The role of interleukin-4-590 (C>T) gene polymorphism and its relationship with lung cancer risk in the Iraqi population

El papel del polimorfismo del gen interleucina-4-590 (C>T) y su relación con el riesgo de cáncer de pulmón en la población iraquí

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Abstract

Background: Interleukin-4 (IL-4) is a pleiotropy cytokine that plays a role as an immune regulator and anti-inflammatory. The study was conducted to determine the polymorphisms of the IL-4 gene and its relationship with lung cancer by using ARMS-PCR (Amplification refractory mutation system technique). The study included 240 samples; their ages ranged from 37 to 72 years. This case-controlled study was performed including 160 Lung cancer patients (mean age 58.92±1.2 years) and 80 controls (mean age 46.91±1.6 years).

Results: The results show that in Lung cancer patients samples high frequency for allele C of IL- 4-590 (C>T), was ratio 74.38% as compared with T allele was ratio 31.87%, and associated with an etiological fraction (EF) of Lung cancer patients, as well dependent on values of odds ratio (OR) was 6.20 and confidence intervals (CI 95%) was 4.09 to 9.40, while T allele associated with a preventive fraction (PF) with Lung cancer patients. The statistical analysis results present CC homozygote genotype of IL- 4 -590 (C>T) gene was higher than in Lung cancer patients and its ratio 61.25% and showed associated with an etiological fraction (EF) in Lung cancer, while CT and TT genotypes present in high frequency in control was ratio 36.25% and 50% sequentially, and present associated with a preventive fraction (PF) in lung cancer patients.

Conclusions: Our findings demonstrate that the IL-4 gene in position -590 (C>T) may represent a risk factor and is associated with lung cancer development in the Iraqi population.

Keywords: IL-4 -590 (C>T) gene, Lung cancer, Polymorphisms, and ARMS PCR.

Resumen

Antecedentes: La interleucina-4 (IL-4) es una citocina pleiotrópica que desempeña un papel como regulador inmunitario y antiinflamatorio. El estudio se realizó para determinar los polimorfismos del gen IL-4 y su relación con el cáncer de pulmón mediante el uso de ARMS-PCR (técnica de amplificación del sistema de mutación refractaria). El estudio incluyó 240 muestras; sus edades oscilaban entre 37 y 72 años. Este estudio de casos y controles se realizó con 160 pacientes con cáncer de pulmón (edad media 58,92 ± 1,2 años) y 80 controles (edad media 46,91 ± 1,6 años).

Resultados: Los resultados muestran en que en las muestras de pacientes con cáncer de pulmón existe una alta frecuencia para el alelo C de IL-4-590 (C>T), que fue del 74,38 % en comparación con el alelo T del 31,87 %, y se asoció con la fracción etiológica (EF) de pacientes con cáncer de pulmón, también dependiente de valores de odds ratio (OR) que fue de 6,20 e intervalos de confianza (IC 95%) fue de 4,09 a 9,40, mientras que el alelo T se asoció con la fracción preventiva (FP) con pacientes con cáncer de pulmón. Los resultados del análisis estadístico presentan que el genotipo homocigoto CC del gen IL-4-590 (C>T) fue mayor en los pacientes con cáncer de pulmón y su proporción fue del 61,25 % y se mostró asociado con la fracción etiológica (FE) en el cáncer de pulmón, mientras que los genotipos CT y TT presente en alta frecuencia en control y su ratio fue de 36,25% y 50% secuencialmente, y asociado a fracción preventiva (FP) en pacientes con cáncer de pulmón.

Conclusiones: Nuestros hallazgos demuestran que el gen IL-4 en la posición -590 (C>T) puede representar un factor de riesgo y estar asociado con el desarrollo de cáncer de pulmón en la población iraquí.

Palabras clave: Gen IL-4 -590 (C>T), Cáncer de pulmón, Polimorfismos y ARMS PCR.

Background

Data associated with lung cancer indicate an increase in incidence and mortality[1]. Lung cancer is the leading cause of cancer-related death in most parts of the world[2], it accounts for about 17.5% of all cancer deaths globally[3]. The data indicates about 14 million new cancer cases in 2012 the number is expected to rise to 22 million in later years. Overall cancer-related deaths were around 8 million in the year and the rate is expected to rise to 13 million by 2032[4]. The estimated death rate for lung cancer was 610.2 per 100,000 in China people in 2015[5]. Globally more than one million people die of lung cancer each year, and it is estimated that cigarette smoking explains almost 90% of lung cancer risk in men and 70 to 80% in women[6]. Tobacco smoking is an epidemic problem in Iraq, and many lung cancers were associated with smoking risk [Ibrahim et al., 2018]. The inflammatory cytokines play a role in inducing or exacerbating some diseases, and the protection is associated with the secretion of some anti-inflammatory cytokines produced by Th2 cells that produce important cytokines such as Interleukin-4 (IL-4) and Interleukin-10[6-8]. The concentrations of serum cytokines are associated with lung cancer survival, such as (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12[9,10]. Interleukin-4 (IL-4) is a potent regulator of antitumor immune responses with both tumor-promoting and tumor-inhibiting properties, since it has both immunosuppressive and anti-angiogenic functions[11], its expression has been linked to breast, kidney, prostate, and colon cancer as well as lung cancer[12]. Interleukin-4 is a cytokine regulator of immune responses that stimulate carcinomas and works to inhibit tumors[13]. IL-4) was originally discovered in 1982 as a low molecular weight (15 kDa) T cell–derived polypeptide of 129 amino acids, which is encoded by the IL-4 gene on chromosome 5q23.31. It is critical for immunoglobulin G subclass 1 (lgGl) production from mitogen-activated B cells. It is secreted by CD4 T cells, helper T cells type 2 (TH2) lymphocytes, and natural killer T cells, and by cells of the innate immune system, including mast cells, basophils, and eosinophils. The source of IL-4 is Th2 cells and is also produced by macrophages, NK cells, mast cells, eosinophils, and basophils, and IL-4 targets many T cells and B cells[14,15]. The phenotypic polymorphism of the IL-4 gene plays a role in regulating the expression of cellular kinematics and is important as a protective or causative part of some diseases. For example, replacing the nitrogen base C with the nitrogen base T at site -590 of the IL-4 gene causes a reduction in the expression of the IL-4 gene, also CC genotype increases in lung cancer patients, and shows an association with the etiological fraction of the disease, while the TT and CT genotypes shows an association with the protective fraction[2]. However, the IL-4 gene polymorphism may be associated with a reduced risk of lung cancer among the Portuguese and Chinese populations[16]. This study aims to assess the phenotypic variation of the IL-4 -590 (C> T) gene using the Amplification refractory mutation system technique (ARMS-PCR) in lung cancer of Iraqi patients.

Methods

Study population

The study included 240 samples; their ages ranged from 37 to 72 years. The study population consisted of 160 of Lung cancer patients (mean age 58.92±1.2 years) and 80 individuals (mean age 46.91±1.6 years) as control. Blood the patients' Samples were collected from Al-Amal National Cancer Hospital in the Medical City in Baghdad. Samples were collected during the period from December 1, 2020, to October 15 of the same year. They had an established diagnosis of lung cancer by the senior board-certified investigators based on specific interviews, clinical examination, medical records (hospital and patient clinic case observations), and family data. The control group involved healthy volunteers. The study was approved by the Ethical Committee of the College of Medicine, Al-Iraqi University. Baghdad, Iraq.

DNA genomic extraction and gel electrophoresis

Two ml of blood from each Lung cancer patient and control (healthy) by using venipuncture, later, 2.5 ml was added into EDTA tubes then DNA was extracted by DNA isolation kit (Promega USA, the according to manufacture instructions manual). DNA purity was qualified by Nanodrop and it was about 1.6 ±1.8. All samples were kept at <22 C° for further study. IL-4 gene polymorphism in position -590 (C>T) was examined by using the amplification refractory mutation system-polymerase chain reaction technique (ARMS-PCR). The primers (Alpha DNA-Canada) that were designed according to Alsaid et al., 2013[16], and 20 µl were the total volume of reaction mix from Pio-Neer in Korea, including 5 µl premix master mix (including Taq, dNTP, Buffer, and Mg2+), 1.5 µl of each outer primer and 1.5 µl of each inner primer, and 5 µl DNA, and 4 µl of RNase-free double distilled water), and the molecular marker size (Pro-Mega-USA) 100-1500 base pair. ARMS-PCR programs of IL-4 gene polymorphism in position -590 (C>T) were summarized according to Alsaid et al., 2013[16]. The genotypes were established by analyzing electrophoresed 1.5% gel of agarose stained with diamond dye (Pro-Mega).

Genotyping of IL-4 -590 (C>T) gene and ARMS-PCR Program

The primers for IL-4 -590 (C>T) gene are as follows according to the published article by Alsaid et al., 2013[16]. The sequences of used primers are Specific: 5`GAATTTGTTAGTAATGCAGTCCTCC-3 for T allele, 5`ACACTAAACTTGGGAGAACATTGTC-3` for C allele and 5`GAATTTGTTAGTAATGCAGTCCTCC-3 for Reverse. Primers Size for IL-4 -590 (C>T) gene are 220 bases per. The ARMS PCR reaction was conducted in 20 µL volume (containing DNA sample, Master Mix, primers, and RNase-free double distilled water) to the published article Alsaid et al., 2013[16]. The ARMS-PCR amplification was done at, initial denaturation of 95 °C for 5 min, followed by 10 cycles at 95 °C for 15 s, 65 °C for 50 s and 72 °C for 40 s, and second initial denaturation at 95 °C for 5 min, followed by 20 cycles at 95 °C for 15 s, 55 °C for 50 s and 72 °C for 40 s and at final extension 72 °C for 10 min.

Statistics

Differences in the frequencies of IL-4 gene in position -590 (C>T) for Lung cancer in this study with control were analyzed by Fisher's test (value P<0.05). The OR (Odds Ratios) and CI (Confidence Intervals) were calculated by Compare 2 Ver.3.04 software Abramson, J. (2003-2013). The Preventive Fraction (PF) and Etiologic Fraction (EF) results were compared with Hardy-Weinberg equilibrium and according to the software within the following website www.had2know.com. The equations for statistical analysis of IL-4 -590 (C>T) gene polymorphisms data in patients and control are:

Relative risk (RR) = (a) × (d)/(b) × (c).

a = number of patients with expression of allele or genotype.

b = number of patients without expression of allele or genotype.

c = number of controls with the expression of allele or genotype.

d = number of controls without expression of allele or genotype.

Etiologic Fraction (EF):

EF = (RR-1)f/RR

Where f = a/a+c

Preventive Fraction (PF):

PF = (1-RR) f/RR (1-f) + f

Where f = a/a+c

According to Schallreuter et al.[17], and Savejgaard et al.[18].

Results

It is shown the genetic frequency of IL-4 -590 (C>T) gene polymorphisms in Lung cancer patients with age 58.92±1.2 years, and eighty healthy individuals as a control sample with a mean age of 46.91±1.6 years, by using ARMS-PCR (amplification refractory mutation system-polymerase chain reaction). Three genotypes CC, CT, and TT are present in Lung cancer patients (Figure 1).

Figure 1. Electrophoresis of genotypes for IL-4 -590 (C>T) in Lung cancer patients, lane M DNA marker, size (100 to 1500 bp), lane 1 and 5 TT genotype, lane 2, 3, 4, and 6 TT genotype, lane 7 CT genotype

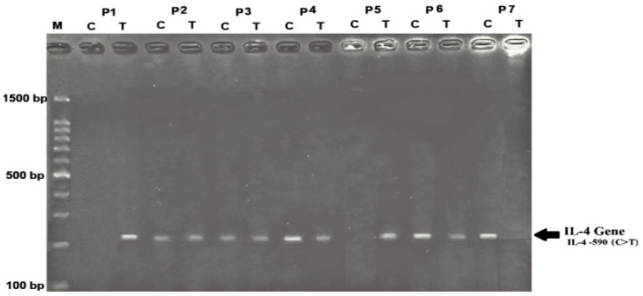
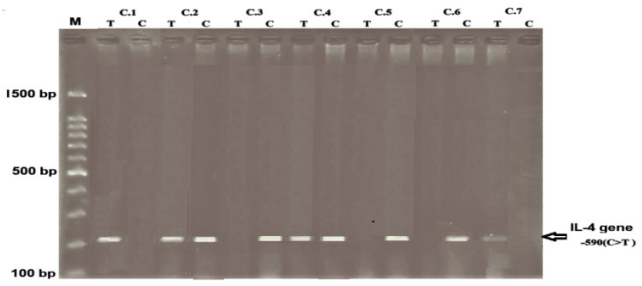


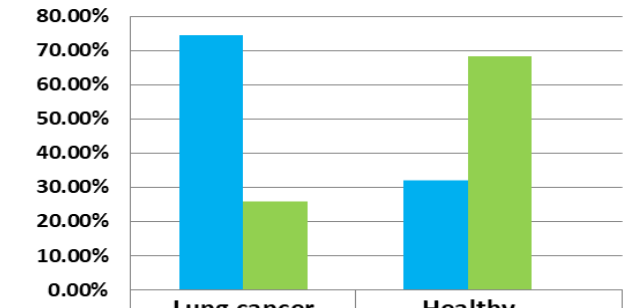
Table 1 showed the alleles frequencies of IL-4 -590 (C>T) gene polymorphisms in Lung cancer patients and control individuals. The frequency of the C allele was present associated with lung cancer patients and significantly higher in Lung cancer patients (p= **0.0001) when compared with controls, the odds ratio (OR) was 6.20, and confidence intervals (CI 95%) was ratio 4.09 to 9.40, in addition, the C allele frequency was higher in lung cancer patients as compared to the control was ratio 74.38% vs 31.87% sequentially (Figure 2).

Figure 2. Electrophoresis of genotypes for IL-4 -590 (C>T) in Control, lane M DNA marker, size (100 to 1500 bp), lane 1 and 7 TT genotype, lane 2 and 4 CT genotype, lane 3, 5, and 6 CC genotype.



The T allele frequency in the control group was higher in comparison with Lung cancer patients' group was a ratio of 68.13% vs 25.62% sequentially (Figure 3), therefore the C allele has etiological fraction (EF) was 62.4% with Lung cancer patients, while T allele has preventive fraction (PF) was 57.1% with the patients.

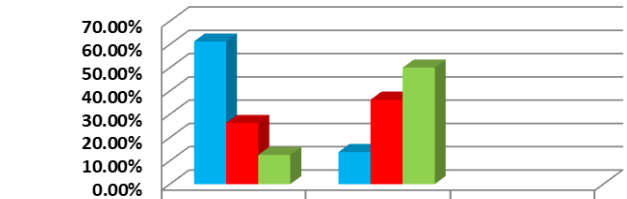
Figure 3. Allelic frequencies of IL-4 590 (C>T) Polymorphism in lung cancer patients and controls.



	Lung cancer patients	Healthy (control)
C allele	74.38%	31.78%
T allele	25.62%	68.13%

The polymorphisms study of genotyping demonstrated a significant difference in genotypes distribution frequency of the IL-4 -590 (C>T) gene by using ARMS-PCR in Lung cancer patients compared with and control group. Table 1 shows a significant variation of CC genotype in Lung cancer patients with control (P= **0.0001) with a higher frequency ratio of 61.25% vs13.75% sequentially as illustrated in (Figure 4). The odds ratio (OR) and confidence intervals (CI 95%) of CC genotype were OR= 9.9 and CI at 95% were 4.89 to 20.11, and CC genotype present associated with an etiological fraction (EF) in Lung cancer patients, while in control CT and TT genotypes show higher frequency comparison with Lung cancer patients ware ratio CC 36.25% and TT 50% in control, CC 26.25%, and TT 12.50% in patients (Figure 4).

Figure 4. Genotype frequencies of IL-4 590 (C>T) Polymorphism in lung cancer patients and controls.



	Lung cancer patients	Healthy (control)	
CC genotype	61.25%	13.75%	
CT genotype	26.25%	36.25%	
TT genotype	12.50%	50%	

The odds ratio (OR) and confidence intervals (CI 95%) of the CT genotype were 0.63 and CI was 0.35 to 1.11 and the TT genotype odds ratio (OR) was 0.14 and CI was 0.08 to 0.27, therefore the CT and TT genotypes have a preventive fraction with Lung cancer risk (Table 1).

Table 1. The frequency of allelic and genotypes in Lung cancer and control samples for IL-4 Gene in position -590 (C>T).

Gene	Genotype & Allele	Lung cancer Number (%)	Healthy Number (%)	OR (CI 95%)	P. value
IL-4 -590 (C>T) gene	CC	98 (61.25%)	11 (13.75%)	9.9 (4.89 to 20.11)	0.0001
	E. F	55.1%			
	CT	42 (26.25%)	29 (36.25%)	0.63 (0.35 to 1.11)	0.074
	P. F	13.6%			
	TT	20 (12.50%)	40 (50%)	0.14 (0.08 to 0.27)	0.0001
	P. F	42.9%			
	C allele	238 (74.38%)	51 (31.87%)	6.20 (4.09 to 9.40)	0.0001
	E. F	62.4%			
	T allele	82 (25.62%)	109 (68.13%)	0.16 (0.11 to 0.24)	
	P. F	57.1%			

Notes: OR (Odds ratio), CI (Confidence Interval), E.F (Etiological fraction), P.F (Preventive fraction), P<0.05 by Fisher's

Discussion

The polymorphisms in some cytokine's gene may be associated with different types of cancer, including lung cancer risk. Genetic changes in the genome may increase cancer risk[19,20]. The genotypic contribution of IL-4 to cancer is not well-studied. As for lung cancer, IL-4 rs2243250 single nucleotide polymorphisms have been found to associate with a reduced risk of lung cancer among Portuguese[21]. Interleukin-4 is a regulatory protein that is produced by T cells and has an essential role in inducing antibody production by plasma cells[22]. Also, it is a pleiotropic cytokine also that can be produced by Mast cells and basophils and has an inhibitory role in inflammation and tumor growth in some carcinoid tumors[23]. It also has a stimulating effect on some tumors, including human colon, breast, kidney, and lung cancer; moreover, IL-4 can prevent the formation of blood vessel clots and reduce the risk of developing blood vessel clots[24]. Cytokine gene polymorphisms may affect inflammatory-related pathways, and influence susceptibility to different types of cancer[25]. The IL-4 gene has been ob-

served to be associated with cancer risk in both Caucasian and Asian subjects. The results indicated that IL-4 in positions rs2243250 and rs79071878 polymorphisms have been associated with susceptibility to cancer[26]. The IL-4 gene affects the transcription of some genes, and there is a relationship between the IL-4 -590 C / T gene and the development of lung cancer risk[27]. Some results suggest that genetic and environmental factors may have the potential to interact with human genes, and the IL-4-590 C / T polymorphism (for C / C + C / T frequencies) may be a potential susceptibility marker for lung cancer patients[28]. The study shows that the Single Nucleated Polymorphism in the region of (T-1099G, C-589T, and C-33T) of the IL-4 gene, which was evaluated in Chinese with lung cancer, showed that the TT genotype was significantly associated with a lower risk of lung cancer, whereas CC type was associated with a protective factor in the risk of infection. This study is in agreement with our results, so the IL-4 gene can play an important role in determining the susceptibility of lung cancer [13, 29-31]. A study showed by Chang et al., 2015 indicates that the percentages of CC, CT the TT of the IL-4 C-589T genotypes was differentially distributed and they account for 69.0%, 26.5%, and 4.5% in lung cancer Patient respectively, and 61.3%, 30.4%, and 8.3% in control group, respectively (p = 0.0156). TT genotype Carriers had a lower risk of developing lung cancer (odds ratio (OR) = 0.48, 95% confidence interval (CI) = 0.27-0.86, p = 0.0106) of the CC genotype carriers[2], these results are consistent with the results of our study.

Conclusions

There was a significant difference in the genotypes of the IL-4 gene at the site -590 (C>T) in Iraqi patients diagnosed with lung cancer, demonstrating a significant correlation between the IL-4 gene for the CC genotype and the risk of lung cancer.

References

1. Zeng H, Zheng R, Zhang S, He J, Chen W. Lung cancer incidence and mortality in China, 2008. Thorac Cancer. 4: (1). 53-58.
2. Chang W, Shou-Cheng W, Chin-Liang C, Hong-Xue J, Chieh-Luh H, Chin-Mu H, Chia-Wen T, Shih-Pinp L, Pei-Chen H, Yen-Li L, Da-Tian B. 2015. Contribution of Interleukin-4 Genotypes to Lung Cancer Risk in Taiwan. Anticancer Research 35: 6297-6302.
3. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. 2007. Estimates of the cancer incidence and mortality in Europe in 2006. Ann. Oncol. 18: 581–592.
4. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-E386.
5. Chen, W, Zheng, R, Baade PD, Zhang S, Zeng H, Bray F, He J. Cancer statistics in China, 2015. CA-A Cancer Journal for Clinicians, 2016;66, 115–132.
6. Pauza ME, Neal H, Hagenbaugh A, Cheroutre H, Lo D. T-cell production of an inducible interleukin-10 transgene provides limited protection from autoimmune diabetes. Diabetes. 1999;48:1948–1953.
7. Phillips JM, Parish NM, Drage M, Cooke A. Cutting edge: interactions through the IL-10 receptor regulate autoimmune diabetes. J. Immunol. 2001;167:6087–6091.
8. Sharif S, Arreaza GA, Zucker P, Delovitch TL. Regulatory natural killer T cells protect against spontaneous and recurrent type 1 diabetes. Ann. NY Acad. Sci. 2002;958:77–88.
9. Enewold L, Leah E, Elise D, Yun-Ling Z, Zhipeng Y, Glenwood T, Anthony J, Curtis C. Serum Concentrations of Cytokines and Lung Cancer Survival in African Americans and Caucasians. Cancer Epidemiol Biomarkers Prev. 2009 January; 18(1): 215–222.
10. Wang HW, Joyce JA. Alternative activation of tumor-associated macrophages by IL-4: Priming for protumoral functions. Cell Cycle. 2010;9: 4824-4835.
11. Shurin, MR, Lu L, Kalinski P, Stewart-Akers AM and Lotze MT. Th1/Th2 balance in cancer, transplantation and pregnancy. Springer Semin Immunopathol, 1999;21: 339-359.
12. Wang HW, Joyce JA. Alternative activation of tumor-associated macrophages by IL-4: Priming for protumoral functions. Cell Cycle, 2020;9: 4824-4835.
13. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, Mahony L, Palomares O, Rhyner C, Quaked N, Schaffartzik A, Veen W, Zeller S, Zimmermann M, Akdis C. Interleukins, from 1 to 37, and interferon-g: Receptors, functions, and roles in diseases. J. Allergy Clinic. Immunol. 2011;127:701-721.
14. Ryan AW, Thornton JM, Brophy K, Daly J, Mcloughlin R, Morain C, Abuzakouy M, Kennedy N, Mcmanus R. Chromosome 5q candidate genes in coeliac disease: Genetic variation at IL-4, IL-5, IL-9, IL-13, IL-17B and NR3C1.Tissue Antigens J. 2005;65(2):150-155.
15. Gomes M, Coelho A, Araújo A, Teixeira AL, Catarino R, Rui M. Influence of functional genetic polymorphism (– 590C/T) in non-small cell lung cancer (NSCLC) development: The paradoxical role of IL-4. Gene, 2012;504, 111–115.
16. Alsaid A, El-Missiry M, Hatata E, Tarabay M, Settin A. Association of IL-4 -590 C>T and IL-13 -1112 C>T gene polymorphisms with the susceptibility to type 2 diabetes mellitus. Disease Markers. 2013;35(4):343-247.
17. Schallreuter KU, Levenig C, Kuhnl P, Loliger C, Hohl-Tehari M, Berger J. Histocompatibility antigens in vitiligo: Hamburg study on 102 patients from Northern Germany. Dermatology. 1993;187:186–92.
18. Savejgaard A, Platz P, Ryder LP. HLA and disease 1982-A survey. Immunol Rev. 1982;70:193–218.
19. Eaton KD, Perrin E, Gary E, Mark D, Matt J, Effie W. Inflammatory Gene Polymorphisms in Lung Cancer Susceptibility. Journal of Thoracic Oncology. 2018; 13(5): 649-659.
20. Gomes M, Coelho A, Araujo A, Teixeira AL, Catarino R and Medeiros R. Influence of functional genetic polymorphism (- 590C/T) in non-small cell lung cancer (NSCLC) development: the paradoxical role of IL-4. Gene. 2012;504: 111-115.
21. Lee JM, Yanagawa J, Peebles KA, Sharma S, Mao JT, Dubinett SM. Inflammation in lung carcinogenesis: new targets for lung cancer chemoprevention and treatment. Crit. Rev. Oncol. Hematol. 2008a;66, 208–217.

22. Yannopoulos A, Nikiteas N, Chatzitheofylaktou A, Tsigris C. The (–590 C/T) polymorphism in the interleukin-4 gene is associated with increased risk for early stages of colorectal adenocarcinoma. 2007;21: 1031–1035.

23. Vairaktaris E, Yannopoulos A, Vassiliou S, Serefoglou Z, Vylliotis A, et al. Strong association of interleukin-4 (–590 C/T) polymorphism with increased risk for oral squamous cell carcinoma in Europeans. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 2007;104, 796–802.

24. Leibovici D, Grossman HB, Dinney CP, Millikan RE, Lerner S, Wang Y, Gu J, Dong Q and Wu X. Polymorphisms in inflammation genes and bladder cancer: From initiation to recurrence, progression, and survival. J Clin Oncol. 2005;23: 5746-5756.

25. Yingxian J, Xiaochuan X, Xiaohan S, Shangwea L. Associations of common IL-4 gene polymorphisms with cancer risk: A meta-analysis. MOLECULAR MEDICINE REPORTS. 2017;16: 1927-1945.

26. Lancaster A, Nelson MP, Meyer D, Thomson G, Single RM. PyPop: a software framework for population genomics: analyzing large-scale multi-locus genotype data. Pac. Symp. Biocomput. 2003:514–525.

27. Gu J, Yingying S, Yongjun Z. Association between interleukin-4 polymorphisms and environment and nonsmall cell lung cancer in Chinese population. Journal of Cancer Research and Therapeutics. 2014;10(2): C135-C139.

28. Walser T, Xiaoyan C, Jane Y, Jay M, Eileen H, Gina L, Sherven S, Steven M. Smoking and Lung Cancer. Proceedings of The American Thoracic Society. 2008;5. 811-815.

29. Ibrahim, B., Saif A., and Mohammed A. 2018. Tobacco Smoking, Lung Cancer, and Therapy in Iraq. Frontiers in Public Health. 6: 1-5. doi: 10.3389/fpubh.2018.00311

30. Hernández PA, Ramírez EG, Soto AP, Alzate CA, Pereira ML, Jimenez CF, Gonzalez DY. Fisioterapia y rehabilitación integral de personas con discapacidad: revisión narrativa. Archivos Venezolanos de Farmacología y Terapéutica. 2021;40(6):648-55.

31. Conde CG, León-Méndez D, León-Méndez G. Desarrollo de un cosmético tipo gel con propiedades antioxidante usando como activo aceite esencial de Citrus sinensis. Archivos Venezolanos de Farmacologia y Terapéutica. 2021;40(1):101-8.