Role of *ALAD* and *VDR* genotypes on the association of low blood lead level with serum uric acid and blood pressure in automobile paint workers of Karachi, Pakistan

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Abstract: Lead is an environmental pollutant having nephrotoxic effects even at low level. Its continuous exposure is associated with increased serum uric acid level that resulting in renal insufficiency. This research was conducted to see the effects of delta-aminolevulinic acid dehydratase (*ALAD*) and vitamin D receptor (*VDR*) genotypes on biochemical parameters and blood pressure (BP) of automobile workers having low blood lead level (BLL) with continuous lead exposure. Automobile paints workers with *ALAD* 1-2 genotype showed the positive association of BLL with diastolic BP ($p<0.05$) whereas, a genotypic combination of *ALAD* 1-2/*VDR* BB showed the negative association of serum uric acid with BLL ($p<0.05$). Similarly negative effects of *VDR* BB genotype ($p<0.01$) and *ALAD* 1-2 genotype ($p<0.05$) were observed in the association of serum uric acid with BLL at the mean age ≥30 years. This suggests that automobile paint workers having *ALAD* 1-2 genotypes are at the risk of increased diastolic BP. The research also foretells that combination of *ALAD* 1-2/*VDR* BB may play a significant role against lead induced nephrotoxicity at low BLL with continuous lead exposure.

Keywords: Lead poisoning, *ALAD* and *VDR* genotypes, serum uric acid, blood pressure, occupational lead exposure, automobile paints workers.

INTRODUCTION

Occupational lead exposure is a serious health problem for the workers. Lead intoxication could occur even at low levels due to continuous exposure (Tong *et al*., 2000). In the developed countries the use of lead is prohibited from exceeding its defined limits in different items. Lead-based paints are one of the primary sources of lead exposure therefore it was banned in the developed countries (Herbert and Needleman, 1998; Gilbert and Weiss, 2006; Meyer *et al*., 2008). In the UN International Conference on Chemical Management held in 2009, 120 countries voted for the removal of lead from paints (*International Conference on Chemical Management*, 2009) however, in the developing courtiers lead is still in use.

In lead-exposed workers, lead mostly enters the body through the respiratory tract and, gets stored in blood, tissues, and bones. The remaining gets excreted from the body through urine (Philip and Gerson, 1994; Markowitz, 2000). Lead is a nephrotoxic agent which enters into the renal cells as free ions (Vander *et al*., 1979; Bennett, 1985). It induces nephropathies by interacting with calcium ions as both acts as an antagonist of each other (Nolan and Shaikh, 1992; Lyn Patrick, 2006). Chronic kidney disease (CKD) and renal failure are associated with high BLL but lead can also cause renal insufficiency even at low BLL (IPCS, 1995; Lin *et al*., 2003; Ekong *et al*., 2006). Continuous lead exposure can also induce cardiovascular diseases (CVD) in the individuals even with low BLL (Kopp *et al*., 1988; Iqbal, 2012). Increased BP is also observed in middle age lead exposed workers as compared to younger individuals (Pocock *et al*., 1988; ATSDR, 2007).

Exposure to lead induces the lead based anemia in exposed workers due to decreased production of Hb. Delta-aminolevulinic acid dehydratase (ALAD) is an enzyme that catalyzes the second step in porphyrin and heme biosynthesis pathway (Wetmur, *et al*., 1991). ALAD is a metalloenzyme that is made up of 8 identical subunits and each subunit possesses zinc ion which is essential for the enzymatic activity of ALAD (Jaffe, 2000). Lead replaces the zinc and inhibits the enzymatic activity of ALAD (Jaffe, 2000; Chatterje, 1995). Polymorphism in *ALAD* gene is produced due to the change of single nucleotide from G to C at the 177th position that creates *Msp*I restriction site (Wetmur *et al*., 1991). This substitution may leads to the conformational change in the enzyme that may increase its affinity towards lead binding and induce intoxication in the exposed individuals with different genotypes (Wetmur *et al*., 1991).

As lead mimics the calcium ions, therefore, it has the ability to bind to the vitamin D receptors (*VDR*) and in this way, it replaces calcium and increases lead burden in the body (Schwartz *et al*., 1986; Fullmer, 1990 and 1992). Like polymorphism of *ALAD*, *VDR* gene is also found to be associated with the difference in lead toxicokinetics

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(Schwartz *et al*., 1995; Onalaja and Claudio, 2000). Vitamin D receptor gene has *Bsm*I restriction site which helps in differentiating *BB*, *Bb* and *bb* genotypes (Schwartz *et al*., 2000).

The objective of this study was to determine the effects of delta-aminolevulinic acid dehydratase (*ALAD*) and vitamin D receptor (*VDR*) genotypes on biochemical parameters and blood pressure (BP) of automobile workers having low blood lead level (BLL) with continuous lead exposure.

METHODS

Data collection

A cross-sectional study was conducted on 160 male automobile paint workers and 40 healthy controls engaged in different occupations with no known lead exposure. Survey of different workshops was done in the different areas of Karachi, Pakistan. Workers of automobile workshops were involved in the study after their written consent with prior information about the research.

Data collection was done on the basis of the questionnaire consisting of personal information regarding occupational experience, use of chewing materials, diet and general health status including weight, height, blood pressure etc.

Blood sample collection

About 4ml venous blood was collected from cubital fossa using sterile disposable plastic syringes of which 2ml each was transferred into the glass vacutainers (ATLAS-LABOVAC) containing K3 EDTA as an anticoagulant and 2ml in a plain glass tube for serum separation.

Analysis of biochemical parameters

The biochemical parameters were analyzed on the same day of collection. Serum uric acid and serum creatinine levels were analyzed on an automatic biochemistry analyzer (ERBA, XL200) using commercial diagnostic kits (Merck).

Atomic absorption spectrophotometry

The BLL of automobile painters and control individuals were determined by atomic absorption spectrophotometry with graphite furnace (Perkin-Elmer, Analyst 700) according to Sole *et al*., (1998).

PCR-RFLP analysis

The genomic DNA extraction was done from whole blood using commercially available DNA extraction kit (Promega). Amplification of *ALAD* was done in 50ul reaction mixture according to the method described by Schwartz *et al*., (2000) with the modification of performing simple PCR instead of nested PCR, using reported internal primers by Wetmur *et al*., (1991) 5'-CAGAGCTGTTC-CAACAGTGGA-3' (Sense) and 5'-CCAGCACAATGTGGGAGTGA- 3' (Antisense).

Briefly, the reaction mixture was prepared by using 2x master mix (Merck), 0.5ug template and 200ng of each primer. The PCR cycles consisted of initial denaturation at 94°C for 2min, 40 cycles of denaturation at 94^{o}C for 1 min, annealing of primers at 60°C for 45 sec and extension at 72°C for 1min with the final extension at 72^{o}C for 10min. The amplification reaction generated 839 base pairs as per NCBI Accession No NG_008716.

The amplification product was analyzed by agarose gel electrophoresis. RFLP analysis was performed by using *Msp*I restriction enzyme (Thermo Scientific) according to Wetmur, *et al*., (1991). All the results were analyzed on 1% agarose gel under UV light in gel doc system (SCIE-PLAS).

The amplification reaction of *VDR* gene was performed in a final volume of 50ul as per the method of Schwartz *et al*., (2000). Amplification generated 822 base pair fragments which was confirmed by agarose gel electrophoresis whereas the RFLP analysis was performed using *Bsm*I restriction enzyme (Thermo Scientific) as described in Schwartz *et al*., (2000). The digested product was analyzed on 1% agarose gel.

STATISTICAL ANALYSIS

Statistical analysis was performed by using SPSS version 20.

RESULTS

Analysis of biochemical parameters

The mean BLL of automobile paint workers was found to be μ=0.462ug/dl which was increased significantly ($p< 0.0001$) compared to the mean BLL μ= 0.173ug/dl of the control group.

The group of automobile paint workers having increased systolic BP also showed significantly increased BLL ($p< 0.05$) as compared to the BLL of control group showing increased systolic BP.

Correlation analysis for Serum uric acid and serum creatinine with BLL

The correlation analysis of automobile paint workers having serum uric acid level ≥7mg/dl showed increased correlation (r=0.10) with BLL as compared to the workers with the level below than 7mg/dl (r=0.03). The correlation of automobile paint workers with serum creatinine level of ≥1.30 mg/dl with BLL showed increased correlation (r=0.37) as compared to the workers with values below 1.30 mg/dl (r= 0.25).

Allelic frequency of ALAD and VDR genes

Allelic frequency of *ALAD* and *VDR* genotypes in our samples were determined by the banding pattern and size of DNA fragments generated by PCR-RFLP as shown in figs. 1 and 2 respectively. The allelic frequency of *ALAD 1-1* was found to be (84.41%), *ALAD 1-2* (14.9%) and *ALAD 2-2* (0.66%) in automobile painters whereas the allelic frequency of *VDR Bb* was found to be (48.58%), *VDR bb* (34.15%) and *VDR BB* (17.27%).

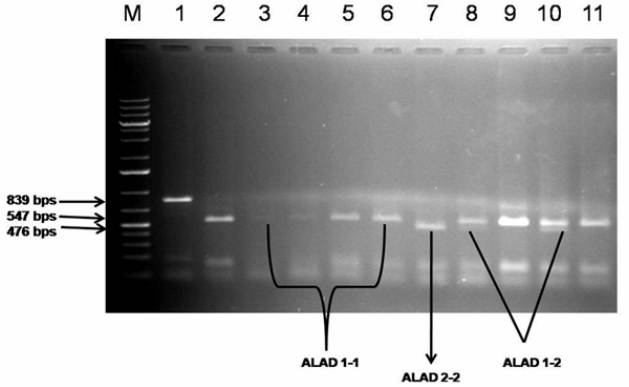

Fig. 1: Image of gel electrophoresis of RFLP analysis after PCR is showing ALAD1-1, ALAD 1-2 and ALAD 2-2 genotypes. M is DNA Marker (Fermentas 1kb plus general ruler). Lane 1 is showing amplified undigested fragment of ALAD. Lane 2 is showing digestion of control DNA with MspI that is representing ALAD 1-1 genotype. Lane 3- 11 is showing digestion of samples DNA with MSPI where ALAD 1-1 homozygotes are present in lane number 3-6, 9 and 11, ALAD 1-2 heterozygotes are present in lane number 8 and 10 while ALAD 2-2 homozygote is present in lane number 7.

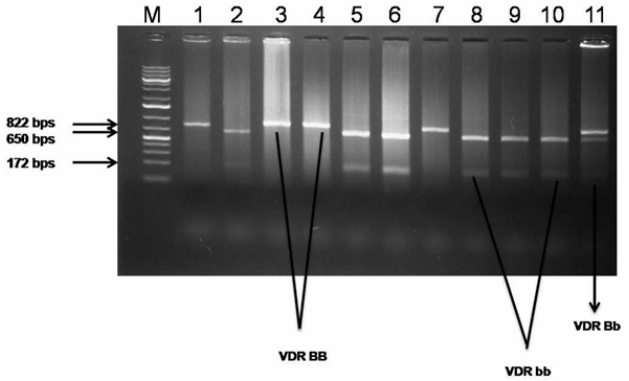

Fig. 2: Image of gel electrophoresis of RFLP analysis after PCR is showing VDR BB, VDR Bb and VDR bb genotypes. M is DNA Marker (Fermentas 1kb plus general ruler). Lane 1 is showing amplified undigested fragment of VDR. Lane 2 is showing digestion of control DNA with BsmI that is representing VDR bb genotype. Lane 3- 11 is showing digestion of samples DNA with BSMI where VDR BB homozygotes are present in lane number 3, 4 and 7, VDR Bb heterozygote is present in lane number 11 while VDR bb homozygotes are present in lane number 5-6 and 8-10.

Association of diastolic BP and BLL with ALAD genotype in automobile paint workers

The multiple linear regression models (table 1) showed the association of diastolic BP and BLL with *ALAD* genotypes in automobile paint workers. In model 1 the association was found to be negative (β= -0.14) with *ALAD 1-1* genotype at insignificant level whereas in model 2 the significant positive association (β= 0.54, $p< 0.05$) was observed with *ALAD 1-2* genotype. The coefficient of determination R^2 between the diastolic BP and BLL of *ALAD 1-2* genotype was 0.29 but it was found to be increased as compared with the *ALAD 1-1* genotype (R^2= 0.02).

Association of serum uric acid level and BLL with the genotypic combination of ALAD and VDR genes in automobile paint workers

Simple linear regression models (table 2) showed the association of serum uric acid level and BLL with the genotypic combination of *ALAD* and *VDR* genes in automobile paint workers. All combinations of *VDR* genotype showed the negative association of serum uric acid level and BLL with *ALAD 1-2* genotype but only *ALAD 1-2/VDR BB* showed significant negative effects on the association ($p< 0.05$, β= -0.99) whereas the rest were found to be insignificant. The coefficient of determination between serum uric acid and BLL of *ALAD 1-2/VDR BB* was also found to be strong (R^2= 0.99) as compared to the other genotypic combinations. *VDR bb* genotype in model 3 of table 4 showed the positive association with *ALAD 1-1* (β= 0.04) as compared to the other models of *ALAD 1-1* genotype whereas the greater negative association was observed with *ALAD 1-1/VDR Bb* genotype (β= - 0.02) but at the insignificant level.

Association of serum uric acid level and BLL with ALAD genotype in automobile paint workers dichotomized by their mean age

Multiple linear regression models (table 3) showed the association of serum uric acid level and BLL with *ALAD* genotypes in automobile paint workers dichotomized by the mean age. Automobile paint workers with mean age <30 years showed the positive association of serum uric acid level and BLL with both *ALAD 1-1* and *ALAD 1-2* genotypes (model 1 and model 3 of table 3) but the p-value was found to be insignificant. However, the greater effect of *ALAD 1-2* genotype (β= 0.08) was observed on the association of serum uric acid level with BLL as compared to *ALAD 1-1* genotype where (β= 0.06).

The automobile paint workers at the mean age ≥30 years showed the negative association of serum uric acid level and BLL with both *ALAD* genotypes (model 2 and 4 of table 3) however, the significant association was observed only with *ALAD 1-2* genotype ($p<0.05$). The coefficient of determination between serum uric acid and predictors of *ALAD 1-2* genotype of the automobile paint workers at

the mean age ≥30 years also showed the strong relationship (R^2= 0.99). The greater effect of *ALAD 1-1*genotype (β= -0.11) was observed on the association of serum uric acid level with BLL as compared to *ALAD 1-2* genotype (β= -2.33).

Association of serum uric acid level and BLL with VDR genotypes in automobile paint workers dichotomized by their mean age

The multiple linear regression models (table 4) showed the association of serum uric acid level and BLL with *VDR* genotypes in automobile paint workers dichotomized by the mean age. The automobile painters with mean age of <30 (model 1 and model 5 of table 4) showed positive association of serum uric acid level and BLL with *VDR bb* and *VDR BB* genotypes but insignificantly. The models also showed the greater effect of *VDR BB* genotype (β= 0.04) as compared to *VDR bb* genotype (β= 0.01).

Models 3 and model 4 of table 4 although shows the negative association of BLL and serum uric acid level in automobile paint workers with *VDR Bb* genotype of both <30 and ≥30 age groups but with the insignificant p-value. Similarly greater association of *VDR Bb* is observed at <30 years (β= -0.01) as compared to the ≥30 years (β= -0.02).

The automobile painters having age ≥30 years (model 2 and model 6 of table 4) showed the negative association of serum uric acid and BLL with *VDR BB* and *VDR bb* genotypes whereas the association was found to be significant with *VDR BB* genotype ($p<0.01$, R^2= 0.83). The models also showed the greater effect of *VDR bb* genotype (β= - 0.18) on the association of serum uric acid with BLL as compared to the *VDR BB* genotype (β= -0.72).

DISCUSSION

Lead poisoning is an occupational disease that occurs because of accumulation of lead in the body due to lead exposure. One of the main sources of lead exposure is the paints used in houses, automobiles, and industries. Despite all the claims of lead free paints, our result shows the average presence of 110.82 ppm lead with the range of 2.5-702 ppm in the randomly obtained automobile paint samples from local markets (Sadaf and Ansari, 2016). A previous study of Clark *et al*., (2009) showed the presence of lead ranging from 6,988-31,960 ppm in the enamel household paints samples collected from different countries of Asia, Africa, and South America.

Continuous exposure of lead to workers with even low BLL is known to be toxic to their health (Tong *et al*., 2000). According to U.S. Public health officials, there is no safety level for the lead exposure (Gottesfeld, 2015). Studies also show that lead does not have any threshold limit and mortality is found to be reported even at 3.6µg/dl (Menke *et al*., 2006; Tong *et al*., 2000; Rossi, 2008).

The U.S. Department of Health and Human Services established 10µg/dl blood lead level as permissible for adults whereas the highest BLL acceptable for the lead exposed workers by standards of the Occupational Safety and Health Administration (OSHA) is 40µg/dl (CDC, 2013). Results of atomic absorption spectrophotometry of automobile paint workers and control group in our earlier study showed the mean BLL values to be µ=0.462ug/dl and µ= 0.173ug/dl respectively (Sadaf and Ansari, 2016) which was not only found to be lower than the permissible limits for unexposed adults and the lead exposed workers but also lower as compared to other similar type of studies by Enander *et al*. (2004), Vitayavirasuk *et al*. (2005), Adela *et al*. (2012), Gottesfeld, (2015). The reason for this low BLL could be the use of chewing materials which included betel leaves and gutka that contains calcium carbonate as one of the ingredients that are a rich source of calcium. The study by Nair, et.al, (2004) also reported the presence of slaked lime in betel leaf (paan masala) and gutka. Vitamin D receptor absorbs calcium from the gastrointestinal tract (Josephson, 2000) and thus decreases the rate of lead absorption as it was reported that decrease rate of lead absorption is strongly associated with the increased intake of dietary calcium (Mahaffey *et al*., 1980). Almost 70.63% of the total automobile paint workers in our study were found to be using the mentioned chewing materials whereas around 90% of the workers above the age of 40 were found taking milk regularly.

The cardiovascular diseases along with certain death cases are also observed at the low level of lead exposure (Kopp *et al*., 1988; Ibrahim *et al*., 2006; Lim *et al*., 2012). Studies by Harlen, (1988), Hertz-Picciotto and Croft, (1993), Cheng *et al*., (2001) and Prozialecks *et al*., (2008) showed that lead-induced hypertension is not only a problem for lead-exposed workers but is also a public health problem through environmental exposure. Our result shows increased mean systolic and diastolic blood pressure in automobile painters with low BLL as compared to the control group though insignificantly. The study by Vupputuri *et al*., (2015) also showed elevated BP due to increased BLL in black males (µ=5.4ug/dl) as compared to the white males (µ= 4.4ug/dl) which were significantly different ($p<0.001$). Thus on the basis of these results it can be concluded that increase in systolic and diastolic BP may be associated with BLL <10ug/dl despite the fact that it considered being the safe level for adults by the CDC USA and OSHA (CDC, 2013).

The genetic makeup of individuals also plays an important role in lead toxicity as the difference in genetic

makeup makes one susceptible or resistant towards lead poisoning (Wetmur *et al*., 1991; Josephson, 2000). Our result shows the significantly positive association of BLL and diastolic blood pressure in automobile painters with *ALAD 1-2* genotype ($p<0.05$) whereas negative association is observed with *ALAD 1-1* genotype but at the insignificant level. A normative aging study by Wu *et al*., (2003) on lead unexposed individuals has shown the significant increase ($p<0.021$) of diastolic blood pressure in the individuals with *ALAD 1-2* genotypes. However, to the best of our knowledge, no study has been done on the occupational lead exposed workers for the determination of association of *ALAD* genotype with BP. Our study also shows that *ALAD 1-2* genotype may play a significant role in the increase of diastolic BP in lead-exposed workers even at low BLL. It can be said that there is an increased risk of cardiovascular diseases and hypertension associated with *ALAD 1-2* genotype even at low BLL.

Increased serum uric acid could induce acute and chronic kidney diseases and is also an indicator for renal failure (Ben-Dov and Kark, 2011). The automobile paint workers in our study had low BLL, therefore, the correlation of serum uric acid with the BLL was analyzed only for those automobile painters who had serum uric acid level of ≥7 mg/dl. Our result showed the positive correlation of serum uric acid with low BLL whereas Anetor, (2002) and Wang *et al*., (2002) also showed the positive correlation of serum uric but with high BLL in lead-exposed individuals. Therefore it is an important finding based on our study that increase in the serum uric acid level can be caused even by the low level of lead in the blood.

The polymorphism in *ALAD* and *VDR* genes is found to be associated with the difference in bioaccumulation of lead in blood and bone respectively (Onalaja and Claudio, 2000). To our knowledge, no study was conducted to see the effect of the genotypic combination of *ALAD* and *VDR* genes on the association of serum uric acid and BLL in automobile paint workers. Therefore we analyzed the role of *ALAD* and *VDR* genes on the association between serum uric acid and BLL. Our results showed the significant negative association between serum uric acid and BLL with *ALAD 1-2/VDR BB* genotype ($p< 0.05$). The other genotypic combinations also showed the negative association but at insignificant level except for *ALAD 1-1/VDR bb* genotype which showed positive association at the insignificant level. Therefore it may possible that *ALAD 1-2/VDR BB* genotype plays a significant role against lead induced nephrotoxicity at low BLL with continuous lead exposure in automobile painters.

The linear regression analysis also showed the negative association of BLL and serum uric acid with all *VDR* genotypes in combination with *ALAD 1-2* genotype as compared with the *ALAD 1-1* genotype where the *VDR* bb shows the positive effect on the association in automobile paint workers. On the observation of negative association of serum uric acid and low BLL with *ALAD 1-2* genotype, it is suggested that the *ALAD 1-2* genotype may have the stronger effect on the association of serum uric acid and low BLL as compared to the *VDR bb* genotype.

A study on Korean lead exposed workers by Weaver *et al*., (2005) showed negative association of BLL and serum uric acid with *ALAD* genotypes in younger lead-exposed workers (<40 years) whereas our study showed positive but insignificant association of serum uric acid with both the *ALAD* genotypes in young automobile paint workers (<30 years). Our result also shows positive association of serum uric acid and BLL with *VDR BB* and *bb* genotypes and negative association of *VDR Bb* genotype is observed in workers at <30 years whereas the study by Weaver *et al*., (2005) shows positive association with *VDR BB/Bb* and negative association with *VDR bb* genotypes in lead exposed workers with median age <40.6 years. This difference in association at the younger age could be due to the period of exposure as the younger Korean lead workers with both *ALAD* and both *VDR* genotypes had lead exposure of 3-4 years whereas younger automobile paint workers in our study had 8-9 years of exposure. The difference in the association by *VDR* genotype also could be due to the difference in genotypic frequency in our study as compared to the Weaver *et al*., (2005). It is predicted from the multiple linear regression models that positive association of serum uric acid with low BLL can induce the renal insufficiency. It is suggested that the association of serum uric acid level and low BLL could be changed due to the continuous and prolong period of lead exposure and the period of exposure may play an important role on the association of serum uric acid and BLL as compared to age factor.

Weaver *et al*., (2005) showed that the *ALAD* genotypes modifies the association of serum uric acid with high BLL in older lead workers (≥40 years), *ALAD1-1* genotype showed significantly positive while *ALAD 1-2* genotype showed negative association in older lead workers. Our results show the negative association of BLL and serum uric acid with *ALAD* genotypes whereas *ALAD 1-2* genotype showed significantly negative association ($p<0.05$) in automobile paint workers. Our young automobile paint workers showed positive but the insignificant association with both *ALAD* genotype and the older workers showed negative association whereas the duration of exposure was significant increased as compared to the younger workers but the older workers were consuming the twice amount of chewing material containing calcium carbonate and also taking milk in their diet. Therefore, it is suggested that the association is also dependent on the nutritional intake of the workers.

Table 1: Simple linear regression models to analyze the modifying effects of *ALAD* genotypes on association of BP and BLL in automobile paint workers

Linear Regression Model of Diastolic BP with ALAD Genotype		Unstandardized Coefficients		Standardized Coefficients	t-value	P-value	Sig	R^2
		B	Std. Error	Beta				
ALAD 1-1	(Constant)	1.922	0.005	--	357.19	0.0001	Sig	0.02
	Blood Lead Level	-0.014	0.008	-0.141	-1.65	0.100	NS	
ALAD 1-2	(Constant)	1.895	0.005	--	344.76	0.0001	Sig	0.29
	Blood Lead Level	0.027	0.010	0.540	2.72	0.014	Sig	

Data was normalized for *ALAD 1-2* genotype by log transformation. Simple linear regression models show negative association of BLL and serum uric acid with *ALAD 1-2* genotype at $p<0.05$ in automobile painters.

Table 2: Simple linear regression models for association of serum uric acid and BLL with combination of *ALAD* and *VDR* genotypes in automobile paint workers

Linear Regression Model of Serum Uric Acid Levels with ALAD-VDR Genotypes		Unstandardized Coefficients		Standardized Coefficients	t-value	P-value	Sig	R^2
		B	Std. Error	Beta				
ALAD1-1 VDRBB	(Constant)	5.01	0.23	--	21.74	0.0001	Sig	0.02
	Blood Lead Level	-0.25	0.29	-0.16	-0.86	0.39	NS	
ALAD1-1 VDR Bb	(Constant)	5.61	0.27	--	20.20	0.0001	Sig	0.001
	Blood Lead Level	-0.06	0.43	-0.02	-0.15	0.87	NS	
ALAD1-1 VDR bb	(Constant)	5.11	0.26	--	19.30	0.0001	Sig	0.002
	Blood Lead Level	0.16	0.46	0.04	0.35	0.72	NS	
ALAD1-2 VDR BB	(Constant)	7.00	0.69	--	101.51	0.006	Sig	0.999
	Blood Lead Level	-0.86	0.05	-0.99	-14.99	0.04	Sig	
ALAD1-2 VDR Bb	(Constant)	6.70	1.24	--	5.39	0.012	Sig	0.32
	Blood Lead Level	-3.86	3.22	-0.57	-1.20	0.31	NS	
ALAD1-2 VDR bb	(Constant)	5.22	0.46	--	11.20	0.0001	Sig	0.002
	Blood Lead Level	-0.17	1.41	-0.40	-0.12	0.90	NS	

Simple linear regression models show significant negative association of BLL and serum uric acid with *ALAD 1-2/VDR BB* genotypic combination at ($p<0.05$) in automobile paint workers.

Table 3: Multiple linear regression models for association of serum uric acid and BLL with *ALAD* genotypes in automobile paint workers dichotomized by their mean age

Linear Regression Model of Serum Uric Acid with ALAD Genotypes		Unstandardized Coefficients		Standardized Coefficients	t-value	p-value	Sig	R^2
		B	Std. Error	Beta				
Age <30 Years *ALAD1-1*	(Constant)	0.417	0.194	…..	2.149	0.035	Sig	0.033
	Blood Lead Level	0.015	0.028	0.062	0.539	0.592	NS	
	Exposure	0.001	0.002	0.035	0.296	0.768	NS	
	Systolic B.P	0.002	0.002	0.157	1.343	0.183	NS	
	Weight	0.000	0.001	0.035	0.293	0.770	NS	
Age ≥30 Years *ALAD1-1*	(Constant)	1.852	1.834	…..	1.010	0.318	NS	0.163
	Blood Lead Level	-0.470	0.580	-0.113	-0.809	0.423	NS	
	Exposure	0.024	0.016	0.207	1.452	0.153	NS	
	Systolic B.P	0.004	0.015	0.044	0.292	0.772	NS	
	Weight	0.039	0.019	0.303	2.026	0.049	Sig	
Age <30 Years *ALAD1-2*	(Constant)	5.449	6.478	…..	0.841	0.425	NS	0.146
	Blood Lead Level	0.149	0.607	0.087	0.246	0.812	NS	
	Exposure	0.046	0.068	0.261	0.676	0.518	NS	
	Systolic B.P	-0.014	0.055	-0.098	-0.254	0.806	NS	
	Weight	0.021	0.030	0.248	0.706	0.500	NS	
Age ≥30 Years *ALAD1-2*	(Constant)	42.750	1.556	…..	27.483	0.023	Sig	0.999
	Blood Lead Level	-17.297	0.611	-2.337	-28.304	0.022	Sig	
	Exposure	0.095	0.005	0.693	17.887	0.036	Sig	
	Systolic B.P	-0.453	0.018	-2.260	-25.850	0.025	Sig	
	Weight	0.298	0.011	2.865	26.996	0.024	Sig	

Note: ^{Sig} Significant difference, ^{NS} Non significant difference

Multiple linear regression models show positive association of BLL and serum uric acid with both *ALAD* genotypes in automobile paint workers below mean age whereas the negative association is observed at the mean age with both *ALAD* genotypes. However, p-value is found to be significant at $p<0.05$ automobile paint workers at the mean age with *ALAD 1-2* genotype. Data was normalized for *ALAD 1-1* genotype for the group of automobile painters with age <30 years by log transformation.

Table 4: Multiple linear regression models for association of serum uric acid and BLL with *VDR* genotypes in automobile paint workers dichotomized by their mean age

Linear Regression Model of Serum Uric Acid with VDR Genotypes		Unstandardized Coefficients		Standardized Coefficients	t-value	p-value	Sig	R^2
		B	Std. Error	Beta				
VDR BB <30 years	(Constant)	5.294	1.174	…….	4.508	0.0001	Sig	0.017
	Blood Lead Level	0.108	0.595	0.044	0.181	0.858	NS	
	Exposure	-0.028	0.055	-0.128	-0.518	0.611	NS	
	Weight	0.000	0.019	-0.005	-0.019	0.985	NS	
VDR BB ≥30 years	(Constant)	20.845	0.971	…….	2.931	0.026	Sig	0.839
	Blood Lead Level	-3.399	0.886	-0.722	-3.838	0.009	Sig	
	Exposure	-0.120	0.025	-0.965	-4.835	0.003	Sig	
	Weight	0.090	0.020	0.999	4.570	0.004	Sig	
VDR Bb <30 years	(Constant)	4.400	1.207	…….	3.646	0.001	Sig	0.024
	Blood Lead Level	-0.047	0.428	-0.015	-0.109	0.913	NS	
	Exposure	-0.012	0.018	-0.107	-0.679	0.500	NS	
	Weight	0.023	0.021	0.168	1.067	0.291	NS	
VDR Bb ≥30 years	(Constant)	2.254	1.390	…….	1.621	0.117	NS	0.356
	Blood Lead Level	-0.109	0.621	-0.029	-0.176	0.862	NS	
	Exposure	0.063	0.019	0.541	3.354	0.003	Sig	
	Weight	0.026	0.021	0.206	1.241	0.226	NS	
VDR bb <30 years	(Constant)	5.569	1.774	…….	3.139	0.004	Sig	0.061
	Blood Lead Level	0.043	0.487	0.016	0.087	0.931	NS	
	Exposure	0.065	0.049	0.261	1.312	0.200	NS	
	Weight	-0.009	0.033	-0.055	-0.275	0.785	NS	
VDR bb ≥30 years	(Constant)	2.195	2.424	…….	0.905	0.380	NS	0.232
	Blood Lead Level	-0.918	1.205	-0.184	-0.762	0.458	NS	
	Exposure	-0.011	0.028	-0.093	-0.383	0.707	NS	
	Weight	0.060	0.034	0.409	1.788	0.094	NS	

Note: ^{Sig} Significant difference, ^{NS} Non significant difference

Multiple linear regression models show positive association of BLL and serum uric acid with *VDR BB* and *bb* genotypes while negative association is observed with *VDR Bb* genotype in automobile paint workers below mean age whereas the negative association is observed at the mean age with all *VDR* genotypes. However, p-value is found to be significant at $p<0.01$ automobile painters at the mean age with *VDR BB* genotype.

The automobile paint workers also show significant negative association with *VDR BB*, *VDR Bb,* and *VDR bb* genotypes but the association was found to be significantly negative only for *VDR BB* genotype ($p<0.01$) at ≥30 years. Weaver *et al*., (2005) also shows the negative association of with *VDR BB/Bb* but the association was positive for *VDR bb* genotype ≥40 years of age. It may possible that the difference in the association of serum uric acid with BLL with *VDR bb* genotype could be due to the difference in genotypic frequency. Furthermore, the positive association may associate with only with *VDR BB* genotype but not with *VDR* Bb genotype as Weaver *et al*., (2005) has combined *VDR BB* and *VDR Bb* genotype due to the decreased frequency of alleles in Korean lead exposed workers.

Our study shows the dual role of *ALAD 1-2* genotype as it has positive effects on diastolic BP at low BLL on automobile paint workers but in combination with *VDR BB* genotype *ALAD 1-2* genotype reduces the serum uric acid level. Further studies with greater sample size will confirm this observation and will further define the roles of the *ALAD* and *VDR* genotypes.

CONCLUSION

Our results predict that individuals even with low BLL with continuous lead exposure have high health risks. The positive association of *ALAD 1-2* genotype is significantly associated with the increased diastolic BP with low BLL. The combination of *ALAD/VDR* genotypes shows that *ALAD 1-2* and *VDR BB* genotypes have greater effects on decrease serum uric acid level at low BLL in automobile paint workers. It shows that *ALAD 1-2* genotype may be playing dual role at low BLL because on one hand, it is significantly increasing diastolic BP whereas on the other it is significantly reducing serum uric acid level in the automobile paint workers having *VDR BB* genotypes, therefore, the combination of *ALAD 1-2* and *VDR BB* may protect the automobile paint workers from renal insufficiency. The positive association between serum uric acid and low BLL with *ALAD* and *VDR* genotypes in younger automobile painters is indicating the risk of renal insufficiency. However in the case of workers with age ≥30 years significant negative association between serum uric acid and BLL was observed with increase duration of exposure as compared to the young automobile paint

workers. This may be because of increase intake of calcium through chewing material and milk intake in automobile paint workers having age ≥30 years as compared to the <30 years.

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