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The High Lead Level Role in the DNA Repair RAD 18 and OGG1 Gene Polymorphism in Gasoline Station Workers

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Abstract: The DNA repair enzymes–heavy metal interaction is an interesting project that can help elucidate several diseases. The current study aims to assess the lead effect in the two DNA repair genes RAD-18 Arg302Gln (rs373572) and OGG1 Ser326Cys in Gasoline station workers. The output showed that work types were Gasoline supply worker with a high percentage (73.52%) than other groups (11.76% and 14.7%) for a maintenance worker and station employee, respectively, significant lead (p 0.010) increasing in station worker than the control group, and significant lead changes among work types groups (p 0.000), the station employee has a low level of lead than other groups, while the Gasoline supply worker has a higher level than other groups, the RAD 18 gene showed two polymorphisms Gln/Gln and Gln/Arg, and OGG1 showed two haplotypes, single and double haplotypes, non-significant association although of high frequent of Glu/Arg in the gasoline supply worker, and significant association of allele frequency, significant association with station worker that have two types of haplotypes (single and double haplotypes) while lack of tri-haplotypes which prepared in higher percentage in control group. The lead level according to RAD 18 genotyping show non-significant (p 0.454) elevation in Gln/Arg genotyping, and according to OGG 1 haplotype lead level was non-significant, changed (p 0.481) between single and double haplotypes. From these outputs, it can be concluded that the lead level is a significant elevation in gasoline station worker and it did not affect RA18 and OGG 1 genotyping, the RAD18 did not associate with workers while OGG strongly associated with them.

Key words: Lead level, DNA repair, RAD 18, OGG1, gene polymorphism, gasoline station workers.

Introduction

Lead is one of the heavy metals that is highly poisonous, it exists in the environment as a nondegradable susbtance and cannot be metabolised in the body, the accumulation of lead in the body by chronic exposure is considered as an occupational risk factor, and leads to a serious threat to human health (Brewster et al., 2004; Chen et al., 2012; Zhang et al., 2014). The entrance of lead in the body, through respiratory and digestive tracts, leads to toxic impacts to different body organs and systems (Pasha et al., 2004). The genotoxicity of lead have been established in recent year (Borghini et al., 2016; Garcia-Leston et al., 2010, 2012; Kasuba et al., 2012; Nersesyan et al., 2016; Ustundag et al., 2014), and has lead to different DNA lesions that can

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be repaired by multiple DNA repair genes, as well as APE 1 XRCC1, XPD and hOGG1. These repair systems include base excision repair, double-strand break repair and nucleotide excision repair (Spencer et al., 2008).

One of the repair enzymes is RAD18 which has a main role in the post-replication repair process in some organisms and humans (Kanzaki et al., 2007). The function of RAD 18 is to bypass DNA damage and post-replication repair at stalled replication forks and in homologous recombination in mammalian cells that repair the double-strand breaks. The current study deal with polymorphism at codon 302 SNP (rs373572) helps in an amino acid substitution from arginine to glutamine (Kanzaki et al., 2008). However, the function of this SNP is still under investigation (Kanzaki et al., 2007).

Another gene in the present study is 8-oxo guanine DNA glycosylase 1(OGG1), which plays a major role in DNA repair pathways after ROS exposure. Investigations detected about 20 variants in the OGG1 gene sequence, the most functional polymorphism is Ser326Cys, and the function of OGG 1 is mediated in the cleavage of the glycoside bond between alteration base and the corresponding sugar in a DNA molecule. The C/G variant of rs1052133 causes serine to cysteine substitution at codon 326 that leads to enzyme dysfunction (Das et al., 2016).

Notably, the DNA repair genes inactivation leads to DNA repair deficiency and DNA damage accumulation that promotes different health problems (Lahtz et al., 2011).

The current study aims to focus on the high lead level role in RAD 18 and OGG1 gene polymorphism in gasoline station workers.

Methodology

Study Subjects

About 40 station workers and 30 healthy individuals were enrolled in the present study. Ethical approval of the Ministry of Higher Education and Scientific Research was considered for blood sample collection, which was thereafter divided into two parts for serum isolation to lead detection and DNA extraction.

Amplification and Electrophoresis Conditions

The DNA was extracted by favorgen extraction kit and detection purity and concentration, the Oligos selected in the current study were: The RAD-18 Arg302Gln (rs373572) primers: F1: 5'-ATA CCC ATC ACC CAT CTT C and R1, GTC TTCTCT ATA TTT TCG ATT TCT T for the Gln allele (146 pb), F2, TTA ACA GCT GCT GAA ATAGTT CG and R2, CTG AAA TAG CCC ATT AAC ATA CA (106 bp) Arg allele. A 206 bp band was the common band by allele-specific PCR (16). The allele-specific PCR of RAD-18 was detected by electrophoresis using 1% agarose gel, 100 V, 25 mA for 50 min), then the gel was documented using a photo-documentation tool with ethidium bromide stain.

The OGG1 Ser326Cys primers F-GGTGGCCCTAAAGGACTCTC, R-AAGGTGCTTGGGGAATTTCT to amplified 295 bp (Das et al., 2016). Then PCR product was analysed to determine haplotypes using single strand conformation polymorphism (SSCP), in briefly; 40% of polyacrylamide with glycerol, TBE (5×) dH_2O, ammonium-per sulphate and TEMD used to gel preparation, samples were mixed with loading dye consist of (xylene cyanol, formamide, bromo- phenol blue with EDTA) 1:1 V/V, mixture incubates at 95 to 7 min, then chilled in ice to 2 min, after this, samples were electrophoresis running at 100V, 0.5× TBE to 40 min, then the gel was stained with ethidium bromide and visualised under UV light.

Lead detection: the lead level was detected by atomic absorption apparatus after the serum was digested with nitric oxide (1:5 V/V) at 100ºC with diluted by dH_2O.

Statistical Analysis

The results represented by mean ±SE or SD, the lead level and genotyping analysed using an independent t-test, ANOVA one way, and odds ratio (CI95%) at $p<0.05$. allele frequency was calculated according to Hardy–Weinberg equilibrium.

Results

The present research implemented to determine the relation of lead with RAD 18 and OGG1 genotyping in station workers, three station work types were enrolled in the present study (gasoline supply worker, maintenance worker and station employment) in addition to the control group, the gasoline supply worker was a higher precentage (73.52%) than other groups (11.76% and 14.7%) for maintenance worker and station employee, respectively (Figure 1).

The means of the age of study groups were significantly different (p 0.000), whereas non-significant for station workers (p 0.088) (Table 1).

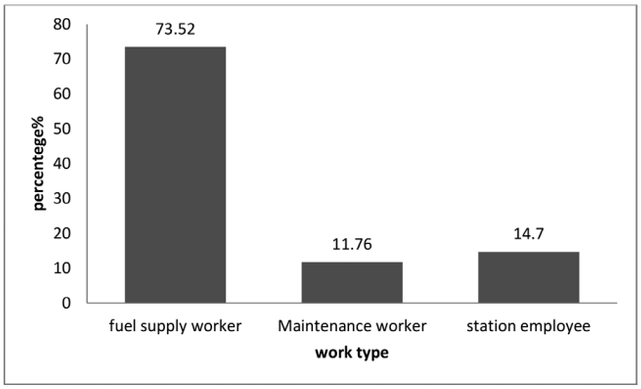

Figure 1: Distribution of study subjects according to station work type.

Table 1: The mean differences in age and work duration of study subjects

Work type	*Age (year)*	*Duration (year)*
Gasoline supply worker	37.72±1.56a	14.48±1.103
Maintenance worker	45.00±1.73b	11.50±4.55
Station employee	41.60±2.063b	12.20±0.96
Control	23.81±0.95c	-
Sig	0.000	0.088
ANOVA one way, mean±SE, P <0.05		

The level of lead showed a significant (p 0.010) increase in station workers than the control group (Figure 2).

The lead level in the study subjects according to work type was detected, which showed that there was a significant difference among groups (p 0.000). The station employee has a low level of lead than other groups, and the gasoline supply worker has high level than the other groups (Figure 3).

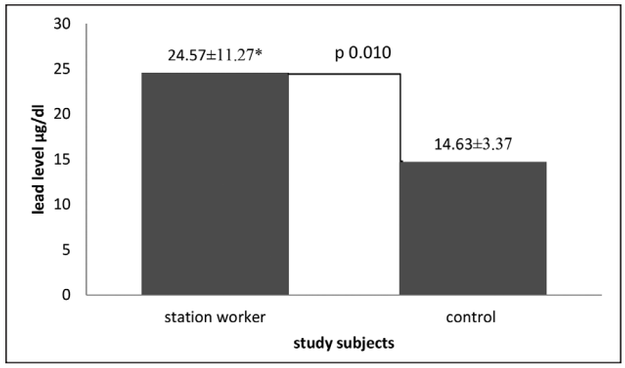

Figure 2: The level of lead in station worker and control group (independent T test, p<0.05, mean±SD).

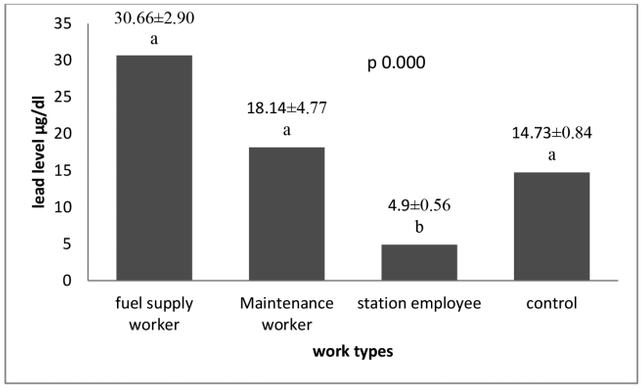

Figure 3: The lead level in study groups (ANOVA one way, p<0.05, mean±SE).

The DNA repair genes polymorphism in the current study represented by RAD 18 and OGG 1 were studied using allele-specific PCR and PCR-SSCP techniques, the RAD 18 gene showed two polymorphisms (Gln/Gln and Gln/Arg), and OGG1 showed two haplotypes (single and double haplotypes) (Figure 4).

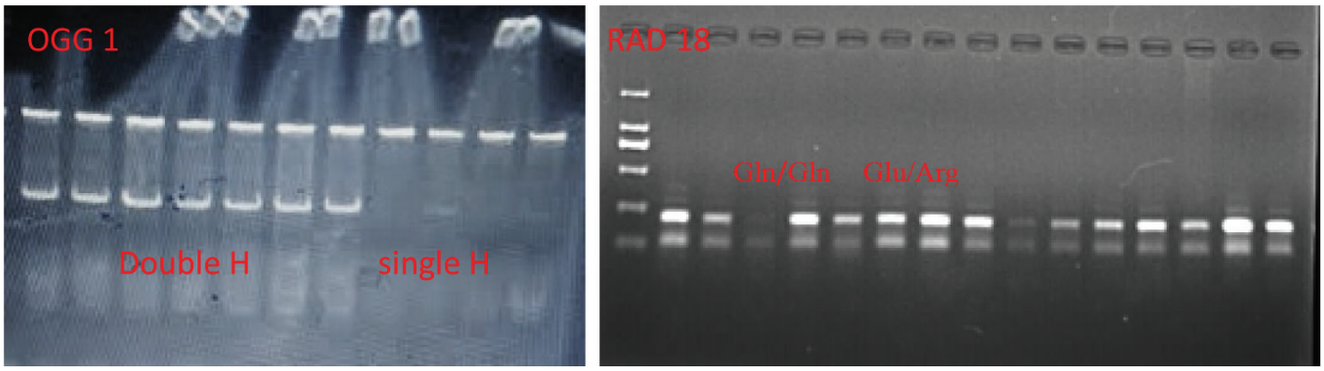

Figure 4: The OGG 1 electrophoresis pattern of SSCP technique show single and double haplotypes (1;1, v:v of loading dye and PCR product respectively, polyacrylamide 40%, glycerol, TBE5 X, TEMED and ammonium per-sulfate 0.1 gm/ml 100 V, 20 mA, ethidium bromide staining). The RAD 18 genotyping electrophoresis pattern of study groups, the RAD 18 has two genotypes Gln/Gln and Gln/Arg (1% agarose, 70 V, 20 mA, 40 min) DNA ladder (100, 250, 500) bp.

Non-significant association of RAD18 genotype with station workers, the frequency of Gln/Arg was higher in study groups, significant association of allele frequency with study groups (p 0.000) (Table 2).

The OGG 1 haplotypes detected by the SSCP technique, the result shows a significant association (p 0.000) with station workers that have two types of haplotypes (single and double haplotypes) while lacking tri-haplotypes which was observed in a higher percentage in the control group (Table 2).

The lead level according to RAD 18 genotyping shows non-significant (p 0.454) elevation in Gln/Arg genotyping (Figure 5).

The lead level in station workers, according to OGG 1 haplotype shows non-significant differences (p 0.481) between single and double haplotypes (Figure 6).

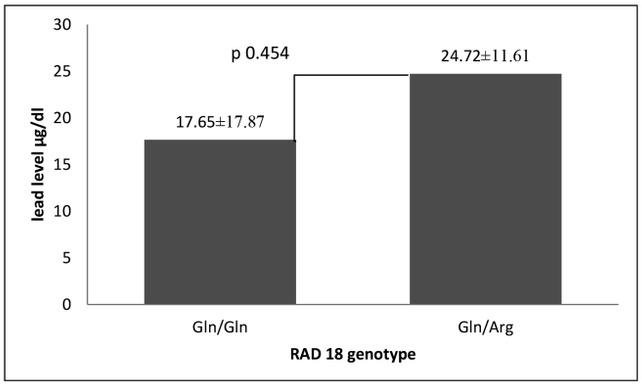

Figure 5: The lead level in station workers, according to RAD 18 genotyping (independent t test, mean±SD at p<0.05).

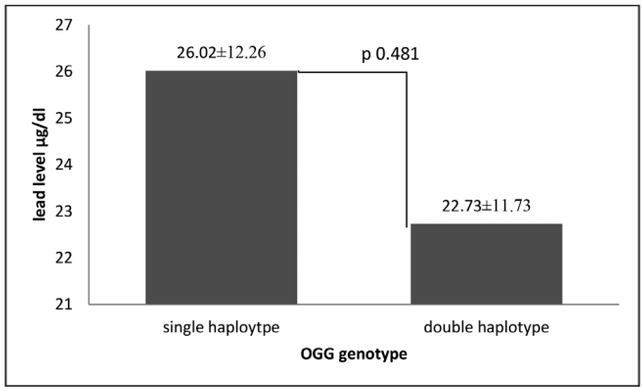

Figure 6: The lead level in station workers, according to OGG 1 haplotypes (independent t test, mean±SD at p<0.05).

Discussion

The current finding showed a significant elevation in lead levels in station workers (p 0.010) and this result agrees with other documents (Alhaj, 2020; Khadairi et al., 2021). The elevation of lead in workers may be because of neglected face masks and protective equipment while working with benzene that lead to inhalation of lead airborne, also the dermal absorption contributed to lead increment (Moline et al., 2005). In Baghdad and Beirut, results were reported (Hikmet et al., 1987; Nuwayhid et al., 2001), but in another study, the lead level in the petrol station workers was within normal limits, this can be clarified because of instability in the job (Mahdi et al., 1997). The level of

Table 2: The statistical analysis of the RAD 18 gene polymorphism and OGG 1 haplotype in study groups compared with control group (X^2 test, p<0.05)

Genotyping	*Gasoline supply worker%*	*Maintenance worker%*	*Station employee%*	*Control*	X^2	*Sig*
RAD18						
Gln/Gln	8	0	20	6.66	1.321	0.724
Gln/Arg	92	100	80	93.33		
Allele frequency						
Gln	0.56	0	0.60	0.53	99.416	0.000
Arg	0.43	1	0.40	0.46		
OGG 1						
Single haplotype	36.84	0	60	30	36.628	0.000
Double haplotypes	63.15	100	40	6.66		
Tri haplotypes	0	0	0	63.33		

lead, according to station work type showed the high level in gasoline supply workers because of the direct deal with gasoline.

Lead classified as a human carcinogen by The International Agency for Research on Cancer (IARC), researchers found that the genotoxic effects of Pb are DNA mutation, chromosome aberration strand breakage, and inhibition DNA inhibition (Ibrahim et al., 2020; Johnson et al., 1998). The genotoxic effects mechanisms of Pb are still unclear, however, the indirect mechanisms also underline the Pb genotoxic like oxidative stress induction that contributes to the inhibition of DNA repair, DNA damage, the DNA and/or protein crosslinks formation, and the regulation of some genes (Hartwig et al., 1990,1994; Li et al., 2001; Méndez-Gómez et al., 2008; Silbergeld et al., 2003).

Non-significant association between RAD18 and station worker, the effect of lead in DNA repair have been studied using different methods with some pathways, the current finding agrees with, Abdullah et al. (2014) who did not find significant differences in the gene expression of several DNA repair included ERCC3, XRCC14, and RAD 51 in stem cells after 24 h of lead nitrate 160 μM exposures. We found a significant association of OGG1 haplotypes with station workers, and this agrees with the findings of Liu et al. (2018) who studied that the methylation levels of some DNA repair including, hOGG-1, BRCA1, LXRCC1 and XPD were significantly elevated in TK6 cells exposed to lead. Also, Gadhia et al. (2012) assessed the expressions of genes responsible for DNA repair alteration in mouse embryonic stem (mES) cells exposed to lead acetate, their results showed a significant lowering in OGG1mRNA expressions and Rad18 at IC50 concentration for 1 h.

On the other hand, the indirect genotoxic of lead is induced oxidative stress that contributed to protein changes, DNA mutation (Pi et al., 2010), the role of OGG-1 in inhibiting 8-OHdG increment by repairs and DNA oxidative injury (Kuznetsov et al., 2011).

The lead level did not affect in the RAD18 genotyping and OGG1 haplotypes. The high level of lead may be affected by the gene expression, and protein structures of RAD18 and OOG1 enzyme but maybe did not impact their genes, Hemmaphan et al (2022) suggested that accumulation levels of Pb may be associated with the expression level of DNA repair genes. The current results need more investigations to detect other SNPs in a related gene with lead toxicity.

Conclusion

The lead level varied significantly in gasoline station worker types, especially in the Gasoline supply worker that elevated, and it did not get effected by RA18 and OGG 1 genotyping, the RAD18 did not associate with workers while OGG strongly associated with them.

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