

Molecular Analysis of CYP1A1 Gene Polymorphisms among Thermal Power Plant Workers in Thi-Qar, Iraq: A Case-Control Study

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ABSTRACT

Background: The industry, including electricity, plays the main part in civilised societies and through it, the development of the society can be evaluated in our current time. And it is also the backbone of the economy and the principal component in stabilising the countries to save their security and independence **Purpose:** This study examined CYP1A1 gene polymorphisms among thermal power plant workers in Thi-Qar, Iraq, exposed to polycyclic aromatic hydrocarbons (PAHs) and electromagnetic fields (EMF). **Methods:** A total of 100 samples were collected: 50 samples from workers who were exposed to occupational hazards directly in thermal power plants and 50 samples from apparently healthy people with no occupational exposure history to these materials. All blood samples were used for the extraction of genomic DNA, and then the polymerase chain reaction (PCR) technique was used to amplify the CYP1A1 gene in all samples. **Results:** Ala/Ala was reported in 74% of workers and 94% of controls, while Ala/Val was observed in 24% and 6%, respectively. The Ala/Val genotype was significantly more frequent among workers than controls (OR = 5.08, 95% CI: 1.33–19.33). The Val/Val genotype was observed in 2% of workers and was not detected in controls (OR = 3.80, 95% CI: 0.15–95.98). Among workers, the ORs for Ala/Val according to employment duration, residence, and educational level were 2.35, 0.61, and 0.90, respectively. For Val/Val, the corresponding ORs were 3.51, 0.60, and 7.86. However, these subgroup associations were not statistically significant. **Implications:** CYP1A1 genotype frequencies differed between thermal power plant workers and healthy controls, particularly for the Ala/Val genotype. Larger studies are needed to confirm these findings and clarify their potential implications for occupational exposure.

Keywords: Polycyclic aromatic hydrocarbons, Electromagnetic Fields radiation, CYP1A1 gene.

Article Information

Received: July 20, 2026; Revised: August 18, 2026; Online: September 2026

INTRODUCTION

The industry, including electricity, plays the main part in civilised societies and through it, the development of the society can be evaluated in our current time. And it is also the backbone of the economy and the principal component in stabilising the countries to save their security and independence. Despite all

that, negative and dangerous effects come from the electricity power on the environment. It is known that the electricity production by the fossil fuel (oil, coal and natural gas) leads to high levels of harmful emissions as carbon monoxide, nitrogen oxides, sulphur oxides and tiny particles like dust, in addition to

hydrocarbons. The main fuel source used in the Nasiriya Thermal Power Plant is black oil, which is polycyclic aromatic hydrocarbons (PAH) which is one of the main causes of environmental pollution and enters the human body through inhalation of small particles, as well as, with water and food and through exposure to the occupational environment, causing mutagenic and carcinogenic effects (1,2). Biotransformation of PAH occurs by cytochrome P450, mainly the *CYP1A1* gene, which is located on approximately 6.068 kilo base pairs (kbp) and contains seven exons that are separated by six introns (3). This gene, *CYP1A1*, encodes *CYP1A1* enzyme responsible for metabolism of carcinogens especially PAH to more soluble materials in the water to be easy to remove from the body to be secreted with sweat and urine after being conjugated with phase II enzymes but PAH be converted toxic or carcinogenic metabolites (4).

In addition, electromagnetic radiation produced by electric power generation stations can have various effects, such as differentiation and abnormalities in cell reproduction, blood disorders, congenital defects in cellular components and chromosomal abnormalities. Through exposure to magnetic fields that generate electric currents and fields, these waves can disrupt the body's physiological balance, increase the lifespan of free radicals, and cause DNA damage in individuals (5). In light of the above, the present study aimed to compare the distribution of *CYP1A1* genotypes between workers at the Nasiriya Thermal Power Station and an unexposed control group. The study also examined whether *CYP1A1* genotype

distribution among workers varied according to duration of employment, residence, and educational level.

METHODS

●Samples collection

A total of 100 blood samples were collected, including 50 samples from workers at Thermal Power Station representing the experimental group and 50 samples from individuals not exposed to the inputs and outputs of the station (such as university faculty, students, and self-employed individuals) representing the healthy control group. Blood samples (2–3 ml per person) were collected from both groups and preserved in tubes containing EDTA at a temperature of -20°C for DNA extraction. An information form was used for both the workers and healthy control groups, which included data on duration of work, residence, and educational level.

●DNA Extraction

Based on the protocol provided with the DNA Extraction Kit manufactured by Geneaid (Korean origin) extract the deoxyribonucleic acid (DNA).

●Polymerase Chain Reaction (PCR)

The PCR technique was used to amplify the *CYP1A1* gene. The primer sequences used are shown in table (1). the procedure was carried out using a reaction mixture of 20 µl as described in table (2), based on the protocol provided with the green master mix manufactured by Bioneer. after all components were added, the samples were mixed using a vortex device and then transferred to the thermal cycler, which was operated according to the program shown in table (3).

Table (1): Oligonucleotide primer sequences used for PCR amplification CYP1A1.

Gene		Primer sequences	Length	T _M	T _A
CYP1A1	F	CAGTGAAGAGGTGTAGCCGCT-3'5'-	21	62	57
	R	5'-TAGGAGTCTTGTCTCATGCCT-3'	21	62	57

TM=Melting Temperature, *TA*=Annealing Temperature, *R*=Reverse, *F*=Forward.

Table (2): The Reaction Mix (20μl) for CYP1A1 gene.

Chemicals	Volume
Master Mix	5 μl
Primer Forward	1μ 1
Primer Reverse	1μ 1
DNA	μl5
D.W.	μl8
Total volume	μl20

Table (3): PCR condition for amplification CYP1A1 gene.

No. of Stages	Steps	Temperature	Time	No. of Cycle
1	Denaturation 1	C94 °	5 min	1
2	Denaturation 2	C94 °	Sec.30	35
3	Annealing	C57°	Sec.30	
4	Extension 1	C72 °	Sec.45	
5	Extension 2	C72 °	10 min	1

After the completion of the Thermo Cycler program, the PCR products were subjected to electrophoresis, and the bands were visualized using ultraviolet (UV) light. A distinct band was observed at 360 base pairs (bp), indicating the presence of the *CYP1A1* gene, as confirmed by comparison with a DNA marker ranging from 100 to 1000 bp.

The *CYP1A1* gene was digested using the restriction enzyme Hpa II, which recognizes a specific tetra nucleotide sequence, in accordance with the protocol provided in the enzyme datasheet from Promega.

Recognition Sequence: 5'-C↓CG G-3'
3'-G GC↑C-5'

Table (4): The Reaction Mix (20μl) for cut CYP1A1 gene.

Material	Volume
PCR reaction mixture	μl1
RE 10x buffer	μl2
Acetylated BSA	μl0.2
Sterile, deionized water	μl16.3
Hpa II (Restriction Enzyme)	μl0.5
Total	μl20

The reaction mixture was then incubated at 37°C for four hours. Although the enzyme is effective within a range of one to sixteen hours the best results were obtained after four hours of incubation. The samples were subsequently subjected to electrophoresis. The appearance of a band at 360 bp indicated the wild-type

genotype (Ala/Ala), while the presence of bands at 140 bp, 220 bp, and 360 bp corresponded to the heterozygous mutant genotype (Ala/Val). In contrast, bands appearing at 140 bp and 220 bp represented the homozygous mutant genotype (Val/Val) (6).

Statistical Analysis

Statistical analysis was performed for all studied samples using the Chi-square test (χ^2) at a significance level of $P \leq 0.05$. The odds ratio (OR) test was also used to analyze the genotype frequencies of the *CYP1A1* gene. All analyses were conducted using SPSS software (7).

RESULTS

Distribution of worker and healthy control samples based on (duration of employment, residence, and educational level). The worker

group was categorized based on duration of employment into several groups, as shown in Table (5). It was observed that the highest percentage of employment duration was 38% within the 10–19 years' category, while the lowest was 2% in the 40–49 years' category. Both the worker and healthy control groups were also classified based on residence, as illustrated in Table (6). It was found that 68% of workers and 88% of healthy individuals lived in urban areas, while those living in rural areas constituted 32% of workers and 12% of healthy controls. Furthermore, the two groups were classified based on educational level, as shown in Table (7). The percentage of educated individuals was 72% among workers and 80% in the healthy group, while uneducated individuals represented 28% of workers and 20% of the healthy group.

Table (5): Distribution of the group of employees according to the duration of work.

Duration of work (year)	Number of workers	
	N	%
1-9	15	30%
10-19	19	38%
20-29	10	20%
30-39	5	10%
40-49	1	2%
Total	50	100%

Mean = 16.25.

Table (6): Distribution of the two groups residence area.

Residence area	Healthy group		Workers group	
	N	%	N	%
rural areas	6	%12	16	%32
urban areas	44	%88	34	68%
Total	50	%100	50	100%

$\chi^2 = 5.82$, $df = 1$, $P = 0.016$.

Table (7): Distribution of the two groups according to educational level.

Education level	Healthy group		Workers group	
	N	%	N	%
Educated	40	80%	36	72%
Uneducated	10	20%	14	28%
Total	50	%100	50	100%

$$\chi^2 = 0.87, df = 1, P = 0.35.$$

■Study of Genotype Frequencies of the *CYP1A1* Gene

The results of the current study, as shown in Table (8), indicate differences in *CYP1A1* genotype frequencies between workers and healthy controls. The frequency of the wild-type genotype (Ala/Ala) was 74% in the worker group and 94% in the healthy control group. The frequency of the heterozygous mutant genotype (Ala/Val) was 24% in workers and 6% in healthy individuals, with an odds ratio (OR) of 5.08.

Meanwhile, the frequency of the homozygous mutant genotype (Val/Val) was 2% in workers compared to 0% in healthy controls, with an OR of 3.80. Among workers, the ORs for the Ala/Val genotype according to employment duration, residence, and educational level were 2.35, 0.61, and 0.90, respectively. For the Val/Val genotype, the corresponding ORs were 3.51, 0.60, and 7.86. However, these subgroup associations were not statistically significant.

Table (8): Frequency of genotypes for *CYP1A1* gene for the healthy and working groups.

Genotypes	Healthy group (%)n=50	Workers group (%)n=50	OR	95% CI
Ala / Ala	47 (94 %)	37(74%)	1.0	————
Ala / Val	3(6%)	12(24%)	5.08	1.33–19.33
Val / Val	0(0%)	1(2%)	3.80	0.15–95.98
Total	50(100%)	50(100%)		

OR= Odds Ratio, 95 % CI= Confidence Interval

Table (9): Frequency of genotypes for *CYP1A1* gene in samples of workers according to length of work

Genotypes	Less than 15 years (%)n=24	15 years and over (%)n=26	OR	95% CI
Ala / Ala	20 (83.3 %)	17(65.40%)	1.0	————
Ala / Val	4(16.7%)	8 (30.76%)	2.35	0.60–9.19
Val / Val	0(0%)	1(3.84%)	3.51	0.13–91.87
Total	24(100%)	26(100%)		

OR= Odds Ratio, 95 % CI= Confidence Interval.

Table (10): Frequency of genotypes for the *CYP1A1* gene in workers by residence.

Genotypes	(%)urban areas n=34	Rural areas (%)n=16	OR	95% CI
Ala / Ala	24(70.58%)	13(81.25%)	1.0	———
Ala / Val	9(26.47 %)	3(18.75 %)	0.61	0.14–2.67
Val / Val	1(2.95%)	0(0%)	0.60	0.02–15.98
Total	34 (100%)	16 (100%)		

OR= Odds Ratio, 95 % CI= Confidence Interval.

Table (11): Frequency of genotypes for *CYP1A1* gene in samples of workers by education.

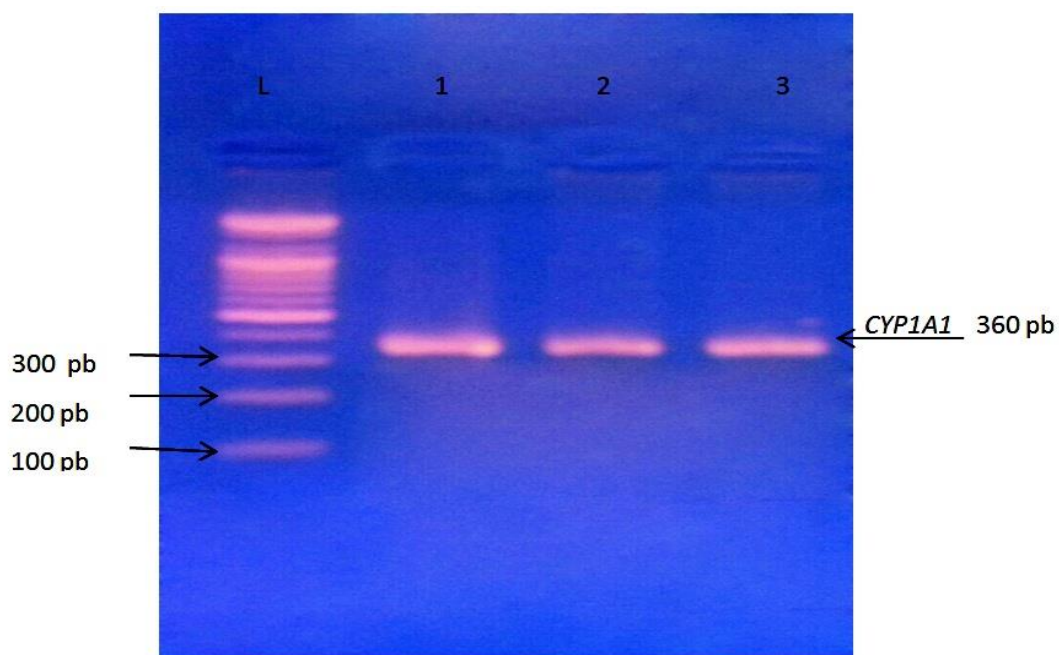
Genotypes	Educated (%)n=36	Uneducated (%)n=14	OR	95% CI
Ala / Ala	27(75.00%)	10(71.43%)	1.00	———
Ala / Val	9(25.00%)	3(21.43%)	0.90	0.20–4.01
Val / Val	0(0%)	1(7.14%)	7.86	0.30–208.54
Total	36(100%)	14(100%)		

● Molecular Genetics Study

The presence of DNA bands extracted from workers and healthy individuals was detected using electrophoresis on 0.8% agarose gel following the method of (8), as shown in Figure (1).

After amplification of the *CYP1A1* gene using PCR technique, its presence was confirmed by electrophoresis of the PCR products on 2% agarose gel, where the gene bands appeared at 360 base pairs (bp), as shown in Figure (2). Subsequently, the PCR products of the *CYP1A1* gene were analyzed using the restriction enzyme *HpaII*, as illustrated in Figure (3).

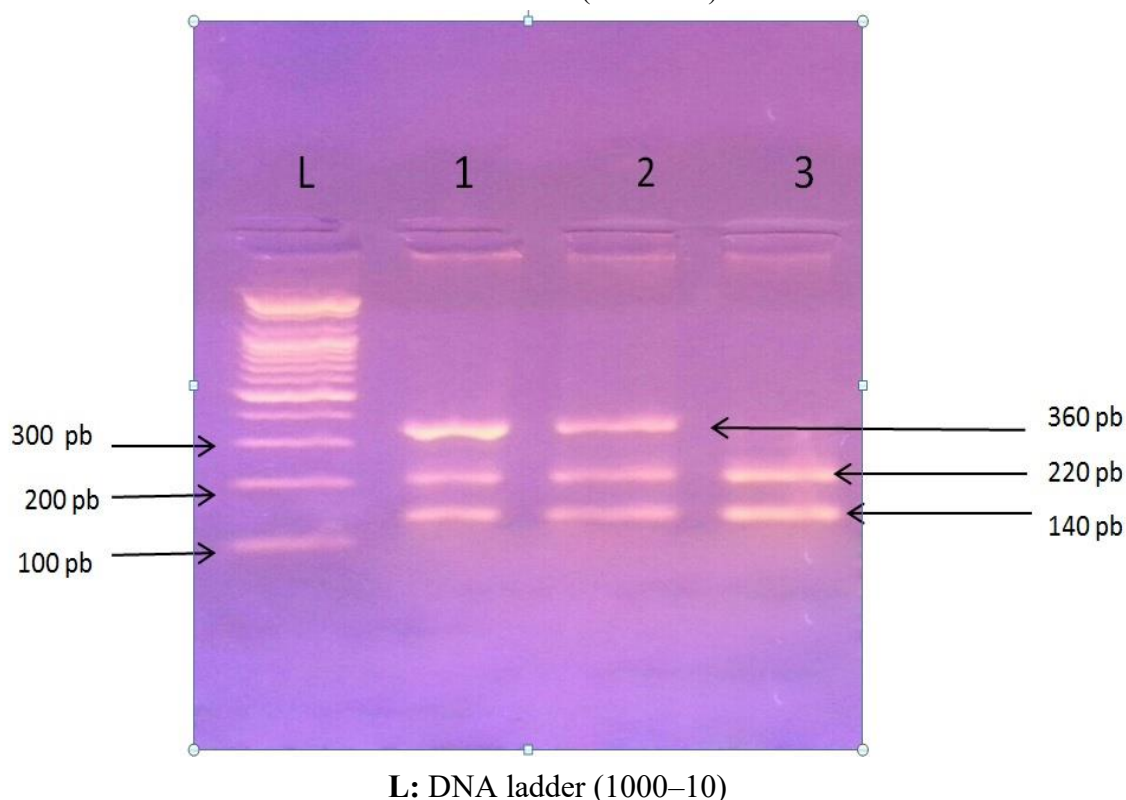
**Figure (1):** Electrophoresis of deoxyribonucleic acid (DNA) on 0.8% agarose gel.



Lane 1, 2, 3: represent the homozygous wild-type genotype Ala/Ala

Figure (2): electrophoresis of PCR products on 2% agarose gel

L: DNA ladder (1000–100).



L: DNA ladder (1000–10)

Lane 1, 2: represent the heterozygous mutant genotype Ala/Val

Lane 3: represents the homozygous mutant genotype Val/Val

Figure (3): analysis of PCR products of the *CYP1A1* gene using the restriction enzyme HpaII.

DISCUSSION

Both environmental and genetic factors contribute to the development of cancer and hereditary diseases, as some cancers are not inherited, but rather occur as a result of exposure to some factors called carcinogens, and these carcinogens can be environmental factors or chemical factors that result from cellular metabolism, such as oxygen species (9). ROS, which has a tendency to interfere with DNA and thus lead to mutation (10). In recent years, a new field in cancer research has focused on the interaction between genes and the environment to understand the pathogenesis of cancer. The initial focus of studying this interaction is attributed to genes related to metabolism, as it is known that most carcinogens require effective metabolic processes in the human body (11). Infectious diseases remain a public health concern in Thi-Qar (12).

The CYP1A1 gene plays an important role in the metabolism of carcinogens, particularly polycyclic aromatic hydrocarbons such as benzo (a) pyrene. CYP1A1-mediated metabolism can generate reactive metabolites that may contribute to DNA damage and carcinogenic processes (13). In May 2011, the International Agency for Research on Cancer (IARC) classified electromagnetic field (EMF) radiation as a possible carcinogen that may transform normal cells into cancerous ones. Human epidemiological studies have shown evidence of an increased risk of glioma and acoustic neuroma. Additionally, EMF radiation has been found to affect protein synthesis, impacting enzymes and altering the binding process of amino acids, which leads to a reduction in their catalytic activity (14). High environmental levels of electromagnetic fields can increase the lifetime of free radicals, resulting in damage to DNA (15). In addition to various effects on Cellular components, including disorders of cell proliferation and differentiation (16). chromosomal

abnormalities, birth defects and various genetic mutations associated with exposure, Long-term exposure to electromagnetic fields (5).

These findings suggest that occupational exposure may be associated with differences in CYP1A1 genotype frequencies. Previous studies have also reported associations between occupational PAH exposure, CYP1A1-related alterations, and oxidative DNA damage (17). which showed the effects of the *CYP1A1* gene on DNA damage as a result of exposure to environmental and occupational hazards. Another study also showed that exposure of workers in thermal electric power plants, PAH and magnetic field radiation cause an increase in chromosomal abnormalities (18).

The results of our study also agreed with the results of another study that indicated a strong relationship between the incidence of malignant diseases and brain tumours among workers in thermal electric power plants and exposure to electromagnetic field radiation (18,19).

While the results of our study do not agree with the study by (19), which showed no clear associations between exposure to electromagnetic field (EMF) radiation and the development of brain tumors, breast cancer, or hematologic malignancies. A study conducted in Sweden found no evidence that emissions from petrochemical industries increased overall cancer incidence in the studied area (20).

Regarding duration of employment, workers with 15 years or more of employment showed higher OR estimates for the Ala/Val genotype (OR = 2.35) and the Val/Val genotype (OR = 3.51) compared with those employed for less than 15 years. However, these associations were not statistically significant, as shown in Table (9). These observed differences may be related to the genotoxic effects of environmental pollutants, such as polycyclic

aromatic hydrocarbons, dependent on the dose. Many studies indicate that prolonged exposure of PAH leads to the accumulation of its metabolites in the blood, which results in a series of cellular changes that ultimately lead to mutations in DNA (21).

These results showed, as shown in Table (10), that there is no significant difference when comparing workers who live in urban areas and rural areas, as the variant mutant type (Ala/Val) created a risk rate (OR=0.61), as for the mutant model. The homozygous (Val/Val) had a odds ratio (OR=0.60). In contrast, a study conducted in Benin reported significantly higher levels of DNA adducts among urban populations exposed to air pollution compared with rural and less-exposed populations (22).

As for the role of educational level and its relationship to the development of mutations in the *CYP1A1* gene, our results indicated that there is no statistically significant difference between educated and uneducated workers in the heterozygous genotype (Ala/Val) (OR=0.90), while a higher odds ratio was observed for the homozygous genotype (Val/Val) (OR = 7.86), this association was not statistically significant, as shown in Table (11).

The reason for this is that the educational level is not only important in improving work-related experiences, but it is also necessary in improving the ability of the workers themselves on how to deal with hazardous chemicals, which in turn enhances the worker's ability to solve health problems related to occupational exposure to hazardous materials (23). This is consistent with the results of another study that indicated that workers who had more education had fewer health problems compared to workers who were less educated or who had not received any education (23). A study of automobile mechanics occupationally exposed to PAHs reported increased markers of genetic damage and emphasized the importance of occupational hazard awareness (24).

CONCLUSION

The present study found differences in CYP1A1 genotype frequencies between thermal power plant workers and healthy controls. The genotype of Ala/Val was more frequent among workers than controls, suggesting that this genotype may be associated with increased susceptibility in occupationally exposed individuals. Although differences in distribution of genotype were also observed according to employment duration, residence, and educational level, significantly statistically different and should be interpreted cautiously. These results concentrate on the importance of occupational health monitoring among workers of thermal power plants exposed to environmental pollutants. Regular medical examinations, appropriate occupational safety measures, and health education are recommended to eliminate potential health risks. Other studies with larger sample sizes and more detailed assessments of occupational exposures are needed to clarify the relationship between CYP1A1 polymorphisms and occupational exposure.

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