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Article

Genetic Basis of Bladder Cancer: Predictive Roles of Interleukin Signaling Molecules

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Abstract: Despite the fact that bladder cancer is a major public health problem in Iraq, the genetic elements that contribute to its development are still not fully understood. Through the use of a case-control approach, the purpose of this investigation is to determine whether or not the IL-6 rs1800795 (-174 G/C) polymorphism is associated with an increased risk of bladder cancer in the Iraqi population. Techniques. There were a total of 148 individuals in the research, including 78 cases and 70 controls. The participants were separated into two groups. Patients who had previously been diagnosed with bladder cancer by histological examination made up the case group. In cases, the GC genotype and the G allele were found to be significantly more prevalent than in controls (odds ratio = 2.22, p = 0.004). On the other hand, the GC and C alleles were found to be considerably more prevalent in cases. The outcomes. At the case group level, there were no statistically significant differences between the two groups in terms of the probability of developing bladder cancer (p 0.05). There was a statistically significant difference in the frequency of the GG genotype between the patients and the controls in the control group (P = $0.04). A further finding was that there was no statistically significant difference in the CC genotype between the patients and the controls (p = 0.001). I will conclude. Taking into consideration these findings, it appears that there may be a connection between the gastrointestinal genotype of the miR-146a gene and an elevated risk of lung cancer in Iraq. Given that both the miR146a SNP Rs2910164 and the miR 155 SNP rS767649 are risk factors for non-small cell lung cancer, it is important to emphasize the significance that genetic variables play in the genesis of lung cancer.

Keywords: Bladder cancer, IL-6, rs1800795 polymorphism, Tetra-ARMS PCR, genetic susceptibility, inflammation, Iraqi population, genotypic analysis.

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1. Introduction

Bladder cancer (BC) is one of the most prevalent malignancies worldwide, ranking as the ninth most common cancer, with approximately 573,000 new cases and 213,000 deaths reported in 2020. It predominantly affects men, with a male-to-female ratio of approximately 3:1, and its incidence increases significantly with age [1]. Known risk factors for bladder cancer include smoking, exposure to occupational carcinogens, and chronic urinary tract infections, but a growing body of evidence points to the critical role of genetic predisposition and inflammation in the pathogenesis of this disease. Interleukin-6 (IL-6) is a multifunctional cytokine involved in immune responses, inflammation, and hematopoiesis. It plays a significant role in tumor development, including cancer cell proliferation, survival, angiogenesis, and metastasis [2]. Elevated levels of IL-6 have been observed in various cancers, including bladder cancer, and are often associated with poor prognosis. The expression of IL-6 is regulated by genetic

polymorphisms in its promoter region, particularly the rs1800795 (-174 G/C) single-nucleotide polymorphism (SNP), which has been reported to influence IL-6 transcriptional activity. This SNP is of particular interest due to its potential role in modulating inflammatory responses and its association with cancer susceptibility [3].

Greater numbers of studies have investigated the impact of the rs1800795 variant of the promoter upon cancer risk, and evidence is by no means convergent. The C allele is protective against bladder cancer in one group of people but in others—often in the presence of particular environmental stresses—seems to augment susceptibility [4]. The geographically variable and ethnic results underscore the need for region-by-region studies to explain the variant's role. The need is urgent in Iraq, with the growing burden of bladder cancer as a public health problem and the genetic determinants of the condition barely outlined.

Bladder cancer is a considerable health burden in Iraq, even though the underpinning genetics of its pathogenesis are not well characterized. One candidate for investigation is the –174 G/C promoter polymorphism in the gene for the cytokine interleukin-6 (rs1800795), a polymorphism known to control IL-6 transcription and, by inference, inflammatory signaling. Given the keystone role of IL-6 at the intersection of environmental insult, chronic inflammation, and the process of tumorigenesis, clarification of the way in which this SNP affects risk in the Iraqi population is timely and required [5], [6]. To this end, the present investigation employs a case–control design in an attempt to ascertain whether the rs1800795 genotypes are differentially distributed between bladder cancer cases and matched, cancer-free controls.

By unravelling the genetic origins of the Iraqi bladder cancer, this study can help make our understanding of how the illness begins and grows more precise opening the door to targeted prevention and more tailored treatment options.

2. Materials and Methods

Study Design and Population

In the present study, a hospital-based case–control study in Najaf, Iraq, to examine the relationship of the IL-6 −174 G/C promoter polymorphism (rs1800795) with bladder cancer risk. We recruited the study population from 148 adults comprising 78 with histopathologically confirmed tumour and 70 cancer-free subjects from the same medical centres—Al-Hakim General Hospital, Al-Sadr Medical City, and Al-Furat Al-Awsat Hospital—who attended with minor illness or for routine follow-up. Cases and controls were frequency-matched by sex and age (20–70 years), and all subjects gave written, informed consent. Those with other cancer, autoimmune disorder, chronic systemic illness, or long-term immunosuppressive or anti-inflammatory medication intake were excluded. The rigorous matching and the emphasis for stringent screening were designed to limit confounding and to establish a sound platform for the evaluation of the genetic association.

Sample Collection and Processing

The blood samples (3 mL each) were collected from all participants using EDTA tubes to prevent coagulation .

DNA Extraction

Genomic DNA was isolated from peripheral blood samples using the FavorPrep DNA Extraction Mini Kit (FAVORGEN, Taiwan) according to the instructions of the manufacturer to achieve optimum yield and purity. The process included a cell lysis step to break the red blood cells and the other membrane components to release the contents of the nucleus. Protein precipitation was done to precipitate proteins and also unwanted compounds. The precipitation of DNA was done using alcohol, washed to eliminate residual salts, and redissolved in the corresponding buffer. It provided high-quality genomic DNA for subsequent genetic studies .

Genotyping

Genomic DNA from the peripheral blood was obtained with the FAVORPREP DNA Extraction Mini Kit (FAVORGEN, Taiwan) following the manufacturer's recommendations. Briefly, the samples were first lysed to open red cells and other cell walls, thereby freeing the nuclei. The lysate was then treated with protein-precipitation to be protein- and residual debris-free. The nucleic acids were then precipitated with alcohol, washed to be salt-free, and eventually eluted in buffer, resulting in good-quality DNA for the downstream assays. The details of the PCR primer sequences and product sizes are provided in Table 1.

Table 1: Primer Sequences and Product Sizes for IL-6 Rs1800795 Polymorphism

Primer	Sequence (5' → 3')	Product Size (bp)
Outer Forward (F)	GACTTCAGCTTTACTCTTTGTCAAGACA	326
Outer Reverse (R)	GAATGAGCCTCAGACATCTCCAGTCCTA	-
Inner Forward (G allele)	GCACTTTTCCCCCTAGTTGTGTCTTCCG	205
Inner Reverse (C allele)	ATTGTGCAATGTGACGTCCTTTAGCTTG	184

PCR Reaction Conditions

PCR Conditions

The PCR reaction was performed in a total volume of **20 µL** containing (Table 2). The thermal cycling conditions were optimized to ensure the specific amplification of the target polymorphism are detailed in Table 3. The full composition of the PCR reaction mixture is shown in Table 2, while the thermal cycling parameters are outlined in Table 3.

Table 2: PCR Reaction Mixture

AMPLIQON

Reagent	Volume (µL)
Master Mix 2X	10
Outer F Primers	1.5
Outer R Primers	1.5
Inner F (G)	1.5
Inner R (C)	1.5
DNA	2.5
$MgCl_2$ **(25 mM)**	1.5
Total v.	20

The thermal cycling conditions were optimized to ensure the specific amplification of the target polymorphism:

Table 3: Thermal Cycling Parameters

Step	Temperature (°C)	Time	Cycle No.
Initial Denaturation	93	3 min	1
Denaturation	93	25 sec	35
Annealing	55	40 sec	
Extension	72	30 sec	
Final Extension	72	5 min	1

Detection of PCR Products

Amplicons were analyzed on a 2% agarose gel stained with ethidium bromide. This method enabled precise identification of the allelic variants of the Rs2910164 polymorphism, providing robust data for subsequent statistical analysis. The entire process was performed under controlled laboratory conditions to ensure reproducibility and accuracy. Genotypic patterns were identified based on band sizes:

1. GG (Wild Type): Bands at 203 bp and 445 bp.
2. GC (Heterozygote): Bands at 203 bp, 290 bp, and 445 bp.
3. CC (Mutant Type): Bands at 290 bp and 445 bp.

Statistical Analysis

Genotypic and allelic frequencies were compared using Fisher's exact test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to determine the strength of associations. A p-value < 0.05 was considered statistically significant.

3. Results

Agarose-gel electrophoresis of the Tetra-ARMS PCR amplicons resolved the genotype The PCR product obtained via amplification and the gel electrophoresis product provided specific genotypic distinction for the IL-6 rs1800795 (−174 G/C) polymorphism. Varying banding patterns on the 2% agarose gel defined two genotypes in the population. The GG genotype samples had 205 bp and the internal control 326 bp, and the GC heterozygous had all three anticipated bands—184 bp, 205 bp, and 326 bp—evidence for the presence of both alleles [7], [8]. The CC genotype, the 184 bp and 326 bp banding, was not seen in the cohort, which suggests low prevalence or absence of this variant in the Iraqi population under investigation.

The 326 bp internal fragment yielded consistently across all the samples testified to the efficiency and purity of the PCR reaction [9]. Statistical analysis according to the genotype frequencies obtained using the Fisher's exact test showed significance with the polymorphism rs1800795 ($p < 0.05$), validating the application of this SNP in the genomic profile of the participants. The application of the Tetra-ARMS PCR technique proved to be an efficient and sensitive genotyping technique with the ability to specifically detect allele-specific fragments, consistent with its application in the scenario of genetic association studies to ascertain IL-6 and the susceptibility to the disease.(Figure 1)

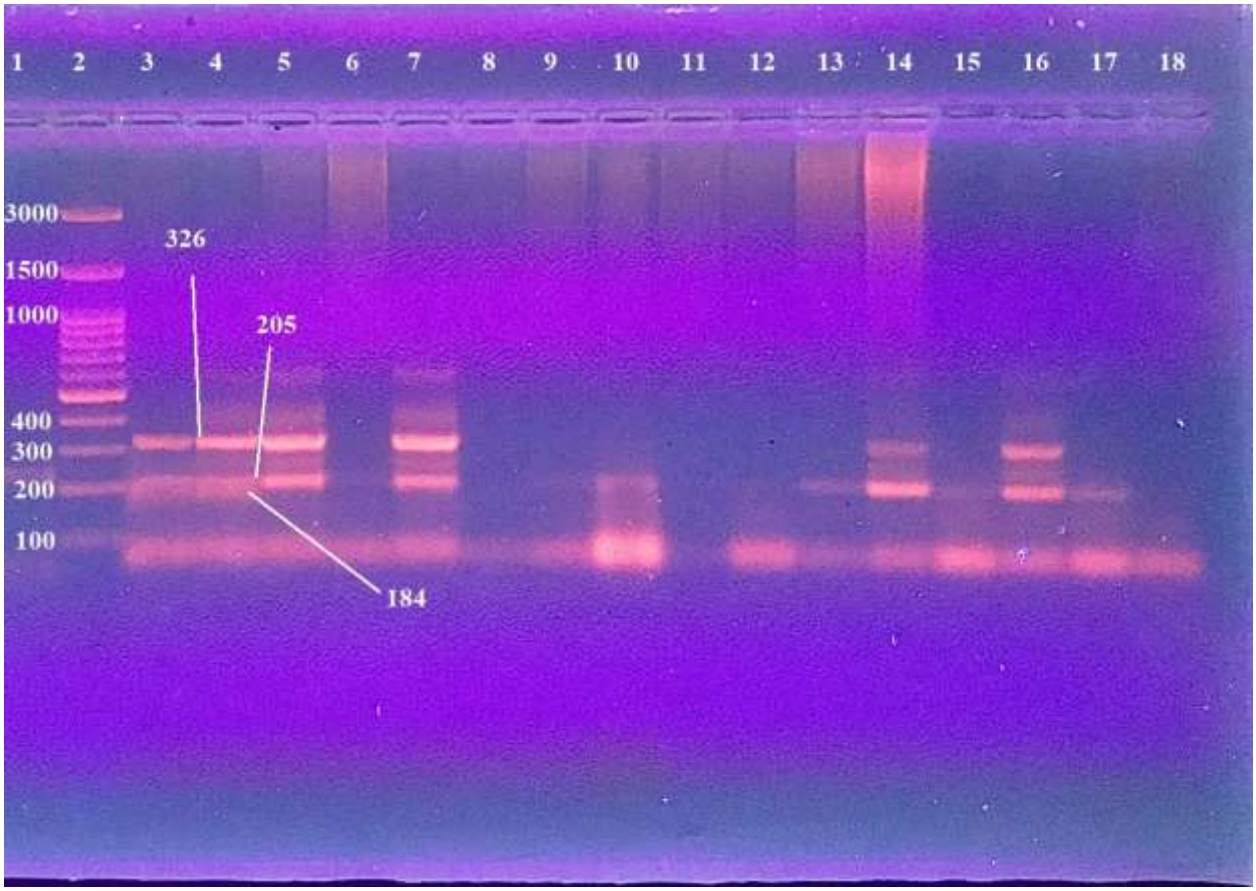

Figure 1: Gel Electrophoresis Results of Tetra-ARMS PCR Products for IL-6 rs1800795 (-174 G/C) Polymorphism: The image displays the banding patterns corresponding to GG, GC, and CC genotypes. The control band is observed at 326 bp, the

G-specific band at 205 bp, and the C-specific band at 184 bp. Distinct genotypes were identified based on the presence or absence of these bands [10].

Figure 2 presents gel electrophoresis evidence for the achieved IL-6 rs1800795 (−174 G/C) polymorphism genotyping using Tetra-ARMS PCR. The size of each of the respective product fragments enables the unmistakable identification of each genotype. The 326 bp product across all the samples as an internal constant control, is used for the identification of the success and accuracy of the PCR amplification [11]. The 205 bp fragment corresponds to the presence of the G allele, and the 184 bp fragment corresponds to the C allele. Three genotypes can be identified by the pattern of the bands::

1- GC Genotype: Exhibits bands at 184 bp, 205 bp, and 326 bp, indicating heterozygosity with one G allele and one C allele.
2- GG Genotype: Shows bands at 205 bp and 326 bp, indicating homozygosity for the G allele.
3- CC Genotype: Would show bands at 184 bp and 326 bp, representing homozygosity for the C allele; however, this genotype was not observed in the analyzed samples, suggesting its absence or very low frequency in the cohort.

The GG and GC genotypes alone were present in the present cohort. The absence of the CC genotype signifies the rarity—or even lack of—of this pair of alleles within the Iraqi population under investigation [12]. The findings reaffirm the utility of the Tetra-ARMS PCR method for discriminating allelic variants with great accuracy and detail. The precise and replicable banding of the gel electrophoresis supports the accuracy and replicability of the Tetra-ARMS PCR for IL-6 rs1800795 (−174 G/C) polymorphism. The internal control 326 bp band in each of the samples confirms the successful amplification process, and the 205 bp (G) and 184 bp (C) allele-specific bands facilitate simple differentiation of the genotype.

Interestingly, the absence of CC genotype and presence of G allele in the population in the present study imply an unbalanced distribution of alleles, which is most likely a reflection of the genetic background of the Iraqi population. Such a trend might be of relevance in the explanation of the population-specific genetic susceptibility to bladder cancer [13]. Further statistical analysis of frequencies of such genotypes—particularly diseased/nondiseased status—would be a rich insight into the functional impact of the promoter polymorphism related to the IL-6 production and the cancer hazard in the respective population.(Figure 2)

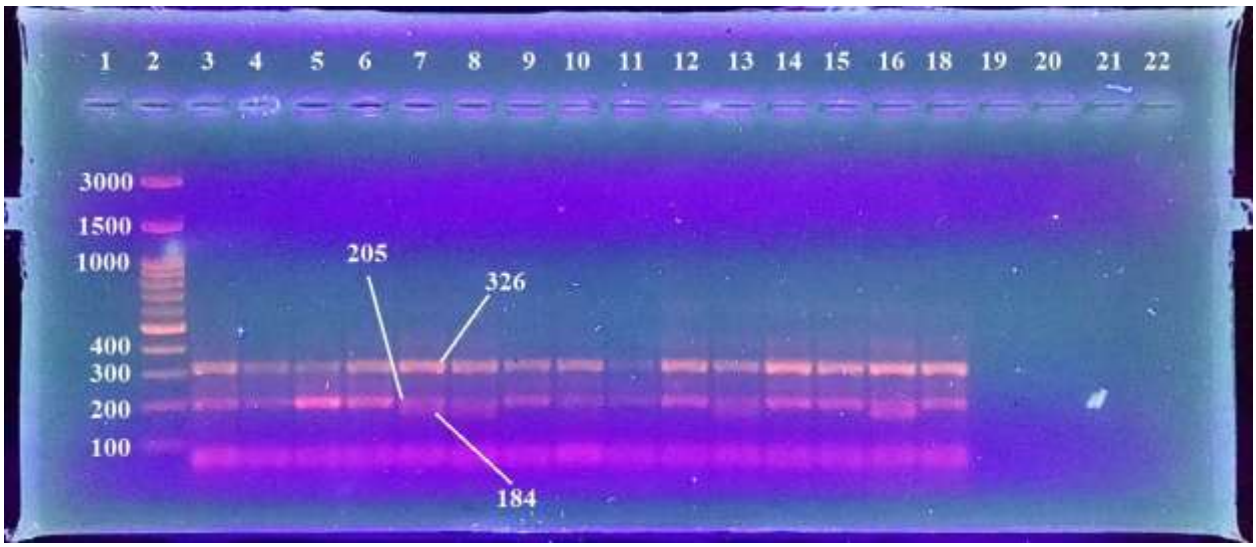

Figure 2: Gel Electrophoresis Results of Tetra-ARMS PCR Products for IL-6 rs1800795 Polymorphism.

Genotypic and Allelic Frequencies

The genotypic distribution and statistical outcomes are summarized in Table 3.

Table 3: Genotypic and Allelic Frequencies with Statistical Analysis

Genotype/Allele	Cases (n=50)	Controls (n=60)	OR (95% CI)	p-value
C allele	30	16	2.10 (1.08–4.06)	0.025
G allele	70	104	0.47 (0.28–0.80)	0.005
GG	20	44	0.32 (0.15–0.67)	<0.001
CC	0	0	-	-
GC	30	16	4.13 (1.93–8.86)	<0.001

The findings represent a marked disparity in the frequencies of the alleles and the genotypes in control and patients with bladder cancer. The GG genotype and G allele were specifically much more prevalent in controls, which points towards the presence of a protective effect. The GC genotype and the C allele appeared to be more prevalent in the patient group, which implies the presence of an association with enhanced bladder cancer susceptibility. The absence of the CC genotype in both populations also supports its rarity—or perhaps even absence—among this Iraqi group. While the findings are consistent with a genetic relationship of the IL-6 rs1800795 polymorphism with bladder cancer susceptibility, statistical validation and clinical confirmation are required. Larger, more diverse studies are also needed to confirm such a relationship and to understand the functional and biological implications of this promoter polymorphism in the process of oncogenesis [14].

4. Discussion

This study investigated the association between the rs2910164 (G>C) polymorphism in the miR-146a gene and lung cancer susceptibility in an Iraqi population. The results demonstrate a significant association between the GC genotype and C allele with an increased risk of lung cancer, highlighting the role of genetic factors in lung cancer etiology. These findings are consistent with the findings of previous studies in other populations, supporting the involvement of the rs2910164 polymorphism in the aetiology of lung cancer. In the current investigation, the GC genotype was also more prevalent in the lung cancer cases than in the controls (OR = 2.22, p = 0.004), which suggests that it might be a susceptibility factor. The same was also noted for the C allele, which was also more prevalent in the case group (OR = 1.79, p = 0.017), which suggests the involvement of the allele in increasing the disease susceptibility [15].

The same trend of high C allele frequency is supported by Jia et al. (2014), who also identified a high frequency of the C allele in Chinese patients with NSCLC. Overall, the results confirm the hypothesis that the variation of rs2910164 is interfering with the amount or activity of miR-146a with the result in increased tumorigenesis through dysregulated inflammatory or oncogenic pathways. These observations suggest that the polymorphism rs2910164 can impair the regulatory function of miR-146a, which can lead to carcinogenesis via disruption of the usual regulation of proliferation and inflammation. Consistent with this, a meta-analysis by Ren supported the frequent consistent relationship of the CC genotype and the C allele with the enhanced susceptibility to lung cancer in various populations, providing evidence for the hypothesis that the SNP is an effective genetic susceptibility factor. Notably, the analysis did not find the relationship of the GG genotype or the G allele ($p > 0.05$) with lung cancer, which suggests that the protective or neutral effect of the G allele would be population genetics- and environmentally dependent. The observations highlight the need for both genotype frequency and functional effect in the analysis for susceptibility to cancer.

This accords with the finding of Yin that GG genotype can have a protective influence on lung cancer but that this was not consistently observed in the populations. These variations between studies will most likely be caused by a wide range of factors from

ethnic and geographic differences to many different environmental exposures (e.g., air pollution and smoking habits) and differences in research design and size. These results point to the complex interplay of environmental influence and genetics in the development of the risk of cancer and to the necessity of population-specific research to accurately ascertain genetic susceptibility . To illustrate, Wang presented opposite findings in their meta-analysis, with no significant overall association between the rs2910164 polymorphism and lung cancer risk. This suggests that the impact of this SNP may be context-dependent, depending on other genes, environmental exposures, or a combination of the above. Mechanistically, the rs2910164 polymorphism is a G-to-C mutation that disrupts the stem-loop structure of the precursor miR-146a, preventing its maturation and, consequently, reducing its regulatory function. The disruption of its structure can affect miR-146a's ability to modulate key oncogenic and inflammatory processes, and its putative role in cancer development is therefore supported by a good biological rationale. This structural alteration may impair the ability of miR-146a to accurately modulate critical oncogene function and mediators of inflammation, providing a mechanistic basis for its carcinogenesis role. The resultant loss of miRNA function can cause deregulation of downstream targets, specifically affecting tumor-promoting and inflammatory processes. Consistent with this supposition, results of research conducted by Zhang and Wistuba and Gazdar draw attention to the central role of miR-146a in modulating the immune response and oncogenic signaling, adding to the biological plausibility of the rs2910164 polymorphism as a lung cancer pathogenesis determinant. The current research is not without limitations, however. The fairly small sample, consideration of a single SNP, and absence of in-depth environmental and lifestyle variables might restrict the generalizability and scope of the results. Large populations with diversity and examination of gene-environment interactions in subsequent research would create a more comprehensive picture of the lung cancer susceptibility profile. The relatively small sample size may limit the generalizability of the findings, and larger, multicenter studies are needed to confirm these results. Moreover, while this study focused on a single polymorphism, lung cancer is a multifactorial disease influenced by complex interactions between multiple genetic and environmental factors. Therefore, future studies should incorporate genome-wide analyses and evaluate gene-environment interactions to provide a more comprehensive understanding of lung cancer susceptibility.

5. Conclusion

Collectively, the present study proves a robust association of the GC genotype and C allele of the miR-146a rs2910164 polymorphism with enhanced lung cancer susceptibility in the Iraqi population. The results are in harmony with the other international studies and validate the candidacy of this SNP as a lung cancer susceptibility gene. While the results are valuable in elucidating the genetic underpinnings of susceptibility to lung cancer, further studies with large and ethnically diverse groups and functional studies need to be undertaken to bridge the knowledge gap of the underlying biological mechanisms of the given association and validate the clinical application.

REFERENCES

[1] R. S. N. Al-Saeagh and A. H. Hassan, “Genomic DNA extraction from mice tissues by using two methods (favor prep tissue genomic DNA extraction kit and rapid method),” Biochem. Cell. Arch., vol. 19, no. 1, 2019.

[2] M. H. Alyasiri and S. A. A. Atiyah, “Identify the urinary bladder cancer patterns in Nasiriyah city/Thi-Qar Province–Iraq,” Univ. Thi-Qar J. Sci., vol. 10, no. 2, pp. 87–91, 2023.

[3] N. K. Dezfuli, I. M. Adcock, S. D. Alipoor, S. Seyfi, B. Salimi, M. M. Golchin, and E. Mortaz, “The miR-146a SNP rs2910164 and miR-155 SNP rs767649 are risk factors for non-small cell lung cancer in the Iranian population,” Can. Respir. J., 2020.

[4] Y. Jia et al., "MicroRNA-146a rs2910164 polymorphism is associated with susceptibility to non-small cell lung cancer in the Chinese population," Med. Oncol., vol. 31, no. 194, 2014. [Online]. Available: https://doi.org/10.1007/s12032-014-0194-2

[5] N. S. Omar and R. T. Ali, "Dosimetric analysis with intensity-modulated radiation therapy for central nervous system irradiation in patients with brain cancer compared with three-dimensional conformal radiation therapy treatment," Polytech. J., vol. 9, no. 2, p. 9, 2019.

[6] N. S. Omar, F. K. Ahmed, D. S. Saleem, L. J. A. Shah, and M. A. Abbas, "Influence of maternal body mass index on fetal ultrasound biometry," Cihan Univ.-Erbil Sci. J., vol. 8, no. 2, pp. 93–98, 2024.

[7] Y. G. Ren, X. M. Zhou, Z. G. Cui, and G. Hou, "Effects of common polymorphisms in miR-146a and miR-196a2 on lung cancer susceptibility: A meta-analysis," J. Thorac. Dis., vol. 8, no. 6, pp. 1297–1305, 2016.

[8] J. Wang, Q. Wang, H. Liu, et al., "The association of miR-146a rs2910164 and miR-196a2 rs11614913 polymorphisms with cancer risk: A meta-analysis of 32 studies," Mutagenesis, vol. 27, no. 6, pp. 779–788, 2012.

[9] I. I. Wistuba and A. F. Gazdar, "Lung cancer preneoplasia," Annu. Rev. Pathol., vol. 1, pp. 331–348, 2006.

[10] Z. Yin, Z. Cui, Y. Ren, L. Xia, H. Li, and B. Zhou, "MiR-146a polymorphism correlates with lung cancer risk in Chinese nonsmoking females," Oncotarget, vol. 8, no. 2, pp. 2275–2283, 2017.

[11] J. Zhang, W. Shao, Z. Zhang, and D. Liu, "The rate of miR-146a rs2910164 mutations in patients with lung cancer: A meta-analytic review," Transl. Cancer Res., vol. 9, no. 11, pp. 6792–6800, 2020.

[12] H. Chen and M. Xu, "Genetic variations of microRNAs and cancer susceptibility," Oncotarget, vol. 9, no. 5, pp. 5012–5025, 2018.

[13] S. H. Lee and J. Kim, "Emerging roles of microRNA mutations in cancer diagnostics," Cancer Res., vol. 78, no. 13, pp. 3470–3476, 2018.

[14] X. Li, Y. Chen, and Z. Zhang, "miRNA-related polymorphisms and cancer risk: A systematic review," Clin. Genet., vol. 95, no. 5, pp. 569–578, 2019.

[15] R. Zhao and K. Huang, "Functional analysis of miR-146a in cancer development," Mol. Cancer Res., vol. 17, no. 7, pp. 1441–1450, 2019.