

Relationship between Nickel Exposure and the Level of Carcinoembryonic Antigen among Welders in an Automotive Plant

¹Chiang Lee Juan, ¹Shamsul Bahri Mohd Tamrin, ¹Syazwan Aizat Ismail, ¹Ridhuan Md Dan, ²Mohd Roslan Sulaiman and ¹Zailina Hashim

¹Department of Community Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

²Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Abstract: Background: Study on the effect of nickel exposure in the occupational setting and its association to carcinoembryonic antigen (CEA) had been studied before. Questions were raised whether the result from previous study would also show the same pattern of the urinary nickel concentration in this study. **Objective:** A cross-sectional study was conducted to determine the association between urine nickel concentration and CEA level among welders in automotive part workers in Malaysia. **Methodology:** There were 56 welders and 44 administrative workers were recruited in this study. Urine and blood samples were collected at the end of shift towards the end of workweek. CEA was assayed in serum using TECO Diagnostic ELISA Kit. Urinary nickel was determined by Graphite Furnace Atomic Absorption Spectrophotometer. **Results:** The mean carcinoembryonic antigen concentrations of the exposed group (4.07 ± 2.60 ng mL⁻¹) was significantly higher ($p < 0.001$) than the control group (1.99 ± 0.97 ng mL⁻¹). Twenty seven percent of the exposed group showed values above the reference range. The mean urinary nickel level was also significantly higher ($p < 0.001$) in the exposed group (1.99 ± 0.91 mg L⁻¹) than the control group. Result showed correlation between serum carcinoembryonic antigen concentration and urinary nickel concentration ($r = 0.206$). **Conclusion:** The significant elevation of urinary nickel levels in the welders compared with controls suggests that the welders were exposed to nickel fumes during welding. It is suggested that elevation of serum carcinoembryonic antigen was due to exposure to nickel during welding processes after controlling other confounding factors.

Key words: Urinary nickel, CEA, welders, automotive industries.

INTRODUCTION

Nickel (Ni) had been used widely in stainless steel industries throughout the world especially activities involved with welding. The chemical hazards of welding occur when fumes arise from stainless steel being welded. Welding fumes contain varying concentrations of potential toxic gases and metals such as manganese, copper and nickel. Although Nickel, chromium, arsenic and beryllium are human carcinogens, only nickel and chromium hexavalent appear to have significant concentrations in the welding

environment^[14]. Studies of nickel exposure were well documented when blood, tissues and in urine samples showed higher concentrations in occupation with direct nickel exposure^[7,11,21-23].

In occupational settings a major route of exposure is direct inhalation of nickel fumes and dust^[15]. Approximately 30% of inhaled nickel reaches the lungs and 20% of inhaled nickel is absorbed into the circulation and Ni²⁺ has the ability to enhance DNA methylation. Through this process, tumor cells may form as a result of inactivated expression of tumor suppressor gene^[6], thus increasing the

Corresponding Author: Syazwan Aizat Ismail, Department of Community Health, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia
Tel: +603-89472358 Fax: +603-89472395

Carcinoembryonic Antigen (CEA) level in the blood. With the abnormal elevation of CEA, we are able to screen and diagnose tumors derived from nickel exposure. Analyses of the relationship between serum CEA level and urine nickel concentration are appropriate for the appraisal of long term inhalation of Ni compounds. Therefore, the main purpose of this study was to determine the association between serum CEA and urinary nickel in welders.

MATERIALS AND METHODS

Study design and location: A cross sectional study design was conducted in a metal based automotive parts manufacturing plant with welding and stamping activities. The plant had 700 employees working in two shifts. Of the 700 workers, 43% were welders. Preliminary questionnaires were distributed to select and control confounding variables such as smoking, alcohol consumption, medical and employment history. Of the 700 workers only 56 welders were selected as the exposed group while 44 workers from administrative department selected as the control group. Consent from respondents was obtained prior to the interview. Sample size calculation was used to determine adequacy as described by Rubinson and Neutens^[24]. The ethical committee of Faculty of Medicine and Health Sciences, University Putra Malaysia had given ethical clearance to this study.

Questionnaire: Detailed questionnaires comprised several sections which include questions regarding demographic information (age, marital status and annual income), occupational information (work duration and number of years of employment as a welder), smoking activities (daily number of cigarettes smoked and years of smoking) and information on the medical history of the respondents.

Sampling and analyses of urinary nickel: Urine was collected into a 50 mL acid-washed (50% nitric acid) polyethylene container at the end of an 8 h shift on Fridays. All the samples were analyzed using the methods described by Sunderman *et al.*^[26] and Nixon *et al.*^[20]. All urine analysis of Nickel was performed by Graphite Furnace Atomic Absorption Spectrophotometry with Zeeman background correction (Hitachi Z-5700) in Industrial Hygiene laboratory, University Kebangsaan Malaysia (UKM) Medical Center.

Sampling and analyses of CEA: Blood (5 mL) was collected from each respondent by a qualified nurse. The serum was analysed for CEA level with CEA

ELISA test kit. Absorbance was measured using spectrophotometry at 450 nm^[27]. The blood specimens were centrifuged in a desiccated centrifuge with hermetically sealed trunnion cups.

RESULTS

Demographic information: Fifty-six Malay male welders (exposed group) and a control group consisted of 44 Malay males from the stamping operations (n = 34) and office workers (n = 10) were examined for this study. Face-to-face interviews were conducted with all the 100 respondents to obtain their demographic information.

Table 1 shows the comparison of demographic data between the exposed and control group. Statistical analysis showed no significant difference in the level of education and number of cigarettes smoked per day between the groups ($p > 0.05$). However, there was significant difference in age, marital status, basic income, duration of work and smoking years between the groups ($p < 0.05$). Each of the categorical of income, duration of working and smoking years was divided into categories.

Distribution of urinary nickel and serum CEA: Table 2 shows that the mean urinary nickel concentration was higher ($1.99 \mu\text{g L}^{-1}$) in the exposed group than the control group ($1.28 \mu\text{g L}^{-1}$). Using the 95th percentile of the control group as the reference limit, 10.7% of the exposed group was higher than the reference limit. Mann-Whitney U test showed a significantly higher urinary nickel concentration ($Z = -4.160$, $p < 0.001$) in the exposed group compared with the control group.

Result showed that the mean of CEA concentration was less than standard level (5.0 ng mL^{-1}) but using the 95th percentile of CEA concentrations in the control group as the upper reference limit, 53.6% of the exposed group was above the reference limit. A total of 35.7% of the exposed group had CEA concentrations of less than 2.5 ng mL^{-1} , while 26.8% of the exposed group had CEA concentrations above 5 ng mL^{-1} . Mann-Whitney U test showed a higher CEA concentration in expose group compared to control group ($Z = -4.736$, $p < 0.001$).

Relationship between serum CEA with U-Ni and selected factors: There was a significant correlation between CEA with U-Ni concentrations and duration of welding ($p < 0.05$), but no correlation was found with the number of cigarettes smoked. There was an inverse association between CEA level and age (Table 3).

Table 1: Demographic data of the respondents (N = 100)

Demographics	Exposed N = 56 (%)	Control N = 44 (%)	χ^2 value (p-value)
Age (years)			
19-24	46 (82.1)	20 (45.5)	15.26
25-30	9 (16.8)	19 (43.2)	(<0.001)**
31-35	1 (1.8)	5 (11.4)	
Marital status			
Single	50 (89.3)	26 (59.1)	12.316
Married	6 (10.7)	18 (40.9)	(< 0.001)**
Education			
UPSR	1 (1.8)	0 (0)	3.62
SRP/PMR	10 (17.9)	10 (22.7)	-0.36
SPM/STPM	43 (76.8)	29 (65.9)	
College/University	2 (3.6)	5 (11.4)	
Basic income (RM)			
<600	29 (51.8)	8 (18.2)	13.561
600-700	15 (26.8)	14 (31.8)	(0.001)**
>00	12 (21.4)	12 (50.0)	
Duration of working (years)			
<2.5	43 (76.8)	50 (50)	7.771
>2.5	13 (23.2)	50 (50)	(0.005)**
Number of cigarette smoked per day			
<10	35 (62.5)	27 (61.4)	0.014
>10	21 (37.5)	17 (38.6)	-0.907
Duration of smoking (years)			
<5	30 (64.3)	19 (43.2)	4.434
>5	26 (35.7)	25 (56.8)	(0.035)*

** : Significant at $p < 0.01$; * : Significant at $p < 0.05$

Table 2: Distribution of urinary-nickel and CEA concentrations of exposed and control group

Statistic	U-Nickel Concentration		Serum CEA Concentration	
	Exposed (N = 56)	Control (N = 44)	Exposed (N = 56)	Control (N = 44)
Mean (\pm SD)	1.99 (0.91)	1.28 (1.05)	4.07 (2.60)	1.99 (0.97)
Median (Inter quartile range)	1.69 (0.71-4.00)	1.09 (0.09-5.52)	3.67 (1.20-15.22)	1.92 (0.43-4.97)
95th Percentile	3.85	3.58	8.96	3.43

Mann-Whitney U: 630.00; Z: -4.160, $p < 0.001$ *; *: p-value is significant at $p < 0.01$

Table 3: Correlation between CEA concentrations with selected variables

Parameters	CEA concentration	
	r-value	p-value
U-Ni concentrations	0.206	0.040*
Age	-0.256	0.010**
Duration of welding	0.365	<0.001***
Number of cigarette smoked per day	-0.002	0.981

***: p-value is significant at $p < 0.001$; *: p-value is significant at $p < 0.05$

DISCUSSION

Distribution of urinary nickel concentration: Urinary Nickel (U-Ni) concentrations in the exposed group in our study were compared with the urinary nickel concentrations reported in other studies^[1,7,11,21-23]. From Table 4, it can be seen that all the previous studies reported higher U-Ni concentrations than our study. The nickel electroplating workers in the study by Sunderman *et al.*^[26] had the highest average U-Ni

concentrations ($27.1 \mu\text{g L}^{-1}$) while a study in Finland^[11] reported the lowest levels ($8.7 \mu\text{g L}^{-1}$). The mean urinary nickel concentration of stainless steel welders in the Finland study^[11] was at least 5.8 times higher than in our study. When the respondents from previous studies were divided into 3 categories; nickel electroplating workers^[7,23] stainless steel welders^[1,11] and high-Ni alloy welders^[23]. The nickel electroplating workers had the highest U-Ni concentrations, followed by stainless steel welders and high-nickel alloy welders.

Factors such as the amount deposited and particle solubility, surface area and size will influence the behavior of nickel particles deposited in the respiratory tract and will probably account for the differences in retention and clearance via absorption. Based largely upon experimental data, it can be concluded that the more soluble the compound, the more readily it is absorbed from the lung into the bloodstream and excreted in the urine^[19]. According to Oliveira *et al.*^[21],

Table 4: Comparison of U-Ni concentrations of exposed group in previous studies and present study

Authors	Country	Occupation	Sample size	Mean ($\mu\text{g L}^{-1}$)
This study	Malaysia	Automotive parts welder	56	1.99±0.9
Oliveira <i>et al.</i> ^[21]	Brazil	Nickel electroplating worker	10	20.6±18.1
Angerer and Lehnert ^[11]	Germany	Stainless steel welder	103	18.5±28.5
Sunderman <i>et al.</i> ^[26]	United States	Nickel electroplating worker	16	27.1±21.2
Åkesson and Skerfving ^[28]	Sweden	High-nickel alloy welder	11	8.7*
Rahkonen <i>et al.</i> ^[29]	Finland	Stainless steel welder	7	11.5±1.6

*: Standard deviation was not stated

the nickel electroplating workers were exposed to soluble nickel compounds, while the stainless steel welders and high-nickel alloy welders were exposed to extremely low solubility of nickel aerosol^[23]. This phenomenon explains why the Nickel electroplating workers recorded higher U-Ni concentrations than stainless steel welders and high-Ni alloy welders.

Carcinoembryonic Antigen (CEA): For exposed group, the CEA concentrations ranged between 1.20 and 15.22 ng mL⁻¹. The comparison of CEA concentrations for the exposed group with other studies could not be made as there has not been any study of CEA on welders or subjects exposed to nickel. The clinical value of CEA was evaluated prospectively in 118 patients with small cell lung cancer. Seventy percent of patients had levels less than 5.0 ng mL⁻¹ and only 19% had levels greater than 20.0 ng mL⁻¹^[16]. Carcinoembryonic antigen was measured in samples of serum coming from 105 non-small cell lung cancer patients. The median and inter quartile range were 3.0 and 7.0 ng mL⁻¹. CEA was higher than 5.0 ng mL⁻¹ in 38% of patients^[17]. For this study, a total of 15 (26.8%) welders had CEA concentrations above 5 ng mL⁻¹. Carcinoembryonic antigen concentrations of 30 welders (53.6%) were higher than the upper reference limit of this study.

Concentrations of 2.5-5.0 ng mL⁻¹ are commonly considered as cut-off points for distinguishing normal from abnormal levels of serum CEA^[10]. Values more than 2.5 ng mL⁻¹ may be found in associations with cancers^[18]. Similarly, raised serum CEA levels could be detected in cigarette smokers, which were why the respondents of this study consisted of smokers for both exposed and the control group, to eliminate the confounding effect of tobacco smoking on serum CEA levels.

In this study, there were several reasons for employing serum CEA among the numerous tumor markers for lung cancer monitoring. In an ideal tumor marker should increase pathologically in the presence of a neoplasm (high sensitivity) and not increase in the absence of neoplasm (high specificity). It should also possess constant serum levels with no major

fluctuations and easy and inexpensive^[8]. Serum CEA has the mentioned characteristics. Serum CEA has been reported for its high sensitivity in several studies. In a prospective study that aimed to assess the diagnostic value of serum CEA, cytokeratin 19 fragment marker (CYFRA 21-1) and Neuron-Specific Enolase (NSE) in the differentiation of malignant from benign solitary pulmonary lesions, serum CEA recorded the highest sensitivity (27.2%) and accuracy (40.4%) when used as a tumor marker alone^[25]. In another study^[12], the highest sensitivity (85.3%) was found in serum CEA. The diagnostic accuracy was 74% for serum CEA, which was higher than NSE (66%) and CA-50 antigen (62%)^[4].

The standard diagnostic procedures in the evaluation of suspected lung cancer include sputum cytology, bronchoscopy and transthoracic needle aspiration. These procedures are complex, expensive and time-consuming in clinical practice^[4,5]. However, tumour marker analysis has the advantage of simplicity in sampling technique (only blood sample is required), which makes it applicable to the respondents. Furthermore, the serum test is inexpensive^[5] and proves to be useful as a complementary tool if standard diagnostic procedures are not applicable to patients^[4]. In patients affected by lung cancer, abnormally elevated values of the marker can be found in 30-70% of the samples^[5].

Relationship between CEA and urinary nickel concentrations: Spearman's rho correlation coefficient demonstrated a significant correlation ($r = 0.206$; $p = 0.040$) between urinary Nickel and CEA concentrations. As mentioned earlier, there has not been any study looking at the relationship between these two parameters. This finding facilitates appraisal of the correlation between lung cancer and occupational exposure to nickel.

Nickel forms Ni²⁺ ion in body fluids and has the ability to enhance DNA methylation specifically in regions bordering heterochromatin. Through this process, many tumors can arise as a result of inactivated expression of a tumor suppressor gene by hypermethylation of their promoter region^[6]. Thus, an

increased level of urinary Nickel can accelerate the DNA methylation and produce more tumors in the body. As a result of this activity, tumors in the blood circulation can release more CEA^[18].

It should be noted that inorganic compounds of arsenic, beryllium, cadmium and chromium might be present in welding fumes^[14]. However, only nickel and chromium appear in significant concentrations in the welding environment^[14]. These agents may interfere with each other to produce synergistic or perhaps antagonistic effects. Pretreatment with nickel has earlier been shown to protect against cadmium intoxication in experiments^[13]. No concrete information seems to be available for the evaluation of synergism or antagonisms of nickel and other carcinogens present in welding fumes. According to Furst^[9], an infinite number of possibilities exist for studies on how Nickel compounds are involved in the cancer process.

Nevertheless, this study has shown that both the urinary nickel concentrations and CEA concentrations of exposed group were significantly higher than the control group. However, no significant relationship ($p > 0.05$) between urinary chromium and CEA concentrations was observed. As only nickel and chromium appear in significant concentrations in the welding environment^[14], this non-correlation indicates that the raised Nickel levels in urine of the welders appear to be the contributory factor to the elevated CEA levels in the welders. Hence nickel compounds pose a higher risk of CEA elevation than chromium in the body.

Relationship of CEA with other variables:

Duration of welding: A significant correlation ($r = 0.365$; $p < 0.001$) was observed between CEA concentrations and duration of welding. Duration of exposure to low dosages of carcinogens is a critical factor^[2]. Nickel, chromium and other carcinogenic elements in welding fumes can induce carcinogenic effects on welders and are capable of causing elevated CEA levels in serum. Duration of welding should represent the cumulative exposure to carcinogenic elements in the welding fumes. It should be noted that Spearman's rho correlation coefficient demonstrated no correlation between CEA concentrations and duration of work ($r = -0.142$; $p = 0.160$). This non-correlation indicated that the CEA concentration was independent of duration of work at the study location, but correlated with the number of years of exposure to welding which shows chronic exposure and long term body burden. About 20% of the welders had worked as welders at different places before being employed at this study location.

Age: Spearman's rho correlation coefficients showed an inverse relationship ($r = -0.256$; $p = 0.01$) between CEA and age. The inverse relationship was unexpected and may be due to the large number of young workers in this study. In a review of CEA by Gold and Goldenberg^[10], who reported raised CEA levels in older subjects compared to younger individuals. Nevertheless, the influence of sex is more important than that the age in CEA determination^[3]. This finding is supported by the conclusion that the influence of age in CEA determination is of minor importance.

Cigarettes smoking: The study found a non significant correlation between CEA concentrations and number of cigarettes smoked per day. This finding contradicts the results of finding by Gold and Goldenberg^[10] who found that CEA was raised in smokers. Since the homogeneity of smoking was control in both groups, the increase of serum CEA level in the exposed group may be caused by exposure to nickel fumes.

CONCLUSION

Our study had shown a relationship between Nickel exposure and the level of serum CEA. Although it is not specific, it may serve as a useful biomarker for cancer screening associated with chemical carcinogen exposure. The detection of this marker may prove useful for early intervention and prevention activities. For example, in persons with highly elevated serum CEA, further diagnosis testing is recommended. Therefore, more studies of CEA levels should be conducted in the future to evaluate the potential role of determination of CEA level among the high-risk groups such as welders who are exposed to hazardous fumes.

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