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**ROLE OF PREDICTIVE VARIABLES AND THEIR INTERPLAY TO DEVELOP  
ANEMIA IN TRAFFIC WARDENS**

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Received 25<sup>th</sup> Jan. 2018; Revised 26<sup>th</sup> Feb. 2018; Accepted 22<sup>nd</sup> March 2018; Available online 1<sup>st</sup> May 2018

DOI: <https://doi.org/10.31032/IJBPAS/2018/7.5.4440>

**ABSTRACT**

Objective of this study was to evaluate the role of lead present in the traffic environment induce hemolytic anemia in the traffic wardens who were highly exposed to the traffic exhaust. Cross sectional comparative study. Biochemical analyses were performed in Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore-Pakistan. 100 traffic wardens with diagnosed anemia and 100 healthy individuals were taken as controls. MDA, SOD, GSH, GPx, GR, NO,  $\delta$ -ALAD, CAT, Vit-A, Vit-C and Vit-E were measured through spectrophotometer. Levels of C-reactive protein (CRP) and lead (Pb) were measured through Elisa Kit by following their respective protocols. Significantly high levels of MDA, NO and Pb (B) were observed in anemic traffic wardens ( $4.59 \pm 0.76$  nmol/ml,  $p=0.011$ ), ( $53.22 \pm 5.74$   $\mu$ mol/ml,  $p=0.022$ ) and ( $29.76 \pm 3.76$   $\mu$ g/dl,  $p=0.039$ ) respectively as compared to the healthy controls, whereas levels of ALAD, SOD and GSH were decreased significantly in subjects ( $11.09 \pm 1.87$  U/L,  $p=0.017$ ), ( $0.43 \pm 0.06$   $\mu$ g/ml,  $p=0.037$ ) and ( $3.09 \pm 1.26$   $\mu$ g/ml,  $p=0.038$ ) in

comparison with controls. High exposure of traffic exhaust increases the levels of lead that raise the MDA levels and reduces the levels of antioxidants resulting in oxidative stress. Lead reduces the levels of ALAD and Hb that results in hemolytic anemia. High exposure of lead in traffic wardens also induces oxidative stress by increasing the levels of MDA and NO. Moreover it might be concluded that lead that is used as an anti-knocking agent in traffic vehicles should be prohibited or maximally reduced in petroleum.

**Keywords: Lead, Malondialdehyde, aminolevulinic acid, hemoglobin, superoxide dismutase**

## INTRODUCTION

Lead, a toxic compound can induce adverse effects in each biological system by damaging hematopoietic, nervous, renal, cardiovascular, immune and reproductive system [1,2]. Engine exhaust from traffic vehicles is the primary source of toxicity of lead because it contains various toxic chemicals that are hazardous for the human body [3]. Among various routes significant amount of lead enters through inhalation and oral route while minute quantity is absorbed through skin [4]. Whereas, respiratory tract not only depends on the size of particles but also on the metabolic activity that leads to DNA damage, protein and lipid toxicity [5]. Lead absorbed in the form of lead diphosphate and transported to blood and erythrocytes [6]. Primary amount of lead is excreted through the urine (>90%) whereas minor amounts are eliminated via the sweat, feces, nails and hairs [7, 8]. Among various protein metallothionein has highest binding affinity with lead, and it can synthesized in

liver as well [9]. Moreover, in erythrocytes enzyme  $\delta$ -aminolevulinic acid dehydratase (ALAD) boosts up the condensation of  $\delta$ -aminolevulinic acid (ALA) to synthesize pyrrole porphobilinogen. Condensation of two ALA molecules generates porphobilinogen (PBG). PBG converts into Hydroxymethylane that leads to the activation of Co-protoporphyrin-3. Decarboxylation of Co-protoporphyrin-3 generates protoporphyrin IX. Protoporphyrin IX takes two  $\text{Fe}^+$  and synthesizes heme. During Red Blood Cells (RBCs) synthesis ALAD plays a key role in heme synthesis, but survives abrasion during maturation of erythrocyte. ALAD remains extreme sensitive in case of divalent lead ions that are indirect pathway to measure blood lead levels (BLL) [10]. Surprisingly, clinicians doesn't have general acceptance of the following test, where as it helps them to detect the concentration of Lead in the body by spectrophotometer. On the other hand advantage of this test that ALAD is

inactivated by Lead that can be measured in blood. Other heavy metals, such as mercury also have the ability to inactivate the human erythrocyte enzymes, but because of its ubiquitous nature lead is believed to be the most significant cause of erythrocytes enzyme inhibition [11, 12].

Lead poisoning arises due to absorbance of inorganic lead particles or through transdermal absorption of organic lead. Several studies evaluate the role of lead in induction of toxicity in the membrane components and a direct correlation has been found between lead induced-lipid peroxidation and these effects. Lead induced-toxicity involved a well-known mechanism that is metal-induced reactive oxygen species. Fenton-like reactions are involved in the induction of oxidative stress through redox-active metal toxicity. Heavy metals such as lead, mercury and cadmium have the electron sharing affinities leads to the formation of covalent bonds between lead and sulfhydryl group of proteins. Glutathione (GSH) present in the millimolar concentration in biological system and accounts for upto 90% of sulfur in non-protein compounds. So interaction of toxic metals with GSH metabolism is the major response of induction of toxicity [13]. However, the major concern of this study

was to evaluate the role of lead in the induction of anemia in traffic wardens that were highly exposed to traffic exhaust.

## **MATERIALS AND METHODS**

From 200 participants, 100 healthy traffic wardens were taken as controls and 100 diagnosed anemic traffic wardens as subjects. Five (5) ml of venous blood sample was taken from the antecubital vein of each participant. The sample centrifugation was done within one hour of collection at 4000 rpm, after which the serum was separated and stored at -70°C until assayed. All chemical reagents of analytical grades were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

## **INCLUSION AND EXCLUSION CRITERIA:**

Traffic wardens with clinically diagnosed anemic patients along with age and sex matched controls were included in present study whereas subjects with any congenital diseases and metabolic diseases i.e., AIDS, HCV, Cancer or any kind of diabetes were excluded out of the study.

## **BIOCHEMICAL ASSAY**

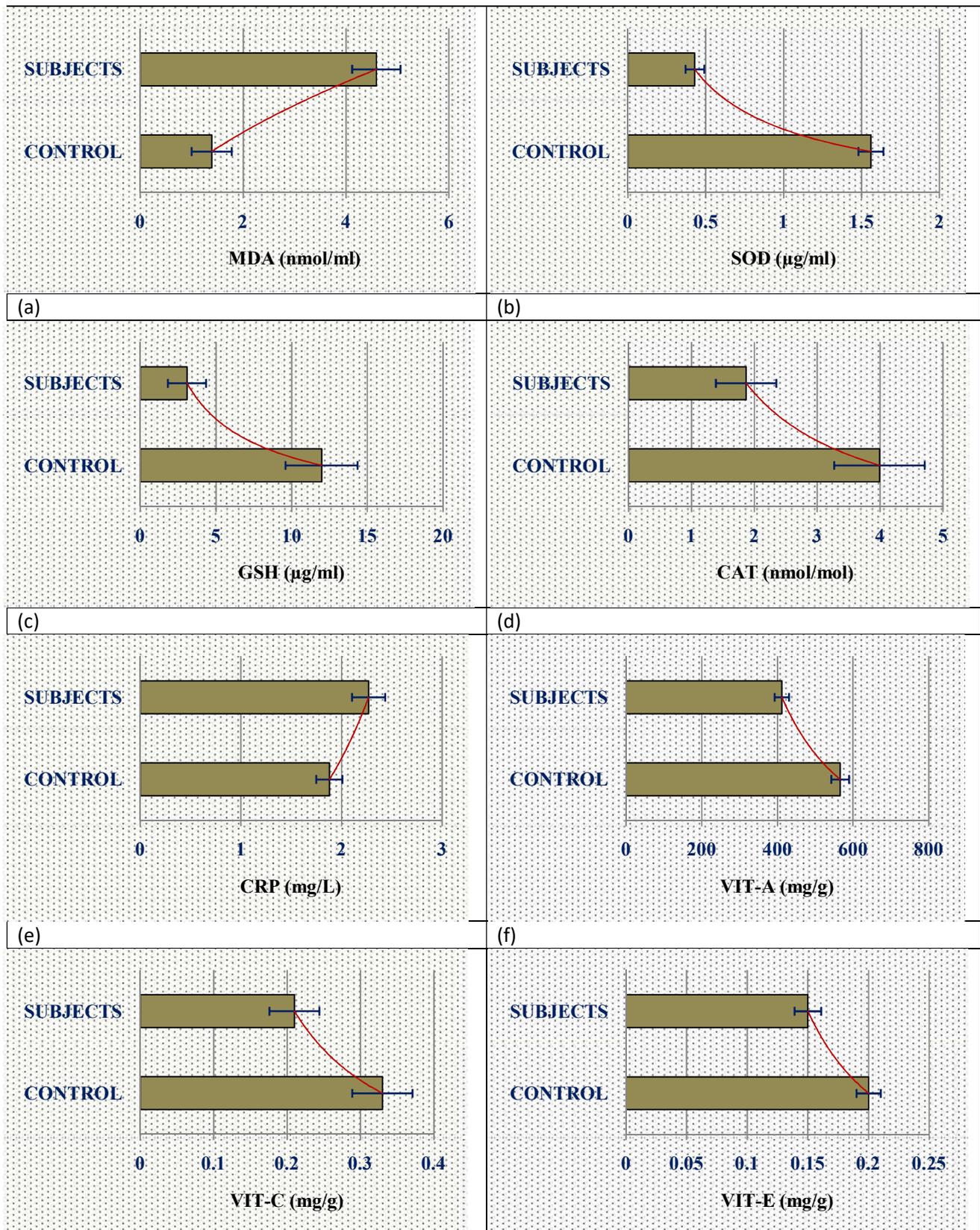
From 5ml of blood sample, 2ml of serum was collected in an EDTA coated wile and performed hemoglobin and neutrophils count. The remaining 3 ml of blood was used in the evaluation of biochemical

assays. While oxidative stress and antioxidant parameters were evaluated by their respective methods: Glutathione [14], CATALASE [15], Superoxide dismutase [16], Malondialdehyde [17], GPX [18], GR [18], VIT-A [19], VIT-C [20], VIT-E [21], NO [22] and  $\delta$ -ALAD [23]. C-reactive protein (CRP) is measured by a turbid metric method by using Synchron<sup>®</sup> ELISA Kit. Levels of lead in urine and blood were also measured using ELISA Kit by following their respective protocols.

## RESULTS

Study subjects comprised of 100 on duty traffic wardens (30-40 years of age) and 100 age and sex matched healthy subjects served as control. They were then distributed with respect to their demographic and information relating to their work place. Fig 1 described an increased level of lead in blood ( $29.76 \pm 3.76$   $\mu\text{g/dl}$ ) in case of duty traffic wardens as compared to healthy control ( $14.54 \pm 1.98$   $\mu\text{g/dl}$ ). Fig 1 depicts that subjects have elevated level of MDA ( $4.59 \pm 0.76$   $\text{nmol/ml}$ ,  $p=0.011$ ) as compared to controls. There was significant increase in level of NO ( $53.22 \pm 7.98$   $\mu\text{mol/L}$ ,  $p= 0.022$ )

in present study. There was a significant decrease in the level of their antioxidants i.e., GSH ( $3.09 \pm 1.99$   $\mu\text{g/ml}$ ,  $p= 0.036$ ), SOD ( $0.43 \pm 0.06$   $\mu\text{g/ml}$ ,  $p= 0.037$ ) and CAT ( $1.87 \pm 0.89$   $\text{nmol/mol}$ ,  $p= 0.019$ ) respectively when compared to the healthy control subjects. Other antioxidants such as vitamin A, E and vitamin C levels were significantly reduced ( $411.98 \pm 18.94$   $\text{mg/g}$ ,  $0.15 \pm 0.011$   $\text{mg/g}$ ,  $0.21 \pm 0.034$   $\text{mg/g}$  respectively) in subjects as compared to controls. Lead toxicity induced inflammation in the body. C- Reactive Protein (CRP) levels remained significantly high ( $2.27 \pm 0.08$   $\text{mg/L}$ ,  $p=0.032$ ) in the traffic wardens as compared to normal healthy subjects. Significant declined in the amount of ALAD ( $11.09 \pm 1.87$   $\text{U/L}$ ,  $p=0.017$ ) in lead toxic subjects as shown in the fig 1 that cause decreased heme production that declined hemoglobin level. Significant reduction in the level of glutathione peroxidase (GPx) ( $4.87 \pm 1.98$   $\text{U/L}$ ) was result of oxidative stress in the body whereas levels of glutathione reductase (GR) were raised ( $2.39 \pm 0.88$   $\text{U/L}$ ) in subjects that were highly exposed to traffic exhaust.



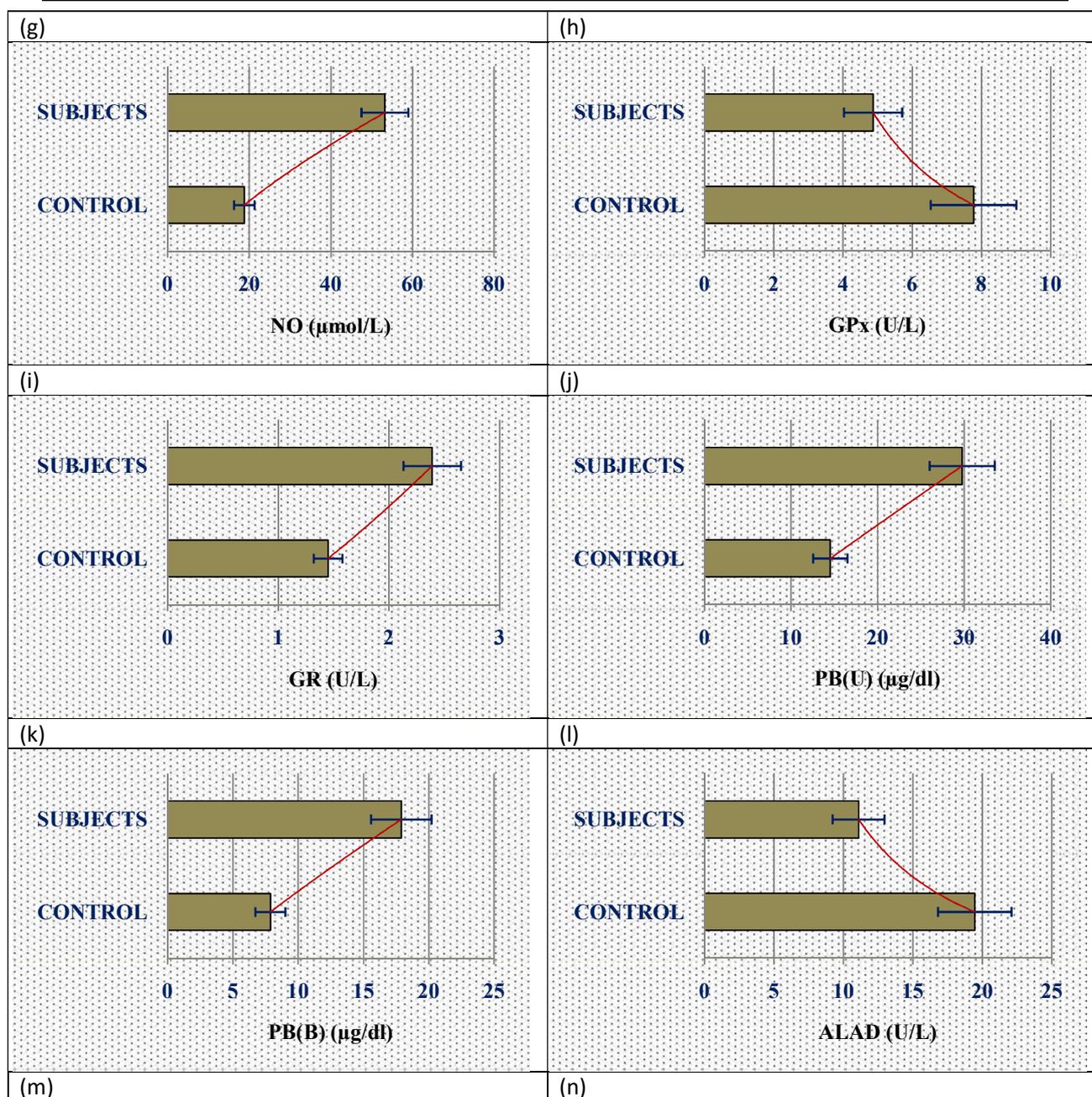


Figure 1: Levels of Circulating Biochemical Variables In Traffic Wardens

Table 1: Demographic and hematological variables in anemic traffic police wardens

VARIABLES	CONTROL (N=100)	SUBJECT (N=100)	P VALUE
WEIGHT	72.78±4.56	79.67±3.78	0.936
AGE (YRS)	31.97±4.26	30.65±3.98	0.235
BMI	22.76±4.78	23.87±4.99	0.118
RBC	4.76±0.89	4.87±0.87	0.055
WBC	8.51±0.75	8.99±0.98	0.039
HB	13.99±1.98	10.87±1.44	0.014
PLT	285±3.87	299.87±4.88	0.234
HCT	41.76±4.65	51.87±5.66	0.033
NEUTROPHILS (%)	71.66±6.26%	111.98±5.84%	0.007

RBC: Red blood cells; WBC: White blood cells; Hb: Hemoglobin; PLT: Platelets; Hct: Hematocrit, BMI: Body mass index

Table 2: Pearson's correlation coefficient's matrix of different variables in traffic wardens

VARIABLES	SOD	GSH	CAT	CRP	VITMIN-A	VITAMIN-C	VITAMIN-E	NO	GPx	GR	PB(b)	PB(U)	ALAD
MDA	-0.535	-0.456	-0.623	0.756	0.165	0.489	0.654	.0548	0.145	0.325	0.348	-0.324	-0.596
SOD		0.568	0.425	0.265	0.158	-0.857	0.0685	0.745	0.235	0.2350	0.235	0.468	0.525
GSH			0.125	0.356	0.253	0.235	0.235	-0.748	0.125	0.252	0.236	0.235	0.632
CAT				0.458	0.065	0.552	0.326	0.125	0.326	-0.548	0.326	0.235	0.635
CRP					0.1256	0.1258	0.235	0.326	0.125	0.562	0.635	0.325	0.124
VITAMIN-A						0.5356	0.235	0.236	0.568	0.235	0.156	0.325	0.215
VITAMIN-C							0.625	0.235	0.125	0.235	0.235	0.325	0.532
VITAMIN-E								0.568	0.156	0.235	0.2356	0.125	0.425
NO									0.125	0.2560	0.235	0.568	0.132
GPx										0.425	0.165	0.123	0.235
GR											0.532	0.125	0.235
PB(B)												0.235	-0.626
PB(U)													0.445

Significant at (0.05)

## DISCUSSION

Various metals affects the biological system in different ways, by inducing DNA damage and inflammation [24] which can be determined by the concentration of C-reactive protein (CRP). It has been explained in the table-02 there is a direct correlation ( $r=0.635$ ) between (Pb Vs CRP). Inflammation leads to the production of reactive oxygen species (ROS) that directly effects on the enzymatic as well as non-enzymatic antioxidants. One of the important stress biomarker is Superoxide dismutase (SOD) that is synthesized as a byproduct during the metabolism of aminolevulinic acid (ALA) in the blood, converts superoxide radical ( $O^{\cdot}$ ) into Oxygen ( $O_2$ ) and Hydrogen peroxide ( $H_2O_2$ )[25]. Reduced concentration of SOD reduces the clearance of superoxide radical, while reduced CAT impairs the scavenging activity of superoxide ( $O_2^{\cdot}$ ) [26]. Secretion of digestive enzymes, cell necrosis and specifically damage of parenchymal tissue

are process that results in increase level of MDA. Present studies also shows similar with previous study [27] that (CRP Vs MDA) have positive relation ( $r=0.756$ ). In this study declined level of antioxidant such as Catalase, Superoxide dismutase, Glutathione has been observed due to lead present in the traffic exhaust. A significant negative correlation ( $r=-0.548$ ) has been observed between (CAT Vs GR). Depletion of antioxidant leads to upregulate RNS including nitric oxide (NO), that is a small molecule having unpaired electron on the antibonding  $2\pi^*$  y orbital. NO radical is produced in the tissue by specific enzyme nitric oxide synthase (NOS's) that form NO radical by metabolize arginine and citrulline. It can diffuse both in aqueous and lipid media, and rapidly diffuses in plasma membrane and cytoplasm[28]. Present studies also explain increased levels of NO leads to the depletion of antioxidants including GSH (NO Vs GSH) that has antagonistic effect on each other ( $r=-0.748$ ).

A strong inverse correlation ( $r=-0.745$ ) between (NO Vs SOD) has been observed that leads to the oxidative stress. Decrease in levels of SOD increases the level of Vit-C as described in the table-02 (SOD Vs Vit-C,  $r=-0.857$ ). Moreover, lead induces interruptions in the antioxidant defense system by increasing the level of ROS and alters the action of antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione (GST) and many content of the GSH in the biological system. Depletion of ALAD also decreases the CAT levels that shows a direct correlation between these two (ALAD Vs CAT,  $r=0.635$ ). Mechanism of lead behind the effect of these enzymes is very complexes; lead has specific function it can either stop bio-elements absorption or replace with the active site of enzyme or bind to thiol (-SH) group of the proteins. On the other hand lead can also induce apoptosis in the kidney as well as in the liver [29]. Similarly, the present study signifies significant positive relationship (CRP Vs Pb,  $r=0.635$ ).

In 2015, Shamshad *et al*, [30] and his companions concluded in their study that chronic exposure to traffic pollution that contains lead particles can increase the cardiovascular and respiratory disease risk

and may result hypoxemia. Results from the Shamshad *et al*, [30] studies are similar to the present study that, a positive correlation ( $r=0.568$ ) between (Pb Vs NO). Table-02 depicts strong negative correlation ( $r=-0.626$ ) between (Pb Vs ALAD) that leads to cause hemolytic anemia in the traffic wardens. Lead induces oxidation to hemoglobin (Hb) that results in hemolysis of the RBC. Reduced level of heme is due to inhibition of ALAD that leads to cause elevated level of ALA substrate in blood and urine. Rapid production of ALA leads to the generation of ROS including hydrogen peroxide and superoxide radicals that attach with oxyhemoglobin that resulted in the generation of hydroxyl radicals and hemolytic anemia [31, 32].

## CONCLUSION

Elevated concentrations of oxidative stress maybe due to increased lead in environment that reason for high level of MDA and Nitric oxide. Increased levels of lead have negative correlation with antioxidants and vitamins i.e., A, C and E. Present study also concluded that lead have positive correlation with the oxidative stress within traffic wardens those remain exposed to the traffic exhausts. It suggests the use of anti-knocking agents inhibits in the petroleum to minimize the risk of lead toxicity.

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**ACKNOWLEDGEMENTS**

The authors are highly thankful to Lab-313, Institute of Molecular Biology and Biotechnology, The University of Lahore-Pakistan to provide the innovation and financial support for the project.

**CONFLICT OF INTEREST**

Author declares no conflict of interest.

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