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# Evaluation of Toxic Elements in Scalp Hair Samples of Myocardial Infarction Patients at Different Stages as Related to Controls

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**Abstract** In the present study, the association of arsenic, cadmium, lead, and nickel between scalp hair samples and mortality from myocardial infarction (MI) patients at first, second, and third heart attack was studied. The biological samples of 130 MI patients (77 male and 53 female) age ranged (45–60 years), were collected and 61 healthy persons of same age group (33 male and 28 female) was selected as control subjects. The toxic elements (TEs) in biological samples were assessed by the electrothermal atomic absorption spectrophotometry prior to microwave-assisted acid digestion. The validity of methodology was checked by the certified human hair reference material (BCR 397). It was observed during the study that 78% of 32 patients of the third MI, age >50 years were expired. In these subjects, the level of As, Cd, Ni, and Pb were increased by 10.6%, 19.5%, 15.7%, and 9.8% in the scalp hair as compared to those who tolerated third MI attack ($p$=0.12). The high level of toxic metals may play a role in the development of heart disease in the subjects of this study.

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## Introduction

Atherosclerosis is the most common pathologic process, which is the original cause of cardiovascular disease, and it often manifests clinically as coronary disease, stroke, or peripheral arterial disease [1]. It is a well-known precursor of ischemic heart disease due to accumulation of lipids and fibrous elements in arteries [2]. The development of atherosclerosis depends on a balance between pro-inflammatory stimuli, anti-inflammatory, and antioxidant defenses mechanisms [3]. The toxic metals/ metalloids (TEs) are being increasingly recognized as essential mediators of the development and progression of cardiovascular disease (CVD) [4].

Although there is not necessarily a direct cause–effect relationship between the development of CVD and TEs status, it is generally believed that some of them, arsenic (As), cadmium (Cd), nickel (Ni), and lead (Pb) are CVD risk factors [5]. Thus, it has been suggested that deficiency, lack of homeostatic control, or excess intakes of some elements might lead to cardiovascular mortality [6]. Factors such as smoking, high cholesterol levels, and hypertension have a significant role in the pathogenesis of cardiovascular disease [7].

Fortunately, the cell possesses highly efficient protective mechanisms, including antioxidants such as α-tocopherol, ascorbate, β-carotene, glutathione, and metal-binding proteins such as transferrin, ceruloplasmin, enzymes, manganese superoxide dismutase, copper–zinc superoxide dismutase, selenoenzyme glutathione peroxidase, and iron-containing enzyme catalase [8]. All these metal-binding antioxidants are designed to prevent the occurrence of free-radical-induced injury under normal conditions. However, it has been argued that if these protective mechanisms may be overwhelmed then severe free-radical-mediated injury may occur [9]. When a free radical comes in contact with the inner lining of the arteries, microscopic injuries result. Eventually, the build-up of fat, cholesterol, TEs, and other substances at the site of injury, narrowing the arteries, leads to cardiovascular diseases [10].

Environmental toxicants have been suggested to play a role in atherogenesis [11]. In particular, long-term exposure to As, a documented poison and carcinogen, has been implicated as a risk factor for cardiovascular disease [12]. The As is considered a major risk factor for an endemic peripheral artery disease characterized by severe arteriosclerosis and subsequent gangrene of affected extremities, so-called “blackfoot” disease, a unique peripheral vascular disease that frequently ends with dry gangrene and spontaneous amputation of the affected extremities [13]. In addition to endemic peripheral vascular effects, As exposure has been related to systemic vascular effects. Increased mortality from ischemic heart disease was first reported in copper smelter workers exposed to As [14, 15]. The accumulation of TEs, such as Pb and Cd in adults, might promote atherosclerosis by increasing oxidative stress (e.g., by catalyzing the production of reactive oxygen species or inhibiting their degradation [16] or by affecting other cardiovascular risk factors such as increasing blood pressure levels [17, 18]. The Cd has been reported to have cumulative effects on mortality, cardiovascular, neurologic, renal, and developmental diseases [19]. Several in vitro (cell cultures) and in vivo studies have implicated Cd as an environmental toxic factor for the vascular endothelium and the smooth muscle function of the vascular wall. In particular, it has been hypothesized that Cd can be a risk factor of atherosclerosis [20, 21], elevation of plasma-free cholesterol [22], and hypertension [23]. In the very

comprehensive review of Nomiyama and Nomiyama [24] about the role of Cd in the development (or not) of hypertension, reported that Cd tends not to change the blood pressure of normotensive humans or animals, and probably affects only hypertensive Cd-exposed workers. It was investigated that chronic exposure of Ni leads to accumulation in the human body, which may cause lung fibrosis and cardiovascular diseases [25]. However, the most important concern related to Ni compounds is their activities as carcinogens [26].

Determinations of trace elements in human tissues and fluids were used to obtain information on nutritional status for diagnosis of diseases, indication of systemic intoxication, and to obtain information on environmental exposure. In the majority of cases, whole blood, serum, plasma, and urine were analyzed [27]. Hair can provide a more permanent record of TEs associated with normal and abnormal metabolism as well as TEs assimilated from the environment. In addition, hair is easily collected, conveniently stored, and easily treated. Therefore, the analysis of human hair has become an important way to understand any quantitative change in certain elements inside the body [28].

Determination of TEs in biological samples requires the use of sensitive and selective techniques such atomic absorption spectrometer (AAS). This technique has need of solubilization of the analyte and complete or partial decomposition of the matrix using either convective systems or microwave ovens and dry ashing. The main advantage of microwave-assisted samples pretreatment is its requirement of small amount of mineral acids and a reduction in the production of nitrous vapors. Microwave systems keep blank levels low because only small volumes of reagents are required and allow more samples to be processed per hour than conventional digestion systems [29].

The aim and objective of our present study was to assess the concentrations of As, Cd, Ni, and Pb in the scalp hair samples of myocardial infarction at different stages (first, second, and third attacks) of both genders and correlated with control subjects of matched age group (45–60 years). The variation in levels of TEs under study was evaluated and related to the severity of myocardial infarction (MI) at different stages. The scalp hair samples were prepared by microwave-assisted acid digestion method, and the validity of analytical procedure was checked by corresponding conventional wet acid digestion of certified reference materials [28, 29].

## Materials and Methods

### Apparatus

A Perkin-Elmer Model 700 (Norwalk, CT, USA) atomic absorption spectrometer, equipped with graphite furnace HGA-400, plus autosampler AS-800, and high-intensity deuterium lamp as background corrector. The instrumental parameters are shown in Table 1. A Pel (PMO23, Japan) domestic microwave oven (maximum heating power of 900 W) was used for digestion of the scalp hair samples. Acid-washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

### Reagents and Glass Wares

Ultrapure water obtained from ELGA LabWater system (Bucks, UK) was used throughout the work. Concentrated nitric acid (65%) and hydrogen peroxide (30%) were obtained from Merck (Darmstadt, Germany), and checked for possible trace metal contamination. Working standard solutions of As, Cd, Ni, and Pb were prepared immediately prior to

**Table 1** Measurement Conditions for Electrothermal Atomization AAS 700

| Parameters | As | Cd | Ni | Pb |
|---|---|---|---|---|
| Lamp current (mA) | 10.0 | 6.0 | 3.5 | 8.0 |
| Wavelength (nm) | 193.7 | 228.8 | 232.0 | 283.3 |
| Slit-width (nm) | 0.7 | 0.7 | 0.2 | 0.7 |
| Drying: temp °C/ramp/hold(s) | 140/15/15 | 140/15/5 | 140/15/5 | 140/15/5 |
| Ashing: Temp°C /ramp/hold(s) | 1300/10/20 | 850/10/20 | 1000/10/20 | 700/10/20 |
| Atomization: temp (°C)/ramp/hold) (s) | 2300/0/5.0 | 1650/0/5.0 | 2300/0/5.0 | 1800/0/5.0 |
| Cleaning: temp (°C)/ramp/hold) (s) | 2600/1/3 | 2600/1/3 | 2600/1/3 | 2600/1/3 |
| Chemical modifier | $Mg(NO_3)_2$ + $Pd(NO_3)_2$ | $Pd(NO_3)_2$ | $Mg(NO_3)_2$ | $Mg(NO_3)_2$ |

Sample volume (10 μl), cuvette = cup, carrier gas = (200 ml/min). Background correction ($D_2$ Lamp) used for all elements

their use, by stepwise dilution of certified standard solutions (1,000 ppm) Fluka Kamica (Buchs, Switzerland), with 0.2 M $HNO_3$. The stock standard solution of modifiers, Mg $(NO_3)_2$ (5.00 g $L^{-1}$) was prepared from $Mg(NO_3)_2$ (Merck), while Pd stock standard solution, 3.00 g $L^{-1}$, was prepared from Pd 99.999% Sigma Aldrich (Milwaukee, WI, USA). All solutions were stored in polyethylene bottles at 4°C. For the accuracy of methodology, the certified reference materials (CRMs), human hair BCR 397 (Brussels, Belgium), was used. All glassware and plastic materials used were previously soaked for 24 h in 5 M nitric acid, washed with distilled and finally rinsed with ultrapure water, dried, and stored in a class 100 laminar flow hoods.

## Sample Collection and Pretreatment

The study protocol was approved by the local ethics committee of higher education commission of Pakistan. This was a hospital-based study. The history of MI patients admitted in the cardiac ward (Dewan-e-Mushtaque) of the civil hospital of Hyderabad, Pakistan in (2007–2008) was collected. Patients admitted to the emergency department of hospital within 12 h of the onset of clinical symptoms suggestive of myocardial ischemia were randomly chosen, while we sampled the scalp hair samples of third-attack patients within 15–30 min admitted in hospital. The study was carried out on a sequential sampling of 130 patients, (35 males and 23 females of first MI attack, 25 males and 15 females of second MI attack, 17 males and 15 females of third MI attack patients), age ranged between 45 and 60 years, who were undergoing routine coronary angiography, mainly for stable angina, and who had at least one positive test of MI (including exercise stress test and dobutamine stress echocardiography). These tests and the angiogram were performed at the cardiac ward. During the study period, out of 32 patients of third MI attack, 13 males and 12 females passed away. The major criteria of inclusion for the present study were skeletal muscle damage or trauma, cardiac resuscitation, and infectious or inflammatory diseases.

Exclusion criteria included: established renal or hepatic disease, vascular disease (i.e., peripheral vascular disease, cerebrovascular disease), malignancy, or those on treatment with antioxidants or aspirin. Patients who were on lipid-lowering medication, oral contraceptives, or hormone-replacement therapy were also excluded from the study. None of our subjects had a prior history of coronary angioplasty or coronary artery bypass graft. However, 68 (52%) subjects were taking anti-hypertensive medication and 31 (24%) of the subjects used anti-diabetic drugs, while 26 (20%) were take both medicines (i.e., they were

diabetic and hypertensive). For all patients, anthropometric parameters including weight, height, and waist circumference were measured using standard protocols. Blood pressure, height, and weight were measured using standard methods (Table 2).

Sixty-one healthy subjects (33 male and 28 female), were recruited from the residence of same city, matched age group and socioeconomic status. The criteria of healthy subjects included no history of symptoms of CVD and acute coronary syndrome documented in their medical notes, and no family history of heart disease was defined by a first-degree relative with a MI, or cardiac death before the age of 55 years. All control subjects underwent a routine medical examination including MI test. All patients and controls/referents were requested to complete an interviewer-administered questionnaire, concerning their demographic characteristics, age, health history, lifestyle habits, and diet. They gave written consent to participate in the study.

## Angiographic Assessment

Coronary angiograms were performed using routine procedures. Analysis of the angiograms was performed by a specialist cardiologist. The presence of one or more stenoses 50% in diameter of at least in one major coronary artery (left main, right coronary artery, left anterior descending, and circumflex) was considered as the evidence of significant CVD [30].

## Collection of Scalp Hair Samples

The scalp hair samples of referents and patients were collected from the nape of the neck. The scalp hair samples were washed as reported in our previous study [31]. Before analysis, each individual hair sample was cut into approximately 0.5-cm-long pieces and mixed to

**Table 2** Clinical and Biochemical Characteristics of MI Patients and Controls

| Parameters | Controls | First MI | Second MI | Third MI |
|---|---|---|---|---|
| Male | | | | |
| Height (cm) | 183.2±12.3 | 180.2±8.7 | 186.0±10.5 | 177.4±7.6 |
| Weight (kg) | 82.5±12.5 | 90.5±9.5 | 93.5±8.1 | 97.4±6.3 |
| Waist circumference (cm) | 88.4±12.0 | 95.5±10.1 | 102.4±13.2 | 104.1±10.1 |
| BMI (kg/m$^2$) | 24.58±5.1 | 27.87±5.8 | 27.02±6.9 | 30.9±5.8 |
| Systolic BP (mmHg) | 139.2±6.8 | 149.0±18.2 | 151.0±22.4 | 156.6±20.1 |
| Diastolic BP (mmHg) | 81.7±3.6 | 93.2±10.6 | 98.6±7.6 | 103.2±9.7 |
| Dyslipidemia, *n* (%) | 0% | 18 (80) | 23 (92) | 17 (100) |
| Female | | | | |
| Height (cm) | 149.67±7.8 | 152.72±11.5 | 148.14±8.9 | 150.6±9.3 |
| Weight (kg) | 67.8±9.2 | 70.5±10.1 | 72.5±10.2 | 76.4±8.4 |
| Waist circumference (cm) | 72.5±12.0 | 81.7±10.1 | 85.7±7.9 | 90.2±10.1 |
| BMI (kg/m$^2$) | 30.26±4.4 | 30.23±4.7 | 33.04±5.2 | 33.7±4.5 |
| Systolic BP (mmHg) | 132.3±6.5 | 139.9±15.5 | 143.8±8.6 | 146.9±13.2 |
| Diastolic BP (mmHg) | 79.2±2.3 | 89.2±8.9 | 93.5±6.7 | 98.9±7.8 |
| Dyslipidemia, *n* (%) | 0% | 30 (86) | 14 (91) | 15 (100) |

*BMI* body mass index

allow a representative sub sampling of the hair specimen. After cutting, each sample was washed with diluted Triton X-100, then rinsed with distilled water and deionized water subsequently. The samples were then rinsed three times with acetone [31]. After washing, hair samples were dried at 70°C for 2 h. The dried scalp hair samples were kept in separate plastic envelopes with an identification number of each participant.

### Microwave-Assisted Acid Digestion

A microwave-assisted digestion (MWD) procedure was carried out, in order to achieve a shorter digestion time. Duplicate samples of scalp hair (200 mg) of each MI patients and control individuals were directly placed into Teflon PFA flasks. Two milliliters of a freshly prepared mixture of concentrated $HNO_3$–$H_2O_2$ (2:1, *v*/*v*) were added to each flask and kept for 10 min at room temperature then placed in a covered PTFE container. This was then heated following a one-stage digestion program at 80% of total power (900 W). Complete digestion of scalp hair samples required 5–8 min. After the digestion, the flasks were left to cool and the resulting solution was evaporated to semidried mass to remove excess acid. About 5 ml of 0.1 M nitric acid was added to the residue and filtered through a Whatman no. 42 filter paper and diluted with deionized water up to 10.0 ml in volumetric flasks. Blank extractions were carried through the complete procedure. Blanks and standard solutions were prepared in a similar acid matrix. The validity and efficiency of the MWD method was checked with certified values of human hair CRM 397 and with those obtained from conventional wet acid digestion method [32].

### Analytical Figures of Merit

Statistical analyses were performed using computer program Excel XL State (Microsoft Corp., Redmond, WA) and Minitab 13.2 (Minitab Inc., State College, PA). Calibration was performed with a series of As, Cd, Ni, and Pb standards. Sensitivity (m) was the slope value obtained by least-square regression analysis of calibration curves based on peak area measurements. The linear range of the calibration curve ranged from the quantification limit up to 100 μg/l was used for all TEs. The limit of detection, equal to 15.9 pg/ g, 1.62 pg/g, 10 pg/g, and 24 pg/g for As, Cd, Ni, and Pb, respectively, was defined as 3 s/m, ‘s’ being the standard deviation corresponding to ten blank injections and ‘m’ the slope of the calibration graph. The quantification limits, defined as 10 s/m were calculated as: 56.8 pg/g, 4.8 pg/g, 31.0 pg /g and 70.0 pg/g for As, Cd, Ni, and Pb respectively.

## Results

The present study is based on hospitalized patients suffering from MI, carried out to determine the concentrations of As, Cd, Ni, and Pb in scalp hair samples. It was found that the MI was associated with a pronounced imbalance in under study TEs (Table 3). The analyzed scalp hair samples were categorized according to the MI patients, referents, and gender. The MI patients were further divided into three subgroups according to first, second, and third MI attack.

The concentrations of As in the scalp hair samples of male MI patients of first, second, and third attack were found in the range of (1.8–2.5) μg/g, which were significantly higher than control subjects of same age group (1.07–1.33) μg/g ($p<0.001$), while same trend was observed in females. The elevated level of Cd was observed in scalp hair of first and second

**Table 3** Concentrations of Toxic Elements in Scalp Hair Samples of Controls and Myocardial Infarction Subjects (μg/g)

| Male $n$=110 | | | | Female $n$=81 | | | |
|---|---|---|---|---|---|---|---|
| Referent | First MI | Second MI | Third MI | Referent | First MI | Second MI | Third MI |
| Arsenic | | | | | | | |
| 1.24±0.09 | 1.9±0.3 | 2.2±0.6 | 2.5±0.4 | 1.16±0.12 | 1.8±0.5 | 1.9±0.4 | 2.2±0.3 |
| Cadmium | | | | | | | |
| 1.25±0.31 | 4.5±0.8 | 5.9±1.8 | 6.2±2.4 | 1.2±0.4 | 4.9±1.2 | 6.2±2.1 | 6.4±1.9 |
| Nickel | | | | | | | |
| 5.4±0.9 | 8.7±1.3 | 8.5±2.5 | 8.8±2.0 | 5.2±0.9 | 8.4±1.7 | 8.8±1.8 | 8.9±1.6 |
| Lead | | | | | | | |
| 7.5±0.4 | 9.7±1.2 | 13.6±6.7 | 18.6±5.4 | 6.4±1.6 | 7.9±0.7 | 11.2±5.3 | 14.5±3.9 |

MI attack patients of both genders (Table 3). The range of Cd in scalp hair samples of male and female MI patients at different severity stage (4.5–6.4) μg/g, was found to be significantly higher than those obtained in scalp hair samples of normal subjects ($p$>0.008), while the mean values of Cd in scalp hair samples of third MI attack of both genders were higher as compared to first- and second-attack patients ($p$>0.022). The Ni levels in hair revealed significant difference between referents and patients, were found in the range of (4.2–6.4) μg/g and (8.4–8.9) μg/g, respectively ($p$=0.01). The elevated level of Pb in scalp hair of male and females MI attack patients of first, second, and third were found in the range of (7.9–28.6) μg/g versus controls (4.78–7.75) μg/g. The correlation of TEs in between controls and MI patients were statistically analyzed by multiple linear regression equation and Pearson correlation as shown in Table 4. The unpaired student $t$ test at different degree of freedom between MI and referents of both genders were calculated at different probabilities. Our calculated $t$ value exceeds that of t critical value at 95%

**Table 4** Linear Regression and Pearson Coefficient for Toxic Elements in Referents ($R$) vs. Myocardial Infarction (MI) Patients

| Male | | | Female | | |
|---|---|---|---|---|---|
| $R$ vs. first MI | $R$ vs. second MI | $R$ vs. third MI | $R$ vs. first MI | $R$ vs. second MI | $R$ vs. third MI |
| Arsenic | | | | | |
| $0.38x-2.4$ | $2.12x-0.38$ | $1.32x+0.9$ | $0.81x+0.83$ | $0.845x-2.8$ | $0.72x+0.98$ |
| $r$=0.14 | $r$=0.34 | $r$=0.55 | $r$=0.19 | $r$=0.28 | $r$=0.40 |
| Cadmium | | | | | |
| $0.389x+4.1$ | $1.12x+4.5$ | $2.41x+3.1$ | $0.48x+4.3$ | $1.037x-7.5$ | $1.58x-8.4$ |
| $r$=0.15 | $r$=0.25 | $r$=0.34 | $r$=0.16 | $r$=0.26 | $r$=0.34 |
| Lead | | | | | |
| $0.214x-11.3$ | $8.59x-86$ | $10.4x-104$ | $0.028x+8.1$ | $1.12x-28$ | $0.912x-30.1$ |
| $r$=0.065 | $r$=0.33 | $r$=0.53 | $r$=0.06 | $r$=0.33 | $r$=0.50 |
| Nickel | | | | | |
| $0.292x+10.4$ | $1.024x-14$ | $1.77x-0.75$ | $0.354x+6.7$ | $0.863x-13.4$ | $1.26x-15$ |
| $r$=0.18 | $r$=0.38 | $r$=0.69 | $r$=0.19 | $r$=0.48 | $r$=0.64 |

confidence intervals, which indicated that the differences between means values of all four TEs in referents and MI patients showed significant differences ($p<0.001$).

## Discussion

Despite the lack of accurate mortality data, there is enough evidence to indicate that CVD is increasing tremendously in Pakistan. The MI patients died during study predominantly after the third attack, with the age of <50 years belong to those patient suffering from both diabetes and hypertension. The mean systolic blood pressure and blood cholesterol were higher, while the mean high-density lipoprotein cholesterol was found to be lower among participants dying in the third MI attack than among those who survived after the first and second MI attack (Table 1).

The findings of the present study clearly demonstrate that the concentration of all four TEs varied in the scalp hair samples of MI patients as compared to controls, i.e., the concentrations of these TEs were increased in scalp hair samples, as shown in Table 3. To our knowledge, the pathologic characteristics of coronary disease and stroke associated with high As exposure in Pakistan have not been described in the accessible literature. In Antofagasta, Chile, researchers who conducted histopathologic studies of children and young adults exposed to high levels of As in drinking water (~600 μg/liter) described fibrous intimal thickening of small- and medium-sized arteries [33]. Some of these children had electrocardiographic signs of myocardial infarction before death [34]. In Taiwan, a dose–response pattern has also been reported associating As exposure with carotid plaques and intima-media thickness, which are subclinical markers of atherosclerosis [35]. In addition to As other co-exposures, socioeconomic development, and dietary deficiencies (such as carotenoids, selenium, or zinc) that may interact with As [36–38]. Because of antioxidant properties of selenium and zinc, they have long been hypothesized that they may prevent cardiovascular and other chronic diseases due to increases enzymatic antioxidant activity and decreases lipid peroxidation [27, 36–38]. The possibility that As causes cardiovascular disease is supported by several biologic mechanisms. The As can increase the production of reactive oxygen species like hydrogen peroxide, hydroxyl radicals, and others [39, 40]. Lipid peroxidation increased significantly after 6 months of As feeding in mice [41]. Persons exposed to high As in drinking water in Inner Mongolia had higher levels of lipid peroxides [42], and in Taiwanese subjects, blood As was positively correlated with levels of superoxide radicals [43]. The production of reactive oxygen species has been implicated as the initial step in As-induced endothelial cell proliferation [40] and apoptosis [44]. Arsenic may induce alterations in nitric oxide metabolism and endothelial function [45]. Arsenic may also induce atherosclerosis by enhancing arterial thrombosis and platelet aggregation [46]. In our study, most of the mortality of MI patients after the third attack also have diabetes and hypertension, which is consistent with other investigations, reporting that high exposure of As has been related to diabetes [47] and hypertension [48].

The mean values of Cd in the scalp hair samples of MI patients of both genders were significantly higher ($p>0.001$) than those observed from corresponding referents of matched age group (Table 4). Several mechanisms may explain an increased risk of atherosclerosis with Cd, including the catalysis of reactive oxygen species [49], the promotion of lipid peroxidation [50, 51], the depletion of glutathione and protein-bound sulfhydryl groups [5], production of inflammatory cytokines [52], and the down-regulation of nitric oxide production [49, 53]. The Cd has also induced atherosclerosis and

hypertension in some animal models in vivo [54]. Ecologic studies have found associations of cardiovascular mortality rates with Cd levels in air, soil, and water [55]. A small case–control study found higher blood Cd in subjects with myocardial infarction than in referents [56], but a cross-sectional study in Belgium found no association between blood Cd and the prevalence of cardiovascular disease [57]. Finally, several autopsy studies have found associations between tissue Cd levels and atherosclerotic lesions [58, 59].

The mean values of Pb in scalp hair were higher in MI patients, which is in accordance with the findings of several other reports [60, 61]. Indeed, previous cohort studies of Pb and cardiovascular disease have used blood Pb as the biomarker of exposure. Several mechanisms support a role for Pb in atherosclerosis. In humans, Pb increases blood pressure [54], and experimental studies show that Pb promotes oxidative stress [5], stimulates inflammation [61], and induces endothelial damage [49]. The role of Pb in the development of atherosclerosis, however, needs to be further investigated in mechanistic studies at low levels of Pb exposure and in prospective investigation in humans using appropriate biomarkers of chronic exposure.

The mean values of Ni in the scalp hair samples of male and female MI patients were higher as compared to those found in the control group, especially in the female patients. Nickel is a potent coronary vasoconstrictor in animal models, but there is no direct evidence that exposure to Ni in the work environment increases the prevalence of cardiovascular disease [62]. Hypernickelemia has been reported in patients with acute MI and unstable angina pectoris [63]. Further investigations of lipid peroxidation in heart cells, especially in workers exposed to high levels of Ni in the working environment, and its relation to CVD are needed. Although Ni has not been associated with any chronic disease, this might change if it is confirmed that Ni has an effect on the function of vitamin B12 or folic acid. These vitamins are receiving attention because they both have an influence on the levels of homocysteine in blood, which, in high concentrations, has been associated with an increased risk of CVD [64]. It was reported that Ni accumulates with age and smoking, perhaps explaining why tissue levels are highest in patients who died of CVD [64].

In this study, the levels of TEs at the onset of the disease were found to be different between patients of MI, grouped according to the different attacks. The levels of all four TEs were found to be high in scalp hair samples of third MI attack as compared to first- and second-attack patients ($p>0.022$). It was observed during the study that 78% of 32 patients of third MI aged >50 years passed away. In these subjects, the level of As, Cd, Ni, and Pb were increased by 10.6, 19.5, 15.7, and 9.8% in the scalp hair as compared to those who tolerated third MI attack ($p=0.12$). The changes in TEs levels observed between the different groups as well as the significant correlations between TEs and cardiac markers suggest that their status is related to the clinical outcome. Furthermore, these changes point out a role of TEs in the pathological mechanisms responsible for MI and their progressions.

## Conclusion

The results of this study revealed that the patients with MI have a disturbed TEs balance in scalp hair samples as compared to referents. However, higher levels of As, Cd, Ni, and Pb correlated well with the degree of myocardial damage as indicated by their concentration in scalp hair samples of MI patients at different stages. High level of TEs in scalp hair samples is also indicative of a low-grade infectious/inflammatory process. The present study provides some support for the hypothesis that dietary TEs increases the risk of coronary disease and indicates that the causal link may be stronger among older men, particularly in

older women. We propose that TEs measurements may be performed on patients presenting to the emergency department with MI and other heart diseases, to test whether TE levels serve not only as markers of inflammation in response to myocardial necrosis but also as predictors of adverse outcomes.

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