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題目：鉻暴露作業勞工免疫功能的影響

Topic：Immunologic Effect of Workers exposed to chromium

## 中文摘要

近年來勞工在職業暴露上的免疫危害逐漸受到重視，因為免疫系統是人體對抗外來侵害的一種防禦機轉 ，一旦免疫系統受到傷害就非常容易感染其它疾病，甚至死亡。許多文獻已證實長期暴露於鉻酸霧滴的電鍍工人會導致呼吸及皮膚方面的疾病，至於對免疫功能的影響則尚未有廣泛研究。因此，本研究的目的將調查六價鉻的暴露與作業勞工免疫功能的相關性。針對三種不同的電鍍工廠(硬鉻、裝飾鉻、混合電鍍鉻)的作業勞工進行調查，事前選取三家不同電鍍工廠共計四十位電鍍勞工及對照組接受問卷調查，以了解其一般生活史、過去病史、工作史及過去一年有無使用個人防護具、罹患感冒等上呼吸道疾病…等。並由耳鼻喉科醫師至作業現場為勞工作各項生理檢查及記錄各項檢查結果包括有無鼻中膈潰瘍、穿孔；鼻炎、鼻竇炎；鼻腔阻塞…

等。另外，再配合作業環境測定、其他血液、尿液等生化檢查，以進一步了解電鍍作業鉻暴露免疫功能的情形(包括 IL-1、IL-2、IL-4、IL-6、IL-8、IL-10、 TNF-$\alpha$、INF-$\gamma$、及 CD4、CD8 等免疫指標.)，作為提供政輔導電鍍勞工早期發現及預防的指標，以有效保障勞工的安全與健康。

關鍵詞：鉻、免疫功能、電鍍工廠員工

**Abstract**

The objective of this study was to investigate the immunologic effect of chromic acid exposure among electroplating workers. The study included 46 subjects selected from five electroplating plants in central Taiwan. Each subject was interviewed using a questionnaire and urine-chromium (Cr) concentration was assessed. Immunologic function was evaluated by interleukin count (IL2, IL-4, IL-6, IL-8, IL-10, TNF-$\alpha$, INF-$\gamma$) and levels of lymphocyte subsets (T-cell, B-cell, T4, T8, T4/T8). Results showed that IL6 and IL8 levels were significantly increased in subjects with high urine–chromium concentration, but TNF-$\alpha$ levels decreased. There was no response for IL-4, IL-10 and INF. Flowcytometry was used to determine levels of lymphocyte subsets: only B-cells percentage had a negative correlation with urine-Cr. Smoking was an important factor that influenced levels of lymphocyte subsets. Exposure to chromium has a detrimental effect on the immune system, so it is evident that worker exposure to chromic acid in the electroplating workplace must be reduced to a minimum.

**Keywords:** chromium, immunologic function, electroplating workers

## Introduction

Chromates are well-known carcinogen and mutagen and are capable of inducing a variety of DNA lesions such as strand breaks, DNA-inter-strand and DNA-protein cross-links in both animals and cultured mammalian cells ( Cohen et al.,1993; Mancuso 1997; Zhitkovich A et al. 1998; Werfel et al. 1998). Even before any carcinogenic effect takes place, the immunologic consequences of hexavalent chromium (Cr(VI))exposure have not been studies extensively. The potential immunomudulatory effects of Cr were evaluated using a series of in vitro and in vivo studies (Synder and Valle, 1991). Their results showed that the increased response of cells from Cr-exposed rats indicated Cr-induced sensitization and may possibly be used as biological markers for Cr exposure. Since Cr (VI) can easily pass the cell membranes and it is reduced inside the cells to its trivalent form, Cr (III), intermediates like Cr (V) and Cr (IV) and radicals are suspected to react with DNA and cause DNA damage (Wefel et al., 1998). Moreover, Cr(VI) was also found to induce reduction of lymphocytes in the systemic circulation. Tangawa (1991) found that workers exposed to Cr(VI) showed a marked lymphocytopenia without alteration of blood natural killer (NK) cells. Snyder (1996) studied IL-6 levels among individuals in Hudson County, New Jersey (an area contaminated with chromium) and found that IL-6 levels were significantly lower than IL-6 levels in non-contaminated areas. Boscolo (1997) studied the effect of chromium on lymphocyte subsets and immunoglobulins from normal population and exposed workers. The study showed that urinary chromium had a significant positive correlation with CD16+56+NK, CD5+CD19+ B and HLA-DR+ activated T, B and NK lymphocytes and a negative correlation with all blood lymphocytes. Also, serum chromium was significantly correlated with all blood lymphocytes and HLA-DR+, CD3-HLA-DR+ and CD3-CD25+ lymphocyte subsets. It is important to note that immunologic

indicators fluctuate a great deal after initial exposure to a toxic substance and this makes it very difficult to obtain consistent data. Previous studies by current authors showed that chromium electroplating plants in central Taiwan and found that workers there had a variety of severe health problems such as renal dysfunction (Liu et al, 1998) respiratory problems (Kuo et al., 1997) and increased sister chromatid exchange (Lai et al, 1998) as a result of exposure to Cr. There is no data available in Taiwan concerning the effect of chromium on the immune system. The objective of this study is to investigate to what extent chromium effects immunologic function. Also, this study can be used to create a database of long term exposure to chromium, including health data and environmental monitoring. This study will be used as a reference by the Taiwan government and electroplating plants, for the puposes of improving safety conditions in the electroplating workplace.

**Materials and Methods**

Subjects: The 46 subjects were selected from five Cr electroplating plants in central Taiwan. None of the subjects had had any immunologic disease, prescription medicine pertaining to immune function or organ transplant one month prior to the commencement of the study.

Methods: Each worker was interviewed by questionnaire. Basic data, workplace conditions and health status were determined. A urine sample was taken from each subject at the end of a shift and was analyzed for chromium by atomic absorption spectrophotometry (AAS) within one week. Detailed information has been outlined in a previous study (Kuo, 1997). The data was divided into three groups based on the concentration of chromium in urine: (1) Low urine-Cr, less than 25% (<1.13ug/g cre) (2) Intermediate urine-Cr, 25-75% (1.13-6.41ug/g cre) (3) High urine-Cr, over 75% (>6.41ug/g cre).

Immunologic function test

Enzyme-linked immunosorbent assay (ELISA) was used to determine the interleukin-2, 4, IL-6, IL-8, IL-10, TNF-$\alpha$ and INF-$\gamma$. The standard procedure for determining interleukins (as recommended by R&D systems) was followed. The sandwich immunoassay technique was used. Blood samples were added to the interleukin microtiter plate and a color reagent was then added. Within 30 minutes, an ELISA reader analyzed the samples. To establish a calibration curve, five concentrations of the interleukin standard were prepared. Each sample was duplicated and the coefficient of variation (CV) was less than 10%.

All data were analyzed using SAS/pc+ 6.04 statistical software (SAS/STAT 1986). Analysis methods included analysis of variance (ANOVA), correlation analysis and the general linear model.

**Results**

Table 1 shows three groups divided according to urine-Cr and immune function. Interleukin-2, IL-4, IL-10 and IFN were not detected in any workers urine samples. However, IL-6, IL-8 and TNF-$\alpha$ were all present. IL-6 and IL-8 levels increased in direct proportion to urine-Cr concentration, but only IL-8 had a statistical significance (High urine-Cr group = 64.08. Low urine-Cr group = 23.57). There was a negative correlation between TNF-$\alpha$ and urine-Cr, but there was no statistical significance. There was a negative correlation between urine-Cr and T-cell and B-cell. However, only B-cell had a significant difference (High urine-Cr group = 8.80%. Low urine-Cr group = 12.89%). The T4/ T8 ratio was correlated positively with urine-Cr. (High urine-Cr group = 1.87. Low urine-Cr group = 1.26). The p value however is 0.074. There was no significant difference in the other indicators: T4, T8, lymphocyte, monocyte and granulocyte.

Table 2 shows the correlation of urine-Cr, airborne Cr(VI) and work duration with immune indicators. There is a positive correlation of urine-Cr with IL-6 and IL-8 (only IL-6 was significant). TNF-$\alpha$ and B-cell correlated negatively with urine-Cr (r = -0.19 and –0.24, respectively). T4/ T8 ratio correlated positively with urine-Cr (r = 0.27). Only monocyte correlated positively with airborne Cr(VI) (r = 0.28). Work duration did not correlate with any of the immune indicators.

Table 3 shows the factors that influence the immune indicators using multiple regression. The high urine group compared to the low group for IL-6 and IL-8 has a statistical significance. However, there was no significance for gender, age and smoking. No factors explained levels of TNF-$\alpha$; there was a slight negative correlation with urine-Cr. After adjustment of the other factors, the results showed that the high urine-Cr group and the medium urine-Cr group compared to the low urine-Cr group for B-cell both had negative statistical difference. Smokers had higher T4 levels, granulocyte and T4/T8 ratios than non-smokers. However, lymphocyte levels were lower in smokers than in non-smokers. Males had lower T-cell levels than females. Age and electroplating work did not affect immune function.

**Discussion**

Most electroplating factories in Taiwan are small-scale and there is a lack of provision of personal protective equipment for workers. Previous studies (Kuo et al., 1997; Liu et al., 1998; Lai et al., 1998) have shown that in hard Cr electroplating factories, the absence of adequate safety procedures and the requirement of workers to handle the electroplating materials for an extended period (meaning high exposure to

airborne Cr.), have resulted in the development of serious health problems among workers. Concentration of airborne Cr(VI) in electroplating factories varies greatly. For example, near the electroplating tank Cr(VI) is highest whereas in the administrative office levels of Cr(VI) are very low. There is no existing data in Taiwan to show the effect of chromium on the immune function of electroplating workers. Glaser (1985) reported that respiratory defense and immunologic functions were stimulated or inhibited depending on dose and time of Cr(VI) inhalation. The humoral immune system was still stimulated at sub-chronic low chromium aerosol concentration of 100mg/$m^3$, but significant depressed at 200 mg/$m^3$. Boscolo (1997) studied the effects of chromium on lymphocyte subsets and immunoglobulins (Ig) from normal population and exposed workers. The results showed that in the workers' blood, $CD_4$+ helper-inducer, $CD_5$-$CD_{19}$+B, $CD_3$-$CD_{25}$+ activated B and $CD_3$-HLA-DR+ activated B and natural killer (NK) lymphocytes were significantly reduced (about 30-50%). Boscolo suggests that Cr(III) may be involved in the mechanisms regulating the immune response in humans. Tanigawa (1991) also reported a decrease in Leu-11a negative lymphocytes in relation to NK cell activity in chromate workers. The lymphocyte subpopulation may provide a useful indicator of the effects of exposure to chromium. The current study measured T-cell and B-cell levels because these are commonly used immune function indicators in hospital

immunology tests. Various interleukin levels were also assessed. Interleukins may be described as lymphocyte-activating factors that stimulate the proliferation of murine thymocytes. The above-mentioned immunologic indicators can be used to evaluate the effect of short-term or long-term exposure of electroplating workers to chromium.

It is interesting to note that exposure to chromium had no effect on the levels of IL-2, IL-4, IL-10 and IFN. However, IL-6, IL-8 and TNF were detected in workers' blood samples. Urine-Cr levels correlated positively with IL-6 and IL-8 levels. This data is consistent with Snyder's (1986) study of IL-6 levels among individuals in Hudson County, New Jersey (an area contaminated with chromium). IL-6 works in conjunction with T helper cells and can stimulate the production of antibodies. Therefore, a decrease in IL-6 levels will result in decreased antibody production, which in turn will reduce immune function. Very probably the ability to promote differentiation of B lymphocytes into antibody-secreting plasma cells, and the induction of acute-phase protein synthesis in the liver in response to environmental stresses. In addition, IL6 can act as enhancing signal for various T lymphocyte activities (IL2 production and cell proliferation) and also induce a febrile response in vivo. However, IL-8 plays an important role in the inducing acute or chronic inflammatory response. IL-8 stimulates neutrophil, basophil and T-lymphocytes to move to the damaged site on cells. Inflammation first involves vascular endothelial

cells, macrophages, neutrophils and mast cells. T lymphocytes join in late when foreign antigens are presented to initiate immune responses. These effects are achieved partly by release at the site of injury of chemoattractant agent (such as IL 8). Chromium is the materials most oftens used for joint implantation. Therefore, Wang's study was aimed at investigateing the cytotoxicity of Cr and weather Cr affects T and B cell profiferation and release of cytokines by human peripheral blood mononuclear cells (PBMC) in vitro. The results shed light on how Cr impaired immune response and cytokines release, suggesting that patients with an extensive exposure to chromium may develop immune dysfunctions. Because cytokines are immunoregulatory molecules, primarily synthesized by leukocytes, they play an extremely important role in the communication network that links inducer and effector immune cells, producing effective resistance to infection. So our results have showed that workers with an extensive exposure to Cr may develop immune dysfunction. Consequently, a long-term exposure of workers to Cr may lead to substantial defects in immune functions including disruption of lymphocyte and macrophage differentiation in alveolar macrophage (AM). AMs are important phagocytic and secretary cells that participate in various complex immunologic and inflammatory processes. Gao(1992) have shown that Cr(VI) is able to induce DNA strand breaks in human lymphocytes both in vitro and rat lymphocytes in vitro and

vivo. Furthermore, the formation of the oxidized deoxynucleoside 8-hydroxydeoxyguanosine (8-OhdG) in isolated DNA after treatment with Cr(VI) or Cr(V) compounds suggested that the mechanism of toxicity might involve the production of hydroxyl radicals, probably through Fenton reaction (Faux et al., 1992).

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**Table 1. Comparison of immune function based on urinary Cr concentration**

| | Low urine-Cr group | Moderate urine-Cr group | High urine-Cr group | P value |
|---|---|---|---|---|
| IL-6 | 0.65±0.55[a] | 0.84±0.60 | 1.18±0.71 | NS |
| IL-8 | 23.57±50.16 | 37.15±45.4 | 64.08±35.22 | <0.01 |
| TNF-α | 2.90±4.51 | 1.98±1.82 | 1.90±2.09 | NS |
| T4 | 34.35±6.91 | 36.04±7.83 | 35.40±7.66 | NS |
| T8 | 28.26±6.59 | 28.04±7.33 | 21.6±6.26 | Ns |
| T-cell | 66.31±9.72 | 66.40±10.93 | 56.6±9.96 | NS |
| B-cell | 12.89±3.44 | 9.90±4.18 | 8.80±3.42 | <0.05 |
| Lymphocyte | 29.21±8.07 | 25.04±9.65 | 28.4±9.71 | NS |
| Monocyte | 4.94±1.50 | 5.31±3.06 | 4.60±1.81 | NS |
| Granucyte | 65.84±8.83 | 69.72±10.26 | 66.8±11.2 | NS |
| $T_4/T_8$ ratio | 1.26±0.33 | 1.28±0.52 | 1.87±1.07 | NS |

[a] means±SD

Low urine-Cr group：urinary Cr concentration <1.13(μg/g Cre)

Moderate urine-Cr group：urinary Cr concentration ：1.13～6.41(μg/g Cre)

High urine-Cr group：urinary Cr concentration >6.41(μg/g Cre)

**Table 2. Correlation between urinary and airborne Cr concentrations, work duration and immune function**

| | Urinary Cr conc. ( $\mu$g/g cre) | Airborne Cr conc. ($mg/m^3$) | Work duration (month) |
|---|---|---|---|
| IL-6 | 0.35** | -0.004 | -0.06 |
| IL-8 | 0.22 | 0.13 | -0.12 |
| TNF-α | -0.19 | -0.12 | -0.11 |
| T4 | 0.05 | -0.06 | 0.07 |
| T8 | -0.22 | -0.08 | 0.20 |
| T-cell | -0.18 | -0.008 | 0.19 |
| B-cell | -0.24* | 0.05 | 0.03 |
| Lymphocyte | -0.002 | 0.11 | 0.07 |
| Monocyte | -0.05 | 0.28* | 0.04 |
| Granucyte | 0.01 | -0.16 | -0.07 |
| $T_4/T_8$ ratio | 0.27 | -0.01 | -0.06 |

* p<0.1 **p<0.05

**Table 3. Analysis of risk factors for immune function using a multiple regression model**

| Variables | IL-6 B(SE) [a] | IL-8 B(SE) | TNF-$\alpha$ B(SE) |
|---|---|---|---|
| Urine-Cr | | | |
| Mediate group(Low group=1) | 0.38(0.26) | 16.24(19.50) | -0.63(1.30) |
| High group(Low group=1) | 0.69(0.26)** | 38.74(20.10)* | -0.85(1.34) |
| Gender(Male=1) | -0.05(0.29) | 20.17(21.78) | 1.41(1.45) |
| Age (years) | 0.01(0.01) | 0.98(0.70) | -0.01(0.04) |
| Electroplating task(no=1) | 0.01(0.01) | -0.36(1.26) | -0.04(0.08) |
| Smoking habit(no=1) | 0.05(0.25) | 8.41(18.94) | 0.77(1.26) |
| $R^2$ | 0.28 | 0.24 | 0.14 |

[a]B(SE) * p<0.1 **p <0.05 **p <0.01

**Table 3. Analysis of risk factors for immune function using a multiple regression model (continue)**

| variable | T4 B(S.E)[a] | T8 B(S.E)[a] | T cell B(S.E)[a] | B cell B(S.E)[a] | Lympho B(S.E)[a] | Mono B(S.E)[a] | Grannel B(S.E)[a] | T4/T8 ratio B(S.E)[a] |
|---|---|---|---|---|---|---|---|---|
| Urine-Cr | | | | | | | | |
| Moderate group | -0.03 | -1.78 | -7.81 | -2.87** | -1.10 | 0.94 | 0.16 | 0.07 |
| (low group=0) | (2.54) | (2.28) | (8.55) | (1.41) | (2.93) | (0.90) | (3.18) | (0.19) |
| High group | -0.23 | -6.49 | -8.82 * | -4.29* | -2.48 | -0.67 | 3.03 | 0.53* |
| (low group=0) | (4.00) | (3.59) | (4.93) | (2.23) | (4.63) | (1.42) | (5.02) | (0.30) |
| Gender (male=0) | 3.26 | -0.78 | 1.07 | 2.45 | 1.08 | 0.71 | -1.73 | 0.24 |
| | (3.75) | (3.37) | (4.62) | (2.09) | (4.34) | (1.33) | (4.70) | (0.28) |
| Age (years) | 0.19 | 0.22 ** | 0.36* | -0.01 | -0.20 | -0.06 | 0.26 | -0.01 |
| | (0.11) | (0.10) | (0.14) | (0.06) | (0.13) | (0.04) | (0.14) | (0.01) |
| Electroplating | -0.41 ** | -0.49** | -1.02** | 0.01 | 0.45* | 0.08 | -0.53** | 0.01 |
| task (no=1) | (0.20) | (0.18) | (0.25) | (0.11) | (0.23) | (0.07) | (0.25) | (0.01) |
| Smoking habit | 3.84 | -5.29 | -4.22 | 0.91 | -4.52 | 0.56 | 3.86 | 0.45 ** |
| (no=1) | (3.05) | (2.74) | (3.75) | (1.70) | (3.52) | (1.08) | (3.82) | (0.23) |
| $R^2$ | 0.22 | 0.33 | 0.43 | 0.22 | 0.32 | 0.07 | 0.31 | 0.21 |

[a] B(SE) * p<0.1 **p <0.05 **p <0.01