Comparison of Cadmium (Cd) Levels In Active and Passive Smokers With Type 2 Diabetic In Puskesmas Kedungdoro Surabaya Using Atomic Absorption Spectrophotometer

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ABSTRACT  The degree of smoking habit in the urban community is still high, although carried out health promotion of harmful substances in cigarettes, one of which is cadmium (Cd). The dangers of cadmium in cigarette smoke causes harm to active and passive smokers. Increased exposure of cigarettes will lead to an increase in the burden of disease in groups at risk such as people with Diabetic Type 2 (DMT2). The purpose of this study was to determine the differences in levels of cadmium (Cd) in the blood of active smokers and passive smokers patients with DMT2 in Puskesmas Kedungdoro Surabaya. This study is expected to provide information to the public about the difference in cadmium levels between active smokers and passive smokers in people with DMT2. The type of this research is a comparative with cross-sectional study design conducted in the Toxicology Laboratory of the Department of Health Analyst and Research and Standardization Center of Industry in August 2020-May 2021. The independent variable in this study is active smokers and passive smokers patients with DMT2, and the dependent variable in this research is the content of cadmium (Cd) in the blood. Analysis of the levels of cadmium in the blood using Atomic Absorption Spectrophotometer by the method of acidifying carbon furnaces. The results showed range levels of cadmium in blood in 8 people suffer from Type 2 Diabetic as active smokers are 0.605-1.5535 µg/dL. While the range levels of cadmium in the blood of the 8 people who suffer from Type 2 Diabetic as passive smoking are 0.5648-2.7045 µg/dL. Based on the results of statistical tests using test Independent Sample T-Test, it can be concluded that there is no difference in the levels of cadmium (Cd) in active smokers and passive smokers patients with DMT2 in Puskesmas Kedungdoro Surabaya. From this study, we recommend to all the people will change the lifestyle to be free from cigarettes smoke in reducing DMT2 case in Indonesia.

INDEX TERMS  Cadmium, Active Smokers, Passive Smokers, Type 2 Diabetic, Atomic Absorption Spectrophotometry

I. INTRODUCTION  The degree of smoking habit in urban communities is still high even though various health promotions have been carried out regarding the harmful substances contained in cigarettes. The increase in cigarette consumption will have an impact on the high burden of disease caused by smoking. The risk of health problems due to smoking are cardiovascular disease, lung disease, neoplasms, endocrinopathy such as diabetes, metabolic syndrome and chronic inflammation[1].PERMENKES No. 28 of 2013, declared that cigarettes are a product derived from tobacco (nicotiana tabacum, nicotiana rustica) that is burned, smoked and inhaled. Around 250 chemicals contained in cigarettes are dangerous components, one of which is cadmium metal[2]. Nicotiana species (in cigarettes) release cadmium metal content from polluted soil. Exposure to cadmium results in increased insulin resistance and ultimately increases the tendency to develop type 2 Diabetic[3]. RISKESDAS data in 2018, shows East Java ranks 5th with the highest prevalence of smokers in Indonesia, as much as 28.9% of the 39,500,000 people or about 11,415,500 people in East Java are smokers[4]. One cigarette has almost 1–2 µg of cadmium but 10% of the total cadmium is inhaled from
smoking. Smokers had 4-5 times higher serum cadmium levels compared to non-smokers. WHO recommends a temporary tolerable weekly intake for cadmium metal is 7 μg/kg body weight[5]. Cadmium (Cd) is a highly toxic and radioactive metal, has a silver white with a shiny figure[6]. The danger of toxic substances contained in cigarette smoke not only occurs in active smokers, but also in secondhand smoke that is non-smokers who inhale cigarettes smoke or called passive smokers.

Previous study by Rosita Andriyati (2019) in the community with an age range of 42-63 years showed that there was no significant difference between Cd levels in the blood of active smokers and passive smokers in the bus terminals[7]. So that both active and passive smokers have the same opportunities in exposure of metal Cd. Smoking habit has become a Cd exposure that may be associated with increased risk for developing diabetic[8]. The data of International Diabetes Federation (2020) showed that 163 million Indonesian’s population suffer from diabetic out of 463 million diabetics globally. The incidence of diabetic is expected to increase to reach 212 million people in 2045. To date, the total cases of diabetic in adults reached 10,681,400 people in Indonesia.[9].

Diabetes Mellitus (DM) or diabetic is a chronic metabolic disorder caused by the pancreas can not use insulin produced effectively. As much as 90% of diabetic incidence in the world is affected by type 2 diabetes mellitus in adults to children. Type 2 Diabetes Mellitus (T2DM) is a state of body’s ineffective use of insulin[10]. One of the lifestyle factors that influence the development of T2DM events is smoking habit[11]. It is estimated that there will be an increase in T2DM in the majority of individuals aged 45-64 years in the next 20 years[12]. Based on a preliminary survey conducted on officers UPTD Puskesmas Kedungdoro, it is known that some patients with T2DM in September to November 2020 with an age range of 40-80 still have a habit of smoking and living in areas contaminated with cigarette smoke.

The mechanism of increase in T2DM due to cigarettes begins after Cd in cigarettes enters the human body, Cd will accumulate in the liver, kidneys and pancreas that can damage the metabolic pathways of carbohydrates especially glycolysis, gluconeogenesis and glucose metabolism through modification and decrease in the activity of relevant enzymes[13]. The process of gluconeogenesis, hexokinase and phosphofructokinase inhibited by Cd. Heavy metals inhibit all enzymes based on their high affinity against free electron pairs in the cysteine group -SH, which is important in enzyme function[14]. Research by Tinkov et al. (2017) showed that Cd adversely affects physiopathology of adipose tissue through several mechanisms, thus contributing to increased insulin resistance in improving diabetic[3]. Research by Sabir et al. (2019) showed that exposure to heavy metals exerts an influence on metabolic disorders accompanied by changes in glucose and lipid homeostasis although the relationship between chronic exposure to Cd and diabetic is still no rejection[13].

From some of the results of the studies still have not been conducted analysis of toxicity of cadmium metal on the increase in the incidence of T2DM sufferers significantly, where the public still do not understand about the incidence of T2DM with impaired glucose metabolism due to smoking habits. Therefore, it is necessary to conduct research on the difference in blood cadmium levels in active smokers and passive smokers who suffer from T2DM, especially in the city of Surabaya.

II. MATERIALS AND METHOD
This study was conducted using comparative method with cross sectional research design to find out the difference in cadmium (Cd) levels in the blood of active smokers and passive smokers with T2DM. The population in the study was the community of Kedungdoro and Tegalsari subdistricts that entered into the working area of UPTD Puskesmas Kedungdoro Surabaya with a medical record of suffering from T2DM. This study sample was taken by purposive sampling (based on researcher criteria) as many as 8 T2DM sufferers aged 40-60 years, consuming tobacco cigarettes daily (active smokers) and as many as 8 people with T2DM aged 40-60 years, not consuming cigarettes but exposed to cigarette smoke (passive smokers), have signed an approval sheet to be a sample of the study. The sampling of the research was conducted at Puskesmas Kedungdoro Jl. Kaliasin Pompa No. 79-81, Surabaya. This research was conducted at the Toxicology Laboratory of Health Analysts Of Poltekkes Kemenkes Surabaya, Jl. Karangmenjangan 18 A, Surabaya and The Center for Research and Standardization of Industry Jl. Jagir Wonokromo No. 360, Surabaya. The research was conducted in November 2020-May 2021. The method of collecting research data using primary data taken after checking cadmium levels (Cd) in the blood of respondents.

A. BLOOD SAMPLING
Blood sampling is done by taking venous blood with the respondent's position sitting or lying and the respondent's arm position is straight. Mounted a tourniquet on the upper arm about ± 10cm from the elbow fold, asking the respondent to clench the hand so that the vein is clearly visible. Choose the position to take blood in the median cubiti vein with a syringe. Clean the stabbing area with an alcohol swab, leaving it to dry. Carrying out a stabbing with a needle, make sure the slope between the needle and the skin forms a 15 degrees angle. If the needle manages to enter the vein, blood will appear into the syringe. Then off the ties of tourniquet and the respondent is asked to remove the fist. Pull the end of the syringe (plunger), letting the blood flow into the syringe until finished. If it reaches 3 cc, then remove the needle gently. Then cover the skin of the needle puncture marks with a dry cotton swab, pressing the stabbing part for ± 2 minutes. Once the blood stops coming out, give it a plaster to close the puncture wound. Blood samples are inserted into a vacuum tube containing EDTA anticoagulants and homogenized (flipped 8 times). Blood with

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anticoagulants EDTA can be stored for up to 3 weeks in a temperature of 40°C[7].

B. PREPARATION OF BLOOD WITH WET DIGESTION METHOD
Pick up 1 mL of blood sample and put into nessler tube. Furthermore added 10 mL HNO3 concentrated as solvent. Then put back in the microwave to warm the temperature below 160°C, heating time adjust the sample. Left overnight to dissolve perfectly. Once completely crushed removed from the microwave and added aquades as much as 10 mL. It is then poured on the nessler tube and added metal-free aquadest up to the 50 mL mark. Furthermore, absorbance measurement is carried out using the Atom Absorption Spectrophotometer.

C. PREPARATION OF ANALYTICAL SOLUTION
The preparation of the solution in performing the examination of the sample Cd consists of the preparation of a standard solution and a blank solution.
1. Preparation of Standard Solution (0,5 ppb; 1,0 ppb; 1,5 ppb; 2,0 ppb; 3,0 ppb)
Standard solution used comes from the parent solution cadmium (Cd) merck brand concentration of 1000 ppm, then dilution with aquades until obtained the desired concentration. At the reading of atomic absorption spectrophotometer with the method of acidification of carbon furnaces uses a standard solution with a series of concentrations of 0.5 ppb; 1.0 ppb; 1.5 ppb; 2.0 ppb; 3.0 ppb in accordance with SNI 06-6989.38 – 2005.
2. Preparation of Blank Solution (0 ppb)
Blank solution which is used in Cd metal inspection using metal-free aquadest.

D. ANALYSIS OF STANDARD SOLUTION WITH ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)
The determination of the maximum wavelength is performed in advance by ensuring that the Cd hollow cathode lamp is attached and then turning on the power button on the AAS Thermo Scientific, adjusting the lamp according to the desired metal through the AAS software system. The wavelength is set according to AAS instructions, where the cd metal absorbance measurement at a wavelength of 228.8 nm. Then perform a standard solution measurement against the calibration blanks. The wavelength obtained at this maximum absorbs curve is used for measurement of cd metal concentration (Cd) in samples.

E. ANALYSIS OF SAMPLE SOLUTION WITH ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)
Ensures that the AAS instrument is ready for Cd metal analysis and makes sure that the result of measurements of the series standard solution has shown a good absorbance curve. Then input the identity of the sample in the software tool AAS Thermo Scientific. Next, take sample measurement based on SNI 06-6989.38 – 2005.

F. ANALYSIS OF RESEARCH DATA
The data obtained include data on Cd level measurement in patients with T2DM as active smokers and passive smokers in μg/dL units. Statistical analysis uses Kolmogorov Smirnov to determine whether or not normal distributed data. If the data is distributed normally, followed by an Independent Sample T-Test of Cd levels in T2DM patients as active smokers and passive smokers. If the distributed data is not normal, then continue with the whitney mann test.

III RESULTS
The research was started by filling out questionnaires in obtaining characteristics of respondents that fit the criteria of the study subject, there are consist of the gender, age range, type of study as active and passive smokers. Characteristics of 16 respondents willing to be the subject of research are presented in TABLE 1.

| TABLE 1 | Characteristics Of Respondents |
| --- | --- | --- |
| Gender Characteristics | Frequent | Precentages (%) |
| Male | 9 | 56,25 |
| Female | 7 | 43,75 |
| Age | | |
| 40-50 | 4 | 25 |
| 51-60 | 12 | 75 |
| Type of Smokers | | |
| Active smokers | 8 | 50 |
| Passive smokers | 8 | 50 |

Table 1, which contains the characteristics of 16 respondents by gender, shows that more respondents of male sex than female respondents. However, it can be seen that the ratio of male and female respondents does not differ much. From TABLE 1 it is known that the group of respondents with the age range of 51-60 years is more than the group of respondents with the age range of 40-50 years. So it can be concluded that people with T2DM are more at risk of being suffered by someone in that age range. In the adult age group has a weak immune system, making it more susceptible to exposure to cadmium (Cd). TABLE 1, it can be known that the number of study respondents who suffer from T2DM as active smokers and passive smokers is just as much. All respondents as active smokers had a male gender. While the female gender in the respondent group as a passive smoker was more than the male gender. Gender is not included in the study requirements, but this characteristic will be used in the discussion of the results of the study as a factor that affects Cd levels in the blood.

Based on the results of examination of cadmium levels (Cd) in the blood using Thermo Scientific AAS at a wavelength of 228.8 nm in the Environmental Laboratory of the Surabaya Industrial Research and Standardization Center, the results of the examination are shown in TABLE 2 and TABLE 3.
Based on the results of the examination in TABLE 2, it can be seen that the levels of cadmium (Cd) in the blood of 8 active smokers who suffer from T2DM in the working area of Puskesmas Kedungdoro Surabaya are above the normal limit value of Cd in the blood (Cd > 0.12 μg/dL). The highest cadmium (Cd) level in active smokers with T2DM was 1.5535 μg/dL, which was found in a sample of 05 with age 50 years and a smoking duration of 30 years. The lowest cadmium (Cd) level in active smokers with T2DM was 0.5648 μg/dL, found in a sample of 08 by age 51 years and having been exposed to cigarettes for 20 years. So that the range of cadmium (Cd) levels in passive smokers with T2DM is 0.5648-2.7045 μg/dL.

**A. STATISTICAL ANALYSIS**

1. **KOLMOGOROV-SMIRNOV NORMALITY TEST**
   The data test of cadmium levels in active smokers and passive smokers conducted normality test using Kolmogorov-smirnov test to find out the distribution of normal distribution data or not by using hypotheses namely Ho (normal distribution data) and Hi (normal non-distributing data). The decision-making requirement is that if the significan value > 0.05, then Ho is accepted and Hi is rejected. In SPSS program version 16.0 obtained test results:
   a. Normality test result on Cd levels of active smokers had significance p value or Asymp. Sign (2-tailed) was 0.509 > 0.05, then zero hypothesis (Ho) is accepted. So it meant the value of Cd levels in the blood of active smokers was normally distributed.
   b. Normality test result on Cd levels of passive smokers had significance p value or Asymp. sign (2-tailed) was 0.957 > 0.05, then zero hypothesis (Ho) is accepted. So it meant the value of Cd levels in the blood of passive smokers was normally distributed.

2. **INDEPENDENT SAMPLE-T TEST**
   After the data of cadmium (Cd) levels test results in the blood of active smokers and passive smokers T2DM sufferers known to be normal distribution through the test then continued with the Independent test to find out the differences significantly in both groups. The hypotheses used are Ho (there is no difference in Cd rates in both groups) and Hi (there are differences in Cd rates in both groups). Terms of decision making using the provisions if the significan value > 0.05 then Ho accepted and Hi rejected. After statistical test using Independent Sample T-Test obtained the value of Asymp. Sign (2-tailed) was 0.085 > 0.05. Ho was accepted and Hi was rejected, so it was concluded that there was no significant difference in cadmium (Cd) levels in active smokers and passive smokers with T2DM in Puskesmas Kedungdoro.

**IV. DISCUSSION**

The levels of cadmium metal (Cd) contained in blood samples of 16 respondents can be determined by using an atomic absorption spectrophotometer, but it is necessary to perform the deconstruction stage in the blood sample. This study used wet deconstruction method in decomposition of samples by adding a specific acid reagent (concentrated HNO₃) to the sample to be analyzed. In a spectroscopic elemental analysis sample preparation, using wet/acid digestion has a substantial effect on the recovery of various analyte contents in highly complex matrices. Wet digestion has benefits of being effective on organic substances such as blood, because it has the ability to destroy the sample matrix and consequently minimize the interference [15].
In the process of digestion, after the addition of HNO₃, heating is done using hotplate. The hotplate used by researcher in defusing a sample has a temperature of about 100-110°C. This is in accordance with the research by Badran et al. showed that wet digestion at a lower temperature about 80°C-100°C may result in an incomplete oxidation and incomplete decomposition of the organic matrix [16]. Nitric acid is often use as an oxidant reagent and most efficient method for recovering Cd in some kind of samples [15]. Previous study has explained that nitric acid (HNO₃) has strong oxidizing properties and using nitric acid and heating during the digestion, will accelerate the disconnection of organometallic bonds into inorganic. In the sample digestion process, a thin brown smoke is obtained during heating where the gas is NO₂ due to using HNO₃ as an oxidizing agent. The presence of these brown smoke indicates that organic matter has been perfectly oxidized by nitric acid. This study took about 15 minutes to complete the digestion process in a sample. Digestion process was finished when the sample becomes a clear yellowish orange color adjusted to the volume of the sample. Then, cadmium (Cd) level on samples can be analyzed by Thermo Scientific atomic absorption spectrophotometer (AAS) at the Research and Standardization Center of Industry.

To our knowledge, this is the first report on blood cadmium level in active smokers compared with passive smokers of the people with Type 2 Diabetic (T2DM) case. Data collection begins with the filling of questionnaires which are then presented in the form of tables and diagrams as characteristics of respondents. The data of cadmium (Cd) level examination is presented in the form of a table, then quantitative analysis is carried out using the SPSS program. Based on TABLE 2 and TABLE 3 can be seen that Cd levels in 8 samples as active smokers and 8 samples as passive smokers are above the normal limit value of Cd in the blood. Where the referent values at Cd levels in blood specimens have a normal value of 0.03-0.12 μg/dL[17][18].

The increase in cadmium (Cd) in the blood could be influenced by several factors both from within a person such as age, gender, the amount of cigarettes consumed, lifestyle or factors from outside a person such as their daily environment. Cd levels in respondents of active smokers and passive smokers who are above normal values in accordance with the study by Aoki et. al (2017), where exposure to Cd that enters the body through the main smoke of cigarettes (mainstream) and directly smoke cigarettes in active smokers could result in harm to self-health and the environment[19]. In addition, according to the study by Jung et. al (2015) explained that a person may be exposed to Cd through second-hand on smoking activities in a home or workplace environment where it is more risky to be exposed by cigarette smoke[20].

The test results were related to the high levels of Cd in passive smokers as respondents to this study in accordance with the study by Rosita et. al (2019) which showed a link between exposure of secondhand smoke and Cd levels in the blood by significantly related to the workplace environment. This was demonstrated by the results of Cd levels examination in respondents as passive smokers of 1.24 μg/L to 1.50 μg/L[7]. Based on the results, we found that the range of Cd levels in the blood of passive smokers is greater than the range of Cd levels in the blood of active smokers. However, after analyzing the results of blood Cd examination in active smokers and passive smokers using SPSS, it was found that there was no significant difference in Cd levels of active smokers and passive smokers with T2DM. The results of cadmium (Cd) levels that have been analyzed statistically in accordance with research by Trouiller et. al (2019) which showed the difference of blood cadmium level in smokers and non-smokers was very small. Even this previous study told that high levels of exposure cadmium had been linked to an increase risk of diabetic[21].

Cadmium (Cd) levels in passive smokers that were greater than active smokers could be due to differences in gender. Where in most passive smokers is a woman. The results of high Cd levels in passive smokers were reinforced by research Jain et. al (2020) showing that although women had higher blood Cd levels than men, the difference between Cd levels in the blood both narrows in the deteriorating state of kidney function[22]. Gender could be a factor in the increase in Cd levels in a person. The results of Cd level examination based on gender characteristics in this study in line with research by Goyal et. al (2020), showed that the average Cd levels in female subjects (2.94 ± 1.06 μg/L) were higher than male subjects (2.44 ± 1.02 μg/L)[23]. The increased of Cd levels in non-smoking women can be caused by pre-menopausal status associated with iron deficiency through serum ferritin test results. Iron deficiency in women can increase Cd absorption through its high binding power in iron transporters. Increased absorption, toxokinetics of Cd transport and storage and elimination of different Cd in female and male respondents are also factors to consider[24].

In samples 03 and 11 as female respondents obtained very high Cd levels of 1.9926 μg/dL and 2.0065 μg/dL at short exposure times. This was due to the intensity of exposure to respondents continuously. While in other passive smokers respondents despite having been exposed to cigarette smoke 20-30 years, the intensity of exposure was still rare. This is supported by Rosita et. al (2019) who stated that the level of pain caused by exposure to metal depends on the exposure process i.e. continuous exposure will give a heavier effect than intermittent exposure[7]. The effect of pain obtained by respondents one of them is that the respondent had suffered from T2DM just a few months before the sampling was done.

The environment is a factor that can affect the level of Cd exposure in a person derived from cigarette smoke. The environment faced by respondents based on the filling of questionnaires as passive smokers is in the middle of smoking activities by couples or the public so that it has accidentally inhaled cigarette smoke from active smokers or smoke directly from the rest of cigarette burning as a sidestream smoke. Field conditions in this incident are supported by research of Lee et. al (2017) reporting that non-smoking people have been
exposed to cigarette smoke in an environment where there is accidental smoking activity, and exposure levels can have health effects associated with blood Cd examination[25].

Blood Cd examination in a person who has never smoked by Lener et. al (2021) showed a considerable number, so it was possible that the source of Cd could be obtained from outside smoking activities[26]. Food is a common source of Cd, but in smokers or in the form of smoke as a result of smoking activity can be the dominant source of Cd exposure[27]. The presence of Cd in addition to the environment, can also be found in some foods, especially in a person who does not smoke. Research by Ghoochani et. al (2018) indicates the source of Cd in food comes from rice, cereals, vegetables, fruit juices caused by environmental pollution of farmland[28]. The source of Cd from food in a non-smoking person was also conveyed by Garner et. al (2016) but as a result of smoking habit remains a major contributor in increasing the Cd levels of a person at the age of 20-79 years[29]. So the lifestyle in controlling the exposure level of Cd sourced in food and lifestyle also needs to be considered.

Previous studies have shown that Cd compounds will enter the body through inhalation as smoke in order of 10-50%, depending on the particle size and the chemical form of cadmium. High dose of Cd will penetrate the alveolus and into the blood circulation. Cd will bind to red blood cells or high molecular weight protein in the blood, then transported to the liver, where is bound to metallothionein in the blood. After that, cadmium redistributed to various tissues and organs. According to the study by Sabir et. al (2019), Cd will accumulate in the liver, kidneys and pancreas. Where in the metabolism of these organs, Cd will damage the metabolic pathways of carbohydrates namely glycolysis by decreasing the activity of phosphosruktokinase and hexokinase. While in the pathways of carbohydrates namely glycolysis, will be hampered due to the activity of phosphosruktokinase and hexokinase. Where in the metabolism of these organs, Cd will damage the metabolic pathways of carbohydrates namely glycolysis by decreasing the activity of phosphosruktokinase and hexokinase. While in the pathways of carbohydrates namely glycolysis, will be hampered due to the activity of phosphosruktokinase and hexokinase.

It can be influenced by length of time in consuming cigarettes (active smokers) or long exposure to cigarette smoke (passive smokers), exposure intensity, gender, age, diet and daily environment of respondents. So, the people who are at risk for exposure to Cd hazards especially in people with T2DM need to take control over lifestyle, diet and avoid residential or workplace environments which exposed by cigarettes smoke.

There are several limitations in this study. First, the population size was relatively small because the criteria in this study. Further study with larger sample sizes should be performed. Second, this study could not analyse cotinine level as indicator of tobacco exposure.

IV. CONCLUSION
This study was expected to compare cadmium levels in blood of active and passive smokers with T2DM case in Puskesmas Kedungdoro Surabaya. From the results of the study on the examination of cadmium levels (Cd) in the blood of active smokers with T2DM obtained Cd levels of 0.605-1.5535 μg/dL. Meanwhile, passive smokers with T2DM have Cd levels of 0.5648-2.7045 μg/dL. In conclusion, this study documented that there is no significant difference betweenCd levels in the blood of active smokers and passive smokers with Type 2 DM at Puskesmas Kedungdoro Surabaya.

Advices for further study are recommended to perform with larger sample sizes and recommended to examine the level of cotinine in the urine sample of passive smokers in support of the results of analysis of Cd levels in the blood due to exposure to cigarette smoke as an influence of increased levels of Cd, especially in someone who does not consume cigarettes.

REFERENCES


