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Effect Of Chromium (VI) Exposure On Serum Amylase Activity In Chromium Plating Workers

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ABSTRACT

The effect of chromium (VI) exposure on serum amylase activity has been monitored in chromium plating workers of Bangalore City (India). 50 subjects using chromium (VI) during electroplating formed the study group. An equal number of age–sex matched subjects working in administrative section formed the control group. Urinary chromium levels were determined by using a flameless atomic absorption spectrophotometer. The serum amylase concentration was determined by using the spectrophotometric method with 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) as substrate. Significant increase of urine chromium and serum amylase activity was noted in the subjects of the study group as compared with the subjects in control group. The level of serum amylase activity was positively and significantly associated with chromium levels in urine of chromium exposed subjects. Multiple regression analysis assessed the effect of chromium exposure, life style confounding factors and presence of gastrointestinal problems on serum amylase activity. Analysis showed that the subjects with smoking, BMI and chromium exposure variables were significantly associated with increase of serum amylase activity. The results of the present study suggests that the increased serum amylase level observed in chromium exposed workers could be used as bio-indicator for pancreatic function in chromium exposure.

KEYWORDS

Urine chromium;
Serum amylase acticity;
Chromium plating.

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INTRODUCTION

Electroplating is the process of oxidation of metal articles by using of electrolytes containing acids or bases. The process of electroplating involves three steps: cleaning, plating and post treatment of articles. Chromium is used as chromic acid, in electroplating of different articles used in the automobile manufacturing industry. The workers engaged in this process are exposed to chromium through inhalation, ingestion and dermal contact. Inhalation is the primary route of occupational exposure to metals[1]. The mechanism of chromium toxicity involved in binding to thiol - groups in enzymes and proteins, formation of reactive oxygen species during redox-cycling and metabolic activation of cytochrome P450 in liver[2]. In animal studies it was observed that the chromium exposure to pancreas have shown an increased accumulation of chromium, synthesis of metallothionein, decrease of β-cell activity and increased glucagon secretion[3-4]. Pancreas secretes 0.084-0.112 μmol of chromium every day into duodenum[5]. The risk of pancreatic cancer was significantly associated with worksite exposure to chromium and chromium compounds[6-7]. The studies relating to occupational exposure to chromium during chromium plating process has reported nasal dysfunction, chromosome abnormality, oxidative damage to DNA, immunological effects and renal tubular dysfunction[8-12]. No reports are available regarding occupational exposure to chromium and the effect on serum amylase activity as exocrine pancreatic function. Therefore, the present study was undertaken to investigate the functional integrity of exocrine pancreas by using serum amylase activity in workers exposed to chromium (VI) during chromium-plating process. The determination of serum amylase, serum lipase, urinary amylase and amylase-creatinine clearance ratio tests are used to assess the pancreatic dysfunction. When compared to the serum amylase and urinary amylase tests, the amylase-creatinine clearance ratio and serum lipase are relatively insensitive tests in patients with acute pancreatitis[13-16]. The measurements of serum enzymes are more specific for the pancreas dysfunction[17]. Hence, the present study was assessed the pancreatic dysfunction by using serum amylase activity.

MATERIALS AND METHODS

The study was carried out in 100 male subjects working in chromium plating industry located in Bangalore (India). These subjects were divided into two groups. The first group formed the study group and consisted of 50 subjects involved in chromium plating operation with an exposure period ranging from 15 to 20 years. The second group formed the control group and consisted of 50 subjects working in administrative unit with no exposure to chromium. The control subjects working in 1kilometer away from the place of work of the study group. The control group subjects were matched regarding age and socio-economic status as that of study group. Demographic information, work history and habits of all subjects were collected through a questionnaire. The subjects with a history of diabetes or hypertension were excluded from the study. The ethical committee approved the study. Informed consent was obtained from each of the subjects included in the study.

Whole blood (2 ml) was collected from each subject in test tube and centrifuged at 3000 rpm for 10 min at 4°C. Serum and the red blood cells were separated. The collected serum was used for the determination of amylase activity.

Serum amylase (E.C.3.2.1.1)

The level of serum amylase activity was determined by the method of Gella et al[18]: Amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-maltotrioside to 2-chloro-4-nitrophenol and maltotriose. The serum amylase concentration is determined from the rate of 2-chloro-4-nitrophenol formation (yellow color compound), which is measured at 405 nm by using a UV-Visible-spectrophotometer (Schimadaz -Model-UV-1601-PC). One unit of enzyme activity is defined, as the amount enzyme required to convert one μmol of substrate such as 2-chloro-4-nitrophenyl-malto-trioside to 2-chloro-4-nitrophenol per liter of sample at 37°C. The level of serum amylase was expressed in units/liter.

Urine chromium

Urine samples were collected from each subject in metal-free polyethylene bottles and used for determination of chromium according to the method of Claude et al[19]. Chromium in urine samples was determined by using a flameless atomic absorption spectrophotometer equipped with graphite furnace (GF-3000) and auto sampler (PAL-3000). This method has been recognized as a specific method for direct determination of chromium in human urine and hence is suitable for routine clinical use. Determination of chromium as internal standard added to urine and showed a recovery rate of 98.4%. The levels of urine chromium were expressed as μg/g of creatinine. The urinary chromium was standardized with urinary creatinine concentration measured by the Jaffe reaction method of Husdsan and Rapoport[20].

Statistical analysis

SPSS package, version 7.5 for windows, was used for the statistical analysis of the data. Student's t-test was used to compare the means for age, duration of work, body mass index, urinary chromium, and serum amylase among chromium exposed subjects and control subjects. The χ^2 -test was used to compare the abnormal proportion of serum amylase and urine chromium between chromium exposed subjects and control subjects. The cutoff value for urinary chromium levels more than 10 μg /g of creatinine as per the recommendation of ACGIH-2005 and serum amylase activity (95th percentile of control group) was used to compare the abnormal proportions between study and control groups. Pearson's correlation coefficient was used to find out the correlation between urine cadmium and serum amylase levels. Stepwise multiple regression analysis was used to assess the effect of chromium on serum amylase activity.

RESULTS

Some of the demographic details of study and control group subjects are presented in TABLE 1. The average age, duration of exposure and body mass index of study group subjects and control group subjects were suitably matched.

TABLE 1: Demographic details of study and control groups

Variables	Chromium exposed workers (n=50)	Controls (n=50)
Age (years)	42.5 ± 3.0	42.0 ± 4.3
Work duration (years)	14.2 ± 3.6	13.7 ± 2.4
Body mass Index (Kg/m^2)	26.4 ± 2.96	27 ± 2.83

Values are mean ± standard deviation
Student t-test was used to compare for age, work duration and body mass index; Statistical test showed no differences between the study and control groups

TABLE 2: Levels of urine chromium and serum amylase in study and control group

Variables	Chromium exposed workers (n=50)	Controls (n=50)
Urine Chromium (μg/g of creatinine)	10.4 ±8.3**	3.2 ± 0.8
Serum amylase (U/L)	59.0± 9.0*	55.0 ± 11

Values are Mean± standard deviation
Student t-test was used to compare for urine cadmium and serum amylase; Statistical test indicated significant differences between the study and control groups
****P<0.001 AND * P<0.05**

The average levels of urine chromium and serum amylase of study and control group subjects are presented in TABLE 2. It should be noted that the levels of urine chromium and serum amylase were significantly increased in chromium-exposed subjects as compared with the control group subjects.

The distribution of abnormal proportion of urine chromium and serum amylase activity of chromium exposed subjects and control group subjects are presented in TABLE 3. The cutoff value of urinary chromium levels more than 10μg/g of creatinine was used as per the recommendation of ACGIH-2005[21]. The cutoff value of serum amylase activity was used as 95th percentile (mean + 2 standard deviation) of control group. The value of ACGIH-2005 for urine chromium levls was consistent with normal levels. The proportions of urine chromium and serum amylase among chromium-exposed subjects were showed significantly higher as compared with the proportion of non-exposed subjects. These abnormal proportions were significantly related to chromium exposure.

TABLE 4 presents the correlation coefficients (r) between urine chromium and serum amylase ac-

TABLE 3: Distribution of abnormal proportions of urine chromium and serum amylase in study and control group

Variables	Cutoff values	Chromium exposed workers (n=50)	Controls (n=50)
Urine Chromium (μg/g of creatinine)	>10[a]	20(40)**	2(4)
Serum amylase (U/L)	>77[b]	7(14)*	0(0)

χ^2 -test was used to compare the abnormal proportion of urine chromium and serum amylase
Figure in parenthesis indicates % of abnormal subjects
a = cutoff values as per ACGIH-2005
b= cutoff values as per 95th percentile of control group.
****P<0.001 and * P=0.019(Yates corrected)**

TABLE 4: Correlation coefficient (r) between urine chromium and serum amylase in study and controls

Variables	Chromium exposed workers (n=50)	Controls (n=50)
Serum amylase (U/L)	0.280*	0.033

Pearson's correlation coefficient was used
***Correlation is significant at 0.05**

tivity in chromium exposed subjects and control group subjects. A positive correlation coefficient was found between urine chromium levels and serum amylase activity in chromium exposed subjects and control group subjects. The correlation coefficient (r) was 0.280 in chromium-exposed subjects and 0.033 in control subjects. The correlation coefficient among chromium-exposed subjects was significant at $p<0.05$. The correlation coefficient was not significant in control subjects. The nasal problems (nasal irritation 8 % and loss of smell 8%), respiratory problems (Cough with sputum 8% and cough without sputum 6%) and gastrointestinal problems (gastritis 8%, epigastrites 8%, loss of appetite 6% and abdominal pain 6%) were reported in workers exposed to chromium.

TABLE 5 shows the results of stepwise multiple regression analysis of variables that affects the concentration of serum amylase activity. The variables included in the regression model were different job category (1=Chromium exposed workers and 2=control group), body mass index (1= 18-24.9 Kg/m^2, 2= 25-29.9 Kg/m^2 and 3=>30 Kg/m^2), smoking status (1=No and 2=yes), alcohol consumption (Usually=1, Sometimes=2 and Never=3), and the

TABLE 5: Multiple regression analysis of variables that affects the serum amylase activity (n=100)

Variables	Serum amylase (U/L)β (P-value)	R^2
Chromium exposure	0.163(0.049)*	0.080
Body mass index 2=25-29.9(Kg/m^2)	0.188(0.024)*	0.113
Smoking Habits 2=yes	0.221(0.034)*	0.226
Alcohol consumption 3=never	0.126(0.018)*	0.065
Gastrointestinal problems No=0	0.214(0.000)*	0.218

Stepwise multiple regression analysis was used
***Regression coefficient and p-value significant at p<0.05**

presence of gastrointestinal problems (0=no problems, 1=gastritis, 2=epigastritites, 3=loss of appetite and 4=abdominal pain). Multiple regression analysis showed that the chromium exposed subjects had significant influence (8%) on serum amylase activity. The levels of chromium in control subjects not influenced on serum amylase activity. It was noted that a body mass index of 25-29.9 Kg/m^2 (11%) and smokers (22%) had significant influence on serum amylase levels. The subjects who had gastrointestinal problems and alcohol consumption did not show any significant influence on serum amylase activity.

DISCUSSION

The studies related to occupational exposure to chromium and the effects on serum amylase were limited. The present study assessed the effect of chromium (VI) on serum amylase activity in workers exposed to chromium during chromium-plating process. The levels chromium noted in urine, plasma and organs reflect the body burden of chromium. The determination of urine chromium was considered as an indicator for chromium exposure[22]. The absorption of chromium is quantified in the urine samples of chromium exposed and non-exposed subjects. The levels of urine chromium in chromium-exposed subjects noted in this study closely agree with the ones reported by Lukanova et al[23]. During the present study urine chromium levels showed a high degree of variation among chromium-exposed subjects. This is similar to the findings of Kuo et al[24] who noticed a higher variation (seven times) in chromium-exposed subjects as compared to control subjects.

Since, the estimation of serum amylase is more sensitive than amylase-creatinine clearance ratio and serum lipase, the determination of serum amylase activity was used for assessing the functional integrity of pancreas in chromium-exposed subjects. During the present study, it was noted that serum amylase was significantly increased in chromium-exposed subjects as compared with control group subjects. The level of serum amylase was positively and significantly correlated with the levels of chromium in urine of chromium-exposed subjects. Since the increased levels of serum amylase is associated with life style confounding factors and presence of gastrointestinal problems, the present study assessed the association between life style confounding factors, presence of gastrointestinal problems with serum amylase activity by using stepwise multiple regression analysis.

Carroccio et al[25] reported an elevated pancreatic enzymes were not associated with alcohol consumption, drug use and presence of abdominal pain. Maruyama et al[26] reported that the subjects who consume alcohol did not influence the serum amylase activity. During the present study, the subjects who consume alcohol and presence of gastrointestinal problems did not influence the serum amylase activity. Matsubara[27] reported positive association between serum amylase activity and body mass index (BMI). Similarly the present study indicated a significant association between body mass index of 25-29.9 Kg/m^2 and serum amylase activity. Milnero wicz et al[28] reported elevated levels of serum amylase activity in smokers as compared with non-smokers and suggest that the tobacco smoking had significant influence on pancreatic exocrine function. During the present study it was noted that the subjects who smokes cigarettes had significantly influenced on serum amylase activity. Smoking habit influenced more on serum amylase activity as compared with other variables.

In conclusion, the urine chromium and serum amylase levels were significantly increased in the chromium exposed subjects as compared with the levels in the control group subjects. The level of serum amylase was positively and significantly correlated with urine chromium levels in chromium-exposed subjects. The increased serum amylase was significantly associated with the subjects with chromium exposure, body mass index and smoking. The subjects with gastrointestinal problems and alcohol consumption did not show any significant association with serum amylase activity. The increased levels of serum amylase activity could be used as biomarkers for chromium toxicity in human exposed populations.

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