Biol Trace Elem Res (2015) 164:185–191
DOI 10.1007/s12011-014-0224-0

Increased DNA Damage and Oxidative Stress Among Silver Jewelry Workers

Necmettin Aktepe · Abdurrahim Kocyigit · Yunus Yukselten · Abdullah Taskin · Cumali Keskin · Hakim Celik

Received: 6 November 2014 / Accepted: 29 December 2014 / Published online: 10 January 2015

Abstract Silver has long been valued as a precious metal, and it is used to make ornaments, jewelry, high-value tableware, utensils, and currency coins. Human exposures to silver and silver compounds can occur oral, dermal, or by inhalation. In this study, we investigated genotoxic and oxidative effects of silver exposure among silver jewelry workers. DNA damage in peripheral mononuclear leukocytes was measured by using the comet assay. Serum total antioxidative status (TAS), total oxidative status (TOS), total thiol contents, and ceruloplasmin levels were measured by using colorimetric methods among silver jewelry workers. Moreover, oxidative stress index (OSI) was calculated. Results were compared with non-exposed healthy subjects. The mean values of mononuclear leukocyte DNA damage were significantly higher than control subjects ($p<0.001$). Serum TOS, OSI, and ceruloplasmin levels were also found to be higher in silver particles exposed group than those of non-exposed group ($p<0.001$, $p<0.001$, $p<0.01$, respectively). However, serum TAS levels and total thiol contents of silver exposed group were found significantly lower ($p<0.05$, $p<0.001$, respectively). Exposure to silver particles among silver jewelry workers caused oxidative stress and accumulation of severe DNA damage.

Keywords DNA damage · Silver · Oxidative stress · Comet assay · Ceruloplasmin · Thiol

N. Aktepe (✉)
Department of Nursing, Mardin Artuklu University School of Health, Mardin, Turkey
e-mail: necmettinaktepe@gmail.com

A. Kocyigit
Faculty of Medicine, Department of Medical Biochemistry, Bezmialem Vakif University, Istanbul, Turkey

Y. Yukselten
School of Medicine, Department of Medical Biology, Ankara University, Ankara, Turkey

A. Taskin
Department of Medical Biochemistry, Harran University Faculty of Medicine, Şanlıurfa, Turkey

C. Keskin
Department of Nutrition, Mardin Artuklu University School of Health, Mardin, Turkey

H. Celik
Department of Physiology, Harran University Faculty of Medicine, Şanlıurfa, Turkey

Introduction

Silver is one of the important metals known since ancient times. It is a very ductile, malleable (slightly harder than gold), monovalent coinage metal, with a brilliant white metallic luster that can take a high degree of polish. These properties make the metal desirable in jewelry, coins, and art. Commonly crafted for its beauty into fine jewelry and silverware, silver's value, rarity, and durability inspired its use in coins throughout history. At the present time, silver is also used in dentistry, electronics, photography, mirrors, and in a number of industrial applications that make use of its distinctive properties [1, 2].

Silver's wide variety of uses allows exposure through various routes of entry into the body. Inhalation of particles or fumes containing silver occurs primarily in occupational settings. Our knowledge of the biological activity in the cells of the silver particles or dusts is limited. Trace amounts of silver are taken by different ways in the bodies of all humans

and animals, but it is a non-essential element to human physiology [1–4]. Inhalation of particles or dusts containing silver occurs primarily in occupational health setting. If there is overexposure, silver can accumulate in the skin, liver, kidneys, corneas, gingival, mucous membranes, nails, and spleen [4]. Recent studies have shown silver accumulation in the liver, lung, and kidney cells could induce cytotoxicity via oxidative cell damage [5–8].

In biological systems, reactive oxygen species (ROS) are produced continuously and are eliminated. They play important roles in a variety of normal biochemical functions, and abnormality in their functions results in pathological processes [9]. Nanosilver particles can interact with membrane proteins and active signaling pathways, leading to inhibition of cell proliferation [10]. If silver particles enter the body in excessive amount and various size and shape, their uptake into the major target cells occurs via endocytosis and later accumulate in the mitochondria. It is possible this is the direct cause of mitochondrial damage and the disturbed function of respiratory chain resulting in ROS generation and oxidative stress. As soon as internalized, intracellular AgNPs induce a series of effects including oxidative stress, impairment of the cell membrane, cell cycle arrest, inflammatory responses, DNA damage and genotoxicity, chromosomal aberration, and apoptosis [5, 9, 11]. The production of silver nanoparticle excessive ROS and apoptosis in the cells has been experimentally shown in various cell culture. Examples of these claims are that of BRLA3A rat liver cells [8], human lung cells [5], mouse embryonic stem cells (mES) [12], and human mesenchymal stem cells (hMSCs) [13]. ROS generation has been shown to play an important role in apoptosis induced by treatment with silver [14].

Antioxidants, such as ascorbic acid, tocopherols, uric acid, ceruloplasmin, and thiol-based regulatory switches have important physiological defenses against oxidative damage. Thiols are organosulfur compounds that contain a carbon-bonded -SH group. Thiol-based regulatory compounds play central roles in cellular responses to oxidative stress and changes in the overall thiol-disulfide redox balance. They have also many physiological functions, including antioxidant activity, maintenance of essential thiol status, detoxification, and regulation of growth and death [11, 15]. Assay of plasma thiol levels is important in terms of showing how they are affected by protein oxidation which is mediated from free oxygen radicals. Ceruloplasmin is the major copper-carrying $\alpha 2$ globulin in the blood, and has multiple functions. It has effective antioxidant properties due to ferroxidase activity [16]. Ceruloplasmin is also a family of acute phase proteins that usually plays a protective role in response to immune-provoking stimuli that modulates the inflammatory response and can regulate the concentration circumstances including acute and chronic infections, cirrhosis, malignancies, lymphoma, trauma, neurodegenerative diseases, and pregnancy or other conditions [17, 18]. Willis et al. reported ceruloplasmin is quite useful in monitoring inflammatory process [19]. Oxidative stress index (OSI) provides an objective assessment of the relationship between oxidants and antioxidants.

Silver jewelry art as one of the oldest standing against today's technological advancement has managed to maintain the freedom of art. Silver jewelry workers are quite common in Mardin-Turkey. Silver, wire processing, which means the art of "filigree", created by bending a thin silver wires spilled into a gathering of small motifs recognized. It is an art based entirely on manual labor. In common with the art of filigree tobacco box, cigarette holders, mirrors, trays, belts, earrings, necklaces, buttons, and rings can be made. Silver jewelry workers are under the health threat because of silver particles inhalation during work.

The purpose of this study was to investigate the cytotoxicity and genotoxicity of silver jewelry workers exposed to silver particles or silver nanoparticle (AgNPs). These particles are thought to have possible adverse effects both on workers and consumers. In spite of widespread application of silver and silver nanoparticle (AgNPs), there is serious lack of information concerning the toxicity of this silver particle at the cellular and molecular level. It is important to investigate their potential toxicity in persons who have contact with silver particles, in order to make a reliable human risk assessment. Therefore, the objectives of this study are to perform monitoring of the particles and nanoparticle exposure during the manufacturing process of silver particles. With this purpose, we have investigated whether or not oxidative stress and DNA damage occurs in silver jewelry workers due to inhalation of silver particles.

Material and Methods

The study was conducted in Mardin with silver jewelry workers. Both silver jewelry workers and control groups were selected from the same area and for similar living conditions. The study group included 35 silver workers (17–48 years old, 15 were smoker but did not use any alcohol, without any chronic and infectious diseases) and control group consisted of 41 volunteers (17–45 years old, 16 were smoker, did not use alcohol, without any chronic and infectious diseases). Silver jewelry workers were working at least 4 h in a day. And they are exposed to silver particles by inhalation during the work. The processes of all the experiments on human blood materials were accomplished in accordance with the policies of the local ethics committee. The protocol of the research project was approved by the "Ethics Committee on Mardin Artuklu University (January 21, 2014 and number 2 documents). We have taken written consent of all the patients and their relatives in order to use material in this research project. Socioeconomic statuses of control subjects were

similar to those of silver workers. After an overnight fasting, venous blood was withdrawn into heparinized tubes. One milliliter of heparinized blood was pipette into another tube immediately to measure mononuclear leukocyte DNA damage. Remaining blood was centrifuged at 3000×*g* for 10 min to separate plasma for analysis of total antioxidant status (TAS), total oxidant status (TOS), total thiol (SH), and ceruloplasmin.

For peripheral mononuclear leukocyte separation, an amount of 1-mL heparinized blood was carefully layered over 1 ml Histopaque 1077 (Sigma) and centrifuged for 35 min at 500×*g* and 25 °C. The interface band containing lymphocyte was washed with phosphate buffered saline (PBS) and then collected by 15 min centrifugation at 400×*g*. The resulting pellets were resuspended in PBS. Membrane integrity was assessed by means of Trypan Blue exclusion method.

DNA Damage Determination by Alkaline Comet Assay

Endogenous mononuclear leukocyte DNA damage was analyzed by alkaline comet assay according to Singh et al. with minor modifications [20]. Ten microliters of fresh peripheral mononuclear leukocyte cell suspension (around 20,000 cells) was mixed with 80 μl of 0.7 % low-melting-point agarose (LMA) (Sigma) in PBS at 37 °C. Subsequently, 80 μl of this mixture was layered onto slides that had previously been coated with 1.0 % hot (60 °C) normal melting point agarose (NMA) and covered with a coverslip at 4 °C for at least 5 min to allow the agarose to solidify. After removing the coverslips, the slides were submersed in freshly prepared cold (4 °C) lysing solution (2.5 M NaCl, 100 mM EDTA-2Na; 10 mM Tris–HCl, pH 10–10.5; 1 % Triton X-100; and 10 % DMSO added just before use) for at least 1 h. Slides were then immersed in freshly prepared alkaline electrophoresis buffer (0.3 mol/L NaOH and 1 mmol/l Na_2ETDA, pH >13) at 4 °C for unwinding (40 min) and then was subjected to electrophoresis (25 V/300 mA, 25 min). All of the above steps were conducted under red light or without direct light in order to prevent additional DNA damage. After electrophoresis, the slides were stained with ethidium bromide (2 μ/ml in distilled; 70 μl/slide), covered with a coverslip and analysed using a fluorescence microscope (Olympus, Japan) vied with epi-flourescence and equipped with rhodamine filter (excitation wavelength, 546 nm; barrier filter, 580 nm). The images of 100 randomly chosen nuclei (50 cells from each of two replicate slides) were analysed visually from each subject, as described elsewhere [21]. Each image was classified according to the intensity of the fluorescence in the comet tail and was given a value of either of 0, 1, 2, 3, or 4 (from undamaged class 0 to maximally damaged class 4), so that the total scores of slide could be between 0 and 400 arbitrary units (AU). Photomicrographs of representative samples were depicted in Fig. 1a, b. All the procedures were completed by the same biochemistry staff, and DNA damage was detected by a single observer who was not aware of subject's diagnosis.

Measurement of Total Antioxidant (TAS)

Plasma TAS levels were determined using a novel automated measurement method, developed by Erel [22]. In this method, hydroxyl radical, which is the most potent radical, is produced via Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing of ferrous ion solution and hydrogen peroxide solution. In the assay, ferrous ion solution, which is present in the reagent 1, is mixed by hydrogen peroxide, which is present in the reagent 2. The sequential produced radicals such as brown colored dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, antioxidative effect of the sample against the potent free-radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values, which are lower than 3 %. The results are expressed as millimole Trolox equivalents per liter.

Measurement of Total Oxidant (TOS) Status

Plasma TOS levels were determined using a novel automated measurement method, developed by Erel [23]. In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly

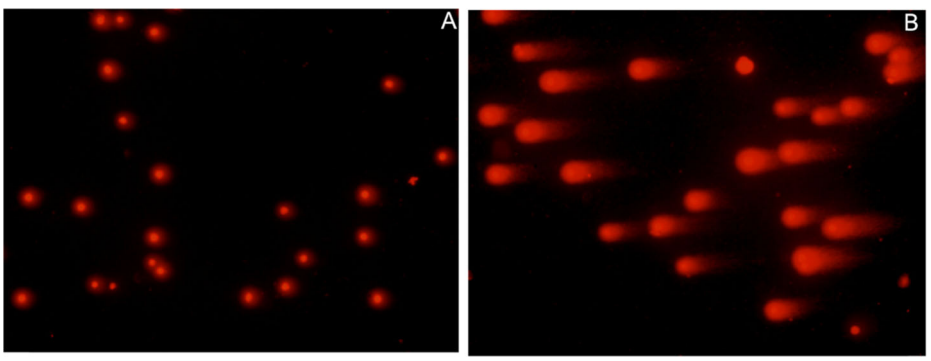

Fig. 1 Representative comet assay images showing DNA damage in peripheral mononuclear leukocyte. **a** Control groups and **b** study groups of silver jewelry workers exposed to silver particles

present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micro molar hydrogen peroxide equivalent per liter (micromole H_2O_2 equivents per liter).

OSI

The percentage of TOS level to TAS level was regarded as the oxidative stress index (OSI) [23]. The unit of serum/plasma TOS and TAS was micromole H_2O_2 equivalents per liter and millimole Trolox equivalents per liter, respectively. The serum/plasma OSI value was calculated as follows: OSI=[(TOS, micromole H_2O_2 equivalents per liter)/(TAS, millimole Trolox equivalents per liter)×100].

Measurement of Total Free Sulfhydryl Groups

Free sulfhydryl groups of serum samples were assayed according to the method of Ellman as modified by Hu et al. [24]. Briefly, 1 mL of buffer containing 0.1 M Tris, 10 mM EDTA, pH 8.2, and 50 μL serum was added to cuvettes followed by 50 μl 10 mM DTNB in methanol. Blanks were run for each sample as a test, but there was no DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer. Sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated using reduced glutathione as free sulfhydryl group standard, and the result was expressed as millimolars [22].

Ceruloplasmin Measurement

Erel's ceruloplasmin measurement method is used. This method is automated, colorimetric, and based on the enzymatic oxidation of ferrous ion to ferric ion Erel [25]. The results were expressed in milligrams per deciliter, and the precision of this assay is within 3 %. For details, see Erel [25].

Statistical Analysis

Differences in DNA damage and plasma parameters between study and control groups were analyzed by Student's *t* test. The data were expressed as mean± standard deviation (SD), and differences were considered statistically significant at $p<0.001$. Statistical analyses were performed using SPSS for Windows Release 11.5 (SPSS Inc., Chicago, IL, U.S.)

Results

Demographic and clinical data of silver workers and controls are shown in Table 1. Mean ages of silver workers and controls 31.7±8.4 and 29.42±7.4. There were no significant differences between two groups with respect to age and body index (BMI).

DNA damage scores and TAS, TOS OSI ceruloplasmin and total thiol levels are shown in Table 2. DNA damage scores were 10.71±3.37 in the control group and 12.91± 3.64 in the silver jewelry workers group. The mean values of mononuclear leukocyte DNA damage were higher than control subjects and it was statistically significant ($p<0.001$). Plasma TOS, OSI, and ceruloplasmin levels were found significantly higher in silver exposed group than those of non-exposed group ($p<0.001$, $p<0.001$, $p<0.01$, respectively) (Figs. 2 and 3). However, serum TAS levels and total thiol contents of silver exposed group were found significantly lower ($p<0.05$, $p<0.001$ respectively).

Discussion

The aim of the study was to analyze the oxidative, cytotoxic, and genotoxic effects among silver jewelry workers exposed to silver particles. We measured peripheral lymphocyte DNA damage and the levels of plasma TAS, TOS, OSI, total thiol concentration, and ceruloplasmin in silver jewelry workers. The major results of the present study are endogenous mononuclear leukocyte DNA strand breaks, which are a well-known type of DNA damage, were significantly higher in silver jewelry workers when compared to the controls (Table 2). TOS, OSI, and ceruloplasmin levels were also higher in silver workers. TAS and total thiol levels were lower in silver jewelry workers. To the best of our knowledge, this is the first study to evaluate genotoxic and oxidative effects of silver jewelry workers.

Usually, the alkaline comet assay was used to quantify, using visual and image analysis, the level of DNA damage in mononuclear leukocytes. Over the last two decades, human peripheral mononuclear leukocytes have been widely used to monitor environmentally induced genotoxicity by a variety of methods, such as micronucleus, chromosome aberration, and sister-chromatid exchange assays [26]. It has also been

Table 1 Comparison of age, body weight, height, and BMI of silver workers and controls

	Patients (n=35)	Controls (n=41)	P
Age (years)	31.7±8.4	29.42±7.4	<0.231
BMI (kg/m^2)	24.95±3.03	23.27±3.22	<0.234
Cigarette smoking (Yes/no)	15/20	16/25	<0.427

Table 2 Comparison of DNA damage, oxidative stress, and antioxidative parameters in silver workers and controls

Parameters	Control group Mean±SD	Silver exposure group Mean±SD	*P*
TAS (millimole Torolox equivalents per liter)	0.96±0.17	0.85±0.15	<0.05
TOS (micromole H_2O_2 equivalents per liter)	12.81±2.9	16.32±4.9	<0.001
OSI (arbitrary unit)	1.33±0.41	1.97±0.80	<0.001
Ceruloplasmin (U/l)	667.5±43.51	702.04±49.05	<0.01
Total thiol (mmol/l)	0.63±0.04	0.59±0.04	<0.001
Mononuclear leukocyte DNA damage (AU)	7.48±5.46	15.37±6.07	<0.001

reported strand breaks arise from DNA damage generated by oxidative stress [27]. We also used this method to measure DNA damage in circulating mononuclear leukocytes. In this study, we investigated possible damage of silver particles in body when it is exposed to excessive amount.

DNAs in native milieu are exposed to various insults. Among these threats, oxidative stress is one of the major causes of damage to DNAs. It is also can be damaged by environmental genotoxic agents and by metabolic activities. Free radicals are key triggers of DNA strand breakage which is related to cell proliferation, cell differentiation, and cell death. Okada suggested prolonged activation of inflammatory cells generates ROS that can damage DNA and contribute to carcinogenesis in chronic inflammatory disease [28]. Besides, metal particles may induce other cellular responses that in turn lead to genotoxicity, such as causing oxidative stress, inflammation, and aberrant signaling responses [29, 30]. In our study, comet assay results indicated silver particles cause significant genotoxicity in mononuclear leukocytes in silver jewelry workers (Figs. 1a, b and 3). Kim et al. [8] and Ahamed et al. [12] proved intracellular AgNPs reduce the mitochondrial function and glutathione level and increase the intercellular ROS level in various cells, which induces oxidative stress and DNA damage [12, 31]. AgNPs were found to increase the DNA tail length in a comet assay, which measures DNA strand breaks as well as alkali labile sites [11]. Prolonged exposure to silver particles or silver particles may increase the generation of ROS. However, excess amounts of these ROS can cause the depletion of TAS, injury to host cells, and induce DNA damage and mutations [7, 12, 32].

Plasma TAS is an accurate index of oxidative stress, which provides a measure of total plasma defenses against ROS [33]. ROS are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as lipids, proteins, or DNA [7, 34]. Increased DNA damage in silver jewelry workers may be due to interaction with DNA of silver particles or nanosilver [9], overproduction of ROS [7], or decreased excretion of oxidant substances [14]. For instance, exposure at 25 μg/ml of AgNPs (6–20 nm in size) induced chromosomal aberrations in 10 % of the IMR-90 normal cell line and in 20 % of the U251 cancer cell line. The DNA damage, chromosomal aberrations, and cell cycle arrest raise the concern

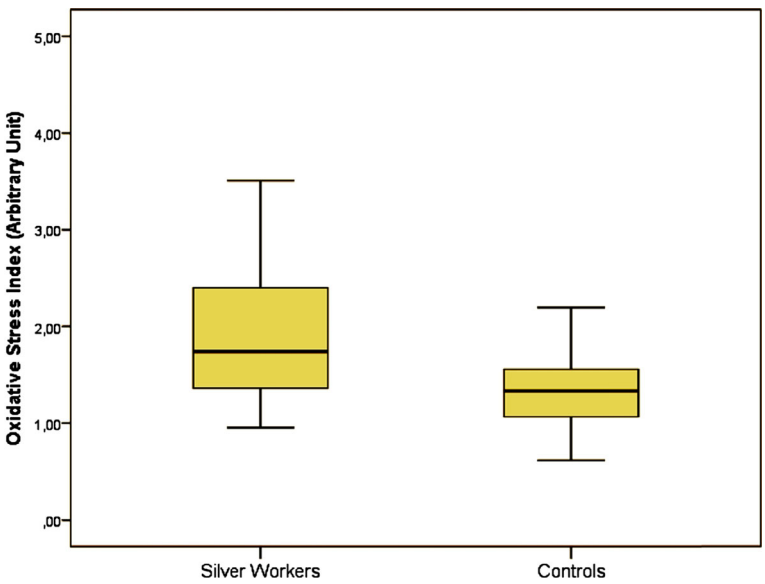

Fig. 2 Boxplot graph of plasma OSI levels in silver jewelry workers and controls

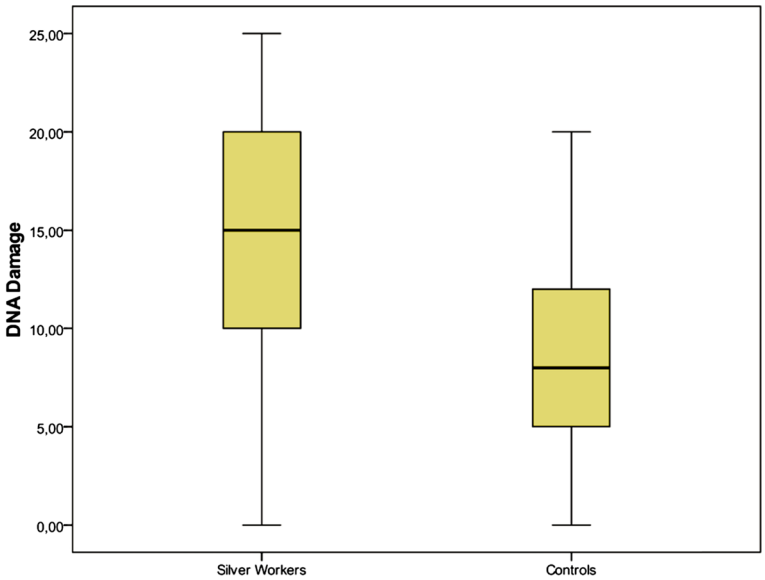

Fig. 3 Mononuclear leukocyte DNA damage levels in silver jewelry workers and controls

about the safety associated with applications of the AgNPs. Ernest et al. in their in vitro study concluded even lower concentration of AgNP changes are detrimental to human cells [35]. The dose response shows an increase in DNA damaging effect with higher the treatment nanoparticle concentration. The findings of the above-mentioned study largely support the findings of our results.

Our study showed silver jewelry worker's total thiol concentrations decreased significantly when compared to the control group. Thiols are the organic compounds that contain a sulfhydryl group and have a protective role against toxic substances such as metal ions. Among all the antioxidants available in the body, thiols constitute the major portion of the total body antioxidants, and they play a significant role in defense against reactive oxygen species [36]. Alterations of the thiol compounds may be an indicator of oxidative stress in cells [14]. The decrease in thiol concentrations in our study may depend on the intensification of turnover between reduced and oxidized glutathione under conditions, which cause increased consumption of peptide for the synthesis of metal-binding proteins. Piao and colleagues [11] have proved in their study that AgNPs can induce generation of ROS and reduce intracellular of GSH. Moreover, Hussain et al. also show in rat liver cell exposure to AgNPs resulted in decreased mitochondrial function accompanied by reduction in intracellular GSH levels [6]. Oxidative stress occurs when the generation of ROS exceeds the capacity of the cellular antioxidant defense system. Depletion of glutathione and protein-bound sulfhydryl groups and changes in the activity of various antioxidant enzymes have been implicated in oxidative damage [37, 38]. An important toxicity mechanism for nanosilver is the interaction of both the ionic and nano-form of silver with sulfur containing macromolecules, due to the strong affinity of silver for sulfur. Silver might affect mitochondrial morphology and respiration by reversible binding to specific thiols. On the other hand, silver is known to strongly react with -SH groups to form stable hemisilver sulfides as well as thiols [38]. Cortese-Krott et al. demonstrated in their study that treatments with low concentrations of $AgNO_3$ strongly decrease the concentration of cellular reduced GSH [38]. Reduced GSH in the cell may be caused by the increased ROS production, by the reaction of silver. As a result, the decreasing thiol levels of silver jewelry workers not only show the deficiency of plasma antioxidant level but also it indicates that there is a protein oxidation.

Our results suggest plasma ceruloplasmin levels in silver jewelry workers were significantly higher compared to control groups. The ceruloplasmin is an acute phase reactant whose concentration mostly as an antioxidant increases in inflammation, infection, trauma, etc. [18, 39]. We predict this increase in plasma ceruloplasmin level is due to its protective effect and acute phase protein properties in silver jewelry workers. Since some studies have shown ceruloplasmin is an acute phase protein such as in neurodegenerative diseases (18), pregnancy [19], infection [17], some autoimmune diseases [39] and it also has a protective effect such as in ulcerative colitis [40], Behçet's disease [41], and herpes infection [17].

In summary, we have concluded silver particles increase oxidative stress and cause DNA damage in silver jewelry workers. In this report, we think DNA damage is not only related to the direct interaction of silver particles but it is also related to the outcome of overproduction of ROS. Additionally, antioxidant therapy may be beneficial for preventing the formation of products leading to oxidative damage and the accumulation of free radicals. So far, very little work has been done in this area. More genotoxicity and antioxidant studies are needed to understand the mode of the risk assessment of silver particles.

References

1. Hadrup N, Lam HR (2014) Oral toxicity of silver ions, silver nanoparticles and colloidal silver —a review. Regul Toxicol Pharmacol 68:1–7
2. Kawata K, Osawa M, Okabe S (2009) In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. Environ Sci Technol 43:6046–6051
3. Lansdown AB (2006) Silver in health care: antimicrobial effects and safety in use. Curr Probl Dermatol 36:17–34
4. Wan AT, Conyers RA, Coombs CJ, Masterton JP (1991) Determination of silver in blood, urine, and tissues of volunteers and burn patients. Clin Chem 37:1683–1687
5. Gliga AR, Skoglund S, Wallinder IO, Fadeel B, Karlsson HL (2014) Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. Part Fibre Toxicol 11:11
6. Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ (2005) In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro 19:975–983
7. Kim S, Choi JE, Choi J, Chung KH, Park K, Yi J et al (2009) Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicol In Vitro 23:1076–1084
8. Kim WY, Kim J, Park JD, Ryu HY, Yu IJ (2009) Histological study of gender differences in accumulation of silver nanoparticles in kidneys of Fischer 344 rats. J Toxicol Environ Health A 72:1279–1284
9. Gurunathan S, Han JW, Eppakayala V, Jeyaraj M, Kim JH (2013) Cytotoxicity of biologically synthesized silver nanoparticles in MDA-MB-231 human breast cancer cells. Biomed Res Int 2013: 535796
10. McShan D, Ray PC, Yu H (2014) Molecular toxicity mechanism of nanosilver. J Food Drug Anal 22:116–127
11. Piao MJ, Kang KA, Lee IK, Kim HS, Kim S et al (2011) Silver nanoparticles induce oxidative cell damage in human liver cells through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis. Toxicol Lett 201:92–100
12. Ahamed M, Karns M, Goodson M, Rowe J, Hussain SM et al (2008) DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol 233:404–410
13. Hackenberg S, Scherzed A, Kessler M, Hummel S, Technau A (2011) Silver nanoparticles: evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells. Toxicol Lett 201:27–33
14. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S (2009) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3:279–290
15. Zhou YT, He W, Lo YM, Hu X, Wu X et al (2013) Effect of silver nanomaterials on the activity of thiol-containing antioxidants. J Agric Food Chem 61:7855–7862
16. Samygina VR, Sokolov AV, Bourenkov G, Petoukhov MV, Pulina MO et al (2013) Ceruloplasmin: macromolecular assemblies with iron-containing acute phase proteins. PLoS One 8:e67145
17. Novikova I, Zlotnikova M (2011) Ceruloplasmin plasma levels in patients with severe forms of herpes infection. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 155:361–366
18. Virit O, Altindag A, Selek S, Yumru M, Bulut M et al (2008) Increased plasma ceruloplasmin levels in schizophrenia. BCP 18:282–287
19. Willis EL, Kersey DC, Durrant BS, Kouba AJ (2011) The acute phase protein ceruloplasmin as a non-invasive marker of pseudopregnancy, pregnancy, and pregnancy loss in the giant panda. PLoS One 6:e21159
20. Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191
21. Cortés-Gutiérrez EI, Dávila-Rodríguez MI, Fernández JL, López-Fernández C, Gosálbez A et al (2011) New application of the comet assay: chromosome-comet assay. J Histochem Cytochem 59:655–660
22. Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37:277–285
23. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–1111
24. Hu M, Louie S, Cross CE, Motchnik P, Halliwell B (1993) Antioxidant protection against hypochlorous acid in human plasma. J Lab Clin Med 121:257–261
25. Erel O (1998) Automated measurement of serum ferroxidase activity. Clin Chem 44:2313–2319
26. Cole J, Skopek TR (1994) International commission for protection against environmental mutagens and carcinogens. Working paper no. 3. Somatic mutant frequency, mutation rates and mutational spectra in the human population in vivo. Mutat Res 304:33–105
27. Rojas E, Lopez MC, Valverde M (1999) Single cell gel electrophoresis assay: methodology and applications. J Chromatogr B Biomed Sci Appl 722:225–254
28. Okada F (2014) Inflammation-related carcinogenesis: current findings in epidemiological trends, causes and mechanisms. Yonago Acta Med 57:65–72
29. Battal D, Celik A, Güler G, Aktaş A, Yildirimcan S et al (2014) SiO_2 nanoparticule-induced size-dependent genotoxicity—an in vitro study using sister chromatid exchange, micronucleus and comet assay. Drug Chem Toxicol 24:1–9
30. Celik H, Celik N, Kocyigit A, Dikilitas M (2012) The relationship between plasma aluminum content, lymphocyte DNA damage, and oxidative status in persons using aluminum containers and utensils daily. Clin Biochem 45:1629–1633
31. Kim JS, Sung JH, Ji JH, Song KS, Lee JH et al (2011) In vivo genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure. Saf Health Work 2:34–38
32. Sarban S, Kocyigit A, Yazar M, Isikan UE (2005) Plasma total antioxidant capacity, lipid peroxidation, and erythrocyte antioxidant enzyme activities in patients with rheumatoid arthritis and osteoarthritis. Clin Biochem 38:981–986
33. Rice-Evans C, Miller NJ (1994) Total antioxidant status in plasma and body fluids. Methods Enzymol 234:279–293
34. Baysal Z, Cengiz M, Ozgonul A, Cakir M, Celik H et al (2009) Oxidative status and DNA damage in operating room personnel. Clin Biochem 42:189–193
35. Ernest V, Gajalakshmi S, Mukherjee A, Chandrasekaran N (2013) Enhanced activity of lysozyme-AgNP conjugate with synergic antibacterial effect without damaging the catalytic site of lysozyme. Artif Cells Nanomed Biotechnol 42:336–343
36. Prakash M, Shetty MS, Tilak P, Anwar N (2009) Total thiols: biomedical importance and their alteration in various disorders. OJHAS 8:1–9
37. Haase A, Rott S, Mantion A, Graf P, Plendl J (2012) Effects of silver nanoparticles on primary mixed neural cell cultures: uptake, oxidative stress and acute calcium responses. Toxicol Sci 126:457–468
38. Cortese-Krott MM, Münchow M, Pirev E, Hessner F, Bozkurt A et al (2009) Silver ions induce oxidative stress and intracellular zinc release in human skin fibroblasts. Free Radic Biol Med 47:1570–1577
39. Nowak M, Wielkoszyński T, Marek B, Kos-Kudła B, Swietochowska E et al (2010) Antioxidant potential, paraoxonase 1, ceruloplasmin activity and C-reactive protein concentration in diabetic retinopathy. Clin Exp Med 10:185–192
40. Bakhautdin B, Febbraio M, Goksoy E, de la Motte CA, Gulen MF et al (2013) Protective role of macrophage-derived ceruloplasmin in inflammatory bowel disease. Gut 62:209–219
41. Oliveira R, Banha J, Martins F, Paixão E, Pereira D et al (2006) Lymphocyte ceruloplasmin and Behçet's disease. Acta Reumatol Port 31:323–329