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REVIEW

Predictive values of some atherogenic risk factors in young workers occupationally exposed to vinyl chloride and heavy metals

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Abstract Traditional risk factors do not explain all of the risk for incident coronary heart disease (CHD) events. Human susceptibility to atherosclerosis and consequently coronary heart disease is maximally exhibited when the environment is unfavorable, especially in workplace. Thus, the present work was undertaken to study the relation of lipoprotein (a) to the other atherogenic risk factors in young workers occupationally exposed to vinyl chloride or some heavy metals by studying the effect of exposure to these agents on the lipid profiles, immunological parameters and the antioxidant defense enzyme system. The results of this study revealed that, in metalists, the cluster features of dyslipidemia, impairment in antioxidant defense mechanism and high levels of Lp (a), CICs, C3 and C4 represent unfortunate events on their cardiovascular system. In VCW, vinyl chloride metabolites caused severe oxidative stress reflected by impairment in the antioxidant defense accompanied by propagation of lipid peroxidation. Additionally, the elevated levels of

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Lp (a), CICs, C3 and C4 may point out to their role as atherogenic risk factors in those workers. In conclusion, young workers occupationally exposed to VC may be at high risk of developing cardiovascular disease in spite of having normolipidemia.

Contents

1. Introduction

The clinical presentations of atherosclerosis mainly involve the coronary and carotid arteries, which remain as the leading causes of morbidity and mortality in both men and women of all racial groups with coronary heart disease (CHD) the leading cause of death worldwide (Cutler et al., 2006). The presence of CHD is considered to be a reliable index for more widespread of atherosclerosis. The disease develops slowly over many years in the intimae layer of large and medium sized arteries, with devastating manifestations usually after the fourth or fifth decade (Griffin, 1999).

Atherosclerosis is a complex disease involving genetic and environmental risk factors, acting on their own or in synergy (Chen et al., 2007). Classical risk factors such as hypertension, diabetes, and obesity are useful as predicators of atherosclerotic disease (Lamon and Hajjar, 2008). However, environmental factors are considered key determinants of cardiovascular disease (CVD). Although lifestyle choices such as smoking, diet, and exercise are viewed as major environmental influences, the contribution of pollutants and environmental chemicals is less clear. Moreover, occupation has been linked to CVD incidence and mortality, but few studies have investigated occupation in relation to early atherosclerotic disease (Fujishiro et al., 2011). However, accumulating evidence suggests that exposure to pollutants and chemicals could elevate the risk of cardiovascular disease. Exposures to arsenic, lead, cadmium, pollutant gases, solvents, and pesticides have also been linked to increased incidence of CVD (Bhatnagar, 2006).

On the other hand, lipoprotein (a) (Lp a) is a low density lipoprotein-like particle synthesized by the liver that consists of an apolipoprotein B100 molecule covalently linked to a very large glycoprotein known as apolipoprotein (a) (Anuurad et al., 2006). The physiological and vascular effects of the particle remain uncertain, but Lp(a) has been shown to enter the arterial intimae of humans (Nielsen et al., 1997); in vitro and animal studies have reported that Lp (a) can promote thrombosis, inflammation, and foam cell formation. (Boffa et al., 2004). Recently, it has been concluded that under a wide range of circumstances, there are continuous, independent, and modest associations of Lp a concentration with the risk of CHD and stroke that appear exclusive to vascular outcomes (Erqou et al., 2009). Moreover, many prospective epidemiological studies have reported positive associations of baseline Lp (a) concentration with CHD risk (Bennet et al., 2008). Furthermore, it has been reported that the risk attributed to Lp a may be reduced by aggressively tackling other vascular risk factors, such as low-density lipoprotein cholesterol (Tziomalos et al., 2009).

However, identification of individuals at risk before the development of atherosclerosis-related diseases is important for preventive cardiology. Accordingly, the present work was undertaken to study the relation of Lp (a) to the other atherogenic risk factors in young workers occupationally exposed to vinyl chloride or some heavy metals. This was achieved by studying the effect of exposure to those agents on the lipid profiles, immunological parameters and the antioxidant defense enzyme system.

2. Methods

In order to achieve these goals, 90 subjects were included in this study and divided into four groups; group (I): 15 healthy subjects with no history of ischemic heart disease with mean age of 33.3 ± 7.4 years, group (II): 15 patients of ischemic heart disease (IHD) with mean age of 46.1 ± 3.6 years who were diagnosed in the Department of Internal Medicine-Cardiology Unit-Medical Research Institute-Alexandria University, group (III): 20 vinyl chloride workers (VCW) with matched age of 37.5 ± 4.5 years and group (IV): 40 metalist workers (MW) with mean age of 35.7 ± 7.8 years. According to the Research Ethics Board of Medical Research Institute-Alexandria University, a signed formal consent was obtained from all the participants in the present study. Healthy subjects, IHD patients and workers were investigated by complete clinical examination (general and systemic).

Blood samples were collected from healthy subjects, IHD patients, VC and heavy metal workers after overnight fast. These samples were divided into two aliquots; first one was used for the determination of serum concentrations of iron, copper and aluminum using a PerkinElmer atomic absorption spectrophotometer-2380 (Brown et al., 1984). Also, serum was utilized for the determination of lipid peroxides (Satoh, 1978), total cholesterol (TC), determined according to Allain et al., 1974 where, free cholesterol was released from its esters after enzymatic hydrolysis and oxidation. The intensity of the color

is proportional to the concentration of cholesterol present in the sample. HDL-cholesterol (HDL-C) (Roeschlau et al., 1974) depending on the usage of Mg^{2+} and dextran sulfate precipitates all fractions of serum lipoproteins except the HDL. After centrifugation the HDL fraction remains in the supernatant. LDL-cholesterol (LDL-C) (Friedewald et al., 1972) and triglycerides (TGs) (Bachorik et al., 1991) were determined after enzymatic hydrolysis by lipase. The indicator is a quinoneimine formed when hydrogen peroxide reacts in the presence of peroxidase with 4-chlorophenol and 4-aminoantipyrine. Lipoprotein a (Lp a) (Walton et al., 1974) by determination of the concentrations present is performed by the turbidimetric measurement of the maximum reaction velocity (peak rate method). Serum immunologic parameters including circulating immune complexes (CICs), complements 3 and 4 (C3 & C4) were also measured (Johnson, 1993) where, circulating immune complexes were determined by precipitation with polyethylene glycol. The concentrations present are determined quantitatively by the turbidimetric measurement of the maximum reaction velocity. Moreover, serum activity level of γ-glutamyl transferase (GGT) was determined depending on the rate of formation of p-nitroaniline (Szasz, 1969). In the second aliquot, erythrocytes were separated and their lyzates were utilized for the determination of glutathione concentration level (GSH) as well as the activity levels of glutathione peroxidase (GPx) and glutathione reductase (GR) (Beutler et al., 1963; Paglia and Valentine, 1967; Beutler, 1969).

3. Results

In patients with ischemic heart disease (IHD), the levels of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), low density lipoprotein-cholesterol (HDL-C) and triglycerides (TGs) were significantly elevated when compared to those in healthy subjects ($p = 0.0001$). Also, the level of lipoprotein (a) significantly raised ($p = 0.001$) (Fig. 1). Serum levels of iron and cupper insignificantly changed when compared to that in healthy subjects, $p > 0.05$ (Fig. 2). Interestingly, serum level of aluminum was significantly higher than that in healthy subjects, $p = 0.002$, as shown in Fig. 2. Immunologic parameters including circulating immunecomplexes (CICs), complement-3 (C3) and complement-4 (C4) significantly elevated, $p = 0.0001$, $p = 0.0001$ and $p = 0.005$, respectively (Fig. 3). Oxidative markers showed a significant increase in the MDA level, $p = 0.0001$ (Fig. 4), accompanied by a significant reduction in the GSH content ($p = 0.0001$) and the activities of glutathione peroxidase (GPx) and glutathione reductase (GRd), $p = 0.015$ and $p = 0.001$, respectively (Fig. 5). Meanwhile, the serum activity level of γ-glutamyl transferase (GGT) was insignificantly different from that in healthy subjects ($p > 0.05$), Fig. 5.

In vinyl chloride workers' (VCW) group, the levels of TC, LDL-C, HDL-C and TGs were insignificantly different from those in healthy subjects ($p > 0.05$). However, the level of lipoprotein (a) significantly increased in those workers when compared to that in healthy subjects, $p = 0.037$ (Fig. 1). Serum levels of iron, copper and aluminum insignificantly changed when compared to that in healthy subjects, $p > 0.05$ (Fig. 2). Immunologic markers, CICs, C3 and C4 were significantly higher in those workers than that in healthy subjects, $p = 0.007$, $p = 0.005$ and $p = 0.024$, respectively, as shown

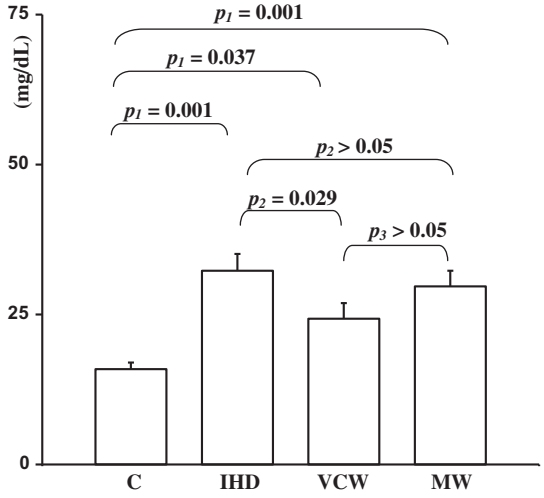

Figure 1 Levels of (mean ± S.E.) of serum lipoprotein a (mg/dL) in different studied groups. $p1$: Comparison with healthy control (C). $p2$: Comparison with ischemic heart disease IHD patients. $p3$: Comparison between vinyl chloride workers (VCW) and metal workers (MW). p: Values were considered significant at level < 0.05.

in Fig. 3. A significant increase in serum MDA level, $p = 0.019$, was observed in those workers when compared to that in healthy subjects (Fig. 4). This was associated with a significant decrease in the GSH content ($p = 0.0001$) and the activities of GPx and GRd, $p = 0.0001$, as shown in Fig. 5. Meanwhile, the serum activity level of GGT was significantly higher than that in healthy subjects ($p = 0.004$) (Fig. 5).

In metal workers' (MW) group, the levels of TC, LDL-C, HDL-C and TGs significantly elevated when compared to those in healthy subjects ($p = 0.002$, $p = 0.002$, $p = 0.002$ and $p = 0.004$, respectively). Moreover, the level of lipoprotein (a) significantly increased ($p = 0.001$) (Fig. 1). Furthermore, serum levels of iron, copper and aluminum significantly increased when compared to that in healthy subjects ($p = 0.044$, $p = 0.0001$ and $p = 0.0001$, respectively) (Fig. 2). On the other hand, immunologic markers, CICs, C3 and C4 were significantly higher than those in healthy subjects, $p = 0.0001$, $p = 0.013$ and $p = 0.004$, respectively, as shown in Fig. 3. For oxidative markers, a significant increase in serum MDA level, $p = 0.0001$, was observed in those workers when compared to that in healthy subjects (Fig. 4). This was associated with a significant decrease in the GSH content ($p = 0.0001$), the activities of GPx and GRd, $p = 0.0001$ and $p = 0.001$, respectively, and the significant elevation in serum activity level of GGT ($p = 0.04$) when compared with that in healthy subjects (Fig. 5).

4. Discussion

The present study was undertaken to investigate the relation of Lp (a) to the other atherogenic risk factors in young workers occupationally exposed to vinyl chloride or some heavy metals.

In the MW group as well as IHD patients, the levels of TC, LDL-C and TGs significantly elevated whereas the HLD-C level significantly reduced. Disturbed lipid profile is one of the most important and potent risk factors in IHD (Maharjan

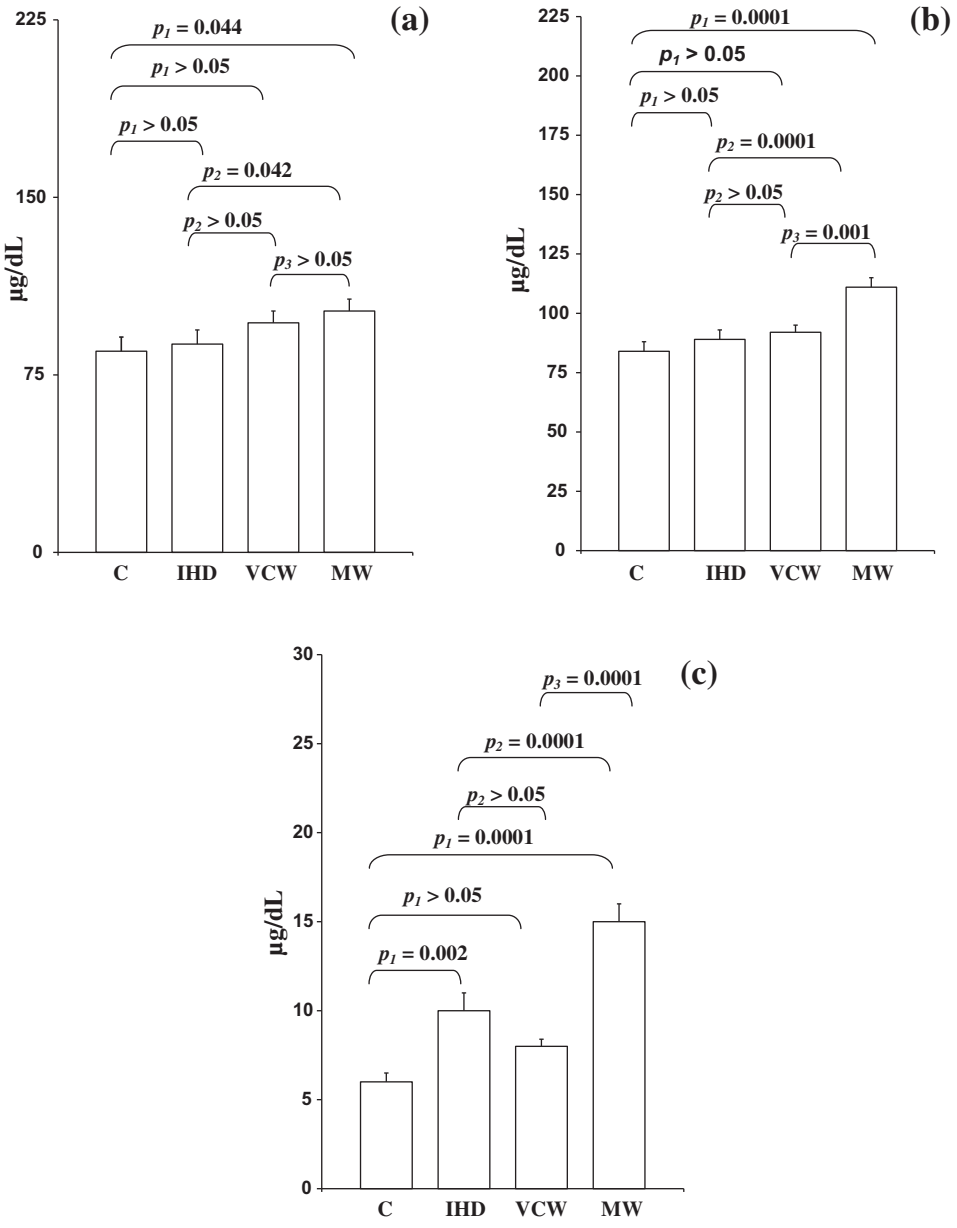

Figure 2 Levels of (mean ± S.E.) (a) serum iron (µg/dL), (b) serum Copper (µg/dL) and (c) serum aluminum (µg/L) in the different studied groups. *p*1: Comparison with healthy control (C). *p*2: Comparison with ischemic heart disease IHD patients. *p*3: Comparison between vinyl chloride workers (VCW) and metal workers (MW). *p*: values were considered significant at level < 0.05.

et al., 2008). The proportionate distribution of cholesterol in HDL and LDL fractions is necessarily important for health equilibrium and that disturbed lipid profile with low HDL-cholesterol and high LDL-cholesterol is an established cogent risk factor in initiation, progression and also in precipitation of IHD (Kasper et al., 2005). Also, elevated triglycerides and triglycerides rich-lipoproteins may have a role in contributing to the progression of mild-moderate atherosclerotic lesions, whereas elevations in cholesterol-rich lipoproteins (e.g., LDL-C) facilitate progression of severe lesions. Patients with elevations in both triglyceride and LDL-C fractions appear to be at increased cardiovascular risk compared to those with isolated elevations in either parameter, and individuals with the lipid triad may derive more pronounced cardio-protective benefits from certain treatments than patients with elevated LDL-C alone (Abdel-Maksoud et al., 2008). Accordingly, the data may indicate that the magnitude of rise of TC, LDL-C and TGs was severe enough to implicate them as major determinants of IHD risk in the metalists group. This is in addition to the significant reduction in HDL-C level in those workers.

Also, the data of the present study indicated a significant increase in the Lp (a) level in metalists and VCW groups as well as in IDH patients. Previous cohort studies have demonstrated an association between elevated Lp (a) levels and increased risk of IHD (Rifai et al., 2004; Danik et al., 2006). Furthermore, a stepwise increase in the risk of myocardial infarction and IHD with increasing levels of Lp (a) has been reported

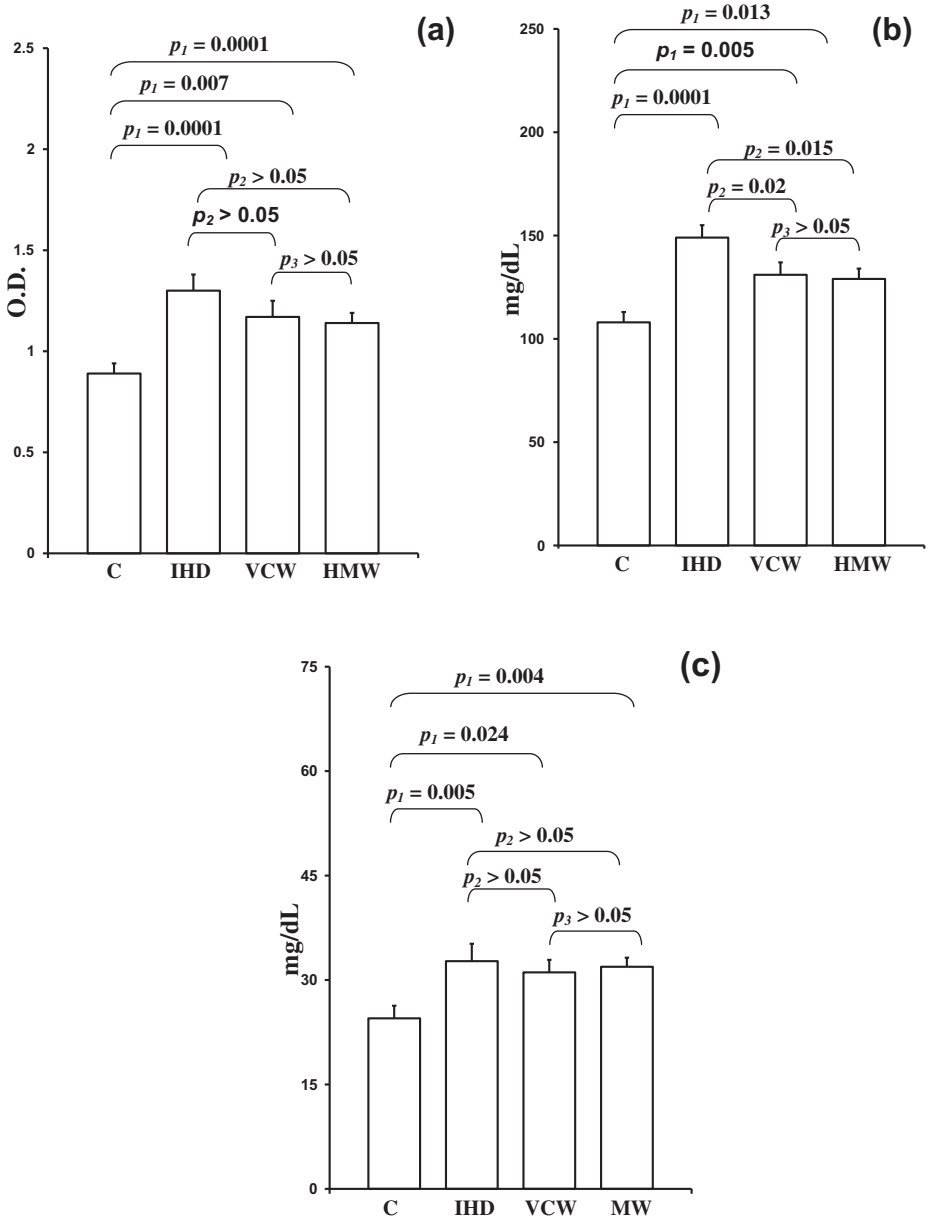

Figure 3 Levels of (mean ± S.E.) (a) serum circulating immune complexes (O.D), (b) serum complement-3 (mg/dL) and (c) serum complement-4 (mg/dL) in different studied groups. *p*1: Comparison with healthy control (C). *p*2: Comparison with ischemic heart disease IHD patients. *p*3: Comparison between vinyl chloride workers (VCW) and metal workers (MW). *p*: values were considered significant at level <0.05.

(Kamstrup et al., 2008). Thus, Lp (a) could influence risk of myocardial infarction and IHD either through promotion of atherosclerosis or increased risk of thrombosis. Previous studies have implicated Lp (a) in the process of atherogenesis including entry into the arterial intima and promotion of foam cell formation, smooth muscle cell proliferation, and plaque inflammation and instability (Nielsen et al., 1997; Boffa et al., 2004; Deb and Caplice, 2004). This may indicate that the metalists and VCW groups are at high risk of developing atherosclerosis and consequently CVD.

In metalists, a significant elevation in serum Fe, Cu and Al concentrations has been observed. This increase could be attributed to occupational exposure to these metals. Anyhow, there is compelling mechanistic evidence for the potential role of iron in atherosclerosis: the role of iron in oxidizing low-density lipoprotein (LDL), iron chelators prevent endothelial cell damage by oxidized LDL, the ability of iron to cause endothelial cell damage, and iron chelators prevent endothelial cell dysfunction and vascular smooth muscle proliferation (Shah and Alam, 2003). Also, circulating copper oxidizes LDL cholesterol (Arai et al. 2005; Ahuja et al. 2006). Low density lipoprotein (LDL) oxidation is a crucial step in atherosclerosis (Brites et al. 2006). The elevated levels of these metals, especially iron and copper, may reflect that metalists have additional risk factor for atherogenesis beside their disturbed lipid profile.

The data of the present work revealed a significant elevation in the aluminum level in IHD patients. It should be noted

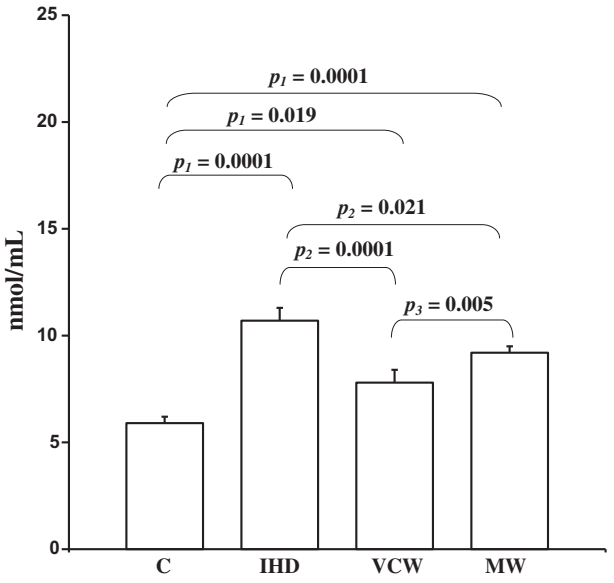

Figure 4 Levels of (mean ± S.E.) of serum MDA (nmol/mL) in different studied groups. *p*1: Comparison with healthy control (C). *p*2: Comparison with ischemic heart disease IHD patients. *p*3: Comparison between vinyl chloride workers (VCW) and metal workers (MW). *p*: values were considered significant at level <0.05.

that no direct evidence has been linked to aluminum as a risk factor for IDH. Also, the level of aluminum in IHD patients has not previously reported. However, aluminum can act as a pro-oxidant by stabilizing reduced iron the initiating species for lipid peroxidation, and by inhibiting the antioxidant action of flavonoid (Yoshino et al., 1999). Furthermore, it has been indicated that the pro-oxidant effect of aluminum may be indirect and concentration independent (Ogasawara et al., 2003). Therefore, additional investigations should be taken to clarify the role of aluminum as pro-oxidant in atherogenesis.

A significant elevation in the levels of CICs, C3 and C4 has been observed in MW, VCW and IDH patients. Previously, the increased levels of CICs in IHD patients have been reported (Kardaszewicz et al., 1991). Atherosclerosis is now recognized as a chronic inflammatory disease and is characterized by features of inflammation at all stages of its development. It also appears to display elements of autoimmunities and several auto-antibodies including those directed against oxidized low-density lipoprotein and heat shock proteins have been identified in atherosclerosis. Circulating Immune complexes (CICs) may form between these antigens and autoantibodies and via Fc receptor signaling and complement activation may modulate the inflammation in atherosclerosis (Burut et al., 2010). The most likely reason for enhanced CICs formation accompanying atherosclerosis in humans seems to be cholesterol (Lecomte et al., 1995), especially LDL-C (Kiener et al., 1995). Also, complement possesses numerous functions related to host defense, plays a role in clearing immune complexes including formation of interfaces between innate and adaptive immunity by recruiting and activating inflammatory cells (Théroux and Martel, 2006). Although the complement system is part of the host defense response, considerable evidence suggests that complement plays an important role in the pathophysiology of IHD (Iltumur et al., 2005). All of these may indicate the involvement of CICs and both of C3 and C4 the atherogenesis in mentalist and VC workers.

The data of the present study showed a reduction in the GSH level in IHD patients. It is widely recognized that vascular endothelial responses to inflammation and oxidative reactions play critical roles in atherogenesis (Libby, 2002). The generally accepted paradigm is that oxidative damage to low-density lipoprotein produces a particle with proatherogenic properties, including the ability to initiate an inflammatory response (Berliner et al., 2001). Inflammation in turn produces reactive oxygen and/or nitrogen species to modulate cell function that can further promote oxidative damage to biomolecules, propagating tissue injury. In this context, adhesion molecules mediate monocyte adhesion to the endothelium, whereas cellular GSH limits/prevents oxidative damage thereby protecting the cell and inhibiting inflammatory reactions (Kevil et al., 2004). In mammalian cells, glutathione and the glutathione peroxidases constitute the principal antioxidant defense system (Ursini et al., 1995). GPx plays an important role in the cellular defense against oxidant stress by utilizing GSH to reduce lipid hydroperoxides and hydrogen peroxide to their corresponding alcohols. Glutathione reductase that catalyzes the reduction of oxidized glutathione (GSSG) to the reduced form of GSH (Meister and Anderson, 1983) is therefore, an important component of GSH-antioxidant system. As previously reported (Motghare et al., 2001), a significant decrease in GPx and GRd was observed in IHD patients. It is known that GPx, as well as GRd, can be inactivated by oxidant species. For instance, 4-hydroxynonenal, an aldehydic byproduct of lipid peroxidation generated during LDL oxidation conceivably in the vascular wall (Esterbauer et al., 1992) has been reported to inactivate GPx (Pigeolet et al., 1990). Furthermore, the catalytic activity of GGT, which is present on the surface of cell membranes and in serum, is responsible for the extracellular catabolism of the antioxidant glutathione. Cysteinyl glycine deriving from the hydrolysis of glutathione performed by GGT has been found to trigger iron-dependent production of reactive oxygen species as well as low-density lipoprotein oxidation. The localization of GGT within the coronary plaque provides a pathological basis for the hypothesis of a direct participation of GGT in low-density lipoprotein oxidation within the plaque and in atherogenesis and coronary artery disease progression (Paolicchi et al., 2004). Accordingly, the elevated GGT activity might be a risk factor for atherogenesis in IDH patients. Meanwhile, it may be suggested that GSH-antioxidant system (GSH and its related enzymes) is a sensitive and reliable index for monitoring oxidative status in atherosclerosis due to its deep implication in atherosclerosis progressing.

For the VCW group, the data showed a reduction in GSH concentration and significant decrease in the activity of GPx and GRd. Meanwhile, a significant elevation in GGT activity level has been observed. It is known that various adverse biological effects of vinyl chloride appear to be dependent upon its metabolic conversion into chemically reactive metabolites mainly in the liver. The mixed function oxidase system is the major metabolic route in which vinyl chloride is first metabolized to chloroethylene oxide. This unstable epoxide is then transformed into chloroacetaldehyde which is further converted to chloroethanol or monochloroacetic acid. Chloroethylene oxide, chloroacetaldehyde and monochloroacetic acid

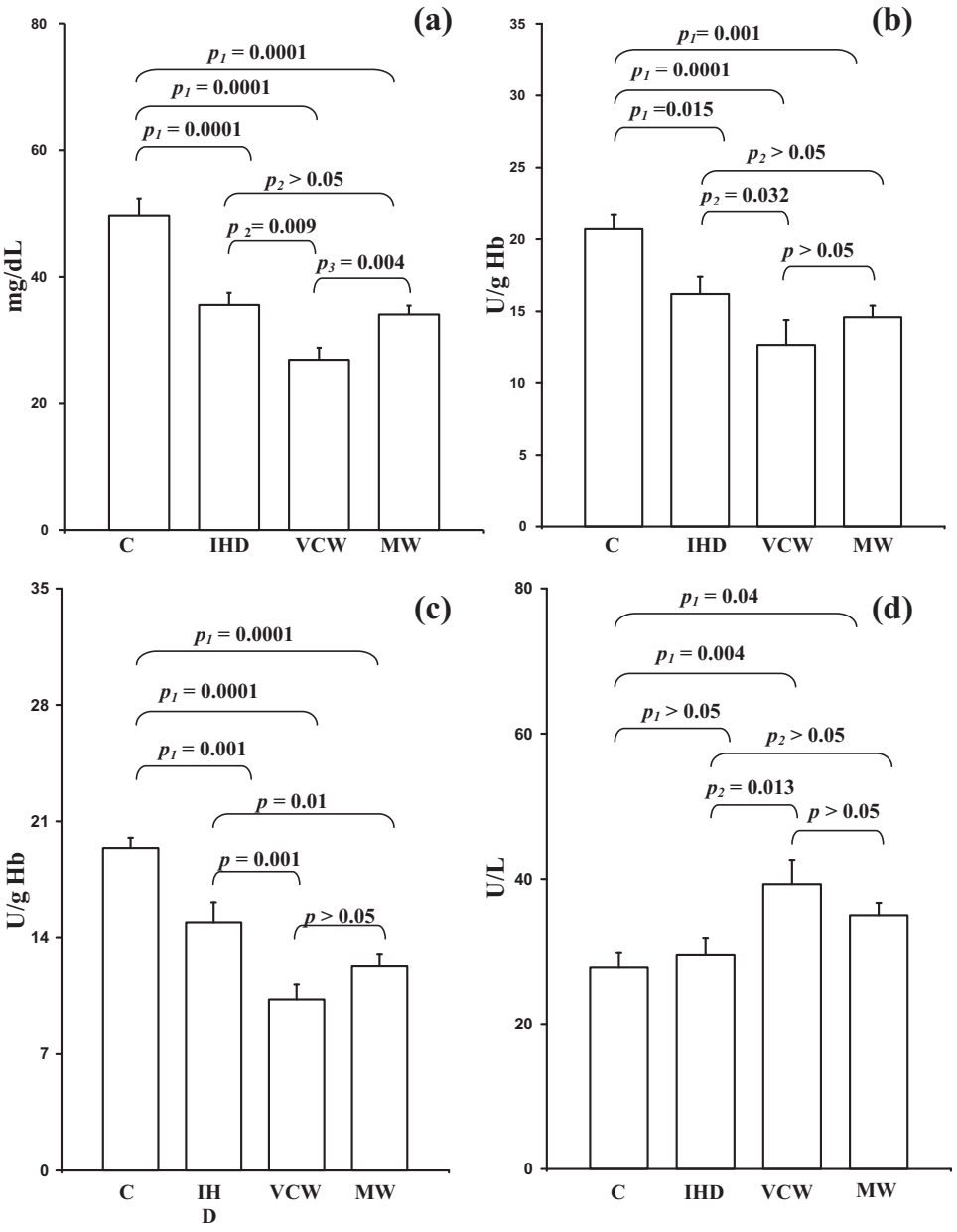

Figure 5 Levels of (mean ± S.E.) (a) erythrocytes GSH concentration (mg/dL), (b) erythrocytes GPx activity (U/g Hb), (c) erythrocytes GRde activity (U/g Hb) and (d) serum GGT activity (U/L) in different studied groups. *p*1: Comparison with healthy control (C). *p*2: Comparison with ischemic heart disease IHD patients. *p*3: Comparison between vinyl chloride workers (VCW) and metal workers (MW) *p*: values were considered significant at level < 0.05.

are the main toxic metabolites of vinyl chloride. It has been reported that chloroacetaldehyde-induced hepatocyte cytotoxicity involved reversible thiol protein adduct formation, mitochondrial toxicity and lipid peroxidation (Christian and O'Brien, 1993). It should be noted that conjugation with glutathione is the main detoxification mechanism for these three compounds (Jedrychowski et al., 1984). Thus, the involvement of GSH in the detoxification of vinyl chloride may explain its depletion. Also, the capability of vinyl chloride to initiate lipid peroxidation may explain the observed significant elevation of serum MDA level in VC workers and the reduction in the activity of the GPx and GRd. VCW group. All of these may indicate that VCW are exposed to oxidative stress due to exposure to vinyl chloride.

On the other hand, accumulating evidence indicates that exposure to chemicals including the work place leads to generation of free radicals which if unaccompanied by available antioxidant leads to oxidative stress (Flora et al., 2008). Thus, metalists are exposed to oxidative stress that is manifested by increased levels of lipid peroxidation and decreased antioxidant capacity of GSH-antioxidant system; decreased level of GSH and the activities of GPx and GRd. Moreover, the elevation in GGT activity which plays a role in generating ROS may contribute to oxidative stress establishment.

5. Conclusion

Conclusively, the herein results proved that the elevated concentration of iron, copper and aluminum in the sera of the metalist group induced severe oxidative stress associated with a salient dyslipidaemia and conspicuous impairment of antioxidant defense, which was manifested by panic decrease in the blood glutathione content level as well as the enzymatic activity level of erythrocytes glutathione peroxidase and glutathione reductase.

For vinyl chloride metabolites either the epoxide or chloroacetaldehyde caused severe oxidative stress and depletion in blood glutathione content as well as the enzymatic activity of glutathione peroxidase and glutathione reductase which exerts impairment in the antioxidant defense. Then it was followed by propagation of lipid peroxidation. Also, the level of lipoprotein (a) highly significantly increased.

In conclusion, metalists are at high risk of developing atherosclerosis and consequently CVD. This could be indicated through the disturbed lipid profile, the elevated levels of metals (Fe, Cu and Al), impaired immunologic parameters and oxidative stress. Also, VC workers are at high risk of developing CVD in spite of having normolipidemia.

References

Abdel-Maksoud, M., Sazonov, V., Gutkin, S.W., Hokanson, J.E., 2008. Effects of modifying triglycerides and triglyceride-rich lipoproteins on cardiovascular outcomes. J. Cardiovasc. Pharmacol. 51, 331–351.

Ahuja, K.D., Kunde, D.A., Ball, M.J., Geraghty, D.P., 2006. Effects of capsaicin, dihydrocapsaicin, and curcumin on copper-induced oxidation of human serum lipids. J. Agric. Food Chem. 54, 6436–6439.

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., Fu, P.C., 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20, 470–475.

Anuurad, E., Boffa, M.B., Koschinsky, M.L., Berglund, L., 2006. Lipoprotein (a): a unique risk factor for cardiovascular disease. Clin. Lab. Med. 26, 751–772.

Arai, H., Berlett, B.S., Chock, P.B., Stadtman, E.R., 2005. Effect of bicarbonate on iron-mediated oxidation of low-density lipoprotein. Proc. Natl. Acad. Sci. U.S.A. 102, 10472–10477.

Bachorik, P.S., Cloey, T.A., Finney, C.A., Lowry, D.R., Becker, D.M., 1991. Lipoprotein-cholesterol analysis during screening: accuracy and reliability. Ann. Intern Med. 114, 741–747.

Bennet, A., Di Angelantonio, E., Erqou, S., Eiriksdottir, G., Sigurdsson, G., Woodward, M., et al, 2008. Lipoprotein (a) levels and risk of future coronary heart disease: large-scale prospective data. Arch Intern. Med. 168, 598–608.

Berliner, J.A., Subbanagounder, G., Leitinger, N., Watson, A.D., Vora, D., 2001. Evidence for a role of phospholipid oxidation products in atherogenesis. Trends Cardiovasc. Med. 11, 142–147.

Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–888.

Beutler, E., 1969. Glutathione reductase: stimulation in normal subjects by riboflavin supplementation. Science 165, 613–615.

Bhatnagar, A., 2006. Environmental cardiology: Studying mechanistic links between pollution and heart disease. Circ. Res. 99, 692–705.

Boffa, M.B., Marcovina, S.M., Koschinsky, M.L., 2004. Lipoprotein (a) as a risk factor for atherosclerosis and thrombosis: mechanistic insights from animal models. Clin. Biochem. 37, 333–343.

Brites, F., Zago, V., Verona, J., Muzzio, M.L., Wikinski, R., Schreier, L., 2006. HDL capacity to inhibit LDL oxidation in well-trained triathletes. Life Sci. 78, 3074–3081.

Brown, S., Bertholf, R.L., Wills, M.R., Savory, J., 1984. Electrothermal atomic absorption spectrometric determination of aluminum in serum with a new technique for protein precipitation. Clin. Chem. 30, 1216–1218.

Burut, D.F.P., Karim, Y., Ferns, G.A.A., 2010. The role of immune complexes in athero-genesis. Angiology 61, 679–689.

Chen, Y., Rollins, J., Paigen, B., Wang, X., 2007. Genetic and genomic insights into the molecular basis of atherosclerosis. Cell Metab. 6, 164–179.

Christian, S.C., O'Brien, P.J., 1993. Molecular mechanisms of chloroacetaldehyde-induced cytotoxicity in isolated rat hepatocytes. Biochem. Pharmacol. 46, 1621–1626.

Cutler, J.A., Thom, T.J., Roccella, E., 2006. Leading causes of death in the United States. JAMA 295, 383–384.

Danik, J.S., Rifai, N., Buring, J.E., Ridker, P.M., 2006. Lipoprotein(a), measured with an assay independent of apolipoprotein(a) isoform size, and risk of future cardiovascular events among initially healthy women. JAMA 296, 1363–1370.

Deb, A., Caplice, N.M., 2004. Lipoprotein (a): new insights into mechanisms of atherogenesis and thrombosis. Clin. Cardiol. 27, 258–264.

Erqou, S., Kaptoge, S., Perry, P.L., Di Angelantonio, E., Thompson, A., White, I.R., et al, 2009. Lipoprotein (a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality emerging risk factors collaboration. JAMA 302, 412–423.

Esterbauer, H., Gebicki, J., Puhl, H., Jürgens, G., 1992. The role of lipid peroxidation and antioxidants in oxidative modifications of LDL. Free Radic. Biol. Med. 13, 341–390.

Flora, S.J., Mittal, M., Mehta, A., 2008. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian J. Med. Res. 128, 501–523.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18, 499–502.

Fujishiro, K., Diez Roux, A.V., Landsbergis, P., Baron, S., Barr, R.G., Kaufman, J.D., et al, 2011. Associations of occupation, job control and job demands with intima-media thickness: The Multi-Ethnic Study of Atherosclerosis (MESA). Occup. Environ. Med. 68, 319–326.

Griffin, B.A., 1999. Lipoprotein atherogenicity: an overview of current mechanisms. Proc. Nutr. Soc. 58, 163–169.

Iltumur, K., Karabulut, A., Toprak, G., Toprak, N., 2005. Complement activation in acute coronary syndromes. APMIS 113, 167–174.

Jedrychowski, R.A., Sokal, J.A., Chmielnicka, J., 1984. Influence of exposure mode on vinyl chloride action. Arch. Toxicol. 55, 195–198.

Johnson, A.M., 1993. A new international reference preparation for proteins in human serum. Arch. Pathol. Lab. Med. 117 (1), 29–31.

Kamstrup, P.R., Benn, M., Tybjaerg-Hansen, A., Nordestgaard, B.G., 2008. Extreme lipoprotein(a) levels and risk of myocardial infarction in the general population: the Copenhagen City Heart Study. Circulation 117, 176–184.

Kardaszewicz, B., Rogala, E., Tendera, M., Kardaszewicz, P., Jarzab, J., 1991. Circulating immune complexes in hypertrophic cardiomyopathy and ischemic heart disease. Kardiol. Pol. 34, 21–24.

Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameron JL. 2005. Harrison's Principle of Internal Medicine, Vol. II, 16th ed., Mc Graw Hill Publishing division, pp. 2152–2182.

Kevil, C.G., Pruitt, H., Kavanagh, T.J., Wilkerson, J., Farin, F., Moellering, D., et al, 2004. Regulation of endothelial glutathione by ICAM-1: implications for inflammation. FASEB J. 18, 1321–1323.

Kiener, P.A., Rankin, B.M., Davis, P.M., Yocum, S.A., Warr, G.A., Grove, R.I., 1995. Immune complexes of LDL induce atherogenic responses in human monocytic cells. Arterioscler. Thromb. Vasc. Biol. 15, 990–999.

Lamon, B.D., Hajjar, D.P., 2008. Inflammation at the Molecular Interface of Atherogenesis: An Anthropological Journey. Am. J. Pathol. 173, 1253–1264.

Lecomte, E., Herbeth, B., Clerc, G., Khalife, K., Siest, G., Artur, Y., 1995. Cholesterol content of circulating immune complexes in patients with coronary stenosis and subjects without evidence of atherosclerosis. Clin. Chem. 41, 1526–1531.

Libby, P., 2002. Inflammation in atherosclerosis. Nature 420, 868–874.

Maharjan, B.R., Jha, J.C., Adhikari, D., Akila, Risal.S., Alurkar, V.M., et al, 2008. Oxidative stress, antioxidant status and lipid profile in ischemic heart disease patients from western region of Nepal. Nepal Med. Coll. J. 10, 20–24.

Meister, A., Anderson, M.E., 1983. Glutathione. Annu. Rev. Biochem. 52, 711–760.

Motghare, K.S., Bhutey, Anil., Murrhar, B.B., Gupta, Madhur., Meshram, A.W., Balsubramanium, Y., 2001. Lipid peroxidation and glutathione peroxidase in ischemic heart disease. Ind. J. Clin. Biochem. 16, 213–215.

Nielsen, L.B., Gronholdt, M.L.M., Schroeder, T.V., Stender, S., Nordestgaard, B.G., 1997. In vivo transfer of lipoprotein (a) into human atherosclerotic carotid arterial intima. Arterioscler. Thromb. Vasc. Biol. 17, 905–911.

Ogasawara, Y., Ohata, E., Sakamoto, T., Ishii, K., Takahashi, H., Tanabe, S., 2003. A model of aluminum exposure associated with lipid peroxidation in rat brain. Biol. Trace Elem. Res. 96, 191–201.

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.

Paolicchi, A., Emdin, M., Ghliozeni, E., Ciancia, E., Passino, C., Popoff, G., et al, 2004. Human atherosclerotic plaques contain gamma-glutamyl transpeptidase enzyme activity. Circulation 109, 1440.

Pigeolet, E., Corbisier, P., Houbion, A., Lambert, D., Michiels, C., Raes, M., et al, 1990. Glutathione peroxidase, superoxide dismutase and catalase inactivation by peroxides and oxygen derived free radicals. Mech. Ageing Dev. 51, 283–297.

Rifai, N., Ma, J., Sacks, F.M., Ridker, P.M., Hernandez, W.J., Stampfer, M.J., et al, 2004. Apolipoprotein (a) size and lipoprotein (a) concentration and future risk of angina pectoris with evidence of severe coronary atherosclerosis in men: The Physicians' Health Study. Clin. Chem. 50, 1364–1371.

Roeschlau, P., Bernt, E., Gruber, W., 1974. Enzymatic determination of total cholesterol in serum. Z. Klin. Chem. Klin. Biochem. 12, 226.

Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chim. Acta 90, 37–43.

Shah, S.V., Alam, M.G., 2003. Role of iron in atherosclerosis. Am. J. Kidney Dis. 41, S80–S83.

Szasz, G., 1969. A kinetic photometric method for serum -glutamyl transpeptidase. Clin. Chem. 15, 124–136.

Théroux, P., Martel, C., 2006. Complement activity and pharmacological inhibition in cardiovascular disease. Can. J. Cardiol. 22, 18B–24B.

Tziomalos, K., Athyros, V.G., Wierzbicki, A.S., Mikhailidis, D.P., 2009. Lipoprotein a: where are we now? Curr. Opin. Cardiol. 24, 351–357.

Ursini, F., Maiorino, M., Brigelius-Flohé, R., Aumann, K.D., Roveri, A., Schomburg, D., et al, 1995. Diversity of glutathione peroxidases. Methods Enzymol. 252, 38–53.

Walton, K.W., Hitchens, J., Magnani, H.N., Khan, M., 1974. A study of methods of identification and estimation of Lp(a) lipoprotein and of its significance in health, hyperlipidaemia and atherosclerosis. Atherosclerosis 20, 323–346.

Yoshino, M., Ito, M., Haneda, M., Tsubouchi, R., Murakami, K., 1999. Prooxidant action of aluminum ion - stimulation of iron-mediated lipid peroxidation by aluminum. BioMetals 12, 237–240.