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Role of matrix metalloproteinase 9 gene polymorphism as genetic risk factor for lung cancer

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***Abstract*---**Lung cancer (LC) is one of the most incident malignancies worldwide. This study investigated the potential role of matrix metalloproteinase9 gene (*MMP9)* single nucleotide polymorphisms (SNPs) in Lung cancer patients. The study population was included 75 Iraqi patients with confirmed Lung cancer and 25 healthy people as controls. In this study, the -1562 C/T polymorphism in *MMP9* gene was detected by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) technique. The genotype distribution results of the -1562 C/T SNP of *MMP9* gene have shown no significant differences (p>0.05) between controls (CC: n= 15, 60%; CT: n=9, 36%; TT: n=1, 4%) and Lung cancer patients (CC: n= 44, 58.66%; CT: n=30, 40%; TT: n=1, 1.34%). Results of the association analysis between genotype distribution of the -1562 C/T SNP of *MMP9* gene and clinicopathological criteria of cases such as histological grading, TNM staging and lymph node metastasis, had shown no significant association (p>0.05). While analysis of the association between genotype distribution of the -1562 C/T SNP of *MMP9* gene and smoking status among patients were statically significant (p<0.05). These results suggested that the SNP in *MMP9* gene was involved in lung cancer progression, tissue invasion and metastasis involvement.

***Keywords*---**lung cancer, MMP9 gene, Iraq,

Introduction

Lung cancer is a disease that affects roughly 230,000 Americans each year. Deaths are estimated at 135,000 patients per year. Lung cancer deaths have

become more numerous than the deaths from prostate, breast, brain, and colorectal cancer combined. It has now become the most common cause of cancer deaths in men and the second most common in women (Clark & Alsubait, 2020). Despite modest improvements in outcomes in the United States, lung cancer survival remains heavily influenced by stage at diagnosis, and most lung cancers (57%) are diagnosed when cancer has metastasized outside the lung (Cronin *et al.*, 2018).

Approximately 85 % of lung cancers are non-small cell lung cancers while 15 % are small cell lung cancers. Histologically, following subtypes of non-small cell cancer are distinguished: adenocarcinoma (38.5 % of all lung cancers), squamous cell carcinoma (20 %) and large cell carcinoma (3 %). Over recent years, the incidence of adenocarcinoma has been increasing. Squamous cell carcinoma is more commonly associated with smoking while adenocarcinoma is the most common histological type in non-smokers (Skřičková *et al.*, 2018).

In Iraq bronchial and pulmonary cancer was the second most common cancer during this four-year period accounting for 8.1% of the total registered cases of cancer during the year 2015, 8.31%, of the total registered cases of cancer during the year 2016, 7.80% of the total registered cases of cancer during the year 2017, and 8.19% of the total registered cases of cancer during the year 2018. Cancer of the bronchus and lung cancer was the most common cancer in males occurring in 6.7/100,000 males in 2015, 7.76/100,000 males in 2016, 8.42\100,000 males in 2017, and 9.50/100,000 males in 2018 (Al-Mosawi, 2020).

Tobacco smoking remains the predominant risk factor for lung cancer development (Bade & Dela Cruz, 2020). Tobacco use has been cited as the cause of approximately 90% of all lung cancers. Patients that currently smoke with 40 pack/year smoking history have twenty times the likelihood of developing lung cancer than a non-smoker (Alberg & Samet, 2003; Schabath & Cote, 2019). The newest and most controversial products potentially influencing lung cancer risk are electronic nicotine delivery systems (ENDS) including electronic cigarettes (e-cigarettes), e-pens, e-pipes, e-hookah, and e-cigars (Dinakar & O'Connor, 2016).

Nontobacco risk factors include environmental and occupational exposures, chronic lung disease, lung infections, and lifestyle factors. Several demographic factors have been identified that influence lung cancer development and outcomes, including gender, age, race, geography, and socioeconomic status (SES) (Bade & Dela Cruz, 2020). Matrix metalloproteinases (MMPs) are a group of zinc dependent enzymes that are involved in tumor cell invasion and metastasis (Kiani *et al.*, 2020). Previous studies have reported that MMPs overexpression and tissue inhibitor matrix metalloproteinases (TIMPs) suppression lead to dysregulation of extracellular matrix (ECM) remodeling causing numerous pathologic conditions such as cancer, neurodegenerative disease, arthritis, and cardiovascular disease (Raeeszadeh-Sarmazdeh *et al.*, 2020).

Although many MMPs are thought to have a role in carcinogenesis, most attention has focused on MMP9, member of the gelatinase protein family. MMP9 is capable of degrading type IV collagen, the most abundant component of the basement membrane, that provides structural support for cells and influences cell signaling

and polarity. Therefore, the destabilization of the basement membrane is an essential step for both the local and metastatic spread of most cancers. These molecules are overexpressed in a variety of malignant tumors and their expression and activity are often associated with tumor aggressiveness and poor prognosis (Bauvois, 2012).

Elevated levels of MMP9 are found in many malignant tumors, such as breast, brain, ovarian, pancreas, colorectal, bladder, prostate, lung cancers, and melanoma (Rydlova *et al.*, 2008; Klein & Bischoff, 2011). The *MMP9* -1562 C>T (rs3918242) polymorphism has been shown to upregulate the promoter activity and the presence of the -1562T allele has also been reported to be associated with the increase in gene expression (Zhang *et al.*, 2012). This study aimed to investigate the possible role of genetic variants in *MMP9* gene as putative susceptibility factors for the lung cancer risk in Iraqi population**.**

Materials and Methods

Seventy-five patients with lung cancer were histologically confirmed and recruited at Middle Euphrates Cancer Center in Al-Najaf governorate. The patients were distributed as 62.67% male and 37.33% female and their ages were ranged from 45 to 89 years, with a mean age of 63.86 years. Among them the number of smoking from about 58 (77.33%) from all cases had been smoking for numerous years while 17 (22.66%) of cases had been never-smoked. The diagnosis was confirmed by histological examination under the supervision of pathologist from the hospital, who determines the lung cancer clinical variables like histological grade, cancer stage (TNM staging system: tumor, lymph node metastasis), depending on the site from which the tumor originated, the tumors with the highest frequency of occurrence were (NSCLC) Squamous cell carcinoma 46.66 % followed by (NSCLC) adenocarcinoma 40% and the smallest proportion for SCLC as 13.34%. Based on grading criteria, the presented lung carcinomas were graded into 5.33% with grade I, 57.33% with grade II, and 37.33% with grade III. While, there were 4%,18.66%, 21.33% and 56% with stage I, stage II, stage III and stage IV, respectively. Just 51 (68%) from all cases were diagnosed with lymph node metastases. All the epidemiological information's about patients like age, sex, and smoking at diagnosis of cancer was collected from patients Data sheets from hospital. During the same period, Twenty-five healthy individual; The exclusion criteria of the control group included previous malignancy, inflammatory disorders or clinical manifestation of any disease. The controls and cases are all Iraqis.

Genomic DNA was extracted from the leukocytes using protocol from Genomic DNA Mini Kit was designed specifically for purifying DNA from blood which was collected in tubes with anticoagulant EDTA from patients and controls. The genotypes of *MMP9*-1562 SNP were determined for all the participants by RFLP-PCR technique. All PCR reactions were uniformly performed with the Thermocycler apparatus (BIO RAD / USA) as initial cycle at 95°C for 3 min; 35 cycles of 95°C for 45 s, 69°C for 45 s, and 72°C for 1 min, and a final extension at 72°C for 5 min. After the PCR, the target single-nucleotide polymorphism (SNP)-containing DNA amplicon were digested with *SphI* restriction enzyme(10u/µl) (10 units is sufficient, generally lµl was used. The source of *SphI*

enzyme (Synthesized by New England Biolabs, Inc, Cat. No. R0182S) is *Streptomyces phaeochromogenes* (NRRL B-3559). The PCR product (5µl) was incubated with 1 µl of *SphI* restriction endonuclease, 5 µl of NEBuffer (10X), and the size was completed by added nuclease free water to 50 µl. Finally, the reaction mixture incubated at 37°C for 60 minutes and incubated in water bath at 65°C for 20 minutes for inactivation. After the *SphI* digestion one of these results was yielded for each sample: one fragments (435 bp) for allele C (homozygous wild genotype, CC), Two fragments (247, 188bp) for allele T (homozygous mutant genotype, TT), and, Three fragments (247, 188 and 435bp) for both C and T allele (a heterozygous genotype, CT).

Following the digestion procedure, each enzyme-digested DNA amplicon was analyzed by agarose gel electrophoresis, pictures were taken, and its individual genotypes were identified. The detailed information of forward and reverse primer residues and the corresponding restriction endonucleases for DNA amplicon is summarized in Table 1.

Table 1: Summary of the primer sequences, restriction enzyme and size of amplicons after enzyme digestion for matrix metalloproteinase-9

Polymorphic site	Primer sequences	Restriction endonuclease	Amplicon size after cutting, bp
MMP9 -1562C/T SNP	Forward 5'- GCCTGGCACATAGTAGGCCC -3' Reverse 5'- CTTCCTAGCCAGCCGGCATC -3'	*SphI*	C: 435 T: 247+188

Statistical analyses

Statistical analyses of all results were carried out by the help of Statistical Package for the Social Sciences (SPSS) version 23 software statistical package using t-test and Chi-square test (with P value at level of significance less than 0.05) to compare value of results between groups. Result values were expressed as mean ± SE, number of patients, or percentages. The associations between MMP2 genotypes and lung cancer risk were estimated by calculating the odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis. Statistically, any difference at $p< 0.05$ was considered significant between any two groups compared.

Results and Discussion

Among the 25 healthy subjects; 15 (60%) had found as homozygous CC alleles, 9 (36%) found as heterozygous genotype (with the C and T alleles (CT), and 1 (4%) had found as homozygous genotype TT alleles; (CC: n= 15, 60%; CT: n=9, 36%; TT: n=1, 4%) (Table 2, figure 1&2).

Table 2: The results of genotypic frequencies of 1562C/T at *MMP9* gene in patients and controls.

Genotypes	Healthy controls (N=25)	Lung cancer patients (N=75)
CC	15 (60%)	44 (58.66%)
CT	9 (36%)	30 (40%)
TT	1 (4%)	1 (1.34%)
P-value	0.688	
Alleles frequency	N(%)	N(%)
C allele	39 (78%)	118 (78.66%)
T allele	11 (22%)	32 (21.34%)
X^2 P-value OR (95%CI)	0.010 0.921 0.961 (0.443-2.087)	

Data were expressed as number and a percentage (N%). *p <0.05 significant. Abbreviations: X^2= chi-square, OR= odds ratio, CI= confidence interval.

Among the 75 Lung cancer patients; 44 (58.66%) had found as wild homozygous CC alleles, 30 (40%) found as heterozygous genotype (with the C and T alleles (CT), and 1 (1.34%) had found as mutant homozygous genotype TT alleles; (CC: n= 44, 58.66%; CT: n=30, 40%; TT: n=1, 1.34%) (Table 2, figure 1&2).

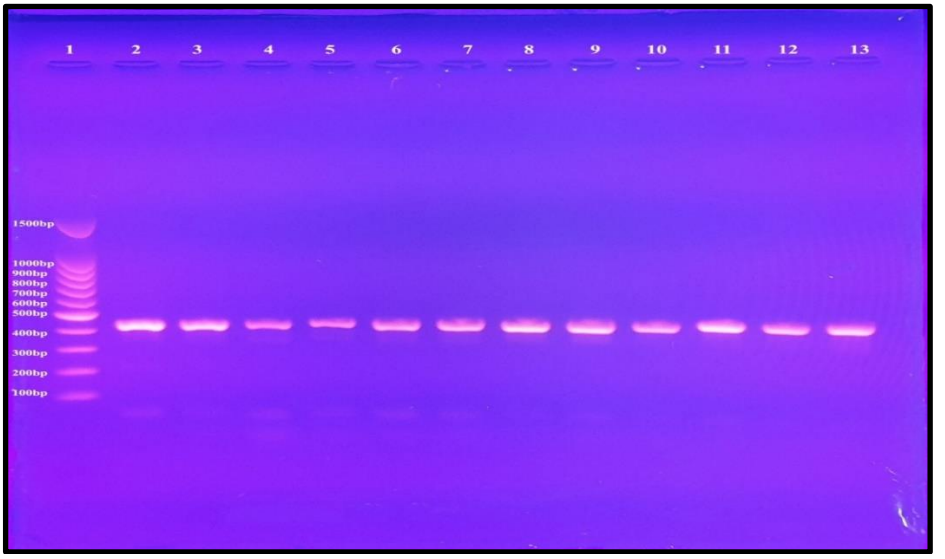

Figure 1: The electrophoresis image of PCR amplification of the *MMP9* gene. Lane 1: 100 bp DNA Ladder; Lane 2 to 13: the 435 bp PCR product.

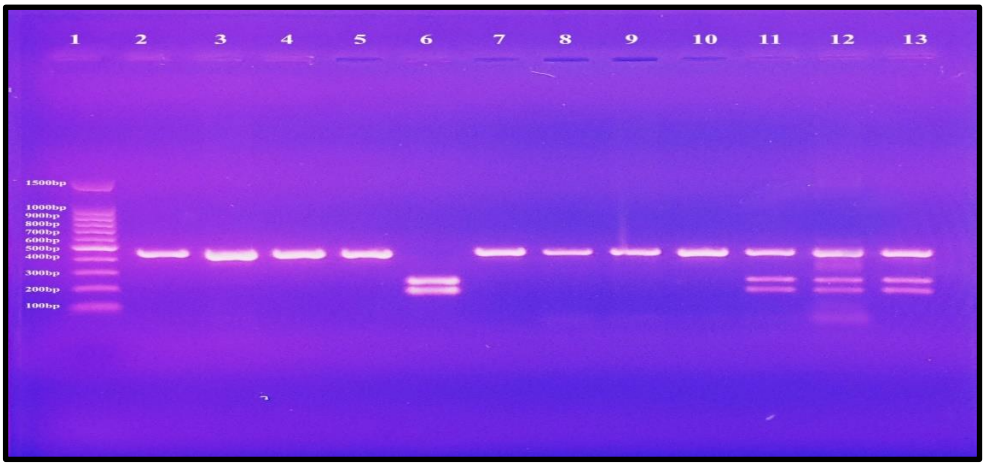

Figure 2: The electrophoresis image of RFLP-PCR analysis of -1562C/T SNP in the *MMP9* gene. Lane 1: 100 bp DNA Ladder; Lane 2,3,4,5,7,8,9, and 10: homozygous genotype, CC (435bp band); Lane 6: homozygous genotype, TT (247 and 188 bp bands); Lane 11,12, and 13: heterozygous genotype, CT (435bp, 247, and 188 bp bands).

That means the frequencies of -1562 C/T SNP in the *MMP9* gene in the 75 Iraqi Lung cancer patients in Al-Najaf province were not significantly differed from that of the 25 healthy controls group ($p<0.05$). The result showed that there were no significant statically differences in the *MMP9* -1562 C/T SNP between lung cancer patients and controls, and they were no significantly likely than controls to have the mutant allele (OR= 0.961, 95%CI= 0.443-2.087, p= 0.921).

In general, MMP9 overproduction or enhanced activity in cancer leads to ECM disintegration, allowing tumor cells to invade other tissues and metastasize to distant organs and progression via their activities in cell death, proliferation, and angiogenesis (Nelson *et al.,* 2000; Hojilla *et al.,* 2003; Gialeli *et al.,* 2011; Klein & Bischoff, 2011). As it was known that MMP9 mediate growth factors activation, release of angiogenic factors or destroy the chemokine gradient development by host immune response and suppress apoptosis of tumor cell (Egeblad *et al.,* 2002).

Previous studies have demonstrated that *MMP9* activity is controlled by the polymorphism in the promoter region of the human *MMP9* gene (Blankenberg *et al.,* 2003; Medley *et al.,* 2004). The C →T functional polymorphism exists at residue 1562 of the *MMP9* gene promoter. The polymorphism produces promoter genotypes with in low (CC) or high (CT, TT) activity, resulting in decreased or increased expression of MMP9 (Cao *et al.,* 2016). DNA-protein interaction assays have revealed that the sequence between nucleotide position 1567 and 1559 relative to the transcription start site of the *MMP9* gene, which encompasses the 1562 polymorphic site, can interact with a nuclear protein whose entity is still unknown. (Zhang *et al.,* 1999). And the latter is located in the gelatinase-specific fibronectin type II domain, which leads to an amino acid exchange (arginine [R] to glutamine [Q]) in the catalytic domain which presumably enhances substrate binding. It is therefore possible that the amino acid conversion associated with

this polymorphism affects the activity of this enzyme (Allan *et al.,* 1995; O'Farrell & Pourmotabbed, 2000).

The previous study explanation was confirmed by Zhang *et al.* (2005) study which demonstrated that the substitution of cytosine for thymine (C→T) in the 1562 position of the *MMP9* promoter generates a high-affinity site for a yet unidentified nuclear protein, leading to an expressive increase in the promoter activity of the *MMP* gene. *MMP9* -1562C/T could be an important SNP for increased expression of *MMP9* in a particular locality. A nuclear repressor protein that generally binds to the *MMP9* promoter in presence of C allele at the position -1562 and keeps the promoter transcriptionally less active, thus no longer able to bind when the C allele is replaced by the T allele and inducing high *MMP9* promoter activity (Zhang *et al.,* 1999; Verma *et al.,* 2015).

In normal physiological conditions, genes are tightly regulated by transcription factors, whereas in cancer, aberrant activities of transcription factors deregulate the gene expression, leading to metastasis (Libermann & Zerbini, 2006). In this study, the genotypes CT and TT were found prevalent in both cases as well as controls, which means that subjects who carry either normal C or mutant T have the same risk to develop lung cancer. On the other hand, the *MMP9* gene was overexpression in several types of tumors than in normal subjects with normal tissue (Huang, 2018)

That means the MMPs enzyme activity is elevated and has an important role in cancer development despite the presence or absence of polymorphism in the promoter region of this gene, particularly may be because the *MMP9* -1562C/T SNP contributed to increasing the expression of *MMP9*. Chaudhary *et al.* (2016) study has proven that *MMP9* mRNA expression of *MMP9* -1562 SNP CT and TT genotypes were 1.5 and 2.5fold increase in Myelodysplastic Syndrome MDS and Acute Myeloid Leukemia AML respectively. In AML, MMP2 -1306 SNP C/T and T/T genotypes showed 2.0-fold mRNA expression.

Li *et al.* (2015) meta-analysis study found that *MMP9* -1562 C/T, a significantly increased risk was found among Asians with model-free approach. According to the findings of Li *et al.* (2019) the subject carrying *MMP9* -1562 CC genotypes showed a significantly increased risk of NSCLC, and the serum levels of MMP9 constitute statistical evidence in support of the notion that MMP9 might function as a key oncogene in NSCLC in Southern Chinese population The NSCLC group had a significantly higher serum level of MMP9 and the results demonstrated an association between the C allele and an increased serum level of NSCLC in *MMP9*-1562 C/T.

While Ghaderian *et al.* (2010) found that plasma levels of MMP9 were associated with its gene polymorphism, which showed a statistically significant difference for the *MMP9* -1562 C/T variant in the patients (p<0.05). The plasma levels were significantly higher in the TT and CT genotypes, compared with CC in the patients (p<0.05). Research by Avci *et al.* (2015) found that there were no significant differences between subjects with the *MMP9* -1562 CC and CT+TT genotypes in the pathogenesis and clinical course of gastric cancer in Turkish subjects conducted with this study.

In a meta-analysis based on 17 case-control studies, Hu *et al.* (2013) reported that *MMP9* -1562C/T polymorphism TT genotype decreases the risk of lung cancer, but not in the Asian population.

A study was done by Matsumura *et al.,* (2005) indicated that *MMP9* -1562 C/T polymorphism may increase the gastric cancer risk among the Japanese population. In Iranian Population Studies of Sadeghi *et al.* (2009) and Hemati *et al.* (2010) already reported a significant association between the *MMP9* -1562C/T promoter T allele and occurrence of breast cancer and found that the *MMP9* promoter T allele increases the MMP9 level in breast cancer patients.

The serum level of MMP9 was markedly elevated in T-Cell Acute Lymphoblastic Leukemia in Chinese Population patients with *MMP9* -1562C/T Polymorphisms in the CT + TT genotype compared to patients with the CC genotype (Lin *et al.,* 2017). A recent case–control study and a minireview by Banday *et al.* (2016) found that the CT heterozygous genotype of *MMP9* -1562C/T SNP showed a significant association with increased risk for the development of colorectal cancer in Kashmiri population, compared with T allele. Other two studies were the first report regarding the association of the *MMP9* -1562C/T promoter SNP with the risk of the development and metastasis of lung cancer (Sienel *et al.,* 2003; Lin *et al.,* 2004).

The high T allele frequency of the *MMP9* -1562C/T which determined in the study Savasoglu and Emin Erdal (2016) can be associated with increased gene expression due to the high promoter activity of T allele. On the other hand, some studies suggested a positive association between -1562 C/T polymorphism in the promoter region of the *MMP9* gene in Caucasian population of Asturias, Northern Spain which associated with the risk of the development of lung cancer (González-Arriaga *et al.,* 2012). Vairaktaris *et al.* (2008) study in Greece, investigated *MMP9* -1562 C/T polymorphism and also reported a strong association with increased risk for developing oral cancer.

In contrast, Lu *et al.* (2016) study found that compared with the CT genotype, the *MMP9* -1562CC genotype might be a marker of increased genotype susceptibility to NSCLC among the South-Central Chinese population. While in the Korean population, the *MMP9* -1562 CC genotype were associated with increased colorectal cancer risks, suggesting that *MMP9* -1562 T allele can influence the outcome of colorectal cancer patients protectively (Park *et al.,* 2011). Although the positive association of *MMP9* -1562 C/T with cancer risk existed in some individual studies (Woo *et al.,* 2007; Vairaktaris *et al.,* 2008). *MMP9* -1562 C/T may not be a major risk factor for most types of cancers (Peng *et al.,* 2010).

Several other studies that reported no association was found between genetic variation in MMP9 and the risk of different cancer types (nasopharyngeal carcinomas and colorectal cancer occurrence (Elander *et al.,* 2006; Nasr *et al.,* 2007; Xu *et al.,* 2007). A recent meta-analysis involving more than 3000 cases and controls were performed to investigate the association between *MMP9* -1562 C/T polymorphism and lung cancer risk. According to their findings, the *MMP9* -1562 C/T polymorphism does not increase the risk of lung cancer and this

polymorphism cannot be considered as a risk factor for lung cancer (Zafari *et al.,* 2021).'

Kubben *et al.* (2006) reported no association between the variation in *MMP9* -1562 C/T polymorphism and gastric cancer in Netherlander population. Another two meta-analysis studies, the first one based on 12 published case with colorectal cancer –control and the second based on nine published case–control studies including 2597 breast cancer cases and 2618 controls studies, which reported no association between *MMP9*-1562 C/T polymorphisms and the risk of cancer (Liu *et al.,* 2011; Zhou *et al.,* 2011). In another meta-analysis based on 12 studies that reported no association between the *MMP9* -1562 C/T polymorphism and ovarian cancer risk (Zhu *et al.,* 2017). Also in another meta-analysis, Meng *et al.* (2018) reported no association between the MMP9 -1562 C/T polymorphism and the risk of urinary cancer.

The association of *MMP9* -1562 C/T SNP and pathological features of the lung cancer cases

The results of this study showed that: among the grade I (n=4) lung cancer cases; 3 (75%) had been found as homozygous CC, 1 (25%) found as heterozygous genotype (CT), and no patient had found as homozygous genotype (TT). In patients whose tumors were grade II (n=43): 23 (53.49%) had found as homozygous CC, 19 (44.18%) were found as heterozygous genotype (CT), and 1 (2.33%) had found as homozygous genotype TT. Finally, in patients whose tumors were grade III(n=28): 18 (64.29%) had found as homozygous CC, 10 (35.71%) found as heterozygous genotype (CT), and no patient had found as homozygous genotype TT (Table 3).

Table 3: The association between *MMP9* -1562 C/T polymorphism and histologic grade of Lung cancer

Subjects		Genotype Distribution Results			P-value
		CC	CT	TT	
Histologic grade	I (n=4)	3 (75%)	1 (25%)	00 (00%)	0.770
	II(n=43)	23 (53.49%)	19 (44.18%)	1 (2.33%)	
	III(n=28)	18 (64.29%)	10 (35.71%)	00 (00%)	

Data were expressed as a number of patients (results values also represented as percentages (%), n=75). *p<0.05 significant.

The study showed no statically significant differences between CC, CT, and TT genotypes frequencies of *MMP9* -1562C/T SNP and the Grade I, Grade II, and Grade III of lung cancer patients. Although the T allele was more frequently observed in higher tumor grade II+III than grade I. Jiang *et al.* (2021) demonstrate that MMP9 overexpression was associated with higher histological grade in breast cancer patients and was more frequent in patients with larger tumor sizes. This result was conducted with the results of Rollin *et al.* (2007) which showed no significant association could be established between a single polymorphism of *MMP9* -1562C/T SNP and differentiation grade.

The association of *MMP9* -1562 C/T polymorphism and TNM staging of lung cancer showed that: among the patients whose tumors were in stage I (n=3); 1 (33.33%) had found as homozygous CC, 2 (66.67%) found as heterozygous genotype (CT), and no patient had found as homozygous genotype TT. In the patients whose tumors were in stage II (n=14): 9 (64.28%) had found as homozygous CC, 4 (28.57%) were found as heterozygous genotype (CT), and 1 (7.15%) had found as homozygous genotype TT. In the patients whose tumors were in stage III (n=16): 9 (56.25%) had found as homozygous CC, 7 (43.75%) were found as heterozygous genotype (CT), and no patient had found as homozygous genotype TT Finally, in the patients whose tumors were in stage IV (n=42): 25 (59.52%) had found as homozygous CC, 17 (40.48%) found as heterozygous genotype (CT), and no patient had found as homozygous genotype TT (Table 4).

Table 4: The association between *MMP9* -1562 C/T polymorphism and TNM staging of lung cancer

Subjects		Genotype Distribution Results			P-value
		CC	CT	TT	
TNM stage	I (n=3)	1 (33.33%)	3 (66.67%)	00 (00%)	0.425
	II(n=14)	9 (64.28%)	4 (28.57%)	1 (7.15%)	
	III (n=16)	9 (56.25%)	7 (43.75%)	00 (00%)	
	IV (n=42)	25 (59.52%)	17 (40.48%)	00 (00%)	

Data were expressed as a number of patients (results values also represented as percentages (%), n=75). *p <0.05 significant. Abbreviations: TNM staging system: tumor, node, metastasis (the stage of the lung cancer).

The study documented no statically significant differences between CC, CT, and TT genotypes frequencies and the 4 clinical TNM stages of lung cancer. Although the T allele is present and was more frequently observed in higher tumor stage (III+IV) compared with low stage.

The result of two studies: Sienel *et al.* (2003) and Lin *et al.,* (2004) suggested that the overexpression of MMP9 protein is related to metastasis in lung cancer, and the circulating MMP9 level also elevated in advanced stages of lung cancer (Jumper *et al.,* 2004). A previous study demonstrated that *MMP9* expression levels elevated in stage III of breast cancer patients (Köhrmann *et al.,* 2009). In Iraq, the results of study by Mahmood *et al.* (2015) showed significant increase of *MMP9* expression levels in the breast tissues at the mRNA and protein levels in 64 cases of stage II-III breast cancer as compared to that in benign tumors in Iraqi women.

Another study in Iraq showed significantly elevated expression levels of MMP9 in the cancerous colon tissues compared with those in benign tissues. Increased levels of and MMP9 were found to be stage-dependent in Iraqi women with colon cancer (Mahmood *et al.,* 2019). Also other recent study showed that *MMP9* overexpression was associated with a higher clinical stage in breast cancer patients and was more frequent in patients with larger tumor sizes (Jiang *et al.,* 2021). This result is consistent with the results of Rollin *et al.* (2007) which showed no significant association could be established between a single polymorphism of *MMP9* -1562C/T SNP and pathological stage of cancer.

Results of Li *et al.* (2019) agreed with the present study and demonstrated no statically difference was founded between *MMP9* -1562C/T polymorphisms and clinicopathological features such as 4 tumor stages and size in lung cancer patients. Regarding the frequency of Lymph node metastases, the results of this study found that in patients whose tumors had metastatic lymph node involvement (n=51): 30 (58.82%) had found as homozygous CC, 21 (41.18%) were found as heterozygous genotype (CT), and no patient had found as homozygous genotype TT. While cases without metastatic lymph node involvement (n=24): 14 (58.33%) had found as homozygous CC, 9 (37.5%) were found as heterozygous genotype (CT), and 1 (4.17%) had found as homozygous genotype TT (Table 5).

Table 5: The association between *MMP9* -1562 C/T SNP and metastatic lymph node involvement of Lung cancer

Subjects		Genotype Distribution Results			P-value
		CC	CT	TT	
Lymph node metastases	positive (n=51)	30 (58.82%)	21 (41.18%)	0 (00%)	0.336
	negative (n=24)	14 (58.33%)	9 (37.5%)	1 (4.17%)	

Data were expressed as number of patients (results values also represented as percentages (%), n=75). *P <0.05 significant.

These results showed no significant differences between the CC, CT, and TT genotypes observed in the lung cancer cases with metastatic lymph node involvement compared to lymph node-negative cases. although the T allele was more frequently observed in the lung cancer cases with positive lymph node metastatic involvement compared with negative lymph node metastatic involvement.

Masson *et al.* (2005) study had revealed that, both MMP2 and MMP9 can degrade type IV collagen and are frequently elevated in human cancer. Additionally, a cooperative effect of MMP2 and MMP9 was demonstrated in an in vivo experimental model establishing the angiogenic phenotype and invasiveness of tumor keratinocytes. Results of Webb *et al.* (2017) confirm that contribution of MMP2 and MMP9 together in cancer angiogenesis through the degradation of ECM components and the activation of pro-angiogenic factors VEGF and TGF-b in diverse cancer tissues.

Two studies with different cancer type (kidney clear cell carcinoma and breast cancer) showed higher expression of *MMP2* and *MMP9* in patients who have metastases when compared to patients without distant metastases (Slaton *et al.,* 2001; Mahmood *et al.,* 2015). Other recent study confirmed that *MMP9* overexpression correlated with lymph node metastasis (Jiang *et al.,* 2021). Matsumura *et al.* (2005) study found that there was a significant association between the *MMP9* -1562 C/T polymorphism and the degree of tumor invasion and lymphatic metastasis in gastric cancer might exist.

Other previous results documented by Xing *et al.* (2007) indicated that the *MMP9* -1562C/T polymorphism affects lymph node metastasis of colorectal cancer However, the risk of lymph node metastasis of CRC was increased in patients with the -1562T allele. This result agreed with the study of Wang *et al.* (2005) that showed the MMP9 genotype frequencies in adenocarcinoma and squamous cell carcinoma patients with lymphatic metastasis were not significantly different from that in lymph node-negative ones. Therefore, the influence of the MMP9 polymorphism on the risk for lymphatic metastasis of NSCLC was not observed at least in the northern Chinese population

This result was consistent with the results of Rollin *et al.* (2007) which showed no significant association could be established between a single polymorphism of MMP9 -1562C/T SNP and lymph node metastasis. Li *et al.* (2019) also agreed with the present study and demonstrated no difference was founded between *MMP9* polymorphisms and lymph node metastasis parameters between lung cancer patients and controls. The mechanism where by MMP2 and MMP9 activity induces cancer angiogenesis involves the cleavage of latent TGF-b in a CD44-dependent manner, which can promote tumor growth and invasion (Yu *et al.,* 2000).

MMP9 promotes endothelial cell migration and triggers the angiogenic switch by releasing VEGF during carcinogenesis (Bergers *et al.,* 2000). Decreased expression of VEGF and *MMP9* in medulloblastoma cells leads to decreased angiogenesis and tumor growth, indicating the pro-angiogenic role of MMP9 in cancer tissues (Bhoopathi *et al.,* 2010). The direct proteolytic cleavage of osteopontin by MMP9 contributes to cancer metastasis, most likely associated with angiogenesis via the regulation of VEGF and angiostatin secretion (Takafuji *et al.,* 2007; Gupta *et al.,* 2013).

The association of *MMP9* -1562 C/T SNP and smoking status among lung cancer patients:

Regarding the frequency of smoking habit, the results of this study found that among patients whose smoking involvement (n=58): 32 (55.17%) had found as homozygous CC, 26 (44.83%) were found as heterozygous genotype (CT), and no patient had found as homozygous genotype TT. While patient without smoking involvement (n=17): 12 (70.59%) had found as homozygous CC, 4 (23.53%) were found as heterozygous genotype (CT), and 1 (5.88%) had found as homozygous genotype TT (Table 6).

Table 6: The association between *MMP9* -1562 C/T SNP and smoking habit involvement among Lung cancer patients

Subjects		Genotype Distribution Results			P-value
		CC	CT	TT	
Smoking status	Current smoking (n=58)	32 (55.17%)	26 (44.83%)	00 (00%)	0.046*
	Never smoking (n=17)	12 (70.59%)	4 (23.53%)	1 (5.88%)	

Data were expressed as number of patients (results values also represented as percentages (%), n=75). *p <0.05 significant.

These result show significant association between different genotypes of *MMP9* -1562C/T SNP and smoking status. The results recorded higher frequency of T allele in current smoker compared with never smoking patients. Carcinogens contained in cigarettes can act through active particles formed during their metabolism in the cells of the bronchial mucosa or through direct binding to receptors and activation of proteins regulating processes such as apoptosis or angiogenesis (Hecht *et al.,* 2003). The irritating effect of tobacco smoking increased the susceptibility to respiratory tract infections and the weakening of local defense reactions may additionally promote carcinogenesis (Wogan *et al.,* 2004).

Direct action on the cell receptors of carcinogens contained in tobacco smoke, especially from the nitrosamine group, may also lead to the activation of cell pathways responsible for the regulation of proliferation, angiogenesis and apoptosis (West *et al.,* 2004; Hope *et al.,* 2007). Experimental studies have shown that exposure to tobacco smoke leads to frequent disturbances of methylation of genes important for life (Belinsky *et al.,* 1998). Among the numerous mutations that occur under the influence of tobacco smoke, there are mutations in the gene P53 (one of the most common). It is found in 80–90% of small cell carcinomas and in 20–60% of non-small cell carcinomas (Greenblatt *et al.,* 1994; Salgia & Skarin, 1998; Jassem *et al.,* 2004). As well as Blons *et al.* (2008) study proved that the mutation of K-RAS gene was strongly associated with carcinogens contained in tobacco smoke, and the gene disorder profile in lung cancer is significantly different than in non-smoking patients with EGFR gene mutation.

The results of Wang *et al.* (2012) study were in agreement with present study which recorded that the -1562 C/T polymorphism of MMP9 gene and smoking have a synergistic effect and are significantly associated with the risk of myocardial infarction in Chinese Uighur population. In accordance with previous study cross-over analyses by Zheng *et al.* (2021) suggested that the combined effect of smoking and CT genotype of -1562 C/T polymorphism contributed to the risk of sepsis.

Conclusions

The genotype distribution results of the -1562 C/T SNP of *MMP9* gene have shown no significant differences (p>0.05) between controls and Lung cancer patients. While the association between genotype distribution of the -1562 C/T SNP of *MMP9* gene and smoking status among patients were statically significant (p<0.05). Results of the association analysis between genotype distribution of the -1562 C/T SNP of *MMP9* gene and clinicopathological criteria of cases such as histological grading, TNM staging and lymph node metastasis, were not significant (p>0.05). These results indicated a possible role of the T allele of *MMP9* gene -1562 C/T SNP in increasing susceptibility to developing lung cancer, with more frequency in poorly differentiated tumors and positive lymph node metastatic involvement cases.

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