WILD TYPE p53 PROTEIN LEVEL AND MICRONUCLEI NUMBER FORMED AS A BIOMARKER OCCUPATIONAL EXPOSURE OF METAL IN DENTAL TECHNICIANS IN SURABAYA

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ABSTRACT

Exposure of occupational metals (Co,Ni and Cr) lead to the formation of ROS, and cause damage to DNA. ROS also cause G-T transversion mutation of p53 gene, and lead to low expression of the wild type p53. Wild type p53 plays an important role in regulating genes in cell cycle, cell growth, and DNA repair. If DNA repair is hampered there will be chromosomal damage, thereby effecting the formation of micronuclei. The aim of this study was to analyze the effects of PPE, on wild type p53 and the level of micronuclei in dental technicians. This study was observational analytic with cross sectional approach. 40 samples were taken by random sampling. Data retrieved through interviews and observations. Wild type p53 from saliva was analized with indirect ELISA and micronuclei was examined by bucal swab, then analised by HPA and stained by HE. Data was analised by Pearson correlation test. Significant value is p <0.05.There was a significant correlation between the use of PPE with wild type p53 and micronuclei level (r 0.436 and r -0,337). The study proved that the use PPE properly is positively correlated with the wild p53 and p53 correlated with micronuclei in dental technicians.

Keywords: PPE; Dental technician; p53 wild; Micronuclei and DNA Repair

INTRODUCTION

Base metal alloy in the prosthetic field of dentistry are selected because they are highly acceptable or biocompatible, high strength, sufficiently tarnish and resistant to corrosion. However, in line with the advantages gained, during the process of making prosthetic dental, alloy metal exposure, and added inaccurate working and shielding conditions to the worker, results in a dangerous exposure to living organisms. Exposure to dental technicians may be dust or smoke derived from grinding during processing of dental restorations [1]. It can cause skin and respiratory disease. The prevalence of dental dermatitis in dental technicians in Australia was 22% and in Denmark was 43% [2]. Also, the dust has the potential to cause lung diseases such as bronchial asthma, cancer, mesothelioma and pneumoconiosis depending on the duration of exposure. In [3] it was reported that there was a high concentration of cobalt, nickel, chromium metal in the dental technician's blood in Surabaya, which are respectively, 27 μg / L, 37 μg / L, and 117 μg / L [3].

Dental technicians are very important to meet the standard of work and safety procedures, including wearing personal protective equipment, work uniforms, protective masks, protective gloves, personal protective equipment, and workplace ventilation. If ventilation, exhauster, filter are adequate then it will be able to reduce the concentration of chromium, cobalt, and nickel in air ambient [4]. Exposure to the metals may increase the number of Reactively Oxygen Species (ROS). Chromium, cobalt, and nickel metal ions can produce hydroxyl radicals (-OH) by Fenton and Haber-Weis reactions. The hydroxyl radical may cause DNA damage [5]. ROS normally exist in all aerobic cells in balance with biochemical antioxidants.

Oxidative stress occurs when this critical balance is disrupted because of excess ROS, antioxidants depletion, or both. ROS can cause Guanine-Thymine transvers mutations of p53 gene [6] and lead to low expression of the wild type p53 protein. Wild type p53 protein involved in many biological processes such as regulation of genes involved in cell cycle, cell growth and DNA repair. If DNA repair is hampered there will be chromosomal damage, thereby effecting the formation of micronuclei. However, occupational exposure to metals in dental technician can be prevented through the use of personal protective equipment (PPE).

A micronucleus (MN) is a small structure that contains chromatin that was excluded from the daughter binucleates following cell division, by chromosomes or late chromosomal fragments [7]. Its usually a sign of genotoxic events and chromosomal instability. The failure occurs when the chromosomal division during the anaphase cycle in the mitotic phase. The study of Rajkokila et al. (2010), mentions that there is an increase in micronuclei in the buccal epithelium in gasoline filling workers compared with the normal group [8].

The p53 gene is considered the most mutated gene in human malignant tumors. The p53 tumor suppressor gene mutates in 50% of human tumors in various organs of the body. The development of molecular biology techniques, can explain that one of the causes of malignancy is the failure or inactivation of tumor suppressor gene p53 [7]. Inactivation of p53, for example in mutated cells or loss of the p53 gene, the expression of p53 protein does not occur or the expression of p53 protein occurs but could not serve as an activator of the transcription process in some target genes such as p21 genes or *Cyclin Dependent Kinase Inhibitor* 1A (CDKN1A) genes and *Growth Arrest and DNA Damage Inducible Protein* (GADD45) [9].

Based on the above background, the authors want to do research on the relationship p53 wild level and Number of Micronuclei formed as a Biomarker Occupational Exposure of Metal in dental technicians in Surabaya. The amount of Mikronuklei is examined through the buccal dental workings of the dental technicians, while the P53 (P53) protein is examined through saliva.

The Examination of wild type p53 protein by ELISA method

MATERIAL AND METHODS

Subject

The research was analytic observational, with cross sectional approach, conducted on 40 dental technicians in Surabaya. Sampling is done by random sampling. Sample criteria are dental technicians who are working on denture prosthesis with metal containing material more than 3 years. Willing their saliva to be taken and answering the questionnaire about the frequency and procedure of the use of personal protective equipment.

Frequency of use the Personal Protective Equipment (PPE) and Occupational environmental Control

The use of Personal Protective Equipment (PPE) was observed and questioned through a questionnaire and then scored. PPE Inspection Covers Frequency and procedures for wearing masks, gloves, protective glasses, lab coats, and shoes by dental technicians.

The category of assessment of the frequency questionnaire of the use of PPE includes three, ie always, rarely, and never with each score of 100, 50, and 0. The frequency of usage of PPE is summed with the score of the usage procedure of each PPE. The score is multiplied by each PPE percentage. Each percentage of consecutive weighting is 30%, 25%, 20%, 15%, and 10% respectively.

The Examination of Micronuclei by buccal swab

MN examination is done after the informed consent was filled. Before starting the sampling, subjects were instructed not to eat, smoke, gargle antiseptic an hour earlier. The subjects were asked to rinse with a 250cc glass of white water to remove debris in the oral cavity. Each subject was taken with a smear method using a wood spatula in the buccal mucosa. The spatula is applied to the object glass for the cell to stick to the glass of the object. Then fixation of swab result. The dried swab result is fixed with 80% alcohol. Furthermore, coloring was done using Hematoxyline Eosine (HE).

Whole saliva is collected by allowing saliva to accumulate and then instructed to spit into the tube.

Then the whole saliva that has accumulated centrifugation done 3.000g for 15 minutes at 4o C until separated between the froth with serum. To evaluate p53 protein level in the saliva, a commercial ELISA Kit was used.

The first time is to coat the microtiter plate with a purified antigen by allowing the solution containing the antigen attached to the wall / surface for 30-60 minutes. Then rinse the antigen that is not bound to the buffer, and coat certain sides that may not be specially attached by antigens with unrelated proteins (such as powdered milk solution). Then the non-stick proteins are rinsed, and add the serum samples to which antibodies will detect and allow specific antibodies to bind to the antigen.

The unbound antibody is rinsed, and adds an anti-Ig which will bind to the Fc region of a specific antibody. Rinse the complex of unbound antibody-enzymes. Added chromogenic substrate: a colorless substrate bound to the enzyme will be converted into a product. Incubate until color appears, and measured with a spectrometer. If the more concentrated the color is detected, the greater the specific antibody levels in the sample

Statistical analysis

The results of PPE scoring and P53 levels were tested for normality using the Kolmogorov Smirnov test. If the data is normally distributed then proceed with Pearson correlation test. If the data is not normal, Spearman correlation test is performed. The significance value or significance of this test if the value of p <0.05 (95% confidence level). All statistical analyzes were performed using the SPSS program.

RESULT AND DICUSSION

RESULT

The research was analytic observational, with cross sectional approach, conducted on 40 dental technicians. Sampling is done by random sampling. Sample criteria are dental technicians who are working on denture prosthesis with metal containing material more than 3 years. Willing their saliva to be taken and answering the questionnaire about the frequency and procedure of the use of personal protective equipment.

Table 1. Exposure of Co, Ni and Cr of Dental Technician on the p53 level and Number of MN

Variable	Significant	Co	Ni	Cr
	Mean±SD	39.84±32.30	69.82±42.33	340.56±137.19
P53	p (T test)	0	0	0
0.25±0.26	r(Correlation)	-0.083	-0.11	0.126
MN	p (T test)	0	0	0
18.03±6.06	r(Correlation)	0.038	0.07	-0.209

Table 2. Mean±SD, Significant Values and Correlation between PPE, p53 level and Number of MN.

Variable	Mean±SD	Significant	Mean±SD	
			PPE	Micronuclei (MN)
			7.22±2.281	18.03±6.06
P53	0.25±0.26	p (T test)	0,005	0,033
		r(Correlation)	0,436	-0,337
MN	18.03±6.06	p(T test)	0,040	0
		r(Correlation)	-0,326	0

Table 1 showed Indicates a significant test value (p<0.05) between p53 level, MN and Ni, Co and Cr, metals contacts in serum of dental technician for all variables but different for each variable. Cr shows a sufficient correlation with p53, whereas Ni and Co show no correlation. Cr value showed negative correlation with Mn which mean when Cr value is high MN was low. Ni and Co showed weak correlation with Mn which mean that if Ni and Co high, then Mn value high too.

Data in table 2 showed the mean of p53 level in dental technician's saliva is lower than p53 level in healthy people which is 0,42±0,13 (Hozzeini et al., 2014). For micronuclei in healthy control is in range 0.05-11.5/ MN 1000 sel and the mean 0.5-2.5/ MN 1000 cell. The data showed that the mean of sample micronuclei is higher than the mean of p53 and significantly correlated with p53 ($p<0.05$). Mean value of PPE is higher than p53 dan Micronuclei and significantly correlated ($p<0.05$). This showed that p53 have positive correlation with PPE and, and negative correlation with Micronuclei.

In Table III showed significant difference ($p<0.05$) between 8 OHdG, p53 level, number of MN in Dental Technician The correlation showed no correlation between p53 level, number of MN and Co, Ni and Cr, dental technician. It showed the mean levels of circulating p53 in saliva (0,27 ± 0,27) lower than normal (0,42 ± 0,13), while the mean level of 8 OHdG higher than Normal (0.25 ± 0.15) and Micronuclei even higher.

Table 3. Mean±SD, Significant Values and Correlation between 8 OHdG, with p53 and Number Micronuclei.

Variabel	Significant	p53	Micronuclei (MN)
8 OHdG 6.62 ± 2.71	Mean±SD	0.25±0.26	18.03±6.06
	p(T test)	0,001	0,000
	r(Correlation)	-0,103	-0,052

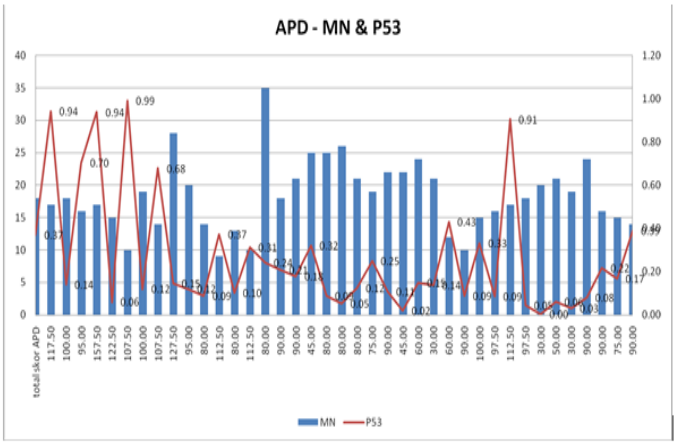

Figure 1. Correlation PPE score with p53 level and number of micronuclei

This figure showed a correlation between PPE score and p53, micronuclei level. Higher PPE score, then higher p53 level and lower micronuclei number.

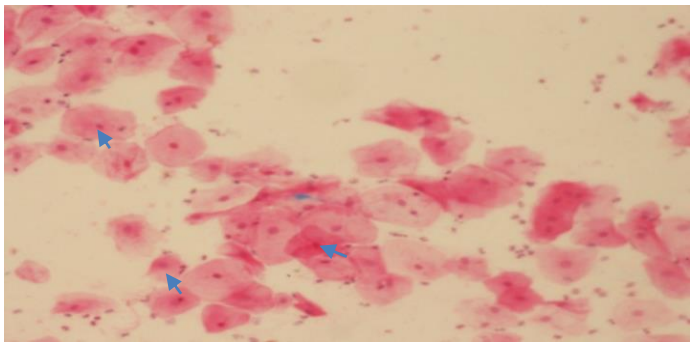

Figure 2. Micronuclei (with 100x magnification).

The fig.2 above is a micronuclei observation of the image with HE staining and is observed using a light microscope with 100x magnification. The image designated by the arrow is a micronuclei formation that is calculated in the field of view.

DISCUSSION

Previous studies have reported high levels of nickel, cobalt and chromium on dental technicians working with metals for more than three years. This is not just caused by exposure to alloy metals, but also inappropriateness of protection and working conditions of dental technicians [3] in the workplace. The study was supported by [4] that dental technicians exposed dust derived from the grinding process during metal casting. Exposure to these genotoxic metals will increase the number of endogenous Reactive Oxygen Species (ROS). Accumulation of metals in the body is not easily destroyed, and excretion takes years. Metal transitions act as catalysts in ROS production, and cell accumulation can cause oxidative stress, which causes oxidative damage to proteins or lipids. Reactive oxygen species (ROS) are known to cause many types of DNA lesions that could be converted into cancer-promoting genetic alterations. An increase in DNA damage causes a gene mutation that regulates cell growth, other than that it is also induces the expression of p53 transcription factor responsible for regulation of cellular response to damage [10] . Evidence showed that tumor suppressor p53 plays an important role in regulating the generation of cellular ROS, either by reducing oxidative stress under physiological and mildly stressed conditions, or by promoting oxidative stress under highly stressed conditions [12]

Activation of p53 may occur in response to a number of cellular pressures, including DNA damage [10], hypoxia and and oxidative stress [11]. In addition to protecting cells from oxidative stress under physiological conditions, p53 also functions in other cellular processes, including gene regulation in cell cycle, DNA repair and cell death. While failure in the process when dysfunctional p53 has the potential to cause genomic instability [12]. Loss of some molecular checkpoints can be found in the development of some tumors, this is due to the development of the cell cycle that goes as it should. The accumulation of genetic change also plays a role in onset kemoresisten, which results in loss of DNA ability to respond to damage. Several changes have been identified, namely changes in tumor suppressor such as p53. With the loss of growth suppression, uncontrolled and result in the development of cell cycle tumorigenesis.

The proper and consistent use of PPE in work will improve p53. However, the dental technicians in this study on average did not use the appropriate PPE during work. Occupational work control is also inadequate to cause high levels of metal in their saliva. Their p53 level in saliva is lower than normal. p53 is a tumor suppressant protein and a transcription factor that can react with DNA damage. If DNA damage is detected, then there are 2 things ordered by p53, which activates DNA repair and stops the cell cycle on G1 until damage can be repaired. If the damage is severe enough, p53 will initiate a cell death program (apoptosis). Cells with mutated or missing p53 genes, p53 protein expression (p53) do not occur or p53 protein expression occurs but can not function as an activator of the transcription process in some target genes.

p53 plays a role in activating the GADD45 target gene that acts as DNA repair. If GADD45 can be activated then damaged DNA can make improvements in order to avoid chromosomal damage that is marked by the emergence of mikronuklei. Micronucleus (MN) is a small structure containing chromatin secreted from the daughter's binucleic after cell division. Usually this is a sign of genotoxic events and chromosome instability. MN is formed during cell division by chromosomes or end chromosome fragments when formed [7]. Failure occurs when the chromosomes divide during the anaphase cycle in the mitotic phase. Analysis of statistical data between p53 level with the number of micronucleus in this study obtained a negative and significant relationship between p53 level with the number of micronuclei which means lower p53 level, the higher the number of micronucleus. Mikronuklei obtained higher than usual. This research is supported by [8], suggests that there is an increase in micronucleus in the buccal epithelium of filling workers compared with the normal group.

the age of 50 years or more, individuals will experience biphasic chromosomal damage and will result in a decrease in the number of micronucleus. Other researcher also reported that a decrease in the number of micronucleus occurred after the age of 40 years. While dental technicians get exposure to metal from the workplace which can further increase the occurrence of micronuclei. Thus the age variable and workplace metal exposure are two opposite variables in affecting micronucleus formation, so there is no significant relationship between age with the number of micronucleus in the dental technician.

CONCLUSION

Free radicals from high concentration of metals Co, Ni and Cr are highly reactive properties and cause further oxidation of molecule in the vicinity. The results of the oxidation will react with molecular complexes within cells, especially chromosome. Chromosome becomes abnormal and nucleobase arrangement changes. Greater toxicity is often associated with high participation and action as a catalyst in generating reactive oxygen species (ROS).

ROS formation lead to oxidative stress conditions. DNA damage also induces the expression of the transcription factor p53 that is responsible for the regulation of the cellular response to damage. The hampered of DNA repaired lead to chromosomal damage, thereby effecting the formation of micronuclei.

Due to their rapid formation and easy detection, MN have become the most prevalent biomarker of chromosomal defects induced by genotoxic agents. The ability to distinguish between chromosome fragments and whole chromosomes lagging behind in anaphase allows the determination of the mechanisms of action of a variety of mutagenic agents. In the last decades, an enormous amount of data has shed light on the mode of MN formation, MN incidence in different cells and tissues among individuals, and the fate of MN in cells damaged by genotoxins. MNi are thought to reflect the initial stage in the development of genomic instability and tumorigenesis. Individuals predisposed to cancer tend to form MN more rapidly than persons without hereditary history. Therefore, MN screening may serve as a valuable method in predicting various diseases, including cancer.

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