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Waterpipe tobacco smoking and gene variants of CYP1A1-Ile^{462}Val and -MspI polymorphisms are possibly associated with the risk of lung cancer in the Iraqi population

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ABSTRACT

Background: Previous studies about the correlation of genetic polymorphisms in the multigene family of cytochrome P450 (CYPs), the effect of tobacco smoking, and the risk of developing cancer have been well investigated in different populations, but not in Iraq. Furthermore, the studies of malignance occurrence relationship with cigarette tobacco smoking revealed the presence of strong association, however, little is known about the risk of Waterpipe (WP) tobacco smoking. Thus, determination two important genetic polymorphisms in CYP1A1, a main member of CYPs, among Iraqi men was our first aim. This is the first study that highlights the correlation of CYP1A1 polymorphisms with the risk of lung cancer in Iraq. The second aim was to evaluate the combined association of WP tobacco smoking and CYP1A1-Ile^{462}Val and -MspI polymorphisms in lung cancer risk.

Methods: This study included 123 lung cancer patients and 129 controls. To determine the variant genotypes, the techniques of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA sequencing were carried out.

Results: The data revealed the possible associations of variant (G) allele of CYP1A1-Ile^{462}Val (OR = 1.6; 95% CI = 1.1–2.4; $P = 0.01$) and variant (C) allele of CYP1A1-MspI (OR = 1.9; 95% CI = 1.3–2.7; $P < 0.01$) with the risk of lung cancer. The variant genotypes of CYP1A1 polymorphisms were significantly correlated in the case of squamous cell carcinoma and synergistically associated in the case of combined effect with WP tobacco smoking ($OR_{Ile/Val}^{462} = 2.0$; 95% CI = 1.0–4.1; $P = 0.04$, and $OR_{MspI} = 2.6$; 95% CI = 1.3–5.5; $P \leq 0.01$).

Conclusion: The results suggest that WP tobacco smoking and genetic polymorphisms in CYP1A1 are most likely important risk factors for lung cancer in the Iraqi population.

1. Introduction

Waterpipe smoking also referred to as hookah, shisha or narghile, is a type of tobacco smoking prevalent in the Middle East, parts of Asia, and Eastern Mediterranean (Fig. 1). The danger of WP smoking is belonged to the prolonged exposure to smoke, where, the exposure time in a single session may last for an average of 1 h. Despite the fact that cigarette smoking is one of the main risk factors of lung cancer, very little is known about the association of WP tobacco smoking with lung cancer. Nevertheless, the limited available studies indicated that WP smokers have a higher tendency to develop lung cancer than non-smokers (Aoun et al., 2013; Awan et al., 2017; Koul et al., 2011). There is sufficient evidence that revealed the presence of many diagnosed chemical toxicants and carcinogens in WP tobacco smoke (WHO, 2014). Indeed, WP smokers are exposed to significant levels of some of these carcinogens, such as carbon monoxide (CO) and polycyclic aromatic hydrocarbons (PAHs), which are greater than what cigarette smokers are exposed to. Indeed, WP smokers are exposed to about 242 to 2359 mg of tar, 1.04 to 7.75 mg of nicotine, and 57.2 to 367 mg of carbon monoxide (CO) in a single smoking session. It was estimated that each session of WP smoking is equivalent to 25, 11, and 2 cigarettes enriched of tar, CO, and nicotine respectively (Primack et al., 2016). Moreover, significant levels of aldehydes, CO, PAHs, and respirable ultrafine particles are emitted directly from WP to the surrounding atmosphere, the second-hand WP smoke represents a further additional risk factor (Fromme et al., 2009).

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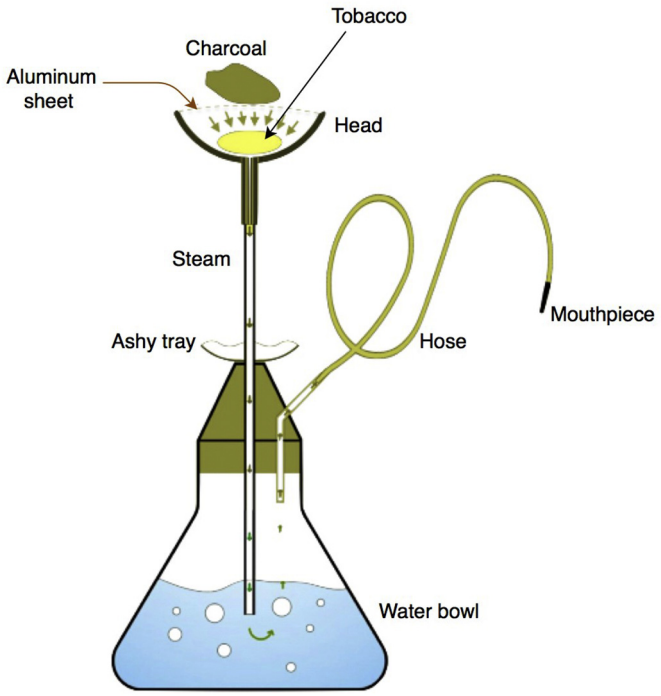

Fig. 1. Schematic diagram of waterpipe tobacco. The tool of Shisha consists of a head where the tobacco is sited. Briquette or charcoal is placed on top of tobacco, usually separated by a perforated sheet of aluminum foil. The bottom of tobacco bowl is also perforated which allows the smoke to pass into a conduit submerged in a second bowl half-filled with water. At the top of the water bowl, a valve is present and connected with a leather hose ended with a mouthpiece part, through which the smokers draw the smoke. The pulled air passes through the coal into the tobacco which consequently leads to bubble the smoke in water before being transferred through the hose to the mouthpiece part.

The exposure to environmental risk factors can increase the individual susceptibility to cancers. Therefore, much attention has been given to explore the genetic variations among phase I of xenobiotic detoxification enzymes. Smoking-related alterations by partial or complete CYPs' promoter methylation were found very common among tobacco smokers (O'Malley et al., 2014). Cytochrome P450 is a multigene family with specificity to different substrates, CYPs represent phase I enzymes required for catalyzing the first oxidative reaction in the metabolism of PAHs, whose compounds have aromatic and cyclic rings and the ability to induce DNA mutations (Anttila et al., 2011; O'Malley et al., 2014).

CYP1A1 which has a crucial role in the metabolisms of PAHs has been identified with several polymorphic forms. Two SNPs, however, have been extensively studied and found to be functionally important; the rs1048943 an (A > G) transition located in the exon 7 which results in an exchange of isoleucine to valine ($Ile^{462}Val$) in the heme-binding site of CYP1A1. The other SNPs, rs4646903 is a T6235C substitution located in the 3′ non-coding area that leads to generating MspI recognition site (Hayashi et al., 1991; Song et al., 2001). The first SNP was found to increase the microsomal activation, while the second has an inducible effect on the activity of hydrocarbon hydrolase (Lin et al., 2000; Smith et al., 2001). Although, several studies have been carried out to assess the effect of genetic variability of CY1P1A1 on the risk of developing lung cancer, inconsistent results among different ethnic populations and even within the same population were reported. Genetic and environmental risk factors in addition to the histological subtypes of lung cancers might be the main reasons behind this discrepancy.

Genetic polymorphisms may alter gene expression or even have deleterious effects on the structure-function properties of genes products, which can increase the risk of cancers. Consequently, extensive efforts have been directed to study the association between genetic polymorphisms, environmental risks factors, and clinical diseases. This study aimed to evaluate the association of genetic polymorphisms of CYP1A1-$Ile^{462}Val$ and -MspI (rs1048943 and rs4646903) with the risk of lung cancer, and to highlights the influence of WP smoking as a genetic modifier agent. Although, several studies have described the association of lung cancer and/or other cancer types occurrence with the genetic polymorphisms of CYP genes, most of these studies were done in populations that used to smoke cigarettes, but not WP tobacco (Duan et al., 2012; García-González et al., 2012; Islam et al., 2013; Ji et al., 2013; Li et al., 2010; Liang et al., 2005; Zhang et al., 2017; Zhu et al., 2018). To the best of our knowledge, no study has examined the combined association of WP tobacco smoking and the genetic polymorphisms of CYP1A1 in the risk of lung cancer, neither in Iraq nor worldwide. However, only one study conducted in Kashmir (India) studied the association of CYP1A1 (rs4646903), WP smoking, and consumption of salted tea with the risk of esophageal cancer (Malik et al., 2010).

2. Materials and methods

2.1. Population of the study

The number of this study subjects was 252, the lung cancer patients, who were diagnosed at the Middle Euphrates Cancer center (MECC) in Najaf city between December 2017 and June 2019, was 123 subjects. One hundred and twenty-nine control subjects, who represented the cancer-free population, were also included in this study. All of the subjects (patients and controls) were male, who have been carefully checked for their medical and smoking history. For lung cancer diagnosis, clinical and routine laboratory examinations along with the cytological or histopathological examination of tumour biopsies were carried out. Regarding the level of WP smoking, this study included only smokers who used to smoke WP at a level of > 240 sessions/day ∗ year and excluded those who used to smoke both WP and cigarette. This study was conducted after getting written consent by each participant to be involved in this study, and the study was ethically approved by the committee of Faculty of Science at the University of Kufa.

2.2. DNA extraction and amplification

To isolate the genomic DNA from patients and controls, 5 ml of peripheral blood were collected from each participant and stored at −20 °C until usage. Extraction of DNA was carried out using “DNA Mini and Blood Mini Kit” supplied by Qiagen. Oligonucleotide primers and

Table 1
Information of PCR-RFLP assay used to analyze the genetic polymorphisms of CYP1A1.

Gene (dbSNPs)	Primers (5′ to 3′)	Restriction enzyme	Alleles (resulted fragment)
CYP1A1 (rs1048943)	Sense: CCCATCTGAGTTCCTACCTGAACG Antisense: CAACCAGACCAGGTAGACAGAGTC	BsrDI	A = digested fragment (114 and 204 bp) G = intact fragment (318 bp)
CYP1A1 (rs4646903)	Sense: GAGGAGGTAGCAGTGAAGAGGTG Antisense: GAGAGGGCGTAAGTCAGCACAG	MspI	T = intact fragment (379 bp) C = digested fragment (144 and 235 bp)

the restriction enzymes used for the PCR-RFLP assay, along with the lengths of generated and digested DNA fragments according to the types of alleles are shown in Table 1.

DNA amplification was carried out using Q5 DNA polymerase, which is known to amplify DNA with ultra-low error rate with ~280 times higher fidelity than *Taq*. The procedure was done according to the instructions supplied with "Q5 High-Fidelity 2X Master Mix kit", which was provided from NEB. PCR reaction was performed in a total volume of 20 µl, each tube contained 50 ng of isolated genomic DNA, 10 µl of Q5 master mix, 0.2 µM of each primer, and nuclease-free water was added to complete the final volume. The melting temperature of all primers was adjusted to be 58 °C. All restriction enzymes were purchased from NEB, and the protocols of digestion were set according to the instructions of the company, which can be accessed using the NEBcloner online tool. Resolving the resulted DNA fragments on 1% agarose gel stained with GelRed (Biotium) was performed. To confirm the results of polymorphisms obtained by PCR-RFLP experiment, DNA fragments were extracted from agarose gel using QIAquick kit (Qiagen) and analyzed for DNA sequencing conducted by Macrogen.

2.3. Genotype determination

The Polymerase Chain Reaction (PCR) combined with the procedure of Restriction Fragment Length Polymorphism (RFLP) was done to analyze the genotypes and alleles of each CYP1A1 single nucleotide polymorphism (SNP). Fig. 2 depicts the CYP1A1 SNPs analysis using PCR-RFLP assay paralleled with a descriptive diagram. Further information regarding reference gene sequences, positions of the recognition sequence of each restriction enzyme, the annealing sites of each primer pair, and the positions of CYP1A1 SNPs are shown in Fig. S1.

2.4. Statistical analysis

Logistic regression was carried out for odds ratio (ORs) calculation with 95% confidence intervals (CIs), this test was used to evaluate the association strength between CYP1A1 gene polymorphisms and lung cancer risks. The univariate estimation of ORs (95% CIs) was used according to Mantel–Haenszel method to evaluate the association between CYP1A1 polymorphisms among smoker and non-smoker or according to the histological type of cancer with the risk of lung cancer. Test of Hardy–Weinberg equilibrium was applied for allele frequencies distribution in both cases and controls, and the Chi-square test was used for significance calculation of any deviation between the expected and observed frequencies. All statistical tests of this study were two-sided with $P < 0.05$ as a level of significance. The Version 23.0 of SPSS software was used for all statistical analyses.

3. Results

3.1. Demographic characterizations

The demographic and clinical data showed no significant difference regarding the age groups of cases and controls, but there was a statistical difference among cases and controls with respect to smoking status. As WP tobacco smoking is not popular among women in the population of Iraqis, thus we did not include women subjects in this study. Histological diagnosis of lung cancer types among patients revealed a following frequency ratio of each type; 47.1% for adenocarcinoma (AD), 43.1% for squamous cell carcinoma (SCC), and 9.8% for the other forms (Table 2).

3.2. CYP1A1 SNPs distribution among patients of lung cancer and individuals of control

Genotypes and alleles distribution of CYP1A1-rs1048943 (CYP1A1-

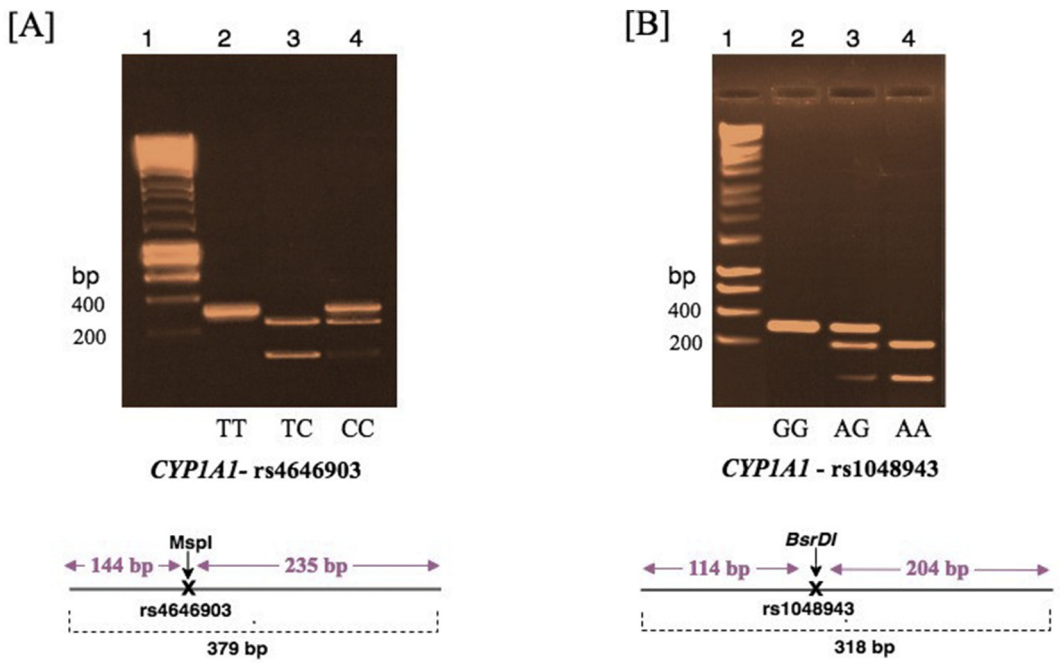

Fig. 2. Single nucleotide polymorphisms analysis of CYP1A1 using PCR-RFLP assay. [A] PCR amplification of rs4646903 containing the region of CYP1A1 was digested with MspI. The cleavage site is only available when the CYP1A1 variant T > C is present, which produces two fragments: 144 and 235 bp. The presence of wild type allele T, however, gives a single amplicon only (379 bp). Lane 1, DNA marker; lanes 2, 3, and 4, are homozygous major TT, heterozygote TC, and homozygous minor CC respectively. [B] The amplicon of CYP1A1 containing rs1048943 (318 bp) was digested with BsrDI. The cleavage site is cut only if the wild allele is present. DNA cleavage results in producing two fragments: 114 and 204 bp. Lane 1, DNA marker; lanes 2, 3, and 4 are homozygous minor GG, heterozygote AG, and homozygous major AA respectively. The diagram below each electrophoresis image depicts the positions of cleavage of each variant by the aforementioned restriction enzymes.

Table 2
Baseline and clinical features of cases and controls.

	Case (n = 123)	Control (n = 129)	P^a
Age group			0.48
≤50 yrs	75 (61.1)	73 (56.6)	
> 50 yrs	48 (38.9)	56 (43.4)	
Gender			
Male	123 (100)	129 (100)	
WP smoking			< 0.001
Non-smoker	37 (30.1)	78 (60.5)	
Smoker	86 (69.9)	51 (39.5)	
Histology			
Adenocarcinoma	58 (47.1)		
Squamous cell carcinoma	53 (43.1)		
Other	12 (9.8)		

[a] Chi-squared *P*-value.

Table 3
Distribution analysis of genotypes and alleles of CYP1A1 variants among lung cancer patients and controls.

	Case (n = 123) n (%)	Control (n = 129) n (%)	OR (95% CI)	P^a
CYP1A1-Ile^{462}Val				0.03
AA	55 (44.7)	79 (61.2)	Reference	
AG	62 (50.4)	46 (35.7)	1.9 (1.16–3.24)	
GG	6 (4.9)	4 (3.1)	2.2 (0.58–7.99)	
Allele				0.01
A	172 (69.9)	204 (79.1)	Reference	
G	74 (30.1)	54 (20.9)	1.6 (1.1–2.44)	
CYP1A1-MspI				< 0.01
TT	43 (35)	72 (55.8)	Reference	
TC	65 (52.8)	49 (38)	2.2 (1.3–3.8)	
CC	15 (12.2)	8 (6.2)	3.1 (1.2–8.0)	
Allele				< 0.01
T	151 (61.4)	193 (74.8)	Reference	
C	95 (38.6)	65 (25.2)	1.9 (1.3–2.7)	

[a] Chi-squared *P*-value.

Ile^{462}Val) and CYP1A1-rs4646903 (CYP1A1-MspI) were analyzed using Hardy–Weinberg equilibrium in both cases and controls. Results showed that the frequencies of CYP1A1-Ile^{462}Val A wild (wt) and G variant (vt) alleles were 69.9% and 30.1% in patients, while 79.1% and 20.9% in controls, respectively. A similar distribution of T (wt) and C (vt) alleles can be seen in the case of CYP1A1-MspI, where the percentages in cases were 61.4% and 38.6%, and in the controls were 74.8% and 25.2% respectively (Table 3).

Statistically significant increases ($P \leq 0.005$) were detected in the distribution of both genotypes AG (OR = 2.1, 95% CI = 1.3–3.6) and GG (OR = 3.6, 95% CI = 1.1–12.4) of CYP1A1-Ile^{462}Val; and also significant rise ($P \leq 0.003$) was observed in the distribution of TC and CC genotypes of CYP1A1-MspI (OR = 2.2, 95% CI = 1.3–3.8) and (OR = 3.1, 95% CI = 1.2–8.0) respectively among lung cancer patients compared to individuals of controls (Table 3).

3.3. CYP1A1 polymorphisms distribution according to the histological types of lung cancer

Two prevalent histological types of lung cancer were diagnosed among lung cancer patients; AD and SCC. When patients were evaluated at the beginning, AD was a bit more prevalent (47%) than SCC (43.1%). Subpopulation of cases according to the histological types and genotypic distribution of CYP1A1 polymorphisms showed that lung cancer patients with SCC had significantly higher frequencies of CYP1A1-Ile^{462}Val AG and GG genotypes (64.2% and 5.6%) than their distribution frequencies among AD (43.0% and 3.5%), the odds ratios were (OR = 3.6, 95% CI = 1.82–7.32) and (OR = 3.6, 95% CI = 1.82–7.32) respectively. Similarly, the distribution of CYP1A1-MspI TC and CC genotypes was higher among patients with SCC (64.1% and 15.1% respectively) than those with AD type (48.3% and 8.6% respectively), with OR = 4.5, 95% CI = 2.10–9.81 and OR = 6.5, 95% CI = 2.04–21.03 respectively (Table 4).

3.4. Relationship of genotypic polymorphisms of CYP1A1 variants and WP tobacco smoking

The association between genotypic polymorphisms and WP smoking with the risk of lung cancer revealed that the distribution of Ile/Val + Val/Val (AG + GG) variants of CYP1A1-Ile^{462}Val among WP smokers was 2.0 folds higher than the distribution of Ile/Ile (AA) genotype (95% CI = 1.0–4.1). Similarly, smokers who were the carrier of the variant genotypes (wt/vt + vt/vt) of CYP1A1-MspI showed a higher tendency to develop lung cancer (2.6 folds) than that of wt/wt (TT). The differences were significant for both CYP1A1 SNPs ($P \leq 0.05$ for CYP1A1-Ile^{462}Val, and $P < 0.01$ for CYP1A1-MspI) (Table 5). The difference between the distribution frequencies of the variant genotypes (wt/vt + vt/vt) of CYP1A1-Ile^{462}Val and -MspI among smokers and the wild genotypes (wt/wt) of the non-smokers is strongly significant ($P < 0.0001$). WP smokers who were the carriers of both variant genotypes of CYP1A1-Ile^{462}Val and -MspI had 5.2- and 5.7-folds increased risk of developing lung cancer in comparison with the non-smokers who carried wild genotypes (Table S1). In contrast, no significant association was noticed between the genotypes of both CYP1A1 SNPs among the non-smokers and the risk of lung cancer (Tables 5, S1).

4. Discussion

Lung cancer is the most common cancer worldwide in the terms of

Table 4
Genotypes distribution of CYP1A1 variants in patients with lung cancer according to the histological types of cancer.

	Control (n = 129)	AD[b] (n = 58)	OR (95% CI)	P^a	SCC[c] (n = 53)	OR (95% CI)	P^a	Other (n = 12)	OR (95% CI)	P^a
	n (%)	n (%)			n (%)			n (%)		
CYP1A1-Ile^{462}Val				0.60			< 0.001			0.53
AA	79 (61.2)	31 (53.5)	Reference		16 (30.2)	Reference		8 (66.7)	Reference	
AG	46 (35.7)	25 (43.0)	1.4 (0.73–2.63)		34 (64.2)	3.6 (1.82–7.32)		3 (25.0)	0.6 (0.16–2.55)	
GG	4 (3.1)	2 (3.45)	1.3 (0.22–7.31)		3 (5.6)	3.7 (0.75–18.17)		1 (8.3)	2.5 (0.24–24.84)	
CYP1A1-MspI				0.27			< 0.001			0.33
TT	72 (55.8)	25 (43.1)	Reference		11 (20.8)	Reference		7 (58.3)	Reference	
TC	49 (38)	28 (48.3)	1.6 (0.86–3.15)		34 (64.1)	4.5 (2.10–9.81)		3 (25.0)	0.6 (0.16–2.55)	
CC	8 (6.2)	5 (8.6)	1.8 (0.54–6.01)		8 (15.1)	6.5 (2.04–21.03)		2 (16.7)	2.6 (0.45–14.55)	

[a] Chi-squared *P*-value.
[b] Adenocarcinoma.
[c] Squamous cell carcinoma.

Table 5
Analysis of association between WP tobacco consumption and CYP1A1 polymorphisms in lung cancer risk.

	Non-smokers			Smokers		
	Ca (n:37)/Co (n:78) n (%)/n (%)	OR (95% CI)	P^a	Ca (n:86)/Co (n:51) n (%)/n (%)	OR (95% CI)	P^a
CYP1A1-Ile^{462}Val			0.73			0.04
AA	23 (62.2)/51 (65.4)			32 (37.2)/28 (54.9)	Reference	
AG + GG	14 (37.8)/27 (34.6)	1.1 (0.5–2.5)		54 (62.8)/23 (45.1)	2.0 (1.0–4.1)	
CYP1A1-MspI			0.53			< 0.01
TT	20 (54.1)/47 (60.3)			23 (26.7)/25 (49.0)		
TC + CC	17 (45.9)/31 (39.7)	1.3 (0.6–2.8)		63 (73.3)/26 (51.0)	2.6 (1.3–5.5)	

[a] Chi-squared *P*-value.

incidence and mortality (Bray et al., 2018). Here in Iraq, according to the cancer report conducted by the ministry of health in 2014, lung cancer is the second most common tumour after breast cancer in both genders (Iraqi Cancer Board, 2018). WP smoking is common in the Middle East, and extremely popular among Iraqi young men. However, we noticed lack of information regarding the correlation between WP tobacco smoking and genetic polymorphisms of phase I metabolic enzymes in the risk of lung cancer. Abnormal alteration in the level of gene expression of those genes responsible for the metabolisms of carcinogens, such as genes encoding the superfamily of CYP and GST proteins, or those that encode DNA repair proteins, might lead to implications that increase the risk of developing cancer (Li et al., 2014; Mclemore et al., 1990; Zhao et al., 2015). Studies of molecular-epidemiology indicate that genetic polymorphisms are a considerable stimulator of lung cancers, and environmental risk factors can induce the occurrence of these polymorphisms. Thus, identifying the genetic polymorphisms in susceptibility markers of lung cancers may provide a better understanding of the mechanisms by which the environmental risk factors affect gene products function. Therefore, the association analysis between the two CYP1A1 polymorphisms and susceptibility to lung cancer among WP tobacco smokers and non-smokers was conducted in this study.

In the current study, the difference in the frequency of distribution of CYP1A1-Ile^{462}Val and -MspI genotypes between cases and controls was statistically significant. Our data also showed a significant prevalence of variant genotypes carriers of both CYP1A1 SNPs in SCC type. Similarly, a study conducted in the Egyptian population in lung cancer patients revealed that the higher frequency of genotypes distribution were among the (wt/vt) and (vt/vt) genotypes of both CYP1A1 polymorphisms (Hussein et al., 2014). Another study also performed in the Egyptian population showed a significant association between CYP1A1-Ile^{462}Val polymorphisms and the risk of lung cancer (Ezzeldin et al., 2017). The results of the current study are also consistent with studies conducted in non-Arab ethnic populations. Motovali-Bashi et al. demonstrated an increased risk of developing lung cancer in carriers of (wt/vt) genotype of CYP1A1-MspI in Iranian population (Motovali-Bashi et al., 2012). Thus, our results are consistent with the Egyptians population, which represent an Arab ethnic population same as Iraqis, and with the Iranians population where an ancient ethnic-mixing had happened between Persians and Iraqis. Other studies carried out in Indian, Chinese, Spanish, Bangladeshi, and Kashmiri populations showed a similar correlation between the distribution frequency of CYP1A1 variants and the increased risk of lung cancer (Girdhar et al., 2017; Islam et al., 2013; Liu et al., 2016; San Jose et al., 2010; Sengupta et al., 2017; Sheikh et al., 2009). The results of this study, in contrast, were inconsistent with results obtained from studies conducted in Portuguese, Swedish, Caucasian, and African-Americans populations (Alexandrie et al., 1994; Mota et al., 2015; Wenzlaff et al., 2005). However, Zhan et al. reported in an updated meta-analysis included 64 studies and comprising of > 18,000 subjects that significant association of CYP1A1 polymorphisms and lung cancer were found among Asians and Caucasians (Zhan et al., 2011). Association of both genetic and environmental factors is an important influencer on the occurrence of tumours. Nevertheless, discovering the existence of familial clustering of particular tumours suggests the presence of genetic factors that play an essential role in tumour occurrence regardless of the environmental risks. This is supported by the fact that populations when exposed to the same external risk factor, they exhibit different degrees of cancer predisposition, which indicates that genetic background plays a crucial role regarding the susceptibility to malignancy (Hemminki et al., 2008).

When we sub-grouped the CYP1A1 polymorphisms according to the histological types of lung cancer, significant distribution of CYP1A1-Ile^{462}Val and -MspI were found with SCC, but not AD or other types. Similar results were found in the population of Egyptians, where the subjects who carried variants genotypes of both CYP1A1 polymorphisms were significantly more susceptible to the risk of SCC (Hussein et al., 2014). A study conducted in the North India revealed that the combined variants genotypes of CYP1A1-Ile^{462}Val and -MspI polymorphism were associated with the risk of lung cancer in patients and was higher in case of SCC type (Girdhar et al., 2017). The results presented here are consistent as well with other previous studies which found a similar association (Le Marchand et al., 2003; Motovali-Bashi et al., 2012; Ng et al., 2005; Sheikh et al., 2009). However, no association was found between the variant genotypes of CYP1A1-Ile^{462}Val and -MspI polymorphisms and the major histological cancer types among the Portuguese population (Mota et al., 2015).

The mechanisms by which lung carcinogens induce the occurrence of tumours are not well understood. The fact that genetic susceptibility of individuals to develop lung cancer represents another important player in malignant occurrence, might explain the variation among different populations. Cigarette smoking is one of the major inducers of lung cancers; nevertheless, not every smoker develops lung cancer. Iraqi population, however, showed a non-rarity distribution frequency of variant allele of CYP1A1-Ile^{462}Val and -MspI polymorphism, unlike the Caucasian population. Thus, further analysis to define the influence of the combined presence of genetic polymorphisms and the exposure to high levels of tobacco smoke provided from WP smoking were performed in the present study. Interestingly, the data of the current study showed that the proportion of individuals who smoke WP tobacco and carried variant genotypes (AG + GG) CYP1A1-Ile^{462}Val was higher among lung cancer patients. Likewise, lung cancer patients who were carriers of variant genotypes (AG + GG) of CYP1A1-MspI and smoke WP tobacco exhibited similarly high proportion. Our results indicated that WP smokers who were carrier of variant genotypes (wt/vt + vt/vt) of CYP1A1-Ile^{462}Val and -MspI polymorphism had > 5 folds increased risk of developing lung cancer than the non-smokers who carried wild genotypes, and 2.0- and 2.6-folds higher than smokers who were carrier of wild genotypes. The association between carrying mutant genotypes of CYP1A1 polymorphisms and smoking WP tobacco in developing the risk of lung cancer presented here is similar to the results of previous studies that showed a synergistic association between these polymorphisms and heavy smoking of tobacco cigarette. Our results are

supported by previous studies conducted among different ethnic backgrounds such as in Kashmiri, Indian, and Chinese populations (Girdhar et al., 2017; Song et al., 2001; Sheikh et al., 2009).

The sample size of the current study was the main limitation, which prevented further statistical analysis as it made further subgroups classification very small to be conducted. The other limitation was the inability to include the other Iraqi ethnic population, i.e. the Kurdish, who are concentrated in the Northern part of Iraq. Further studies are needed to provide more details regarding the distribution of genotypic variants of CYP1A1 polymorphisms among women. In addition, by comparing the combined effect of cigarette smoking and WP tobacco smoking and investigating the genotypic distribution of CYP1A1 polymorphisms may provide more insights. Taking into consideration that assessing the effect of other risk factors; such as environmental factors, diet, place of residence (rural or urban), and history of cancers are also required to validate our findings.

In conclusion, the results of this study revealed that the distributions frequency of CYP1A1-Ile^{462}Val and -MspI polymorphism were elevated among lung cancer patients, especially among SCC type holders. This indicates that these variants might increase the risk of lung cancer in the Iraqi population. Our study for the first time reported the synergistic influence of WP tobacco smoking and CYP1A1 polymorphisms, which were increased by smoking. By comparing the distribution of these polymorphisms among Arab and other ethnic populations, our results supported the statement that the certain cancers susceptibility among individuals may rely on certain ethnic gene polymorphisms.

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Author contributions

B.K.K., I.J.L., and N.N.A. contributed to carried out the experiments and analyzing data. B.K.K in addition conceived the study, supervised the experiments, and wrote the paper. N.N.A. in addition contributed by doing the statistical analysis and designing the schematic figures of this study. All authors contributed to writing the final form of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mgene.2019.100623.

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