Correlation between Haemoglobin Concentration and Malondialdehyde Level in Healthy Middle Age Adults

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Abstract

**Background and Aims:** In every disease, there will be the triggers on the occurrences. One of them is the oxidative stress, an imbalance between free radicals such as reactive oxygen species (ROS) and the antioxidants. The human body manages free radicals through neutralization by the antioxidative defense mechanism, however, when excessive free radicals being produced, it will become less effective. Over 40 years of age, oxidative stress become prominent and may associated with many diseases such as neural disorders and cardiovascular diseases. Oxidative stress can cause damage to lipids, known as lipid peroxidation, producing malondialdehyde (MDA), a marker to detect the degree of oxidative stress. Availability of oxygen (O$_2$) is the main factor of the ROS formation, which could be indicated by haemoglobin (Hb) concentration. Thus, this research is conducted to find out the correlation between Hb concentration and MDA level in healthy middle age adults.

**Methods:** A correlation study was conducted in a private University, Shah Alam from January 2018 until March 2018. Total 16 participants, who are MSU staffs, age between 45 and 65, with no known diseases, are selected after the consents are gained. Pearson correlation was used for statistical analysis of the data.

**Results:** It is found that there is a strong correlation between Hb concentration and MDA level (r: .756, p <0.001). This means that MDA production is significantly increased when the Hb concentration is increased.

**Conclusion:** The MDA level is strongly correlate with the Hb concentration in the middle age apparently healthy adults. It can be postulated that MDA production correlates with oxygen availability at the intracellular respiration in normal middle aged person.

**Keywords:**
Oxygen, Oxidative phosphorylation, Reactive oxygen species, Oxidative Stress, Lipid peroxidation, Malondialdehyde, Haemoglobin
Introduction

All the cells in our body use oxygen molecule during intracellular aerobic respiration at the step of oxidative phosphorylation that involves the electron transport along the chain in the inner membrane of the mitochondria and chemiosmosis (Khan Academy). Energy released from electron transport is stored as an electrochemical gradient that is used to make adenosine triphosphate (ATP), main high energy storage compound for cellular functions. If oxygen is not enough in place, the electron transport along the chain will be delayed and ATP will no longer be produced enough as a consequence. This can lead to reduced cellular functions, and, after enough time, cell or even the whole organism may die.

Reactive Oxygen Species (ROS) is a group of reactive molecules and free radicals derived from molecular oxygen. At the end of the electron transport chain, oxygen splits into two atoms, one accepts electrons and forms superoxide like free radicals and another takes protons (H+) to form water (Khan Academy). The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including: superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and nitric oxide (Held, 2014). They have the potential to cause a number of deleterious events. It was previously thought that only phagocytic cells produce ROS in their defense mechanisms. In healthy condition, however, 1-4% of oxygen inhaled is used to form ROS in mitochondria. As a consequence, nearly one billion ROS molecules are produced from every cells daily in our bodies (Wagner, Venkataraman and Buettner, 2011).

The human body has several mechanisms to manage the free radicals such as by producing antioxidants either produced naturally inside the body, the endogenous or externally supplied through foods or supplements, the exogenous. However, when excessive free radicals are gained or the antioxidants fail to cope the injuries occur in the cell by ROS in so many ways, called oxidative stress which can lead to more cellular damage.

Lipids are vulnerable targets because their molecular structure is abundant with reactive double bonds (Porter, Caldwell and Mills, 1995). According to the study of Barrera (2012), the effect of oxidative stress on cells could be found in lipid peroxidation that is a process where the free radicals attack the lipids containing carbon-carbon double bonds such as the phospholipids or polyunsaturated fatty acids (PUFA) by taking their electron. When one lipid molecule loses its electron, it will attack other neighbouring lipid molecules in the same way, which later propagate the chain reaction. This condition could occur in the cell membranes or other subcellular components, causing the cellular injury or cellular damage. The product of lipid peroxidation could be aldehydes, ketones, alkanes or carboxylic acids. Malondialdehyde (MDA), the three carbons dialdehyde, appears to be the most mutagenic product of lipid peroxidation (Ayala, Muñoz, and Argüelles, 2014). It has been widely used to detect oxidative stress and the antioxidant status in clinical situations.

Along this process, O2 is the main culprit. If oxygen availability is less the production of beta oxidation may be less. Cellular injuries may be less. In the previous studies, some suggested that the percentage of O2 supplied to the body tissue can induce the oxidative stress, through the ROS action (Vento, Moro, Escrig et al, 2009; Jagannathan, Cuddapah and Costa, 2016; Lages, Nascimento, Lemos, et al 2015). However their studies were done on neonates or in vitro cell cultures. Other studies found no different in oxidant production when the cell is exposed to different O2 tension less than 40% (Nagatomo, Fujino. Kondo and Ishihara, 2012). They found excessive oxidative stress when the cells are exposed to more than 40% of oxygen tension. However their research were on the rats.

Middle age is a phase of human life which is in between young and old age. People with age of 40 to 65 years old will fall in this group. It is believed that the human undergoes decline in antioxidant status as they start to age (Adiga and Adiga, 2008). Occurrence of non-communicable diseases such as cardiovascular diseases like coronary heart disease and hypertension, metabolic diseases like diabetes mellitus, starts in this age range and found to be related with oxidative stress (Isaac, 2012). Oxygen availability can be estimated by haemoglobin concentration as it carries oxygen in its haem molecule (Thomas and Lumb, 2012). In the previous studies, the relationship of oxidative stress and occurrence of diseases were studied but little is still known how the amount of available oxygen, correlate with the MDA...
level. In this research the correlation between haemoglobin concentration and MDA production in middle aged adults are studied to estimate the correlation between $O_2$ availability and MDA production in the body.

Methodology

This cross sectional correlation study was done in a private university of Shah Alam, Malaysia on 16 healthy middle age population (45 to 65 years old) with normal BMI, who are the staffs of this university. The BMI was calculated and classified according to the standard classification provided by the World Health Organisation (2016). Subjects were recruited by convenient sampling method. After explaining about the research, informed consents were taken from those who wanted to participate in the research. Physical examination involving the determination of vital signs. Anthropometric measurements were done before taking the venous blood sample of about 2 ml from antecubital vein under antiseptic condition. Haemoglobin concentration was determined by Cyanmethaemoglobin method and MDA was determined by ELISA kit that applies the competitive-ELISA application (Wuhan Fine Biological Technology Co.,Ltd.). Its sensitivity was 1.875ng/mL and detection range is 3.125-200ng/dL. This kit is for quantitative detection of MDA in serum, plasma, tissue homogenates and other biological fluids. Plasma was used to detect MDA in this study.

This research was only conducted after proposal approval was granted by the Research Committee of MSU. Ethical confidentially was ensured by reporting the results of the data obtained in a collected manner with no reference to a specific individual. The data were analysed using Pearson's Correlation methods using Statistical Package for the Social Sciences (SPSS) software, version 22.

Result

In this study, subjects involved were equal member of females (n=8) and males (n=8). Demographic and anthropometric characteristic are presented in the Table 1. The mean age was 52.13 ± 6.86 years with the age range from 45 to 65 years. The BMI of the study group 21.73±2.12 was within normal range for both WHO cut off point (18.5-24.9 kg/m²) and Asia cut off point (18.5-23 kg/m²) (WHO expert consultation, 2004)

The mean Hb concentration and MDA level are shown in table 2 and their correlation is shown in table 3 and figure 1. Haemoglobin concentration was within normal range for both male (14-18 g/dL) and female (12-16 g/dL) of middle aged range (medcinenet.com/haemoglobin). Moreover it was strongly and significantly correlated with MDA level.

Discussion

The availability of oxygen at the tissue level has to depend on oxygen amount in the arterial blood, partial pressure of oxygen, haemoglobin concentration and saturation with oxygen, cardiac output and its distribution. Haemoglobin concentration and cardiac output are quite variable when compared with other determinants that can be similar among peoples staying in the same environmental air pressure and temperature. Cardiac output is significantly higher in people with BMI more than 25 Kg/m² compared to less BMI group (Shitij, Sumit, and Lal, 2016). However, according to the study of Stelfox, Ahmed, Ribeiro, et al. (2006), each 1 Kg/m² increased BMI was associated with only 0.08 L/min increase in cardiac output (p < .001). In this study, cardiac output in the participant may not be too different as the mean BMI is within normal range (21.73 ± 2.12 Kg/m²).

Haemoglobin is a protein molecule responsible for carrying almost all of the oxygen in the blood. About 98% of oxygen is reversibly bound to it and released at the tissue level. Factors influencing the release of oxygen to the tissues such as temperature, pH, $P_{CO_2}$ and 2, 3 diphosphoglycerate (2, 3 DPG) would be similar in the participants as the environmental factors are the same and all are apparently healthy. Thus, oxygen availability for the tissues of the participants in this study seems to be mainly depends on the haemoglobin concentration. McLellan and Walsh, (2004) also stated that the Hb concentration works as one of the indicators for the availability of $O_2$ in tissues, where the $O_2$ supplied to the tissue rises as Hb concentration inclines. Increased $O_2$ at tissue cells may grow the chance to develop into ROS and
subsequently lead to disequilibrium between ROS and antioxidants level since they are started to decline in middle aged adults (Adiga and Adiga, 2008).

The findings of present study is supported by the findings of Vento, Moro and Escrig et al (2009) who had done study on neonates born at 24 to 28 weeks of gestation. They were resuscitated with fractions of inspired oxygen of <30% (n=37) or 90%. (N = 41). The group resuscitated with low-oxygen (<30%) needed fewer days of oxygen supplementation (6 vs 22 days; P <0.01) and fewer days of mechanical ventilation (13 vs 27 days; P < 0.01) and had a lower incidence of bronchopulmonary dysplasia at discharge (15.4% vs 31.7%; P <0.05) than those resuscitated by high-oxygen. They suggested that the percentage of O2 supplied to the body tissue can induce the oxidative stress, through the ROS action (Vento, Moro, Escrig, et al, 2009).

A review by Jagannathan, Cuddapah and Costa (2016) stated that oxygen levels range from 2–9% in different organs in vivo. The cell culture experiments in vitro at atmospheric O2 levels (21%) showed oxidative stress by generating reactive oxygen species (ROS). Deleterious effects such as DNA damage, genomic instability and senescence were involved. The findings of Lages, Nascimento, Lemos, et al. (2015) is in agreement with the findings of this research. They studied the effects of two different oxygen levels 3% and 21% on the human neural progenitor cells derived from pluripotent stem cells. They found that low oxygen concentrations altered the mitochondrial content and oxidative functions of the cells, which led to improved ATP production, while reducing generation of reactive oxygen species (Lages, Nascimento, Lemos, et al, 2015). They postulated that oxygen concentration should be carefully regulated in all living tissues, beginning at the early embryonic stages. Unbalances in oxygen regulation can lead to cell death and disease.

The finding of this study is opposite to that of Almuhana (2013) who conducted a study to verify the relationship between hemoglobin concentration, reduced glutathione (GSH) and malondialdehyde (MDA) levels in 50 healthy women and 100 pregnant anemic women. It was found that significantly high MDA in anemic pregnant women (Almuhana, 2013) that may not only be due to anaemia alone but also due to the stress of pregnancy itself as increased level of lipid peroxidation products can be seen in pregnancy (Basu, Bendek, Agamasu, et al., 2015).

It is also opposite to the hypothesis and suggestion of Zubieta-Calleja and Zubieta-DeUrioste (2017). They found that high altitude residents are perfectly adapted to their environment. The cities of La Paz (3100–4100 m) and El Alto (4100 m) stand as living proof of this with 2.7 million inhabitants living perfectly normal lives, undisturbed by hypoxia and most even unaware of its existence (Zubieta-Calleja, and Zubieta-DeUrioste, 2017). All the cells of the organism adapt to a lower arterial partial pressure of oxygen (PaO2) that is an essential component to increase compensatory hemoglobin at high altitude. Although red blood cell count and haemoglobin are increased in response to hypoxia, amount of oxygen in the atmosphere is only 53% as the sea level at the level of the Mount Everest Base Camp in Nepal (Lovett, 2016). That is the reason for long lives in high altitude because oxygen available at the tissue level may be less as the partial pressure of oxygen is about half of the sea level. It is supported by their conclusion that sea-level residents have the disability of intolerance to hypoxia that grants high-altitude residents an advantage in the ultimate goal of life, longevity.

In this study, O2 partial pressure in the inspired air is not measured as it is assumed normal for sea level that is about 21% in the atmosphere. Partial pressure of arterial oxygen in the participants of this study would be in normal range as they are free from known pulmonary diseases. According to this, the finding of strong correlation between haemoglobin and MDA level in this study showed that even with normal O2 tension in the arterial blood, oxidant activity will be increased according to availability of O2 in the tissues carried by increased haemoglobin. Thus, when haemoglobin concentration is high the amount of O2 carried will be high and tissue can get more O2 (McElhan and Walsh, 2004) and oxidant formation and oxidative stress will be high. If haemoglobin concentration is low the opposite effect may occur but we need to know the optimal haemoglobin concentration that will not lead to oxidative stress in the tissues but effective for cellular metabolism.
Conclusion

The MDA level is strongly correlate with the Hb concentration in the middle age apparently healthy adults. It can be postulated that MDA production correlates with oxygen availability at the tissue cells of the normal middle aged person.

Acknowledgement

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References


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Table and Figure

Table 1: Demographic and Anthropometric Characteristic of the Healthy Middle Age Adults

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.13 ± 6.86</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.73 ± 2.12</td>
</tr>
</tbody>
</table>

level of significant at p<0.05.

Table 2: Mean haemoglobin concentrations and MDA of the Healthy Middle Age Adults

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb Concentration</td>
<td>14.79 ± 1.86</td>
</tr>
<tr>
<td>MDA Level (µmol/L)</td>
<td>1.90 ± 0.37</td>
</tr>
</tbody>
</table>

level of significant at p<0.05.

Table 3: Correlation between Hb Concentration and MDA Level in Healthy Middle Age Adults

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hb Concentration</th>
<th>p - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA Level</td>
<td>r = 0.756</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Pearson correlation test was performed and level of significant at p<0.05.

Figure 1: Correlation between Hb Concentration and MDA Level in apparently healthy Middle Age Adults

![Figure 1: Correlation between Hb Concentration and MDA Level in apparently healthy Middle Age Adults](image-url)