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A rise in the risk of lung cancer in Iraq due to miRNA-423 rs6505162 polymorphisms.

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ABSTRACT

Cancer disease types represent a significant public health challenge globally, with its prevalence on the rise. It is the most frequently diagnosed cancer among patient's cases in human worldwide. Objective: evaluated genotyping and allele frequency of miR-423 via tetra-arm-PCR and highlight the role polymorphism among case-control lung cancer (LC). Results: The miRNA-423 rs6505162 polymorphisms in lung cancer shown three genotypes; AA, AC, and CC were 20%, 36.6%, and 43.3% among patients. Increase in risk is associated with miRNA-423 rs6505162: CC genotype exhibited an odds ratio (OR) of 3.05 with a 95% confidence interval (CI) of 0.96-9.6 (p=0.05). The results indicated a correlation between microRNA-423 and rs6505162. The results indicated a correlation between microRNA-423 and rs6505162 A>C genetic variation and the susceptibility to LC among Iraq. The C allele was found to significantly elevate the risk allele C of LC was showed an OR of 1.5 with a 95% CI of 0.72-5.11 (p=0.2). In conclusion, According to our findings, patients in Iraq who have the microRNA-423 CC genotype and the C allele are more vulnerable and have advanced stages of LC. Additional research with larger sample sizes is required to validate these findings..

KEYWORDS: lung cancer, microRNA-423, Tetra-Arm-PCR, polymorphism.

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INTRODUCTION

Human cancer represents a significant public health issue globally (Siegel et al.,2015). Individuals with different genetic backgrounds who are exposed to the same environmental factors may have different susceptibilities to LC (Mbemi et al.,2020; Al-Aawi,2024;Alwan,2024). SNPs in miRNA have been shown in numerous studies to affect miRNA targeting as well as pri-miRNA transcription, processing, and maturation, which may be linked to the onset and progression of cancer (Morales-Pison et al 2023). The investigation of Al-Musawi et al.,(2025) sought to assess the correlation with SNP of miRNA-423 rs6505162. With rising incidence rates, especially in men, and a high death rate, lung cancer is a serious health issue in Iraq and Arab world. Salim et al.,(2011) reported that 14,788 new cases of LC in people under 65 and 14,788 new cases in people over 65, both male and female, are anticipated in 2020. Evasion of immune detection, overexpression of oncogenes, inhibition of apoptosis, and interactions with proteins that contribute synergistically to carcinogenesis and metastasis (Hanahan and Weinberg, 2011).

MicroRNAs are crucial in the regulatory processes associated with tumor development. Recent studies have shown that microRNA-423 (miR-423) is often expressed at abnormal levels in various human cancers and is involved in several signaling pathways that contribute to cancer progression (Rothe et al.,2011). This contradictory role in cancer could complicate the use of miR-423 as a target for diagnosis and treatment. Additionally, the interaction between miR-423 and lncRNA warrants further investigation (Song, and Chen, 2011).

A number of signaling pathways and the progression of the disease are significantly impacted by the aberrant microRNAs (miRNAs) seen in human malignancies. The expression of particular protein-coding genes is regulated by these little non-coding RNA molecules. (*Calin and Croce 2006)*.

MicroRNA-423 is identified as an oncogenic element frequently up regulated in numerous cancers. However, the relationship between this microRNA and the risk of breast cancer has been inconsistent (Iorio, 2012). Therefore, we undertook a study to

investigate the connection between the microRNA-423 rs6505162A>C gene variant and LC susceptibility in the Iraqi population

MATERIALS AND METHODS:

Sampling population:

This case-control study comprised 30 (15 females and 15 males) clinically confirmed lung cancer patient, and their history were tracked in Marjan tumor center, the 30 (15 females and 15 males) healthy group were confirmed by subjected to periodical laboratory tests, The study population included both sex and age ranged from 38-65years old duration February to April 2024.

Samples collection:

Approximately 3 ml of peripheral blood was collected from each participant, including both the LC patients and the matched healthy controls, using venipuncture into EDTA vials and then stored at -20°C until further steps.

Genomic DNA extraction:

The FavorPrep Genomic DNA Mini Kit (Blood/Cultured Cell) was used to extract genomic DNA. (Taiwan com.) (Al-Jubory and Imran, 2020). The extraction process was conducted following the manufacturer's recommended protocol. Amplification targeted region under interest was performed by conventional PCR machine (Labenat USA com.) For the exploratory analysis, the SNP rs6505162 was genotyped using the Tetra-ARMs PCR technique.

Amplification via tetra ARM-PCR

Using the amplification refractory mutation (ARM-PCR) approach, the genotypes of microRNA-423 rs6505162 A>C in LC illness were found via was done utilizing four primers.
Fo:5′-TTTTCCCGGATGGAAGCCCGAAGTTTGA-3′, Ro: 5′-TTTTGCGGCAACGTATACCCCAATTTCC-3, Fn: 5′-TGAGGCCCCTCAGTCTTGCTTCCCAA-3′, Rn: 5′-CAAGCGGGGAGAAACTCAAGCGCGAGG-3′.(Mir et al.,2018).

polymerase chain reaction (PCR) condition:

Amplification flanking main primers pair region was done by condition: 93°C for 3min. and 32 cycles: 93°C for 3sec. 61°C for 35sec. and 72°C for 35sec. and final extension 72°C for 3min. and cool to 4°C and shutdown the PCR program. The Quality and size of PCR bands for three genotyped was visualized at 2%TBE agarose gel (Himedia com.). The ARMS-PCR was carried out in a 25ul reaction volume using 50ng of template DNA, 12ul of GoTaq® Green Master Mix (cat no. M7122) (Promega, USA), and 1.2ul, of 10 pmol of each primer (Macrogen Lab.). 8ul of nuclease-free ddH2O was added to bring the final volume of 25ul into balance. Lastly, 2ul of DNA for each groups under inserted.

Allele-specific tetra-primer amplification was performed on the genomic DNA for miR-423 rs6505162 A/C using a tetra-primer ARMS PCR method. To determine the miR-423 rs6505162 A/C genotyping, ARMs-PCR was employed.

Statistical analysis:

Direct counting determined the genotype and allele frequencies. MedCal was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). All computations were completed with the P-value of 0.005.

RESULTS:

Verified location of microRNA-423 rs6505162 A>C

The SNP rs6505162 A>C located at 30117165 of microRNA-423 on Chr:17 had reference strain NO. NC_000017.11. The type of the wild allele A mutant to allele C in SNP rs6505162 (Figure 1).

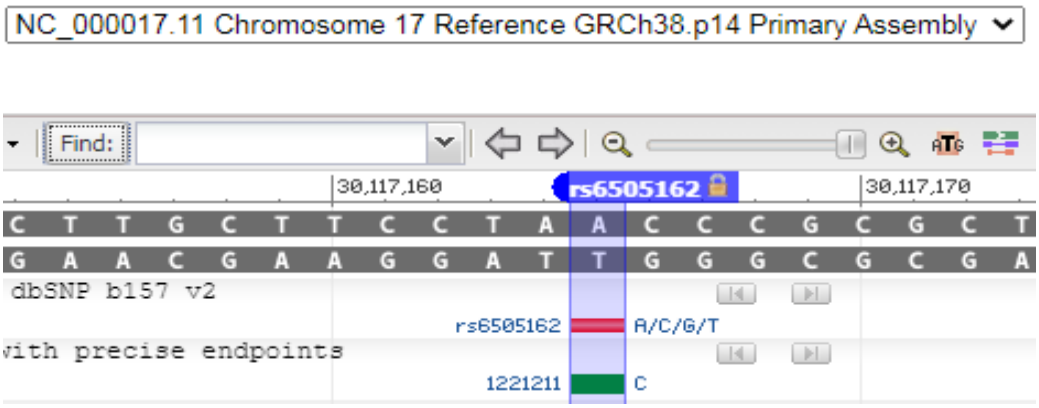

Figure 1: Illustration location rs6505162 SNP at 30117165 of MicroRNA423 gene on Chr:17 reference strain NO. NC_000017.11.

Amplification targeted region of MicroRNA423:

The amplification focused on MicroRNA423 gene by the ARM-PCR technique was successfully amplifying the partial sequence of interest with four primers. The wild-type allele AA exhibited two bands: a main band at 336bp and A allele band at 228bp. In contrast, the heterozygous mutant allele AC displayed three bands: 336bp, 228bp, and 160bp. The homozygous mutant allele CC presented two bands, including the main band at 336bp, and 160bp for the C allele, observed in both patient and healthy groups as illustrated in Figures 2(A&B).

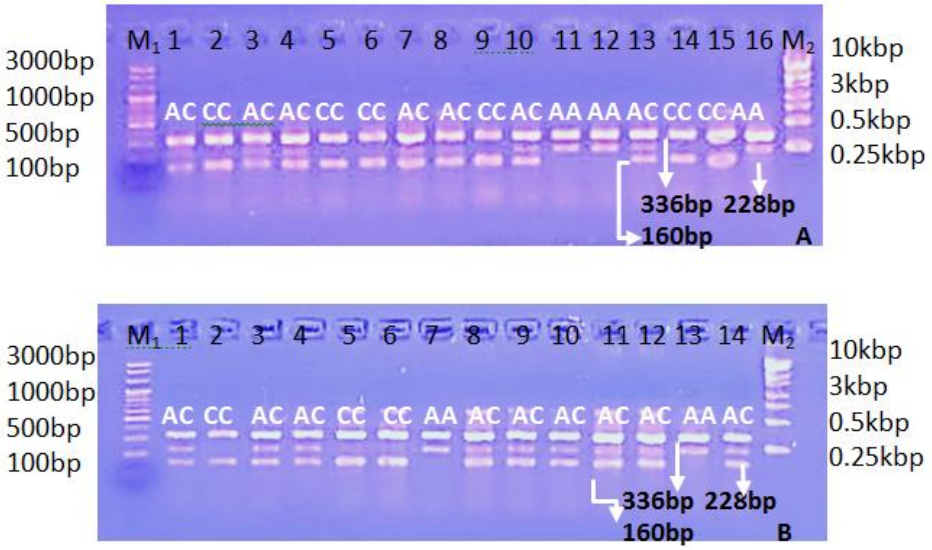

Figure (1): Gel electrophoresis of PCR products of amplification partial sequence of MicroRNA423 gene covering SNP rs6505162: A-Case group, lanes1,3-4,7-8,10,13 AC genotypes;2,5-6,914-15 CC genotype;11-12,16 AA genotype. B healthy group, lane 1,3-4,13 AC genotype; 2,5-6 CC genotype; 7,13 AA genotype, PCR products were visualized by gel electrophoresis on 2% of agarose , 100V for 45 min. M1= Marker 100-3000bp,M2=250-10000bp.

Genotyping and allele frequency:

The genotypes number and allele frequency were summarized in table 1. The results findings three genotypes (AA, AC, and CC). In compartmented with genotyping rs6505162 A>C in LC patients infection. The OR and allele frequency in two groups showed a substantial change. According to the Odd Ratio (OR) values, the C allele in CC was regarded as a risk allele and was associated with the disease of interest. The allele frequency was greater with C allele 37 in the cancer patient group with high value of OR=1.5(0.72-5.11), p value = 0.2, and the allele frequency was lower with C allele 31 in the healthy group Table 1. The Odd Ratio was higher with genotype CC OR= 3.05(0.96 – 9.6) with high significance p value 0.05.

Table (1) Genotype and allele frequency of rs6505162 A>C) associated with/without cancer patients and control.

rs6505162 A>C		**Patients N=10**	**Control N=10**	**OR(95%CI)**	**P-value**
Genotypes Pharyngeal fungal infections	AA	6(20%)	5(16.6%)	Reference group	
	AC	11(36.6%)	19(63.3%)	0-3(0.11 – 0.95)	0.04
	CC	13(43.3%)	6(20%)	3.05(0.96 – 9.6)	0.05
Allele Frequency	A	23(38.3. %)	29(48.3%)	0.66(0.32-1.37)	0.2
	C	37(61.6%)	31(51.6%)	1.5(0.72-5.11)	0.2

DISCUSSION:

A notable difference in genotypes distribution was identified between LC patient and control subjects (p=0.05). This study determined three genotypes AA, AC, and CC were 20%, 36.6%, and 43.3% among patients, compared with 16.6%, 63.3%, and 20% in the control group, respectively. The microRNA-423 CC genotype exhibited an odds ratio (OR) of 3.05 with a 95% confidence interval (CI) of 0.96-9.6 (p=0.05). Additionally, the allele frequency of microRNA-423C showed 37(61.6%) in patients compared with 31(51.6%) with an OR of 1.5 with a 95% CI of 0.72-5.11 (p=0.2) (Table 1). An increased risk of lung

cancer is indicated by the enhanced OR linked to the C allele.

Our findings concurred with those of Jameel (2024), who suggests that miR-423 is linked to a number of cancer types and that, depending on the type of cancer in Iraq, it can play both oncogenic and tumor-suppressive roles.

The elevated OR associated with the C allele indicates an increased risk of developing cancer, compared with AA genotypes and the A allele of microRNA-423 in central Iraq.

However, our findings contradicted those of Zhou et al. (2023), who discovered that MiRNA-423 rs6505162 variants significantly decreased the risk of LC in both recessive (AA vs. CA + CC, adjusted OR = 0.17, 95% CI = 0.03–0.90, P=0.038) and heterozygous (CA vs. CC, adjusted OR = 0.14, 95% CI = 0.03–0.81, P=0.028).

Identifying the genes associated with breast cancer susceptibility is vital for enhancing diagnosis, treatment, and potential prevention strategies. MicroRNAs, which are non-coding RNA molecules, can function as either oncogenes or tumor suppressors (Shivdasani, 2016). More than 1,000 miRNA genes are found in the human genome, and they use sequence complementarity to control the translation or degradation of messenger RNA (Li et al., 2017). More than half of microRNA genes have been identified as oncogenes or tumor suppressors implicated in carcinogenesis, and they are present in fragile locations or genomic areas linked to cancer (Calin et al., 2004). In recent years, the genetic variants in miRNAs have been identified as oncogenes of many different types of cancer and found to play an important role in initiation and development of malignancies (Chang et al., 2016). The SNP microRNA-423 rs6505162 C>A has been linked in studies to the chance of developing a number of malignancies, with varying degrees of success. Similarly, a recent study in a South American population found that pre-micro-423 rs6505162 raises the risk of familial breast cancer in families with a high history of the disease (Morales et al., 2016). A study by Smith et al. (2012) found that miR-423-rs6505162 is linked to a lower risk of breast cancer, while another study found that miR-423-rs6505162 is linked to both overall survival and recurrence-free survival in colorectal cancer (Xing et al., 2012). Additionally, it was noted that smoking people may have a markedly higher risk of esophageal cancer if they have miRNA-423 rs6505162 C>A (Yin et al.,2013). Non-coding RNA molecules known as microRNAs have the ability to function as tumor suppressor genes or oncogenes. (Shivdasani, 2016;Li et al., 2017; Carthew, 2006; Xie and Sadovsky, 2016). Better diagnosis, treatment, and perhaps prevention of LC may result from this study's emphasis on identifying the genes that are sensitive to the disease. A detailed examination of alterations in microRNA expression and the target genes they are linked to is essential to improving our knowledge of cancer. Even while individual research has produced significant findings, an integrated network research is required to gain a firm understanding of the fundamental molecular causes of cancer. This multidisciplinary method provides insightful information about how the genome is reprogrammed under illness conditions. Additionally, it aids in the identification of pertinent biological entities that may be useful targets for cervical cancer treatment or diagnosis Khraibet, M. R., & Kadhim, E. J. (2025), Kazem, A. J., Jorani, L. E., & Al-Azzawi, A. K. J. (2025), Mohsin, M. D., & Edan, B. J. (2024).

ETHICS APPROVAL

Written informed permission was provided by each participant in this investigation. The Marjan Hospital Ethics Committee gave its approval to the study

REFERENCES

1. Al-Alawi,M.T.K.(2024).Anti-Cancer Efficacy of silver oxide nanoparticales synthesized using terephthalic acid:a comprehensive study. Hilla University College journal for medical Science,2(4).
2. AL-Jubory R.J. and Imran Z.K.(2020). Molecular Detection of Abundance of Uncultured Lavage Microbiota and Evaluated the SNPs in Partial Sequence of CARD9 Gene. The Journal of Research on the Lepidoptera: 5(2): 256-269.
3. Al-Musawi, J. M., Al-Shadeedi, F., Shakir , N. A., & Ibrahim, S. Q. (2025). Epidemiological Characteristics of Lung Cancer Patients in the Middle Euphrates Region of Iraq: Retrospective Analysis from the Middle Euphrates Cancer Center (2018-2023). Al-Rafidain Journal of Medical Sciences (ISSN 2789-3219), 8(2), 63–70.
4. Alwan,H.M,Abdallah,H.N.,Hazmzah,A.S.(2024).Molecular analysis of FimH Gene in Biofilm-Proucing and Multidrug Resistant Klebsiella pneumoniae. Hilla University College journal for medical Science, 2(3).
5. Calin GA, Croce CM.(2006). MicroRNA signatures in human cancers. Nat Rev Cancer.;6(11):857-866.
6. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A. 2004;101:2999–3004.
7. Carthew RW. Gene regulation by microRNAs. Curr Opin Genet Dev. 2006;16:203–8.
8. Chang S, He S, Qiu G, et al. MicroRNA-125b promotes invasion and metastasis of gastric cancer by targeting STARD13 and NEU1. Tumour Biol. 2016;37:12141–51.
9. Hanahan D, Weinberg RA.(2011). Hallmarks of cancer: the next generation. Cell. 144(5):646-674.
10. Jameel, Z.I(2024). Four microRNA gene polymorphisms are associated with Iraqi patients with colorectal cancer. Egypt J Med Hum Genet 25, 47.
11. Kazem, A. J., Jorani, L. E., & Al-Azzawi, A. K. J. (2025). Concept of Marginal Fit Accuracy of a Single Crown Prosthesis Using Conventional Impression Techniques and Intra-Oral Scanner. Hilla University College Journal For Medical Science, 3(2), 63-69.

12. Khraibet, M. R., & Kadhim, E. J. (2025). Anti-Lung Cancer Activity of the Herbal Medicinal Plant Cycas revoluta. Hilla University College Journal For Medical Science, 3(2), 25-30.
13. Li C, Zhang Y, Li Y, et al. The association of polymorphisms in miRNAs with nonsmall cell lung cancer in a Han Chinese population. Cancer Manag Res. 2018;10:697–704.
14. Mbemi, A., Khanna, S., Njiki, S., Yedjou, C.G. and Tchounwou, P.B. (2020) Impact of gene-environment interactions on cancer development. Int. J. Env.Res. Pub. He 17, 8089,
15. Mir R, Al Balawi, I A, Abu Duhier, F M,(2018). Involvement of microRNA-423 Gene Variability in Breast Cancer Progression in Saudi Arabia. Asian Pac J Cancer Prev, 19 (9): 2581-2589.
16. Mohsin, M. D., & Edan, B. J. (2024). Impact of Physical Activity and Body Size on Serum Level of Anti-Müllerian Hormone in Women in Babylon Province. Hilla University College Journal For Medical Science, 2(2), 49-57.
17. Morales S, Gulppi F, Gonzalez-Hormazabal P, et al (2023). Association of single nucleotide polymorphisms in Pre-miR-27a, Pre-miR-196a2, Pre-miR-423, miR-608 and Pre-miR-618 with breast cancer susceptibility in a South American population. BMC Genet. 2016;17:109.
18. Morales-Pison, S., Jara, L., Carrasco, V., Gutierrez-Vera, C., Reyes, J.M., Gonzalez-Hormazabal, P. et al. (2022) Genetic variation in MicroRNA-423 ´promotes proliferation, migration, invasion, and chemoresistance in breast cancer cells. Int. J. Mol. Sci. 23, 380,
19. Rothe, F.; Ignatiadis, M.; Chaboteaux, C.; et al.(2011). Global microRNA expression profiling identifies MiR-210 associated with tumor proliferation, invasion and poor clinical outcome in breast cancer. PLoS ONE , 6, e20980.
20. Salim,E, Jazieh,A.R, Moore,A.(2011). Lung Cancer Incidence in the Arab League Countries: Risk Factors and Control. Asian Pacific J Cancer Prev, 12, 17-34.
21. Shivdasani RA. MicroRNAs:regulators of gene expression and cell differentiation. Blood. 2016;108:3646–53.
22. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5-29.
23. Smith RA, Jedlinski DJ, Gabrovska PN, et al. A genetic variant located in miR-423 is associated with reduced breast cancer risk. Cancer Genomics Proteomics. 2012;9:115–8.
24. Song, F.J.; Chen, K.X.(2011). Single-nucleotide polymorphisms among microRNA: Big effects on cancer. Chin. J. Cancer , 30, 381–391.
25. Xie L, Sadovsky Y. The function of miR-519d in cell migration, invasion, and proliferation suggests a role in early placentation. Placenta. 2016;48:34–7.
26. Xing J, Wan S, Zhou F, et al. Genetic polymorphisms in pre-microRNA genes as prognostic markers of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2012;21:217–27.
27. Yin, J., Wang, X., Zheng, L., Shi, Y., Wang, L., Shao, A. et al. (2013) Hsa-miR-34b/c rs4938723 T>C and hsa-miR-423 rs6505162 C>A polymorphisms are associated with the risk of esophageal cancer in a Chinese population. PLoS ONE 8, e80570
28. Zhou J., Meng C., Li Y. (2023)MiRNA-423 rs6505162 and miRNA-6811 rs2292879 SNP Associated with Lung Cancer in Hainan, China. Bioscience Reports (2023) 43 BSR20231152