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Original Article

Effect of the Concentration Levels of Growth Hormone and Insulin-like Growth Factor I on the Polymorphisms of the Il12p40 Gene in Lung Cancer Patients

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Abstract

The prevalence of lung cancer as one of the most common cancers with the highest mortality rate is one of the most important health problems in humans across the world. Molecular research can provide valuable information about genetic changes associated with the pathogenesis of the disease that may be used to improve prognosis and treatment. The current study aimed to examine the genotyping utility of the Il12p40 (IL-12B) gene (rs3212227, A>C) polymorphisms and detect its relationship with the concentration levels of HGH and IGF-1 for the non-small cell lung carcinoma (NSCLC). This study investigated 67 cases with NSCLC (60 males and 7 females) and 28 healthy individuals as controls. The serum level of HGH and IGF-1 was determined using an enzyme-linked immunosorbent assay. Genotyping of the IL-12B gene polymorphisms (rs3212227, A>C) was carried out by polymerase chain reaction-restriction fragment length polymorphism. The serum levels of the HGH and IGF-1 were estimated, and the results of the IL-12B genotyping showed an increased risk of NSCLC. The homozygous wild (AA) genotype of the IL-12 gene showed that the risk of NSCLC was higher than that of the heterozygous (AC) and homozygous genotypes (CC). Moreover, a significant elevation was found in the serum levels of the HGH in the NSCLC patients, compared to the control group. The result showed that the IL-12 gene polymorphism was implicated in the pathogenesis of the NSCLC and directed several metabolic changes.

Keywords: Growth hormone, Insulin growth factor I, IL-12, Non-small cell lung carcinoma, Polymorphism

1. Introduction

Lung cancer is one of the leading causes of cancer mortality worldwide, particularly in developed countries. The most common types are small cell lung carcinoma (15%) and non-small cell lung carcinoma (NSCLC) (1) consisting of the subtypes of squamous cell carcinoma (30%), adenocarcinoma (45%), and large cell carcinoma (9%) (2). Molecular technological advances are providing insight into the biological processes involved in lung cancer. Cancers were caused by the accumulation of inherited and somatic mutations in oncogenes and tumor-suppressor genes (TSGs) (3). Identification of the oncogenes and TSGs involved in the pathogenesis of lung cancer and the promise of improving early diagnosis increase the number of available prognostic pathogenesis (4). Clinical data suggested that lung cancer is caused by various genetic and epigenetic changes, including oncogene activation and inactivation of the TSG (5). Interleukins (ILS) are a cytokine group of immune modulators and extracellular

mediators between cells that play an important role in the immune system and transmission signals between cells regulating their growth, differentiation, and mobility (6). Immune system defects or autoimmune diseases are described as defects in ILS because they are important in stimulating immune responses (7). Research has shown that the production of interleukin 12 (IL-12) is regulated in response to pathogens and their function is differentiated. IL-12 is a pro-inflammatory cytokine involved in the induction or amplification of the T-helper type 1 (Th1) response, which is induced in human dendritic cells (8). The P35 and P40 IL-12 subunits are interconnected by three covalently bonded disulfide bridges that play an important role in innate and adaptive safety immunity (9). Two polypeptide chains (IL-12Rβ1 and IL-12Rβ2) are composed of the IL-12 receptor (10). 14 SNPs have been found in the IL-12B gene based on the HGMD data, and several studies have been concentrated on the chromosome locus of IL-12B, located on a 3' non-traduced region of the chromosome locus 5q31-333 (10, 11). The IL-12B SNP rs321227 affects the production of IL-12 and has been associated with several diseases as an immune mediator (11). There are also studies on the interaction of SNP rs3212227 with cancerous diseases, such as lung cancer (10).

Human growth hormone (hGH) is secreted by the anterior pituitary gland, GH-release hormone-stimulated, and somatostatin inhibited (12). The growth hormone acts on the liver and other tissues to boost insulin production, such as insulin growth factor I (IGF-I), which supports the growth effects and the volume of GH. The circulating IGF-I blood levels tend to decrease as individuals get older and obese (13).

Further understanding of the effects of the IGF-1 signals may lead to a new approach to lung cancer prevention and treatment (13). New reports suggest that a high risk of pulmonary abnormalities is linked to low circulating IGF1 and hGH (12). IGF-1 also stimulates endothelial production-derived NOs, and decreases may play an important role in atherosclerosis development for individuals with low serum hGH levels. The reduction of IGF-1 also leads to the NSCLC risk (12, 14). This study aimed to investigate the association of the SNP rs3212227 with the analysis of HGH and NSCLC levels in Iraqi patients.

2. Material and Methods

2.1. Sampling

The samples were taken after tumor diagnosis in the Department of Oncology in AL-Najaf province, Iraq, from December 2018 to April 2019. The study consisted of 67 cases (60 males and 7 females) and 28 healthy individuals as the control group. All tumors were tested by the Union for International Control of Cancer classification system. The study was carried out in the Biology Department in the central biology laboratory and molecular laboratory, Faculty of Sciences, University of Kufa, Kufa, Iraq. The phenotypic data included body mass index (BMI).

2.2. Plasma Collection and Measurement of HGH and IGF-1

In total, 5 milliliters of blood samples were collected and divided into two parts. One milliliter was collected in an EDTA-containing tube and used for DNA extraction, and four milliliters were placed in a serum tube for enzyme-linked immunosorbent assay (ELISA). The mixture was allowed to coagulate for 15 min at room temperature. Blood was centrifuged at 3000×g for 10-15 min. The serum was collected, divided into three aliquots, and kept at -20ºC until analyses for measuring the concentration levels of HGH and IGF-1 in cancer and normal samples. Measurement of HGH and IGF-1 serum concentrations was performed using ELISA (15).

2.3. Genotyping of SNPs in the IL-12B Gene

Genotyping of the IL-12 gene polymorphisms (rs3212227, A>C) was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The amplification of the intended gene was achieved with specific primers Forward: 5-TTTGGAGGAAAAGTGGAAGA-3, Reverse: 5-AACATTCCAT ACATCCTGGC-3, the thermocycler program for PCR amplification was initial denaturation 94 for 7 min, followed by 35 cycles

with denaturation 94 for one min, annealing at 62°C for one min, and extension at 72°C for one min. The final extension was at 72°C for 5 min (16). PCR products were resolved in a 1.5% agarose gel stained with ethidium bromide to detect the band which is in 300 bp.

The PCR product was digested with restriction enzymes. TaqI restriction enzyme was used for the analysis of the IL-12 gene polymorphisms (rs3212227, A>C). Enzymes were incubated at 37°C for 1 or 14 h overnight. Electrophoresis was in a 2% stained agarose gel with ethidium bromide. Restriction pieces were found as expected: A/A: 300 bp, C/A: 134, 166, 300 bp., and C/C: 134, 166 bp.

2.4. Statistical Analysis

Various statistical analyses were applied to analyze the data. The mean continuous levels of phenotypes and genotypes were analyzed using SPSS software (version 20.0) (SPSS Inc., Chicago, IL). Statistical significance was evaluated using the Student's *t*-test for the single or multiple comparisons of experimental groups; moreover, the relationship between target SNPs and RA risk was assessed using unconditional logistic regression and the Chi-square test. A *P*-value of <0.05 was considered statistically significant, and a *P*-value of <0.01 indicated a highly significant difference. For polymorphisms analysis, data were tested for deviations from the Hardy-Weinberg Equilibrium (HWE) using the software available at the Institute of Human Genetics, Helmholtz Zentrum, München, Germany.

3. Results and Discussion

The estimation of biochemical data showed significant differences in the serum level of HGH and IGF-1 among the NSCLC patients, compared to the control group (Table 1). The data also showed a significant increase in the serum levels of HGH among the NSCLC patients, compared to the control group (*P*<0.001); however, a significant decrease was noted in the NSCLC patients' serum regarding the levels of IGF-1, compared to the control group (*P*<0.05).

Table 1. The estimation of biochemical data in the serum level of HGH and IGF-1 among the NSCLC patients

Parameters	NSCLC patients (n=67)	Control group (n=28)	*P*-Value
	Mean±SD (Range)	Mean±SD (Range)	
HGH (ng/ml)	2.81±1.27 (1.27–6.87)	1.94±0.88 (0.87–4.03)	<0.001
IGF-1 (ng/ml)	55.6±42.7 (1.7–223.1)	64.1±50.2 (12.4–199.1)	<0.05

After the replication of the of IL-12 gene SNP rs3212227 (Figure 1), the genotyping results obtained from the PCR-RFLP method (Figure 2) were found to be consistent with those from HWE. Based on the fragments digested by the TaqI restriction enzymes, the identified genotypes included: A/A: 300 bp, C/A: 134, 166, 300 bp., and C/C: 134, 166 bp.

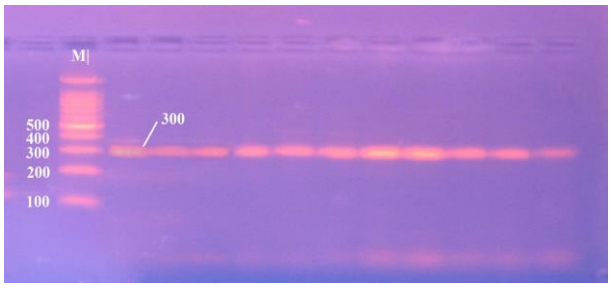

Figure 1. PCR product of the IL-12 gene SNP rs3212227, (A→C) SNP in 2% agarose gel electrophoresis, 5 µL of PCR product loaded in each well. Lane M: DNA Ladder (100-1.5 kb) Lanes 1-10: PCR product (one band 300 bp)

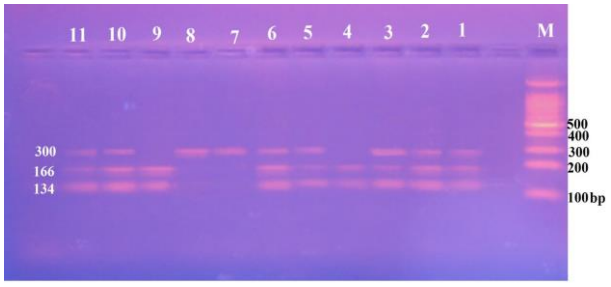

Figure 2. Restriction digestion of PCR product for the IL-12 gene SNP rs3212227, (A→C) SNP in 3% agarose gel electrophoresis, and 12 µL of PCR product loaded in each well. Lane M: DNA Ladder (100-1.5 kb), Lane 7, 8, and 4: One band, the uncut fragments (300 bp) are wild genotype (AA). , Lane 1, 2, 3, 5, 6, 10, and 11: Three bands (300, 166, and 134 bp) are heterozygous genotypes (AC). Lane 4, 9: Two bands (166 and 134 bp) are homozygous genotypes (CC).

Genotype distribution analysis under different inheritance models highlighted significant differences in all SNPs of rs3212227 in the IL-12 gene and the risk of NCSLC in a case-control study (Table 2).

The results showed that the genotyping frequencies of the IL-12 gene were consistent with those obtained from HWE (P>0.05) in the patient (NSCLC) (X2=0.017, allele frequencies A=0.75 and C=0.25, P=0.53) and control groups (X2 =0.19, allele frequencies A=0.75 and C=0.25, P=0.75). The homozygous wild genotype (AA) increased the risk of NSCLC about four times higher than that of heterozygous genotype (AC) (OR=4.7, 95% CI=3.15-7.4, P=0.0017) (Table 3) and about six times higher than that found in the homozygous genotype (CC), after adjustment for age and BMI (OR=6.8, 95% CI=1.23-16.1, P=0.0001). The risk of genotype regarding dominant inheritance model (AC+CC) for patients who are the carriers of the AC genotype was about four folds higher than AA wild genotype allele (OR= 4, 95% CI=2.22-12.4, P=0.0001).

The allele frequencies of A were found to be 75% and 82% in the NSCLC patients and the control group, respectively (Table 4); however, the frequencies of the C allele in the NSCLC cases and the control group were 25% and 18%, respectively. The allele frequency of A was higher than that of C in both groups (NSCLC and the control group). Although the frequency of allele C increased the risk of NSCLC by about one and a half times, compared to allele A, this increase was not significant between the groups.

Table 2. Distribution and Association of the SNP rs3212227 (A→C) SNP Genotypic Frequencies by Hardy-Weinberg Equilibrium between the NSCLC Patients and the Control Group

Genotype	No.	Allele A	Allele C	Allele frequencies	HW-observed frequency	Percentage %	HW-expected frequency	Percentage %	X^2	*P*-value HWE
Cases										
AA	43	86	0		43	64	43.57	67.52	0.017	0.049
				A=0.75						
AC	15	15	15	C= 0.25	15	22	14.87	20.96		
CC	9	0	18		9	14	9.57	12.52		
Total	67	101	33		67	100	67	100		
Control										
AA	18	36	0		18	64.4	16.78	54.39	0.19	0.035
				A= 0.75						
AC	6	6	6	C=0.25	6	21.4	8.44	38.72		
CC	4	0	8		4	14.2	2.78	6.89		
Total	28	42	14		28	100	28	100		

X^2: Chi-square; P>0.05 statistically significant by Hardy-Weinberg Equilibrium. C: Cytosine; T: Thymine; n: number.

Table 3. Genotype frequencies of A→C polymorphism for the IL-12 SNP (rs3212227) gene in cases and controls and their associations with the risk of NSCLC

Genotype	Patients No.	Percentage %	Control No.	Percentage %	Crude model OR[a]	(95% CI)	Adjusted model OR[b]	(95% CI)	*P*-value
A→C	67	100	28	100					
AA	43	64	20	71	1.00				
AC	15	22	6	21	4,76	(3.11-7.31)	4.77	(3.15-7.4)	0.0017
CC	9	14	2	0.07	6.99	(3.9-12)	6.89	(1.23-16.1)	0.0001
AC +CC vs. AA	24	36	8	28	4.01	(2.27-5.9)	4.0	(2.2-12.4)	<0.0001

[a] Multinomial logistic regression. [b] Multinomial logistic regression stratified adjusted for age and BMI; n: number; CI, confidence interval; OR, odds ratio.

Table 4. Allele frequency of the IL-12 SNP (rs3212227) gene A→C SNP in the patients and control group

Allele		Patients	Control	OR (95% CI)	*P*-value
A Allele	No. %	101 75%	46 82 %	1.00 Reference	
C Allele	No. %	33 25 %	10 18%	1.5 (0.68-3.30)	=0.3113
Total Allele	No. %	134 100%	56 100%	-	-

[a] Multinomial logistic regression. [b] Multinomial logistic regression stratified adjusted for age and BMI; n: number; C.I, confidence interval; OR, odds ratio.

The current study also showed the association between genotype AA allele of the IL-12 gene and NSCLC risk as to the cause of increased HGH production. The serum levels of IGF-1 in the AA genotype NSCLC were almost equal to the level of CC genotype NSCLC. Although it was higher than the level in the AC genotype NSCLC patients, this difference was not significant. As a result, the association of the IL-12-A allele and the risk of NSCLC with increased HGH production is questionable. Guler and Zapf (17) measured the highest HGH serum levels in the patients homozygous for IL-12 A alleles; however, other researchers showed the highest HGH levels in patients with the IL-12 CC genotype (18). Delafontaine and Song (19) observed a gradual increase in serum GH levels in cancer patients in terms of the advanced stages of neoplastic disease. This apparent inconsistency between the studies may be in part due to different methods and experimental models that were used.

The most common mechanism behind the present findings is that polymorphisms of 5′′ untraditional region (5′UTR) of IL-12 seem to increase the activity of these genes as a base promoter (18, 20) leading to the growth of angiogenesis and the development of NSCLC (18). Serum HGH concentration showed no significant correlation with age and gender (P>0.05), which was similar to the findings of a study by Liu and Chen (20). However, there was a significant association between HGH level and genotype (P=0.03). All these

indicate that the HGH is an independent risk factor for genetic factors. The results of this study showed that the HGH level was higher in the Planocellular type than the Adenocarcinomas; however, it was not significant (P>0.05). This may be another cause for the higher incidence of proliferative NSCLC in the Planocellular than Adenocarcinomas. To assess the functional relevance of the IL-12 A/C polymorphism on the serum IGF-1, the patients' sera IGF-1 concentrations were measured (21). The present study revealed that serum IGF-1 level in the AA genotype NSCLC was almost equal to the level of CC genotypes NSCLC; however, the statistical analysis was not significant (P=0.617). In addition, serum IGF-1 level was higher than that in the NSCLC patients of the AC genotype; however, this change was not significant either.

4. Conclusion

In conclusion, the rs3212227 IL-12 gene polymorphisms are involved in the pathogenesis of NSCLC, as well as the direction of several metabolic changes. Examination of the wild homozygous (AA) genotype of the IL-12 gene showed that the risk of NSCLC was about four times higher than that of the heterozygous (AC) genotype and about six times higher than that found in the homozygous genotype (CC). The study showed significantly elevated serum levels of HGH in the NSCLC patients, compared to the control group, and significantly low serum levels of IGF-1 in the NSCLC patients, compared to the control group. Further studies should be conducted on larger samples and different populations. Therefore, improving knowledge about genetic factors, immune disorders, and hormone secretion will be an important step in describing the pathogenesis of the inflammatory process leading to cancer and making prevention possible.

Authors' Contribution

Study concept and design: A. A. I.
Acquisition of data: M. S. M.
Analysis and interpretation of data: R. A. N.
Drafting of the manuscript: M. A. Z.
Critical revision of the manuscript for important intellectual content: S.Q. K.
Statistical analysis: I. A.
Administrative, technical, and material support: A. A. I.

Ethics

Sample collection was approved by the Institutional Ethics Committee of the Department of Oncology, and informed consent forms were signed by all participants.

Conflict of Interest

The authors declare that they have no conflict of interest.

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