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Original Article

EVALUATION OF LIVER FUNCTION MARKERS AMONG WORKERS IN JEWELLERY UNITS

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ABSTRACT

Objectives: Jewellery making unit workers are exposed to potent toxic chemicals and heavy metals during the manufacturing process. Various alloys are used during the manufacturing process of gold in which silver is used in higher concentration. The induction of metallothioneins (MTs) might occur due to the occupational exposure to heavy metals. Hence, the relationship of silver or MTs with liver function markers were investigated.

Methods: A cross sectional study was conducted in the jewellery making units located in Coimbatore, Tamilnadu. A total of 211 participants [exposed (n = 158) and control (n = 53)] were included for the study and their liver function markers namely alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein, albumin, total/direct bilirubin and total cholesterol were estimated. Levels of serum silver and MTs were also assessed.

Results: There were significant ($p<0.05$) increases of serum silver, MTs, ALT, AST, total cholesterol, total/direct bilirubin and significant ($p<0.05$) decrease of ALP, total protein and albumin in exposed groups compared to control group. Levels of MTs were positively correlated with ALT, AST, total cholesterol, total/direct bilirubin and were negatively correlated with albumin, total protein and ALP.

Conclusion: The significant changes of liver function enzymes were observed due to the prolonged period of exposure to silver. However releases of these markers were in the normal range which might be due to the induction of MTs. The elevated levels of MT in the serum compared to control group pointed out that it might provide a cellular defense strategy against silver.

Keywords: Silver, Metallothioneins, Occupational exposure,

INTRODUCTION

Metal pollution is a major public health problem in developing countries. Metal pollution affects the physical and mental development of an individual leading to decreased work capacity that in turn affects the development of a country [1]. Jewellery making is one of the world's oldest manufacturing sectors that involve some hazardous processes. Most often, pure gold is alloyed with copper, zinc and silver in varying proportions to produce the wide range of karat gold. According to industrial applications, jewellery, silverware and photographic industries were the largest consumers of silver using 40, 31, 22 percent respectively [2].

Silver is classified as a xenobiotic metal and has no known physiologic function in the human body which is used in higher concentration during the manufacturing process of gold. Silver ions are absorbed into the systemic circulation from the diet, drinking water, by inhalation, ingestion and through intraparenteral administration [3]. Furthermore, silver compounds are ionized in body secretions and body fluids to produce biologically active ions (Ag^+). Ag^+ ions generate reactive species which bind strongly to metallothioneins, albumin and macroglobulins. When silver is absorbed systemically, it is metabolized in liver and mostly excreted via urine and feces. But some of the biologically active ions are deposited in tissues or circulated in the biological system which becomes a toxic factor that causing oxidative damage to the cells [4, 5, 6].

Metallothioneins (MTs) belong to the group of intracellular, low molecular weight and cysteine rich proteins which are involved in detoxification processes of heavy metals with molecular weight from 5-16 kDa. The protein consists of 61 amino acid residues of a polypeptide chain in which 20 residues are cysteine and many lysines and arginines. It has more than 30% of cysteine residues and reduced form of cysteine directs its metal binding properties through mercaptide bonds [7]. MTs can be used as a biomarker in the field of industrial health due to the synthesis of MTs by exposure to heavy metals and the accumulation of metals in the cells and the tissues [8]. Bioavailability of ionic form of silver is more toxic to organisms than any other metal. However, it is very difficult to determine the potential toxicity of silver, because its bioavailability is dependent on the physical, geochemical and biological processes that determine metal uptake by living organisms [9].

There is no specific investigation to assess whether silver affects the liver like other heavy metals in human. Hence the present study was to determine the levels of liver function markers - alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), albumin, total cholesterol, total protein and total or direct bilirubin concentration among workers exposed to silver compounds.

MATERIALS AND METHODS

Target population

A cross sectional study was conducted in the jewellery making units located in Coimbatore (Kuniamuthoor, Saibaba Colony and R. S Puram) Tamilnadu. The study protocol was approved by the Ethical Committee (HEC.2011.27) of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu. The jewellery workers live and work in small rooms which do not have adequate ventilation. They do not seem to wear protective mask, shoes, personal protective clothes and gloves.

The study consisted of inclusion and exclusion criteria in which workers with minimum one year of exposure were included and those with known history of diabetes mellitus, liver diseases, blood transfusion and any other pathological conditions were excluded from the study. Questionnaires were administered to elicit their personal details namely food habits and medical history. Informed written consent was obtained from all participants.

A total of 231 jewellery unit workers aged 20–50 years who are residing and working in Coimbatore for many years were asked to participate in the study among whom 158 participants were included based on the selection criteria and they were considered as

exposed group. Those who are living in the same area with no relation to making of jewellery were considered as control group comprising of 53 subjects. The exposed group was further categorized into 2 groups as occasional (Group B, n=85) and frequent (Group C, n=73) workers. Control group was denoted as Group A. The demographic and socioeconomic status were matched among the exposed and control groups.

Sample collection

Peripheral venous blood samples were collected from the subjects. All samples for serum (Vacutainers - AcCuvet-PLUS with clot activator) and plasma (Na_2EDTA vacutainers -AcCuvet-PLUS) were centrifuged at 3000 rpm at 4°C for 10 minutes. The samples were aliquoted into labeled cryo vials and stored at - 20°C for further analysis. Serum samples were used to assess the levels of liver function markers and metallothioneins. Fraction of the serum samples were diluted three fold with double distilled water before assay by Flame Atomic Absorption Spectroscopy (FAAS). Height and weight were measured by standard method. Systolic and diastolic blood pressure were measured using Omron Blood Pressure Monitor: HEM 7112.

Analytical methods

Serum silver level was determined by FAAS (SHIMADZU, AA- 7000) [10]. Serum metallothionein content was assessed by modified method of Viarengo *et al* (1997) [11]. Activity of serum ALT and AST were estimated by DNPH method [12]. Albumin levels were assessed by BCG dye method [13]. Levels of total protein were quantified by Biuret method [14] and total cholesterol was quantified by CHOD-PAP method [15]. Total and direct bilirubin levels were determined by Diazo method [16].

Levels of random glucose and ALP were estimated by GOD/POD [17] and Kinetic method respectively [18].

Statistical analysis

SPSS package version 16.0 was used for the statistical analysis of data. The statistical significant level was set at P<0.05. Shapiro-Wilk test was carried out to identify the normal distribution of the data (p>0.05). ANOVA was performed for normally distributed data and if the data showed variation at significant level (p< 0.05), post - hoc test was done to know the association or difference between each group. Kruskal Wallis Test was done for non - normally distributed data and further Mann-Whitney test was conducted to know the association or difference between the groups. Spearman's rank correlation was performed to find out the association of metallothioneins or silver levels with liver function indicators.

RESULTS

The blood borne markers of liver function of jewellery unit workers were compared with control group. General profile and anthropometric measurements of exposed and control group are listed in table 1 and 2. There were no significant difference in age distribution, height, weight, diastolic pressure, smoking and alcoholic habit between the exposed and control group workers. Smoking habit of the selected participants was expressed in pack years which is calculated in terms of number of pack years = (number of cigarettes smoked per day × number of years smoked)/20; 20= number of cigarette in a pack. Alcohol consumption of selected participants was stated in units/week (1 unit = 10 ml). Systolic pressure was significantly increased (p<0.05) in exposed groups (B and C) than control group (A). There was no significant difference (p > 0.05) observed in systolic pressure among exposed group workers.

Table 1: General profiles of the selected workers employed in jewellery units

Groups	Age*	Pack Years*	Frequency of alcohol consumption /Week*
Control group (n = 53)	35 (44 - 27)	0.74 (2.5 - 0.00)	3.0 (21.50 – 0.00)
Exposed group (n =158)	36 (42 - 32)	0.76 (2.3 - 0.37)	5.0 (25.00 – 0.00)
p value (>0.05)	0.200	0.549	0.344

*= median value (inter quartile range); Mann Whitney test computed for groups with unequal sized samples

Table 2: Anthropometric measurements of the selected jewellery unit workers

Anthropometric Measurements	Control group (A) (n=53)	Exposed groups		p Value (p<0.05)
		Occasional (B) (n=85)	Frequent (C) (n=73)	
Height (cm)$	156 (161-154)	157 (162-154)	156 (160-153)	0.208
Weight (Kg)$	65 (75-60)	65 (75.5-58)	62 (71-56)	0.115
BMI$(Kg/m²)	26.64 (30.4 - 23.9)	25.8 (29 - 23.4)	24.7 (27.9 - 22.9)	0.075
Systolic pressure $	120 (120 - 114)	121 (130 -115)	123 (130 - 115)	**0.004**⊙
Diastolic pressure ¥	74.94 ± 8.1	78.32 ±10.43	78.20 ± 8.17	0.074

$=median value (Inter quartile range); Kruskal Wallis test computed for groups with unequal sized samples and Mann Witney test computed between the groups; ¥= mean value ±standard deviation; ANOVA computed for groups with unequal sized samples and post hoc computed between the groups ⊙ = The median difference is significant at 0.05 level.

Workers of jewellery units had been exposed to hazardous compounds while processing of gold in which silver is used in higher concentration. Silver is considered as xenobiotic compound with no known trace metal value in the human body. Therefore study was more concentrated on silver exposure of jewellery unit workers who have been exposed to this metal daily over an extended period of time. Figure 1a shows that serum silver level was significantly raised (p<0.05) in exposed groups (B and C) than control group (A).

Among the exposed groups concentration of silver was significantly increased (p<0.05) in group C compared to group B.

In the present study serum MT levels was significantly elevated (p<0.05) in exposed groups (B and C) than control group A. Among exposed groups, MT level was significantly raised (p<0.05) in frequent workers (group C) than occasional workers (Group B) as presented in Figure 1b.

Blood marker of liver function

Serum AST and ALT activities were found to be increased significantly ($p<0.05$) in jewellery unit workers (Group B and C) compared to controls (Group A) of the study. Among the groups of exposed workers, the activities of these liver marker enzymes were found to be increased in frequent workers (Group C) than occasional workers (Group B). Activity of serum Alkaline phosphatase (ALP) was found to be decreased significantly ($p<0.05$) in the jewellery unit workers (Group B and C) compared to control group (Group A).

Among the jewellery unit workers, ALP activity was decreased significantly ($p<0.05$) in frequent workers (Group C) than occasional workers (Group B) as given in Figure 2.

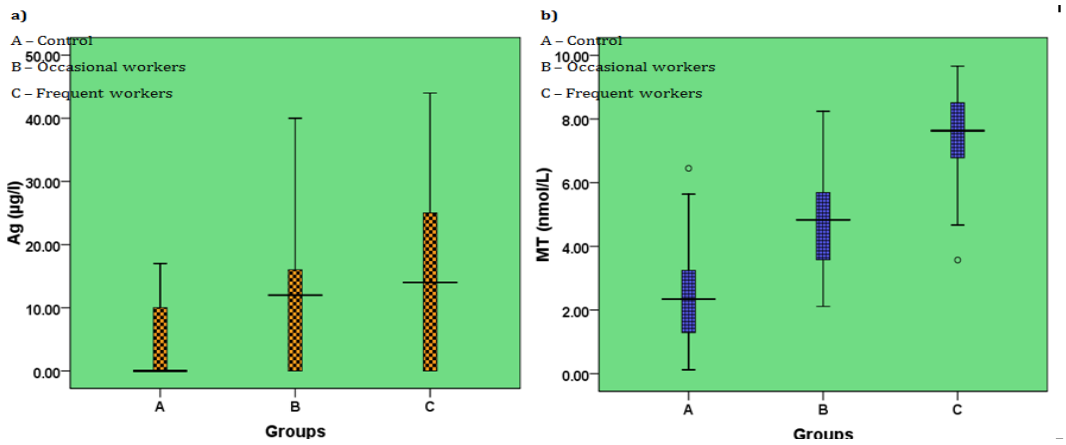

Fig. 1: Levels of serum silver and metallothioneins among workers in jewellery units

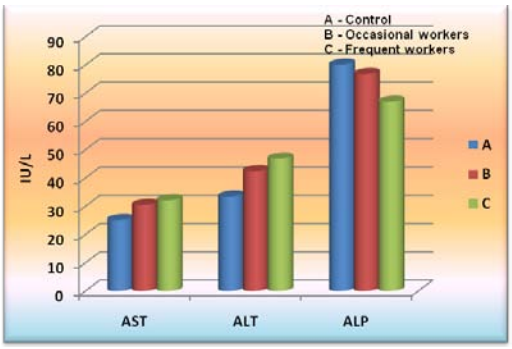

Fig. 2: Mean activities of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and Alkaline Phosphatase (ALP) in serum among jewellery unit workers

The levels of serum albumin and total protein were found to be decreased significantly ($p<0.05$) in exposed workers (Group B and C) than control group workers (Group A). Among the jewellery unit workers, they did not show any significant difference ($p>0.05$) between frequent workers (Group C) and occasional workers (Group B) as shown in Figure 3.

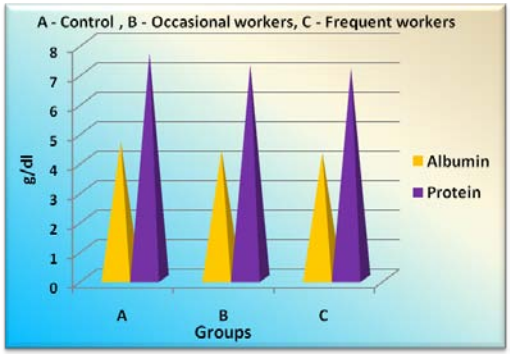

Fig. 3: Mean levels of albumin and total protein in serum among jewellery unit workers

Level of serum cholesterol was significantly increased ($p< 0.05$) in the jewellery unit workers (Group B and C) than the controls (Group A). Among the exposed workers, level of total cholesterol was significantly increased ($p<0.05$) in frequent workers (Group C) compared to occasional workers (group B) as depicted in Figure 4.

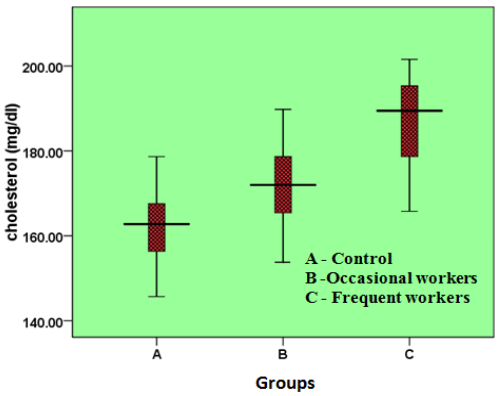

Fig. 4: Level of serum cholesterol

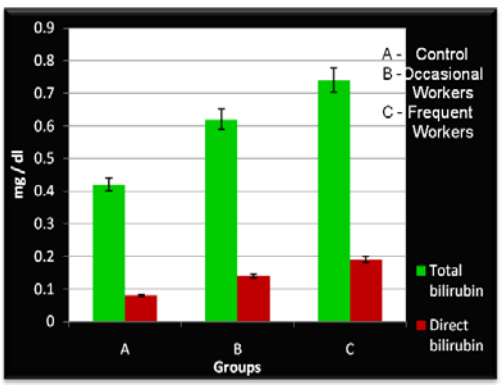

Fig. 5: Serum total and direct bilirubin levels among jewellery unit workers

Serum total and direct bilirubin levels in the jewellery unit workers (Group B and C) were found to be increased significantly ($p<0.05$) compared to control group (Group A). Among the exposed groups, the levels of total and direct bilirubin were raised significantly ($p<0.05$) in group C (Frequent workers) than group B (Occasional workers) as presented in Figure 5.

Correlation of silver and MTs with liver function markers

Diagnostic laboratories assess the functions of the organs through various biochemical parameters. Correlation analysis of silver might help to assess the interactions among the markers of liver function and MTs. Levels of MTs and silver were positively correlated with ALT, AST, total cholesterol and total bilirubin whereas they were negatively correlated with ALP. Level of albumin was negatively correlated with MTs whereas there was no correlation observed between albumin and silver which is given in Table 3.

DISCUSSION

The present study indicated that there was no significant difference in body mass index between the exposed group and the control group workers. According to Kim *et al*. (2008) the oral toxicity of silver nanoparticles was studied in male and female Sprague-Dawley rats in which the rats did not show any significant changes in their body weight [19].

Increase in systolic blood pressure in the studied population might indicate the insufficient sleep in subjects doing extensive overtime work or due to an increase in reactive oxygen species (ROS). Correlation analysis of systolic pressure did not significantly associate ($p>0.05$) with silver exposure, age, years of exposure, BMI, cholesterol, alcohol intake and pack years. Hence the increase in systolic blood pressure might be due to insufficient sleep of the workers which is supported by Tochikubo *et al*. (1996) [20]. Nevertheless, additional objective measures are warranted in order to come to a more reliable conclusion.

In the present study, silver level was increased in exposed workers (Group B and C) than control (Group A) which was supported by an *invivo* study. This suggested that silver nanoparticles do not induce genotoxicity in bone marrow of male and female rats [19]. Silver concentration in lung tissues from Sprague Dawley rats exposed to silver nanoparticles for 90 days was significantly increased ($p<0.01$) with dose. There was also a clear dose dependent increase in silver concentration in blood [21].

According to Eisses and Kaplan (2005), copper trafficking protein (ctr-1) can effectively transport silver into the cells exposed to even low micro molar concentration of silver [22]. [11]$^{0}AgNO_3$ injected rats showed that silver metabolism resembles that of copper with respect to tissue distribution. Silver does not significantly inhibit internal copper absorption but it affects copper metabolism [23]. Hence, estimation of silver concentration is an important parameter in the characterization of individual degree of exposure and the occurrence of adverse consequences.

Chang *et al*. (2006) revealed that MT level in human peripheral blood lymphocytes of workers exposed to cadmium was significantly higher than control groups [24]. A recent animal study confirmed increased hepatic and decreased urinary MT levels after cessation of oral exposure to cadmium [25]. Similar result was noticed in the present study as serum MT level was significantly increased in exposed workers (Group B and C) than control group (A). Also the level of MT was positively correlated with silver.

Table 3: Correlation of liver function markers with serum metallothioneins and silver among jewellery unit workers

Variables	Ag	MTs	ALT	AST	Total Bilirubin	Albumin	ALP	Total Cholesterol
Ag	1.000	0.299**	0.420**	0.209**	0.255**	-0.102 ^{NS}	-0.294**	0.289**
MTs	-	1.000	0.653**	0.340**	0.412**	-0.170*	-0.580**	0.627**
ALT	-	-	1.000	0.229**	0.413**	-0.164*	-0.500**	0.546**
AST	-	-	-	1.000	0.209**	-0.113	-0.226**	0.280**
Total Bilirubin	-	-	-	-	1.000	-0.168*	-0.244**	0.399**
Albumin	-	-	-	-	-	1.000	-0.321**	-0.133
ALP	-	-	-	-	-	-	1.000	-0.429**
Total Cholesterol	-	-	-	-	-	-	-	1.000

Ag = Silver, MTs = Metallothioneins, ALT =Alanine amino transferase, AST = Aspartate amino transferase, ALP = Alkaline phosphatase; ** = Correlation is significant at 0.01 level, * = Correlation is significant at 0.05 level,^{NS}= Not significant.

ALT and AST activities were increased in exposed groups (Group B and C) than control (Group A) and both the enzymes were positively associated with MTs and silver. This finding could be supported by the reports of Kara *et al*. (2005), where ALT, AST activities were increased in cadmium induced rats. This study also postulates that MT can act as antioxidant against cadmium toxicity and lipid peroxidation [26].

In the present study, serum ALP activity was found to be decreased significantly in the jewellery unit workers (Group B and C) compared to control (Group A) which might be due to the inhibition of ALP by silver as supported by Chen *et al.* (2000), who reported that heavy metal ions such as Hg^{2+}, Ag^{+}, Bi^{2+}, Cu^{2+} and Zn^{2+} inhibit the activity of alkaline phosphatase in green crab (*Scylla serrata*) [27]. There was a significant negative correlation ($p<0.05$) noticed in ALP with both MTs and silver in the study.

Serum total protein and albumin levels were found to be decreased significantly in the jewellery unit workers (Group B and C) compared to the control (Group A) of the study. These results were supported by the findings of Saadat and Ansari- Lari, (2005) in gasoline exposed filling station workers [28]. Serum albumin concentration and albumin/globulin ratio were significantly decreased in silver jewellery workers and spray painters as compared with the control group [29].

A study conducted by Oner *et al*. (2008) revealed that an increased total cholesterol level in serum of freshwater fish (*Oreochromis niloticus*) was recorded by the exposure of low level (0.05 mg/L) of silver metal [30]. Larregle *et al*. (2008) observed that cadmium exposure alters directly or indirectly serum lipid content and liver lipid metabolism in adult male Wister rats [31]. Similar result was observed in the present study and also a significant positive correlation ($p<0.05$) was noticed between serum cholesterol and silver (Table 3).

Occupational and environmental metal exposure has been associated with significant changes in hepatic clearance of bilirubin as opined by Shebl and Sarhan (2008) [32]. In the present study, serum total and direct bilirubin levels in the jewellery unit workers (Group B and C) were found to be increased significantly ($p<0.05$) compared to the control group (Group A). A significant positive correlation ($p<0.05$) was noticed between serum total bilirubin and silver of jewellery unit workers.

The result of the present study could be supported by the work of Brodkin *et al*. (2001) [33] who showed that mean direct bilirubin concentration and direct / total bilirubin ratio were increased in styrene exposed workers compared to control. They also demonstrated that direct bilirubin concentration and direct / total bilirubin ratio were associated ($p< 0.001$) with exposure to styrene.

The present study results show that the activities of AST, ALT, levels of total cholesterol and total or direct bilirubin were raised whereas total protein, albumin and ALP activity were decreased in exposed groups compared to control group which might be due to the exposure of silver. These changes occurred within the normal limit range which may be due to the synthesis of metalloproteins or low sample size of the selected population. A survey on Egyptian Tilapia fish farms revealed that high concentration of heavy metals in water and fish tissues alter the liver enzymes, serum proteins and MT gene expression [34].

CONCLUSION

The significant changes in liver function markers were observed due to the prolonged period of exposure to silver. However release of these enzymes was in the normal limit range which might be due to the induction of metallothioneins. The elevated levels of MT and the association of MT with some of the liver enzyme markers revealed that it might provide a cellular defense strategy against silver.

CONFLICT OF INTERESTS

Declared None

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