Evaluation of some blood parameters in parallel with expression of p53 and IL-6 in industrial pollution exposed subject

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Abstract: Industrial pollution has the ability to cause cancer and inflammatory diseases in humans. This study aimed to determine inflammatory and cancer biomarkers in parallel with some other blood biochemical parameters in normal and exposed people. The study subjects 45 workers who were exposed to trace elements and 45 control subjects. Blood biochemical parameters such as liver enzymes were examined by Hitachi 912 analyser and the expression of P53 and IL-6 genes was also detected by Real time PCR technique. Our findings showed that the gene expression of both IL-6 and P53 increased in workers who were exposed to pollution. The result showed that the exposure to industrial pollution has led to increased expression of P53 and IL-6. These evidences may confirm a potential role for pollution and trace elements for developing the risk of inflammatory diseases and cancer in workers.

Keywords: liver enzymes; white blood cells; red blood cells; environmental pollution; trace elements; IL-6; p53.

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1 Introduction

The air quality is of the main environmental concern, because workers in industrial areas and people living in neighbouring residential areas are exposed to the pollution (Lodovici and Bigagli, 2011). Industrial pollution, including particulate matter, gases such as

carbon monoxide, sulphur oxides, nitrogen oxides and other trace elements that are considered as secondary products and waste of factories impose various effects to the human body and therefore threat human health (Urashima and Chang, 2000). Elements such as copper, molybdenum, selenium, chromium, nickel, iron, zinc, vanadium, manganese, silicon, boron, fluorine and arsenic are known as trace elements in the human body because of their necessity and limited quantities (Chan et al., 1998; Fleisch et al., 2012; Naghibalhossaini et al., 2013).

Molybdenum is one of the rare trace elements in the structure of the sulphite oxidase enzymes, aldehyde dehydrogenase, xanthine oxidase enzymes and serves as a cofactor. Molybdenum deficiency causes brain damage, the mental and the shift lens (Hajizadeh et al., 2004). According to the results obtained on mice animal models and humans, liver and esophageal cancers are caused by molybdenum deficiency (Ray et al., 2012; Zafar and Ali, 2013). It has also been evidenced that there exists an inverse relationship between the molybdenum level and female deaths due to oesophagus cancer, and a direct relationship between pancreatic cancer and molybdenum was also documented (Amaral et al., 2012; Schneider et al., 2013). Results of an in vitro study demonstrated that in breast cancer cells apoptosis is induced by molybdenum, however it has been indicated that it is anticancer (Anh Tran et al., 2014). Copper is also another trace element which exists in the structure of some enzymes, including superoxide dismutase, cytochrome oxidase and dopamine hydroxyls as well as ceruloplasmin (Videla et al., 2003; Hajizadeh et al., 2004). Copper ions interfere with oxidation and reduction activities. Copper plays pivotal role in the production of oxygen released from metabolites and also free radicals are able to naturally bind to the components of the cell and lead to lipid peroxidation, protein oxidation, and further nucleic acids are damaged (they are degraded in this way) and lead to carcinogenesis followed by cancer progression (Jomova and Valko, 2011). High levels of copper in parallel with increased oxidative stress boost the risk of cancer progression. The angiogenesis properties of copper facilitate the progression of tumour genesis, in breast cancer (Daniel et al., 2005; Gupte and Mumper, 2009) likewise tumour suppressor genes including NANOG, OCT4 (Karimabad et al., 2017a), P21 BAX, BCL2, the P53 (Karimabad et al., 2017b). P53 has been also described as a marker which is expressed in various cancers and the P53 protein is known as a tumour suppressor protein that prevents cancer progression via different anti-cancer mechanisms. It plays an important role in apoptosis and stability of the genome, and prevents neovascularisation activities (Almeida et al., 2009; Gupte and Mumper, 2009). Copper ions facilitate fitting of the Zn within the right place to P53 protein and generate an abnormal defunct protein. Despite suppressing tumour genesis, P53 significantly increases copper accumulation in the liver and enhances the risk of cancer in mice and humans. In general, the imbalanced copper and zinc causes the P53 up regulation (Formigari et al., 2013).

It has been documented that trace elements affect the expression of IL-6. IL-6 plays key parts in both the innate and acquired immunity (Karadag et al., 2008; Garbers et al., 2011) and is able to induce cell maturation and differentiation (Calò et al., 2003; Chakravarti et al., 2006; Duffy et al., 2008). IL-6 activates intracellular transcription factors by phosphorylation, in various tumours (Bromberg and Darnell, 2000). IL-6 regulatory effects on survival, growth, proliferation, and differentiation of cancer cells is related to tumour, angiogenesis, and cachexia (Wang et al., 2002; de Oliveira et al., 2009; Shinriki et al., 2009; Kayamori et al., 2010).

According to the previous reports, subjects who were exposed to copper and molybdenum for a long time, showed increased measures of these elements in their periphery.

It was also observed that serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) are significantly increased in exposed subjects and lead to cadmium pollution (Ikeda et al., 2000; Goswami et al., 2005). Another study found that subjects who were exposed to arsenic displayed elevated serum levels of liver enzymes and IL-6 (Ray et al., 2012).

Furthermore, a relationship was documented between these elements, and other industrial pollution, leading different changes in the processes of cancer and inflammatory diseases. Thus, according to the aforementioned comments, the present study was designed and aimed to examine the changes in gene expression of P53 and IL-6 as well as the complete blood count (CBC), C-reactive protein (CRP), SGOT, SGPT, alkaline phosphatase (ALP) in subject who were exposed to high concentration of industrial pollution in compare to control.

2 Material and methods

In this cross sectional study specimens were collected from 45 consecutive subjects who were continuously exposed to trace elements and control subjects. The study group contains workers of Sarcheshmeh Copper Mine. Controls were selected from normal population living in Rafsanjan town, in the south east of Iran. A questionnaire including demographic data such as age, sex, smoking behaviour, body mass index (BMI) and a personal work history was also filled out by either exposed workers or controls (Table 1) (Guo et al., 2011). Further, filling a consent form, blood samples were collected by venipuncture method. Approximately, 10 ml of blood was collected aseptically in sterile 10 ml polypropylene tubes without any anticoagulant for serum isolation. Care was taken to prevent any mechanical damage which might cause hemolysis of the blood. The tubes were allowed to stand at room temperature for few minutes and then were put on ice and were then transferred to laboratory for further processing. Coagulated blood was centrifuged at 3,000 rpm for 10 minutes and the clear serum was collected and stored at –80°C for further analysis. The CBC samples were analysed with the Coulometer Sysmex kx-21N and transaminase enzymes (SGOT, SGPT and ALP) were measured by Autoanalyser BT-3000.

Table 1 Shows duration of occupation, age and BMI of both exposed workers and control subjects

Variable	*Subjects*	*Exposed workers*	*P-value*
Age (year)	40.5± 6.6	39.9± 5.6	0.20
Body mass index (kg/m^2)	25.1± 3.1	25.3± 3.6	0.61
Duration of occupation (year)	16.9± 6.1	15.4± 4.1	0.08

3 RNA extraction and cDNA synthesis

Total RNA was extracted from peripheral blood using total RNA extraction Kit (PARS Tous, Iran) according to the manufacturer's instruction. All RNA samples were treated with DNase I at 37°C for 30 min for removal of any genomic DNA contamination. The fidelity of prepared RNA samples was also analysed by agarose gel electrophoresis and with a spectrophotometer, respectively. Approximately, 3–5 µg of purified total RNA was used for cDNA synthesis, using the cDNA PCR reverse transcription kit (PARS Tous, Iran), according to the manufacturer's guidelines. The resultant cDNA was used for the detection of mRNA expressions of IL-6 and P53 genes using specific oligonucleotide primers (Table 2) (Hosseini et al., 2017). The β-actin gene was used as an internal control. The volume of PCR mixture was 20 µg containing 20 ng of cDNA, 10 µg of prime Q-Master Mix with SYBR Green I (GeNet Bio) and 200 nM each of the forward and reverse primers according to the manufacturer's guidelines. Real-time polymerase chain reaction (real-time PCR) was performed using a thermal cycler (Bio-Rad CFX96, Hercules, CA, USA). The PCR reaction conditions were initial denaturation of templates at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 30 s. The amplified products were examined on 1% agarose gel and documented on the Gel-doc system (Bio-Rad) to verify their size and dissociation curve analysis.

Table 2 Demonstrates the primer sequences which were used in this study (F, forward; R, reverse)

Gene	*Primer sequences (5 → 3)*
IL-6	F: TGCAATAACCACCCCTGACC
	R: ATTTGCCGAAGAGCCCTCAG
P53	F: TGAAGCTCCCAGAATGCCAG
	R: GCTGCCCTGGTAGGTTTTCT
β-actin	F: GGGCATGGGTCAGAAGGATT
	R: CGCAGCTCATTGTAGAAGGT

4 Statistical analysis

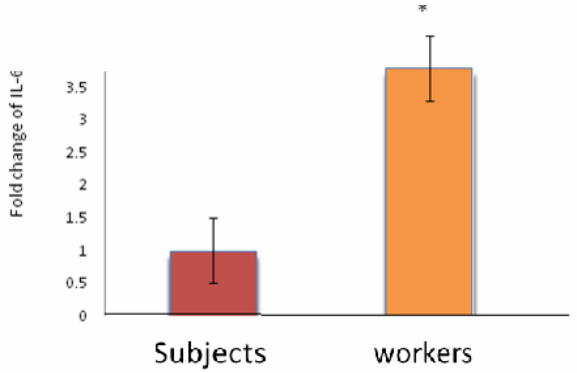
The Mann-Whitney-u-test was performed to examine significant differences in all of studied parameters, including SGPT, SGOT, ALP and gene expression of P53 and IL-6.

The collected data were analysed by SPSS software version 18. The changes in parameters were reported as 'mean ± SD' and independent two tail t-test was used. Differences were considered significant, if $p < 0.05$.

5 Results

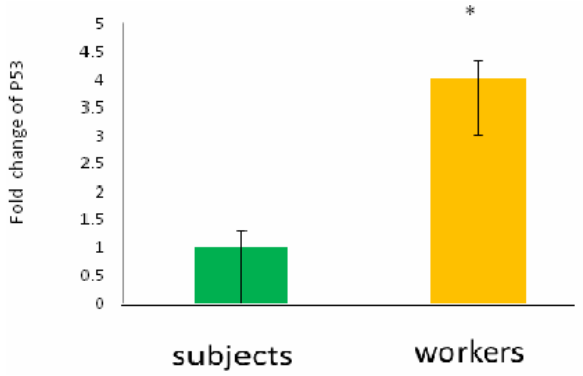
In the present, study 90 subjects were enrolled in two groups and each group consisted of 45 subjects. The first group included workers who were exposed and the second group were the staff of the Rafsanjan University of Medical Sciences. The ages of subjects varied from 39 to 41 years and have been exposed over 15 years. Our result showed that there was not a significant difference between age BMI and duration of working (Table 1). The expressions of genes P53 and IL-6 were examined in subjects and control group (Figures 1, 2).

Figure 1 Demonstrates the changes in mRNA level of IL-6 in exposed workers and control subjects (see online version for colours)



Note: * significant difference with control.

Figure 2 Demonstrates the changes in mRNA level of P53 in exposed workers and control subjects (see online version for colours)



Note: * significant difference with control.

We observed that both P53 and IL-6 were significantly increased in exposure group when compared to control (P53 = 0.00, IL6 = 0.0037).

The serum levels of liver enzymes SGOT and SGPT, were also elevated in exposure group, while ALP level remained unchanged but it was insignificant (Table 3). We also

observed that the number of both white blood cells (WBC) and red blood cells (RBC) population was significantly increased in exposure group in compare to control (Table 4).

Table 3 Demonstrates the levels of liver enzymes in exposed workers in the copper complex and control subjects

Liver enzymes	*Control*	*Exposed workers*	*P-value*
	Mean rank	*Mean rank*	
SGOT (U/L)	47.37	48.52	0.84
SGPT (U/L)	43.52	51.7	0.15
ALP (U/L)	45.02	51.60	0.25

Note: The Man-Whitney-U-test was performed for comparing groups.

Table 4 Demonstrates the number of WBC and RBC in exposed workers and control subjects

Cell type	*Number in control subjects*	*Number in exposed workers*	*P-value*
WBC (10^3/mL)	$6.1\times 10^3 \pm 0.2$	$6.9 \times 10^3 \pm 0.0$*	0.001
RBC (10^6/mL)	$5.3\times 10^3 \pm 0.0$	$5.6 \times 10^6 \pm 0.0$*	0.009

Note: * significant difference with control.

6 Discussion

Industrial pollution is amongst the main sources of air pollution. Pollutants mainly are CO, NO_2, SO_2, particulate matter and trace elements (Urashima and Chang, 2000). Heavy metal pollution is a problem associated with areas of intensive industry. Human life on the earth is almost impossible without heavy metals. Although these metallic compounds are important for human but exposure to them during production, utilisation and their uncontrolled discharge into the environment has caused lots of hazards to man, other organisms and the environment itself. Different heavy metals which are used by man are sustained maintained within the ecosystem and several of these have been reported to exhibit toxic effects.

Despite the paramount importance of some heavy metals as trace elements, their general biotoxicity is of greater health concerns. A large body of evidences has reported that some heavy metals are potential risk factors in the pathogenesis of cancer (Järup, 2003). There exist also evidences that air pollution can induce a subsequent systemic inflammatory response. Alveolar macrophages play an important role in relation to the inflammatory process in the lung and the systemic inflammation (Mukae et al., 2000).

The interaction of macrophages with particulate matter leads to the elevation of their phagocytic activities, oxidant production and release of other inflammatory markers such as tumour necrosis factor-α (TNF-α) and IL-6. It has been indicated that a wide range of inhaled substances stimulate alveolar macrophages to produce pro-inflammatory cytokines such as IL-1, IL-6, and IL-8 and TNF-α and clearing inhaled pollutants (Terashima et al., 1997; van Eeden et al., 2001; Sinden and Stockley, 2010). The present study was carried out in Rafsanjan on workers of Sarcheshmeh industrial copper mine complex. We observed that the expressions of both P53 and IL-6 were elevated in

workers compared to control subjects. These findings are consistent with a previous investigation that reported in subjects who were exposed to pollution some elements such as copper and molybdenum were increased in their serum (Hajizadeh et al., 2004). In the present study, we examined the relationship between industrial pollution and P53 mRNA level in heavy metals exposed subjects that could possibly increase the risk of cancer as well as inflammatory diseases. The ratio of copper to zinc in the plasma is a biomarker which has a direct link with inflammatory markers such as IL-6 and CRP (Malavolta et al., 2010).

Copper as a trace element plays various important roles in body. Copper in low concentrations increases the level of cellular reactive oxygen species (ROS) and in turn destroys germs and eventually serves as an apoptotic factor. Copper in combination with ROS damages DNA and subsequently activates P53 and cell death path way. On the other hand, the increased measures of copper by various mechanisms disrupt the link between zinc and therefore P53 leading to production of P53 mutant form. Mutations in the P53 protein lead to improper packaging and dysfunction of the protein, leading to an increased risk of malignancy (Formigari et al., 2013).

P53 also increases until the induction of apoptosis is achieved. Our findings are consistent with a study that measured P53 gene in workers of a coal mine who faced increased expression of this gene (Zhang et al., 2008). Accumulating evidences are provided by researches on other trace elements and for example increased levels of arsenic may induce the cytosine methyl transfer associated with increased mRNA of the enzyme. This may explains the initial hyper methylation and increased p53 expression following long-term contact with arsenic. Due to excess methyl groups by arsenic or the absence of methyl groups further reduces methylated cytosine then leads to hyper methylation (Mass and Wang, 1997; Salazar et al., 1997; Chanda et al., 2006).

Cobalt and nickel reported to inhibit binding of p53 to single-stranded DNA (PalecĬek et al., 1999). Consistently, studies showed that, the combination of cobalt chloride induces ROS and eventual apoptosis (Stenger et al., 2011). In agreement with present study, a study was performed on gas station workers and oil pollution on p53 and researchers obtained similar finding (Uzma et al., 2010).

This study clearly showed a significant increase in IL-6 expression and the subsequent increase in copper status which indicates poor prognosis and an increased risk of cancer in subjects who were exposed to large quantities of copper compounds. Arsalane et al. (1994) also observed that, nickel hydroxyl carbonate increases IL-6 and TNF-alpha in macrophages.

In this study, a relation was observed between direct copper compounds and numbers of WBC and RBC. There is a direct relationship between the copper and especially the WBC and RBC formation. Inversely, the exposure to benzene decreased the RBC but did not affect WBC. These results are different from the results obtained in this study (Koh et al., 2015). The elevated biochemical parameters such as SGOT, SGPT and the reduction of ALP are other findings of this study that in people exposed to direct contact with copper is more than the control group. Liver enzymes (SGOT, SGPT, ALP) are good tools for detection of liver disorders. Several studies showed that exposure to cadmium and arsenic leads to enhanced levels of these enzymes and further damage to the liver. This is comparable with our results which showed induced levels of SGOT and SGPT. There may be other elements and compounds in industrial pollution that inhibit the other liver enzymes such as ALP.

7 Conclusions

In this study, we found that direct exposure to industrial pollution may cause the reduction of ALP but elevation of some other biomarkers such as SGOT, SGPT, WBC, RBC in industrial pollution exposed subjects. Furthermore, expression of P53 and IL-6 was significantly increased in people who were in direct contact with the contaminant compared to control group. Also there is the possibility of creating unusual interactions and increasing the risk of cancer and related disorders. Therefore, these results may suggest to the managers of these industrial complexes to consider the health status of their workers as well as people who on living in the vicinity of these complexes. To achieve this we recommend application of filters to the pollutant systems to prevent air pollution. Authors may also recommend either exposed workers of this sector or people living in neighbouring areas to use a mask to lessen the risk of exposure at least partially by breathing in safer air and lower the risk of being polluted via pulmonary system or in halation.

Conflict of interest

The authors declare no conflict of interest

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