RESEARCH ARTICLE

Apurinic Apyrimidinic Endonuclease 1 Gene Polymorphism Association with Total Antioxidant Level in Automobile Technicians

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ABSTRACT

The association of apurinic apyrimidinic endonuclease 1 (APE1) gene polymorphism with total antioxidant level in automobile technicians was proposed in the present study, using APE1 Asp148Glu (rs3136820) variation, reactive oxygen species (ROS) and total antioxidant levels (TAO) were depended in a case-control study, the results showed the mean of age (33.30 ± 1.94) years, body mass index (BMI) (26.19 ± 3.53) kg/m^2 and work period (7.68 ± 4.9) years, the ROS level non-significant changes between cases and control (p 0.567) and significant elevation in TAO (p 0.000), The APE1 Asp148Glu (rs3136820) genotyping produced three genotypes (TT, GG and TG). The results of APE1 genotyping distribution showed non-significant association of Asp148Glu (rs3136820) with automobile technicians GG (p 0.8636), GT (p0.0718), also allele frequency didn't associate with cases (p 0.8434), non-significant association of APE1 genotypes with smoker (p 0.1656), hookah (p 0.1001), alcohol uptake (p 0.8012) and chronic disease (p 0.6981), The ROS levels according to APE1 genotypes show non-significant variation (p 0.976) among genotypes. The TAO level according to APE1 genotypes showed significant differences among GG and TG in control group while significant elevation in TAO between TG genotypes in cases and control groups. The current study concluded that the APE1 gene polymorphism APE1 Asp148Glu didn't associate with automobile technicians with long exposure to lead and cadmium, but it was associated with elevation in TAO level in workers.

Keywords: Apurinic apyrimidinic endonuclease 1, Gene polymorphism, Total antioxidant level, Automobile technicians.

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INTRODUCTION

Automobile technicians employees are the most exposed to environmental pollution exposure, different types of work and areas of an automobile like automobile body repairers (panel beaters), auto mechanics, welders, auto painters, vulcanizers, and auto electricians. In automobile technicians, the main source of polluted molecules are exposure to lead and cadmium. Chronic exposure to low amounts of heavy metals for a long term produces chronic toxicity. This is increased among developing and rural populations and individuals living near areas densely polluted without necessary environmental rules.[1,2]

One of the pollution exposures assessed during the work by biological monitoring is induced oxidative stress, which is unbalanced in free radicals production and removing excessive reactive oxygen species (ROS) from cells and tissues to prevent harmful effects on cell components from producing a clinical picture called oxidative stress.[3] That lead to a deterioration of the tissues and cells that originate with an evident loss of efficiency. Oxidative stress is unlike other diseases because it doesn't generate well-defined symptoms. This is more difficult to estimate and observe in the different working environments that might be big and well-ventilated, in addition to small workshops.[4] On the other hand, workers' habits and lifestyles are also different, mainly related to smoking habits.[5]

The base excision repair is a curtail mechanism to correct base lesions, including modifications of oxidative effects that started by a glycosylase-dependent recognition and removal of

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destructed nucleotides from DNA strand to form an apurinic/apyrimidinic site, which is latter ligated by another step of repair step.[6] The apurinic/apyrimidinic endonuclease 1 (APE1) protein is significantly involved in DNA damage in monocytes. The gene encoded to APE1 is located at chr 14q11.2, which accounts for nearly all of the a basic site cleavage activity observed in cellular extracts.[7] APE1 cleaves the phosphodiester backbone immediately at the 50 of a basic site, by a hydrolytic mechanism to make a break in a single-strand DNA, leaving a 30-hydroxyl and 50-deoxyribose phosphate terminus.[8]

MATERIAL AND METHODS

A case-control study was implemented in the DNA lab in Babylon University. A 32 automotive workers were enrolled in the current study, and 30 were healthy, apparently as a control group. Blood samples and data were collected from study subjects using EDTA tube for DNA isolation and gel tube for serum collection. Data included (age, period of work, smoking habit, chronic disease and BMI).

DNA isolated by extraction kit (favorgen) instruction, then detected purity and concentration, DNA repair gene studied in present work was APE1 Asp148Glu (rs3136820) F1′CCTACGGCATAGGTGAGACC, R 1 T C C T G A T C A T G C T C C T C C - 3 ›, F 2 T C T G T T T C A T T T C T A T A G G C G A T, R 2 GTCAATTTCTTCATGTGC CA. The G allele 167 bp, T allele 236, and 360 bp common band at 60ºC.[9] Ito *et al.*, 2004).

The ROS and total antioxidants were detected using the colorimetric method. Data were analyzed using SPSS version 23, and significantly was detected using an independent sample t test, odd ratio (CI95%) and allele frequency according to Hardy-Weinberg. All statistical analyses were performed at a *p-value* 0.05.

RESULTS AND DISCUSSION

The DNA repair mechanisms have a vital role in genome integrity, cells used varied repair processes to maintain the nucleic acid, and this processing is affected by exogenous and endogenous factors, here in present research, we aimed to evaluate the interaction of oxidative stress in the APE 1 Asp148Glu (rs3136820) in automobile technicians, first of all the distribution of study subjects are clarified in the Table 1. Also the factors that may be implicated in the stimulation oxidative stress were estimated, including smoking cigarettes (50%) and hookah (40%), alcohol uptake (3.4%) and chronic disease (23.33%), in addition to the mean of age 33.30 ± 1.94 years, body mass index (BMI) 26.19 ± 3.53 kg/m^2 and work period 7.68 ± 4.9 years, the ROS level non-significant changes (*p* 0.567) and significant elevation in total antioxidant levels (TAO) (*p* 0.000) (Table 1).

The oxidative stress unbalance in workers of auto technicians (auto mechanics, panel beaters, battery repairers, and auto painters) that observed by Omotosho *et al.*[10] They clarified this by chronic exposure to heavy metals like lead and cadmium. Same output were found by Adekola *et al.*,[11] suggested that occupationally exposed auto mechanics appeared to have oxidative stress and may benefit from improvement in antioxidant status. One of the ROS induction factors is exposed to heavy metals as well as lead and cadmium, the worker who contributed in the present study have high level of cadmium and lead (data not shown), this fact also proved by Flora *et al.*,[12] and Ercal *et al.*[13] The chronic exposure to heavy metals that induced unbalance in free radicals production and its removal by de-toxicity lead to trigger disease by different mechanisms, one of this mechanism is impaired in DNA rapier systems.[14,15]

The APE 1 Asp148Glu (rs3136820) genotyping produced three genotypes (TT, GG and TG) (Figure 1, Table 1),

The results of APE1 genotyping distribution show non-significant association of Asp148Glu (rs3136820) with automobile technicians GG (*p* 0.8636), GT (p0.0718), also allele frequency didn't associate with cases (*p* 0.8434) (Table 2). Despite the non-significant association of APE1 Asp148Glu with Automobile technicians that have high level of TAO in present study, other evidence found an association that the APE1 variant genotype decreases repair of 8-OHdG and that arsenic exposure is associated with oxidative stress in women.[16] Also, Hao *et al.*,[17] suggested that APE1 alleviates H/R-induced injury in H9c2 cells by attenuating oxidative stress.

Table 1: The socio-demographic distribution of study subjects

Subjects	*Automobile technicians cases*	*Control*	*p-value*
Age	33.30 ± 1.94	33.0 ± 11.4	0.000
BMI	26.19 ± 3.53	28.07 ± 5.41	0.168
ROS	13.20 ± 0.980	13.20 ± 0.90	0.567
TAO	1190.2 ± 360.40	903.30 ± 130.51	0.000
Smoking			-
Yes	50%	0	
No	50%	0	
hookah	40%		-
Yes	60%	0	
No		0	
Alcohol uptake			-
Yes	3.4%	0	
No	96.6%	0	
Work period	7.68 ± 4.9	0	-
Chronic disease			
Yes			-
No	23.33%	0	
	76.66%	0	

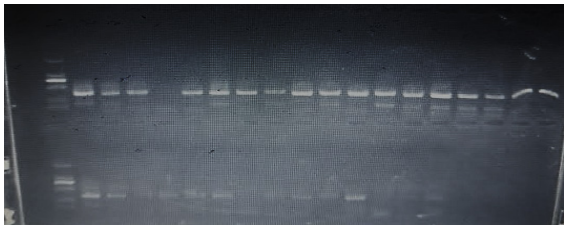

Figure 1: The APE1 genotypes (TT, TG and GG) electrophoresis pattern (1% agarose gel, 100 V for 30 minutes. EB staining.

Non-significant association of APE1 genotypes with smokers (*p* 0.1656), hookah (*p* 0.1001), alcohol uptake (*p* 0.8012) and chronic disease (*p* 0.6981) (Table 3).

Other studies didn't agree with the present study, A statistically significant interaction of smoking status with APE1 Asp148Glu polymorphism was found by Agaçhan *et al.*[18] On the other hand, cigarette smoking may induce DNA damage and individuals with a reduced capacity of DNA repair would be expected to have more carcinogen–DNA adducts in their tissue.[19]

According to APE1 genotypes, the ROS levels show non-significant variation (*p* 0.976) among genotypes Figure 2.

The TAO level according to APE1 genotypes. Significant differences were observed among GG and TG in control group, while significant elevation in TAO between TG genotypes in cases and control (Figure 3).

DNA is exposed to damage according to endogenous and/or exogenous factors. The repair system contributes in the genome integrity against these factors. The defenses in this system lead to triggering diseases; however, the variation in DNA repair genes is implicated in some diseases.[20,21]
Base excision repair genes have a vital role in DNA damage removal induced by oxidation through heavy metal exposure to tobacco smoking (cigarettes or hookahs).[22,23] The *APE1* is the rate-limiting enzyme in the Base excision repair pathway.[24] In spite of not reduce endonuclease activity by the APE1 Asp148Glu polymorphism, cases with Glu allele may have higher sensitivity to ionizing radiation that induced ROS expression.[25,26]

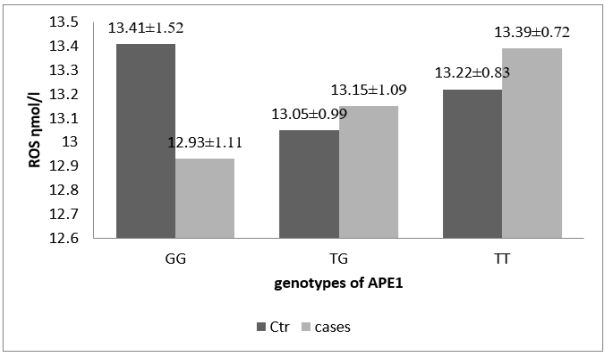

Figure 2: The ROS level in study subjects according to APE1 genotypes (non-significant differences p 0.976)

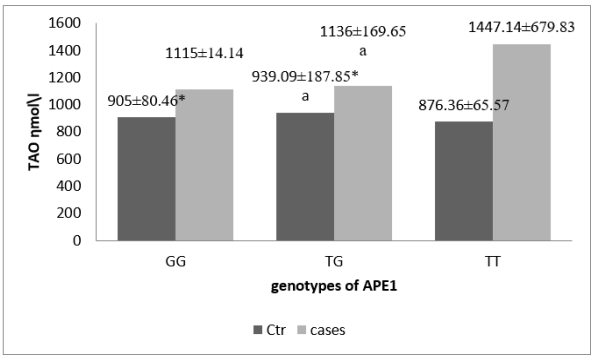

Figure 3: The TAO level in study subjects according to APE1 genotypes (significant differences p 0.002, * referred to significant among APE1 genotyping in the control group, a referred to significant differences between case and control have TG genotype)

The APE1 enzyme stimulates the activity of DNA-binding of redox-sensitive transcription factors by blocking the adjacent cysteine between a thiol group and disulfide bond, which is changed by the intracellular redox status. The biological importance of APE1 is demonstrated by evidence that reported the inability to establish homozygous-deficient APE1 mice or APE1 knockout mammalian cell lines.[27] APE1 have been found to be implicated in RNA metabolism.[28] and in modulation of transcriptional regulation by specific acetylation of the protein itself.[29] However, APE1 has many physiological and pathological processes, like tumorigenesis, angiogenesis, aging and oxidative stress signaling.[30]

The evidence of *in-vitro* studies documented that the APE1 expression is stimulated by oxidative stress and the APE1 knockdown enhanced genotoxicity and cell killing events by ROS, while APE1 overexpressed is protected cells.[30] The excessive generation of ROS causes the accumulation of DNA oxidative lesions, which are repaired by base excision repair. One of these repair enzymes is APE1, which works on genome maintenance and prevents ROS-induced apoptosis.[31] Another study observed the APE1 deficiency causes an elevation in ROS level in HeLa cells, implying that APE1 may play more than one role in the response to oxidative stress in the cells.[32,33] They

Table 2: The genotypes distribution of APE1 in study subjects

Geno typing	*Automobile technicians%*	*Control group%*	*Odd ratio CI 95%*	*p-value*
GG	6.66	12	0.8485, 0.1302–5.5292	0.8636
GT	70	44		
TT	23.33	44	3.0000, 0.9073–9.9197	0.0718
G	0.41	0.34	0.9437, 0.5308–1.6777	0.8434
T	0.58	0.66		

Table 3: The APE1 genotypes distribution according to the smoking (cigarette, hookah) habit, alcohol uptake and chronic disease in automobile technicians

Genotyping	*GG %*	*GT%*	*TT%*	X^2	*P*
Smoker	0	40	6.66	3.59694	0.1656
Non-smoker	6.66	30	16.66		
Hookah	0	36.66	3.33	4.60317	0.1001
Non-hookah	6.66	3.33	66.66		
Alcohol uptake	0	3.33	0	0.44335	0.8012
Non-alcohol uptake	6.66	66.66	23.33		
Chronic disease	0	16.66	6.66	0.718722	0.6981
Non-chronic disease	6.66	53.33	16.66		

suggested that APE1 may regulate the antioxidant system in cells across gene expression regulation of antioxidant enzymes. Moreover, which antioxidant enzymes APE1 controls subset and which mechanisms of the APE1 regulate the expression of antioxidant enzymes have yet to be understood.

CONCLUSION

The current study concluded that the APE1 gene polymorphism Asp148Glu didn't associate with automobile technicians that long exposure to lead and cadmium but was associated with elevation in TAO levels in workers.

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