# Analysis of TP53 Mutants Due to Chromium Metal Exposure on Dental Technicians at Surabaya Laboratory

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**Abstract**

Dental technicians used chromium in base alloys to produced dental prostheses. In the process of manufacturing, metal dust could be absorbed to the body through inhalation, skin and digestion. Chromium could increase Reactive Oxygen Species (ROS) formation which triggered mutations in the P53 gene and increased expression of Tumor Protein p53 (TP53) mutant level. This study was conducted to look at the relationship between chromium metal exposures to TP53 mutant levels in dental technicians in Surabaya Laboratory.

Cross-sectional study was performed on 40 dental technicians and 30 controls after ethical clearance. Blood sampling was conducted for the examination of chromium by *Atomic Absorbance Spectrophotometry* (AAS) and examination of TP53 mutant by ELISA method.

The chromium metal concentration (367.98±141.30) and TP53 mutant level (0.69±0.2) in dental technician's blood samples were higher value than controls (0.09±0.17 and 0.54±0.16). There was significant difference result between dental technicians to controls *P*=0.000 (*P*<0.05). Spearman test showed a positive correlation between chromium and TP53 mutant levels *P*=0.000 and r= 0.41 (*P*<0.05).

Chromium metal plays a role increasing the level of TP53 mutant in dental technicians at the Surabaya Laboratory

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## Introduction

Chromium (Cr) was used as a mixture with other metals in both base alloys, Ni-Cr and Co-Cr alloys, which were popularly used by dental technicians to create partial removable dentures, porcelain fused to metals and bridges.[1] Base alloys were chosen to replace gold alloys type (IV) because of the cheaper cost.[2,3] In the process of prosthesis manufacturing, dental technicians were particularly vulnerable to exposure of residual metal alloy dust or smoke.[4] Hariyani et al. (2015) had stated before that the chromium metal level of the dental technicians in Surabaya was 117 µg/L with an average control value of 0,06 µg/L. The high level of the chromium was motivated by the lack of self-protection equipment used by dental technician that metal particles could be absorbed into the body.[5] This exposure could impair health status because of absorbed dust through inhalation, skin, and digestion.[6,7]

Metal dust or smoke induced respiratory and skin diseases in the form of pneumoconiosis, asthma, contact dermatitis, and also cancers.[8,9] Chromium exposure in some epidemiological studies was associated with the incidence of lung cancer.[8,11]

Chromium could lead human lung cells into malignant transformation within the Reactive Oxygen Species (ROS) formation. Chromium metal could be a catalyst increasing ROS through the Fenton and Haber-Weiss reactions that converted $H_2O_2$ into hydroxyl groups (OH).[10,11]

Increasing the formation of free radicals in the body leaded an imbalance between elimination by antioxidants and the production of free radicals itself, called oxidative stress. Hydroxyl group could react and damaged the

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surrounding biomolecules such as DNA, lipids and proteins.[12]

Hydroxyl radicals (OH) trigger DNA damage, especially characterized by the formation of 8-hydroxy 2'-deoxyguanosine (8-oHdG). Increased ROS production also induced TP53 gene mutations in the form of GC-TA transversions.[13] Transverse mutations are also common in human cancers and mainly occur in TP53 tumor gene mutations.[14] Thus, the aim of this study is to observe the correlation between chromium concentration and TP53 mutant level in dental technicians.

## Materials and methods

Cross sectional study was done after ethical clearance has been approved by Health Research Ethics Committee, Faculty of Dentistry, Airlangga University (Number:269/HRECC.FODM/XI/2017) with two groups of samples, 40 dental technicians and 30 controls. Informed consent was taken before this study established.

## Blood collection procedures

Blood collection was done by a professional nurse. Blood samples was collected 4 ml, 3 ml was used for measurement of chromium metal concentration and 1 ml used for measurement of TP53 mutant level. Blood samples were taken intravenously by using disposable needle. For measurement of chromium metal concentration, blood samples were placed on vacuum tubes with anticoagulants. Meanwhile, blood samples were placed on plain vacuum tubes for measurement of TP53 mutant level.

## Measurement of chromium metal concentration

Measurement of metal level was done at the Regional Health Laboratory in Surabaya using Atomic Absorbance Spectrophotometry (AAS) with a wavelength of 357.9 nm. The result was declared in units of µg/L.

## Measurement of TP53 mutant level

Measurement of TP53 mutant level used ELISA TP53 Kit ELISA Mutant Kit (Competitive ELISA) MyBioSource.com. TP53 ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-TP53 antibody and a TP53-HRP conjugate. The assay sample and buffer are incubated together with TP53-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometric at 450nm in a microplate reader. The value of TP53 mutant levels is expressed in Unit/OD.

## Statistical analysis

Mean and standard deviation was calculated for chromium metal level and mutant TP53 levels in dental technicians and control groups. This data were analyzed using statistical software (IBM SPSS 16, New York). Difference test used Mann Whitney U test and correlation test used Spearman Test.

## Results

The age and gender of subject are shown in Table 1. Dental technician is mostly in the population aged 19-29 years, while slightly present in the age group 41-51 years. Most of dental technician are male while the few women are included, while control respondents are the contrary.

Chromium concentration of dental technicians and controls were 367.98±141.30µg/L and 0.09±0.17µg/L respectively. Chromium concentration in dental technicians was very higher than controls. Mean value of TP53 mutant in dental technicians and the controls were 0.69±0.2U and 0.54±0.16U respectively. The comparative test showed that there was a significant difference with p value 0.000 ($P$<0.05) between the value of chromium levels in the technician's blood compared with the value of blood control level, and vice versa in mutant TP53 (Table 2).

Spearman test on exposure of chromium metal to TP53 mutant expression showed significant value $P$=0.000 ($P$<0.05). This suggested a significant relationship between chromium concentration and TP53 mutant. The strength of significance in chromium metal was moderate to TP53 mutant levels (Table 3).

| Age | Man | | | | Woman | | | |
|---|---|---|---|---|---|---|---|---|
| | Dental Technician | % | Controls | % | Dental Technician | % | Controls | % |
| 19-29 | 16 | 42.1 | 5 | 41.7 | 4 | 50 | 5 | 27,8 |
| 30-40 | 10 | 26.32 | 3 | 25 | 3 | 37.5 | 5 | 27,8 |
| 41-51 | 9 | 25.71 | 2 | 16.7 | 1 | 12.5 | 8 | 44.4 |
| | 35 | 100 | 12 | 100 | 8 | 100 | 18 | 100 |

**Table 1.** Subject Characteristic by Age and Gender.

| Variables | Chromium (µg/L) | | TP53 Mutant (OD/Unit) | |
|---|---|---|---|---|
| | Dental technicians | Controls | Dental Technicians | Controls |
| Mean±SD | 367.98±141.30 | 0.09±0.17 | 0.69±0.2 | 0.54±0.16 |
| Comparative Test (Sig.) | 0.000 | | 0.000 | |

**Table 2.** Mean, Standard Deviation, and Comparative Test using Mann Whitney U Test on the Value of Each Variable to the Value of Control.

| Variables | | TP53 mutant |
|---|---|---|
| Cr | Sig | 0.000 |
| | R | 0.41 |

**Table 3.** Correlative Test Using Spearman Test on the Chromium Level to the TP53 Mutant Level.

## Discussion

The chromium element (Cr) was found easily in the environment and widely used in plating, tannery, pigmentation, dye production, metallurgy, and chemical industry.[15] In dentistry, chromium is used as a mixture of Ni-Cr and Co-Cr alloys with content not exceeding 20%. Chromium in alloy was useful for corrosion resistance in the oral environment because of its oxide layers.[2] The form of chromium known to be toxic is hexavalent chromium [Cr (VI)].[16]

In this study, the chromium concentration in dental technicians and controls were 367.98 µg / L and 0.09 µg/L respectively. That showed that the chromium concentration in dental technicians was 4.088 times than in healthy controls. Those were higher than in previous study by Hariyani et al. (2015) which was 117 µg/L with an average control of 0.06 µg/L.[5] Another study in North Jordan by Al-Hourani et al. showed a chromium concentration was 46.18 µg/dL in dental technicians (the control was 17.84 µg/dL).[17] Such findings were due to environment factors around dental technicians. Dental technicians were more exposed with various chemical materials included metal dust while processing dental prosthesis. Inhalation, skin and digestion were three main route of absorbed metal dust but inhalation route was the most frequently induced health problems.[6,7] Cr (VI) could cause hepatotoxic, dermatitis, enteritis, reproduction, renal and lung toxicity. The main health problem especially was respiratory diseases.[16] This respiratory disease could be pneumoconiosis, asthma, also cancer.

This respiratory disease could be pneumoconiosis, asthma, also cancer. A case report in Denmark showed a case of adenocarcinoma in a dental technician who worked on cobalt-chromium alloy for 20 hours / week.[18] Another study by Sorahan et al. (1998) in the UK and Beveridge et al. (2010) in Monteal, suggested that workers exposed to nickel and chromium increase the risk of lung cancer.[19,20] Menck and Henderson (1976) suggested dental technicians had a higher risk for developing lung cancer compared to the other occupations.[21] Thus, The International Agency for Research on Cancer (IARC) classifies chromium metals as carcinogenic (group 1).[22]

Recent research showed that nickel in Ni-Cr alloys caused apoptosis due to free radical reactions. Likewise nickel, chromium could cause an unfavorable reaction by producing free radicals such as nickel.[23] Cr(VI) could directly crossed the cell membrane and reduced into Cr(V), Cr (IV), Cr(III).[24] The chromium ions reacted with $H_2O_2$ and converted into hydroxyl groups (OH) in the Fenton and Haber-Weiss reactions, resulting in increased Reactive Oxygen Species (ROS).[10,11,23] The hydroxyl group (OH) was the most dangerous and highly reactive radical.[25,26] Under normal conditions, ROS was present at low levels and could be controlled by various antioxidants in the body.[27] Various toxic effects of Cr (VI) associated with generation of reactive oxygen species due to intracellular reduction by cellular reducers such as ascorbic acid, glutathione (GSH), and cysteine.[28] Free radicals could react with deoxyribonucleic acid (DNA) and generated 8-hidroxy 2'-deoxyguanocyn (8-OHdG). The 8-OHdG product was a guanine oxidative product that can cause miss paired with deoxyadenosin (dA) resulting in GC-TA transversion mutation.[29]

In this study, the mean value of TP53 mutant in dental technicians and the controls were significant difference. Also with Pearson test showed significant correlation which was appropriate with the theory that there was high expression TP53 mutant due to higher level of metal. Chromium metals also generated

mutations in the TP53 gene.[8,9] Increased ROS production causes TP53 gene mutations in the form of GC-TA transversion.[13] Mutations often occur in cancerous conditions and 60-70% of mutations occur in TP53 tumor genes with 38% G-T transvers mutations.[14,30,31] Some of the most frequently detected TP53 mutations are at 175, 245, 248, 249, 273, and 282 codons.[32] Different missense mutations on p53 could show different activities.[33]

The wild TP53 gene played roles in DNA repair function, cell cycle arrest, and apoptosis. In performing its function, the wild TP53 served as the transcriptional transactivation of various genes by binding the binding site to its promoter region. Although TP53 mutations were often considered equivalent, other evidence suggested that different mutants exhibited different profiles due to loss of wild-type TP53 activity, ability to inhibit wild TP53 type, and gain of function.[34,35] The role of mutant TP53 in various activities acquired cancer requires further study.

## Conclusions

Increased Reactive Oxygen Species (ROS) in the body of dental technicians was due to elevated levels of chromium metal resulting in increased expression of TP53 mutant.

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## Declaration of Interest

The authors report no conflict of interest.

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