) with the total of IgG.levels Findings also showed significantly difference in the FeNO levels of workers from various work sections (p=0.01). From the multivariate statistics, there were also significant relationships between the high FeNO with their closeness between machines (p=0.03), higher number of machines (p=0.02), high environmental bacteria colonies (p=0.04), longer employment years (p=<0.001) and frequent reporting of respiratory symptom such as cough (p=0.03). **Conclusion:** There were significant correlations between the total IgG antibodies levels with the microbial contaminants of metalworking fluids in metal working process. Besides, there were also significant relationships between work duration, smoking, BMI and environmental contaminants with the total IgG.levels. Risk factors from the workplace such as the number of machines, closeness between machines and high environmental bacteria colonies and longer employment years had significant relationships with the airway inflammation (FeNO). Exposure to MWF also resulted in significantly frequent cough symptom.

**Keywords:** Metalworkings fluids, IgG Antibodies, Microbial Contaminants, Airway Inflammation and Fractional Exhaled Nitric Oxide (FeNO)
Abstrak tesis yang di kemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

HUBUNGAN ANTARA SERUM IgG DAN KERADANGAN SALUR PERNAFASAN SEBAGAI PENENTU PENDEDAHAN MIKROB DALAM BENDALIR KERJA LOGAM ANTARA PEKERJA PEMESINAN DI NILAI, NEGERI SEMBILAN, MALAYSIA

Oleh

NURUL MAIZURA BINTI HASHIM

Disember 2016

Pengerusi : Professor Zailina Hashim, PhD
Fakulti : Perubatan dan Sains Kesihatan

Pengenalan: Bendalir kerja logam (MWFs) yang biasa digunakan dalam industri pemesinan semasa proses kerja logam seperti memotong, membelok, pengilangan, penggerudian dan pengisaran. Bendalir kerja logam (MWFs) yang berasaskan air biasa digunakan dalam industri pemesinan merupakan media yang sangat baik bagi pertumbuhan mikroorganisma. Pekerja pemesinan ini adalah yang paling terdedah kepada bahan cemar logam. Pernyataan Masalah: Industri pemesinan adalah salah satu industri di Malaysia yang pesat pertumbuhan. cecair logam larut (MWFs) yang biasa digunakan oleh industri pemesinan. cecair ini mewakili potensi yang tinggi untuk risiko kesihatan kerana kandungan air yang tinggi di dalam emulsi yang sangat baik untuk pertumbuhan mikro-organisma. masalah pernafasan dan masalah kulit mana yang berkaitan dengan pendedahan pencemaran mikrob cecair logam (MWFs). Upper keradangan saluran udara adalah masalah biasa di kalangan machinists dan ia dilaporkan bahawa, kira-kira 39% daripada pekerja yang terdedah kepada MWFs mempunyai gejala saluran udara. pendedahan MWFs mengandungi campuran ejen reaktif dan pro-radang boleh menyebabkan tindak balas toksik di atas sel-linin, menyebabkan pembentukan penurunan biasanya dinyatakan protein pertahanan imun Objektif: Untuk mengkaji hubungan antara tahap serum IgG dan keradangan saluran pernafasan dengan pendedahan mikrob dalam kalangan pekerja MWFs. Metodologi: Kajian irisan lintang telah dijalankan ke atas 138 pekerja pemesinan yang terdedah kepada MWFs. Maklumat latar belakang, pekerjaan, kesihatan dan kediaman responden telah dikumpulkan dalam satu set soal selidik yang diadaptasi daripada Persatuan Thoracic Amerika (ATS-DLD-78). Dua ml darah telah diambil daripada cubital responden untuk menguji tahap jumlah antibody IgG dalam darah. Pecahan hembusan Nitrik Oksida (Feno) pekerja diukur menggunakan instrumen NIOX-MINO. Penilaian mikrob telah dijalankan ke atas sampel MWF di setiap mesin dan aerosol dalam persekitaran kerja. Intrumen DUO SAS SUPER 360™ telah digunakan untuk persampelan bakteria dan kulat di udara. Data telah dianalisis dengan menggunakan
perisian SPSS Versi 22.0. **Keputusan dan Perbincangan:** Kajian ini mendapati bahawa, nilai minima bagi bakteria alam sekitar dan kulat dalam semua bahagian kerja adalah 285.83 cfu/m³ dan 231.2 cfu/m³. Manakala pencemaran mikrob dalam sampel MWFs adalah 37.9167 cfu/ml³ (bakteria) dan 38.8333 cfu/ml³ (kulat). Purata bagi tahan IgG dalam semua bahagian kerja adalah 13.79 g/liter. Tahap Bakteria di dalam persekitaran mempunyai hubungan yang signifikan dengan jumlah IgG antibodi (p=0.003). Terdapat juga hubungan yang signifikan antara BMI (p=0.044), tempoh kerja (p=0.014), merokok (p=0.014) dan mikrob di persekitar (0.049) dengan tahan IgG responden. Dapatan kajian juga menunjukkan perbezaan dalam tahan Feno pekerja dari pelbagai bahagian kerja (p=0.01). Daripada statistik multivariate, terdapat juga hubungan yang signifikan antara Feno yang tinggi dengan jarak antara mesin (p=0.03), bilangan mesin yang lebih tinggi (p=0.02), mikrob persekitaran yang tinggi (p=0.04), pengalaman bekerja yang lama (p=<0.001) dan laporan gejala pernafasan seperti batuk (p=0.03). **Conclusion:** Terdapat hubungan signifikan antara jumlah antibodi IgG dengan mikrob di dalam sampel berdalir kerja logam dalam proses kerja logam. Selain itu, terdapat juga hubungan yang signifikan di antara tempoh kerja, merokok, BMI dan pencemaran mikrob di persekitaran dengan tahan IgG. Faktor-faktor risiko dari tempat kerja seperti bilangan mesin, jarak antara mesin dan pencemaran mikrob di persekitaran yang tinggi dan tahun pekerjaan juga mempunyai hubungan yang signifikan dengan keradangan saluran udara(Feno). Pendedahan kepada MWFs juga menyebabkan gejala batuk ketara.

**Kata Kunci:** Bendalir Kerja Logam, Antibodi IgG, Pencemaran Mikrob, Keradangan Salur Pernafasan dan Pecahan hembusan Nitrik Oksida (Feno)
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I certify that a Thesis Examination Committee has met on 28 December 2016 to conduct the final examination of Nurul Maizura binti Hashim on her thesis entitled "Relationship between Serum IgG Level, Fractional Exhaled Nitric Oxide and Microbial Contamination in Metalworking Fluids among Machinists in Nilai, Negeri Sembilan, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

**Sarva Mangala Praveena, PhD**  
Senior Lecturer  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Chairman)

**Zamberi bin Sekawi, PhD**  
Professor  
Faculty of Medicine and Health Science  
Universiti Putra Malaysia  
(Internal Examiner)

**Rusli Nordin, PhD**  
Professor  
Monash University  
Malaysia  
(External Examiner)

[Signature]

**NOR AINI AB. SHUKOR, PhD**  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 22 March 2017
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Zailina Hashim, PhD  
Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

Rukman Awang Hamat, PhD  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

Hayati Kadir, PhD  
Senior Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

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Professor and Dean  
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<td>Body Mass Index</td>
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<td>CFU</td>
<td>Colony Forming Unit</td>
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<td>DGBB</td>
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<td>eNos</td>
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<td>Nitric Oxide</td>
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<tr>
<td>NOS</td>
<td>Nitric Oxide System</td>
</tr>
<tr>
<td>Ppb</td>
<td>Part Per Billion</td>
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<tr>
<td>PPE</td>
<td>Personal Protective Equipment</td>
</tr>
<tr>
<td>SABB</td>
<td>Self-Aligning Ball Bearing</td>
</tr>
<tr>
<td>SRB</td>
<td>Spherical Roller Bearing</td>
</tr>
<tr>
<td>TH</td>
<td>T-helper</td>
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<tr>
<td>$X^2$</td>
<td>Chi Square</td>
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</table>
CHAPTER 1

INTRODUCTION

1.1 Background of Study

Metalworking fluids (MWFs) are commonly used in machining industry during metal working process such as cutting, turning, milling, drilling and grinding. These fluids were used extensively as industrial lubricants to provide lubricate cool metalworking operation by reducing friction at tool-work piece interfaces and carrying away heat.

MWFs are also frequently known as coolant, lubricants, machining fluids and cutting oil (NIOSH, 2011). MWFs generally comprised four major categories which are straight oil, soluble oil, semi-synthetic fluids and synthetic fluids. Straight oil contain of highly refined petroleum, marine, vegetable, animal or synthetic oil and not to be diluted with water. Soluble oil is the combination of highly refined lubricant-based oil and emulsifiers which will be diluted with water. Semi-synthetic fluids contain lower amount of highly refined lubricant-based oil and is water soluble. Synthetic fluids is water soluble and contain no petroleum oil (NIOSH, 1998). All of these MWFs are water soluble except for straight oil.

MWFs are usually contains a wide range of additives, such as biocides, surfactants, anti-oxidants, corrosion inhibitor, emulsifiers and stabilizers. Various chemical additives are formulated into MWFs in order to achieve specific performance requirements. Each of these additives has specific function and its own negative effects to the worker’s health (Fornander, 2013).

Since the most frequently used MWFs in machining industry are water-based type, the primary concern is the presence of contaminants that encourage the growth of bacteria and fungi. The fluids contain hydrocarbon, organic substrate, phosphorus, nitrogen and water which are conducive for microbial growth. Biological agents present in the MWFs liquids are emitted in the formed of droplet bioaerosols during a rapid rotation of the working metal tools which are inhaled by machine operator and causing adverse health effects mainly on respiratory system (Cyprowski et al., 2007).

Exposures to MWFs were associated to various health diseases such as cancer, respiratory outcome and skin disease (Park et al., 2009). Dermatitis is the most common complaint associated with MWFs. Respiratory effects such as upper respiratory irritation, asthma and hypersensitive pneumonitis (HP) are caused by exposure to diluted MWFs, microbial contaminants and chemical contaminants of the fluids (Park et al., 2009).
Based on a National Occupational Exposure Survey (NOES) by the National Safety and Health, it is estimated that 1.2 million workers are potentially exposed to MWFs (NIOSH, 1983). Workers were exposed to the MWFs by inhalation of the diluted fluids mist (aerosol) or skin absorption of fluids through contact with the MWFs during the handling with the workpieces coated with fluids (NIOSH, 1998).

Other chemical constituents may also presence in MWFs during their recirculation such as debris, dust, metal swarf, metal fines, hydraulic oil, bacteria and fungi (Burton et al., 2012). The MWFs are still easily contaminated by microbes even after the adding of biocides into the fluids as a treatment and remain in the tanks (Mattsby-Baltzer et al., 1989). The breakdown product of biocides in the MWFs may serve as nutrients for microbial growth. The common contaminants in MWFs are the Gram-negative bacteria (Bakalova et al., 2007).

There is very few biomarkers of exposure or dose have been identified. IgG molecules are composed of two light chain (kappa or lambda) and two gamma heavy chain. Approximately 80% of serum immunoglobulin is IgG and its main task is to defence our body against microorganism. IgG antibodies in serum have been suggested as an indirect marker of exposure of fungi (Burell and Rylander, 1981; Eduard et al., 1992). IgG antibodies serve as excellent indicator and tool in assessing the exposure to microbial contaminants. There is significant elevation of IgG antibodies response to bacteria antigen (Laitinen et al., 1999).

Based on National Safety and Health (NIOSH), there is no recommended or specific limit for bacterial and fungal concentration in contaminated MWFs due to limited and insufficient health data. Thus, management programme of MWFs is conducted in order to protect workers. However, not all of the control measures are successful in eliminating the microbial contaminants. For example, addition of biocides in the MWFs may result in the emergence of other biocides-resistant strain and induced other health impact such as allergy and dermatitis (NIOSH, 1998).

Previous studies in rats show that the levels of endotoxin-specific antibodies, including IgG\textsubscript{2a} and IgE, were increased significantly ($P < 0.05$) in the rats exposed to endotoxins. The results also shows that lung inflammatory responses may induce without changing pulmonary function after repeated exposure to MWFs contaminated with endotoxins. Thus, the study concluded that endotoxin-specific IgG\textsubscript{2a} and IgE may be effective biomarkers for workers exposed to MWFs contaminated with endotoxins in the workplace (Lim et al., 2005).

Based on Clinical Practice Guideline of The American Thoracic Society (ATS), measurement of fractional nitric oxide (NO) concentration in exhaled breath (FENO) is a quantitative, non-invasive, simple, and reliable method of measuring airway inflammation that provides a complementary tool to other ways of assessing airways disease (ATS, 2011). Studies shows that FENO level increased substantially after exposure to MWFs in most subjects. Repeated measurements of FENO in workers seem to be a relevant method to identify subjects with airway inflammation after
exposure to MWFs. When more subjects have been included in the study, FENO may also help to sort out what characteristics of the exposure can be associated with airway inflammation (Andersson et al., 2012).

1.1 Problem Statement

Rapid shift of employment in manufacturing industry from the agriculture industry is due to the globalization phenomena in Malaysia. Machining industry is one of the industries in Malaysia that are rapidly growth (MIDA, 2012). Widespread use of MWFs as lubricants was initiated along with automobile and aircraft manufacturing in the beginning of the 1900s (Suuronen, 2009).

MWFs are critical issue because it causes a variety of health implications to the machinists and are commonly used in machining industry as lubricant during metal working process were used extensively as industrial lubricants to lubricate metalworking operation by reducing friction at tool-work piece interfaces and carrying away heat. The fluids are used to improve the machining performance thus prolong the life of the cutting tool (HSE, 2011).

Soluble metalworking fluids (MWFs) are commonly used by machining industry. These fluids represent a high potential for health risk due to high water content present in the emulsion which is excellent for micro-organism growth (Duchaine et al., 2012). Respiratory problem and skin problem may be implicated by the microbial contaminants exposure of metalworking fluids (MWFs). Biological agents present in the MWFs liquids are emitted in the formed of droplet bioaerosols during a rapid rotation of the working metal tools which are inhaled by machine operator and causing adverse health effects mainly on respiratory system (Cyprowski et al., 2007).

High number of cases of allergic alveolitis reported in the last 15 years (Bernstein 1995; Kreiss, 1997; Zacharisen 1998). Immune response to environmental antigens was associated with this disease. Mycobacteria group was suspected as a causal agent in infected workers. In the first study reporting cases of allergic alveolitis in metal turning plants, IgGs against *Pseudomonas fluorescens* were investigated (Duchaine et al., 2012).

According to Fornander et al. (2013), upper airway inflammation was a common problem among machinists and it is reported that, about 39% of the workers exposed to MWFs had airway symptoms. Some of the examples of airway symptoms are runny nose, sore throat, nasal blockage and allergic rhinitis. The findings also imply effects on the immune system among worker exposed to MWFs which may contribute to the upper airway inflammation. MWFs exposure containing a mixture of reactive and pro-inflammatory agents may induce a toxic response in the upper cell-linin, resulting in decreased formation of normally expressed immune defence protein (Fornander, 2013). Similar finding has been shown by Greaves et al. (1997), which they conclude that
about two-fold higher risk for upper airway symptoms in workers exposed to MWFs compared to unexposed workers.

A study conducted by Laitinen et al. (2007), showed that there is a significant elevation of specific IgG antibodies response to bacteria antigen and serum IgG antibodies may serve as indicator and tool in assessing the exposure to microbial contaminants. The detection of many diseases is dependent upon accurate detection of particular antibodies present in blood. However, the development of biochemical reagents that can reliably detect these antibodies has proved remarkably challenging. The diagnosis of many diseases relies heavily upon the accuracy of antibody detection. Assays to detect antibodies using known antigens are used extensively to diagnose infectious and autoimmune diseases. And antibodies exhibiting unique antigen binding patterns have been shown to occur in diverse human diseases, including oncological, inflammatory, and neurological and psychiatric disorders.

Based on study by John et. al. (2013), the utility of antibodies in diagnostics derives from their intrinsic affinity and specificity, biochemical stability, and abundance in blood. Nevertheless, the identification of rare antibody specificities indicative of disease and the development of reagents for their accurate detection have proved exceptionally difficult. Intersubjective variability of antibody specificities is a major challenge to the development of accurate tests. Specifically, individual genetic and stochastic variations that shape the antibody repertoire introduce heterogeneity in disease antibody subpopulations (polyclonal variation, specificity, affinity, and titer) that hinders uniform antibody detection.

Human exposure may occur via inhalation of airborne spores and hyphal fragments, ingestion of contaminated food products, and skin or eye contact after handling of contaminated material. The presence of antibodies to specific fungi could be used to determine whether specific species of fungi found in a person’s environment are the cause of health concerns a patient may be having. Although antibody reactivity is necessary to identify certain fungi as possible sources of sensitization, the presence of serum antibody against a specific fungus offers only supporting evidence of causation in cases where environmental exposure has been confirmed (Douglas et. al., 2004).

A study by Immonen et. al. (2002), studied specific IgG to 24 fungi in students at schools with identifiable fungal contamination and students at schools without observable fungal contamination. The study found association between IgG to fungi and exposure to fungi in the school. In addition, there was association between fungal IgG levels and asthma, coughing, or wheezing (Immonen et. al., 2002). Another study, compared IgG levels with a variety of fungi between 93 students in schools with moisture problems and 33 students from a school without moisture problems. The authors found relationship between IgG levels and “exposure” status or the presence of asthma among study participants (Immonen et. al., 2002).
1.2 Research Justification

Machining industry is one of the largest machining industries in Malaysia. MWFs are widely used as lubricant in these machining industries. Most commonly used MWFS in Malaysia industry is water-based which are very conducive for microbial growth such as bacteria and fungus. Machining workers are most exposed to the contaminant of metalworking fluids (Duchaine et al., 2012).

Exposures to MWFs were associated to various health diseases such as cancer, respiratory outcome and skin disease (Park et al., 2009). Dermatitis is the most common complaint associated with MWFs. Respiratory effects such as upper respiratory irritation, asthma and hypersensitive pneumonitis (HP) are caused by exposure to diluted MWFs, microbial contaminants and chemical contaminants of the fluids (Park et al., 2009). However, there is limited study related to metalworking fluids exposure yet in Malaysia. Therefore, more studies are required to study the interaction effect between biological contaminants so that prevalence action can be applied to prevent related health implications.

Nowadays, many workers spend at least 8 hours per day or more than 40 hours per week in their workplace. Healthy workplace is an important concern as the environmental exposures in the workplace will influence health, well-being and productivity of the employees. However, there is lack of MWFs research and its association with airway inflammation and also immune response conducted at this country. Thus, MWFs research among machining workers which are exposed to MWFs and its relation to microbial contaminant exposure need to be explored and therefore, control measures to prevent health impact from the usage of metalworking fluids and the intervention to overcome matter can be taken in the future.

1.3 Study Variables

In this study, the independent variable is the exposure of microbial contaminants in metalworking fluids (MWFs) while the dependent variable is the serum IgG level and airway inflammation level of workers.

1.4 Conceptual Framework

Figure 1.1 shows the conceptual framework of the study. In Malaysia, rapid shift of employment in machining industry from the agriculture industry is due to the globalization phenomena. Automotive, electronic and manufacturing are the examples of machining industries in Malaysia that are rapidly growth (MIDA, 2012). Metalworking fluids (MWFs) are widely used as lubricant in these machining industries.
Physical, chemical and biological hazard are main hazards from the usage of MWFs. Most commonly used MWFs in Malaysia industry is water-based which are very conducive for microbial growth such as bacteria and fungus. Thus, in this study we were focused on the biological hazard which comprises bacteria and fungus. All types of hazards could affect human health through inhalation, ingestion and skin contact.

These microorganisms exposure will cause the physiological response such as airway inflammation and respiratory problem to the human. Besides that, this microbial exposure also will trigger immune response. Thus, antibodies in the human blood are one of the biomarkers and indicator for the specific microbial exposure. In this study, specific IgG antibodies in the blood were studied to study the microbial exposure.
Figure 1.1: Conceptual Framework
1.6 Research Objective

1.6.1 General Objective

To study the relationships between serum IgG levels and airway inflammation (FeNO level) with the microbial exposure among MWFs workers.

1.6.2 Specific Objectives

1. To determine the social demography, residential information, employment and workplace characteristics of the respondents in metal working process.
2. To determine the species and levels of bacteria and fungi in MWFs in various work sections.
3. To determine the distribution of total and specific serum IgG levels, FeNO levels, respiratory and skin symptoms of the respondents in various work sections.
4. To determine the correlation between serum IgG and FeNO levels with the microbial levels in MWFs.
5. To determine the relationship between microbial levels with workplace characteristics.
6. To determine the relationship between serum IgG and FeNO levels with individual, residential, workplace characteristics of the respondents and microbial levels.
7. To determine the relationship between reported health symptoms serum IgG and FeNO levels.

1.7 Research Hypothesis

1. There are significant correlation between serum IgG and FeNO level with the microbial levels of MWFs among respondents in metal working process.
2. There are significant relationships between microbial levels with individual and workplace characteristics of the respondents.
3. There are significant relationships between serum IgG and FeNO levels with individual, residential, workplace characteristics of the respondents and microbial levels.
4. There are significant relationships between reported health symptoms with serum IgG and FeNO levels of the respondents.
REFERENCES


