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Original Article

Evaluation of circulating cell-free nucleic acids in plasma as biomarkers of laryngeal cancer

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ABSTRACT

It has been aimed to compare the level of cell-free nucleic acids (DNA, RNA, and miRNA) concentrations in laryngeal cancer patients with the control group composed of healthy individuals. It has been utilized 46 patients with laryngeal carcinoma who were previously diagnosed and treated were included and 46 healthy individuals were included as a control group. Peripheral blood samples were obtained from all participants. DNA, RNA, and miRNA were fluorometrically measured. We applied Mann Whitney U test to compare both groups and the adjusted general lineal model to identify associations between nucleic acid concentrations and tumor stages. Circulating cell-free microRNA and RNA concentrations in the laryngeal cancer patients were significantly different ($p<0.05$). Most importantly, tumor stages were the main factor that altered miRNA concentration in circulation. Our findings support that circulating cell-free miRNA and RNA have potential to be associated with laryngeal cancer. Finally, cell-free miRNAs can be used as a tool to predict different stages of laryngeal cancer.

1. Introduction

Cancer rates increase after the fifth decade and peak in the seventh and eighth decades. The most common type of head and neck cancer is squamous cell carcinoma (85-90%) [1]. Furthermore, laryngeal cancer (LC) is one of the most common cancers of the head and neck cancer. Laryngeal carcinoma accounts for 30% of all head and neck malignancies [2]. In the last 10 years, the incidence of LC has increased and it accounts for 2-5% of all newly diagnosed malignancies in one year. LC was observed in 1.6% of males and 0.4% of females [3].

Tumor stage, anatomical localization, histopathological differentiation, and neck metastasis are general prognostic factors of Laryngeal carcinoma. Especially neck metastasis is accepted as a poor prognostic finding [4]. Therefore, biomarkers are needed for early diagnosis and treatment, and thus, increase the chances of the patients for the treatment. Biomarkers are cellular, biochemical or molecular alterations that can be easily and non-invasively measured in human tissues and are directly or indirectly in the pathway of disease [5].

Recent advancements in molecular techniques provide new investigation areas in different liquid biopsies such as blood, serum, plasma, cell, and urine. Molecular changes in bodily fluids provide a unique opportunity to explore new biomarkers of diseases. In this context, plasma is one of the sources of different molecules including cell-free DNA (cfDNA), cell-free RNA, and cell-free miRNA. cfDNA was first described by Mandel and Metais in 1948 [6].

It has been reported that the amount of cfDNA was higher in cancer patients than the healthy HCs, and the concentration of ccfDNA

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in serum increased after radiation therapy [7]. Furthermore, evidence obtained from cancer patients indicated that cfDNA originates from tumoral tissue since cfDNA has a genetic and epigenetic character similar to tumor DNA [8].

It is accepted that the mechanisms that cause the DNA to become free in the cell are also caused to release the RNA. In this context, cfRNA presents and measures in a variety of bodily liquids. To date, cfRNA has been used to investigate the causes of several diseases such as diabetes, trauma, stroke and myocardial infarction [9-11].

A miRNA is a small, endogenously uncoded RNA molecule of length 19-25 nucleotides in length. Researches have shown that miRNAs play a role in cellular functions, proliferation, apoptosis, metastasis, and cellular changes [12, 13].

Currently, more than 2000 miRNAs in the human genome have been identified [14], which are associated with a number of diseases, including cancer and other diseases [15, 16]. In addition to being in the cell, miRNAs have been determined in bodily fluids. The circulating free or bodily fluid microRNAs (cfmiRNA) have been widely reported to be markers of several human diseases [17, 18].

There are several reasons to use cell-free nucleic acids as candidate biomarkers of disease; 1- they are available in many bodily fluids, 2- bodily fluids can be easily obtained, 3- the measurement of cell-free nucleic acids is direct, sensitive, inexpensive, and relatively simple, 4- the amount of nucleic acids is enough for analysis, 5- they can be analyzed in terms of genomic, epigenomic and epitranscriptomics. Since there is insufficient data regarding the role of circulating cell-free nucleic acids in plasma samples in LC patients, in the present study, we measured the cfDNA, cfRNA and cfmiRNA amounts in the plasma samples obtained from LC patients. Then we compared the findings with the HC group results. By investigating whether these molecules in plasma samples differed between LC patients and healthy individuals, we evaluated cfDNA, cfRNA, and cfmiRNA as biomarkers of LC.

2. Material and Methods

2.1. Study design and participants

The study was approved by the ethics committee of the University (2011-KAEK-27/2018-E.1800185667). Consent forms were obtained from the patients and the study was conducted according to the Helsinki Declaration. A total of 92 individuals (46 patients with laryngeal carcinoma) who were previously diagnosed and treated were included as patients and 46 healthy individuals were included as a control group. The patient group consisted of individuals who applied to the same otolaryngology clinic and were followed up in the same clinic. The patients generally applied with the complaint of hoarseness. The mass was detected in the endoscopic examinations, afterward, the necessary imaging methods were applied to evaluate the neck and mass, and direct laryngoscopy-biopsy was applied to the patients for a definitive diagnosis. Appropriate treatment was planned for patients diagnosed with squamous cells. Clinical history, age, gender, occupation, smoking habits, history of surgery, radiotherapy, and chemotherapy, and physical examination findings of the patients with cancer were recorded. The type of treatment, recurrence and metastasis were recorded. In the study, the HC group included individuals aged 40 years or older, who had no additional disease, did not use any drugs for any reason and had no family history of cancer. Similarly, the demographic characteristics of the HC group were recorded.

2.2. Collection and measurement of nucleic acid amount

For the cfDNA, 5 ml of blood was collected in sterilized tubes containing K3-EDTA. After taking the blood samples, they were immediately centrifuged at 3000 g for 10 min. The plasma samples were then centrifuged at 16000 g for 10 min. The DNA content of the plasma samples was measured directly with a fluorescence-based Quant-iT™ high-sensitivity DNA, RNA, and miRNA assay kits and a Qubit® fluorimeter (Invitrogen, Carlsbad, CA, USA).

The Qubit® 2.0 Fluorimeter is used for quantitation of DNA, RNA, and miRNA. It uses

specific dyes for each type of molecule which has extremely low fluorescence until they bind to their targets. After binding, they give off an intensely fluorescent signal which is directly proportional to the nucleic acid concentration of any solution. In the present study, the cfDNA, cfRNA, and cfmiRNA of each individual were measured using a DNA, RNA, and miRNA curve obtained from the standards of known concentrations. Plasma samples were analyzed in duplicate and the mean of the two values was used as the final nucleic acid amount.

2.3. Statistical analysis

We compared the result of circulating nucleic acids in both groups by using the Mann-Whitney U test. Multiple regression analysis was performed to determine the effects of other factors of populations. Spearman Rho correlation test was used to determine the relationship between the parameters.

We applied general linear model analysis to evaluate the effects of different variables (tumor stages, taking radiotherapy, tumor sites, seconder diseases, and urban or rural living conditions) and continuous variables (age)) on ccfmiRNA, ccfRNA, and ccfDNA amounts in different models. All analyses were performed using IBM SPSS Statistics 19. Graphs were drawn using GraphPad Prism for Windows.

3. Results

The demographic characteristics of both populations are given as supplemental data. All patients were male, and the mean age of the patient group was 64.15. All patients also have a history of smoking. On the other hand, 46 individuals included in the study as the control group were given. 52.2% of the individuals in the control group were male. The mean age of the control group was 50.39.

In this study, the amounts of cfDNA obtained from cancer and the control groups are given in figure 1. Accordingly, the mean cfDNA amount obtained from the cancer group was 1137.9 ng/µl, while it was 1216.2 ng/µl for the control group. When the values obtained in both groups were compared with the Mann-Whitney U test, no statistically significant difference was observed (p>0.05) (Figure 1).

In the study, the amount of cfRNA obtained in the cancer was found to be less in individuals with cancer than the control group. The mean amount of cfRNA was 6221.7 ng/µl and 7064.1 ng/µl in the cancer group and the control group, respectively. When the mean values of both groups were compared with the Mann-Whitney U test, the amount of cfRNA in cancer patients was significantly lower than the control group (p<0.05) (Figure 2).

The distribution of the amounts of cfmiRNA obtained in cancer and control groups is given in Figure 3. The cfmiRNA amount was 5646.5 ng/µl and 5028.3 ng/µl in cancer and control groups, respectively. The amount of cfmiRNA obtained in the cancer group was found to be higher than the control group. When the Mann-Whitney U test was applied to the data obtained in both groups, it was found that the averages were significantly different (p<0.05).

The relationship between cfDNA and smoking is given in Figure 4. Accordingly, no statistically significant difference was found between smoker cancer patients and smoker control individuals (p>0.05). Similar results were obtained between smoker cancer patients, and control non-smokers (p>0.05).

The relationship between cfRNA amount and smoking is given in Figure 5. Accordingly, no statistically significant difference was found in smoker cancer with smoker control individuals (p>0.05). However, a statistically significant difference was found between smoker cancer patients with non-smokers control individuals (p<0.05).

The association between cfmiRNA and smoking habit is given in Figure 6. Accordingly, the difference was found to be statistically significant between cancer patients who smoke with the control group (p<0.05). Accordingly, higher amounts of miRNA were detected in cancer patients. On the other hand, no statistically significant difference was found between cancer patients who smoke with control non-smokers (p> 0.05).

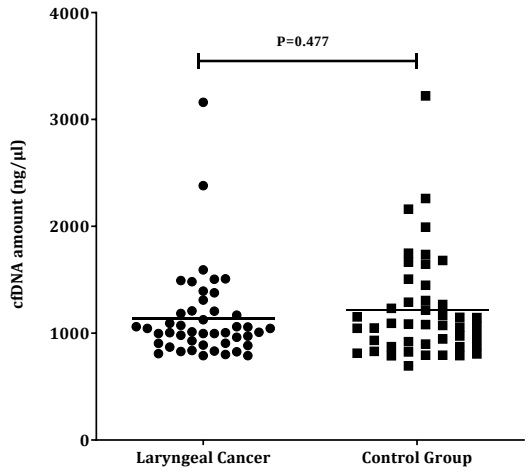

Fig. 1. the amounts of cfDNA in the cancer and the control groups

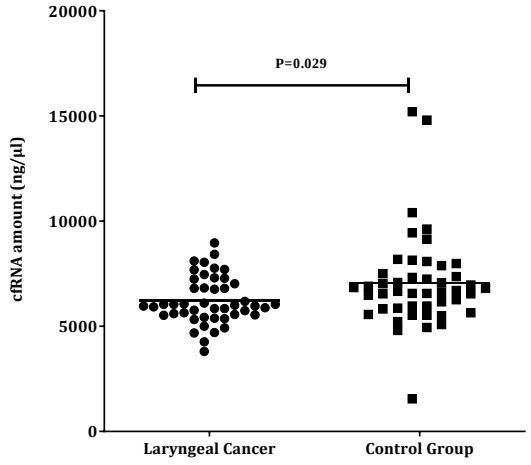

Fig. 2. the amount of cfRNA in the cancer group and the control group

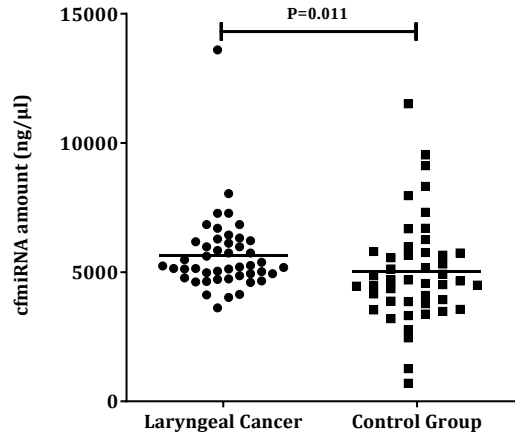

Fig. 3. the amount of cfmiRNA in the cancer group and the control group

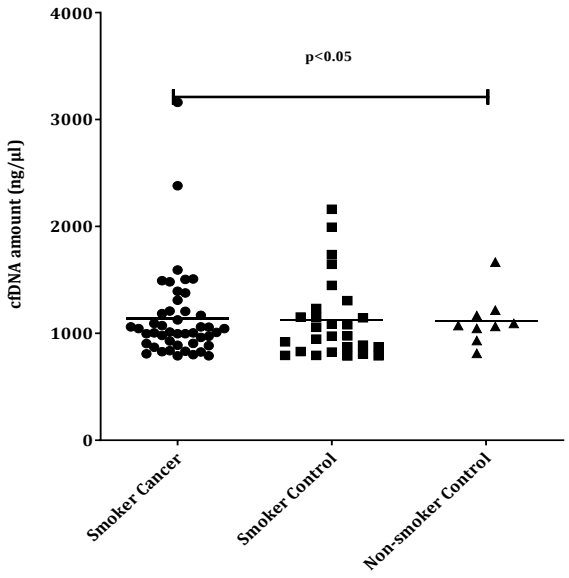

Fig. 4. the amount of cfDNA among the smoker cancer with smoker control and non-smokers control individuals

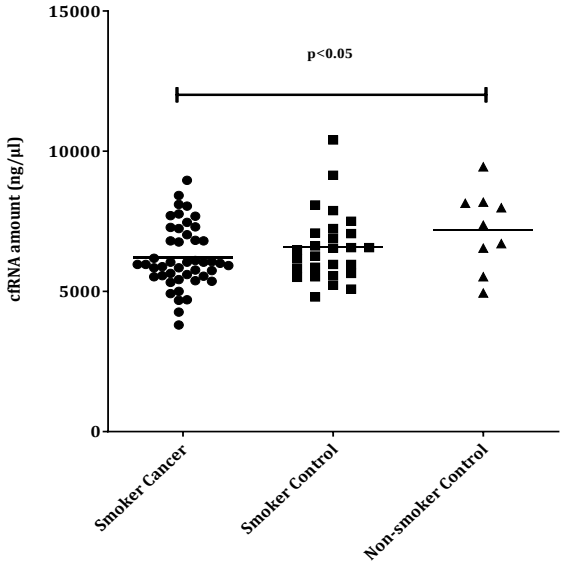

Fig. 5. the amount of cfRNA among the smoker cancer with smoker control and non-smokers control individuals

When the association between the amount of nucleic acid obtained only from cancer individuals was analyzed, significant and positive correlations were observed. According to Spearman rho analysis (Table 1), a positive and statistically significant difference between cfDNA and cfmiRNA was determined (r=0.78, *p*=0.001). Similarly, a statistically significant difference was found

between cfmiRNA and cfRNA (r= 0.394, p=0.007). Finally, a positive and statistically significant difference was found between cfRNA and cfDNA (r = 0.388, p = 0.008). When the Spearman rho correlation method was applied to the amount of nucleic acid obtained in healthy individuals, statistically significant differences were found between cfmiRNA and cfDNA (r=0.36, p=0.014) and cfRNA (r=0.31, p=0.034), (Table 2).

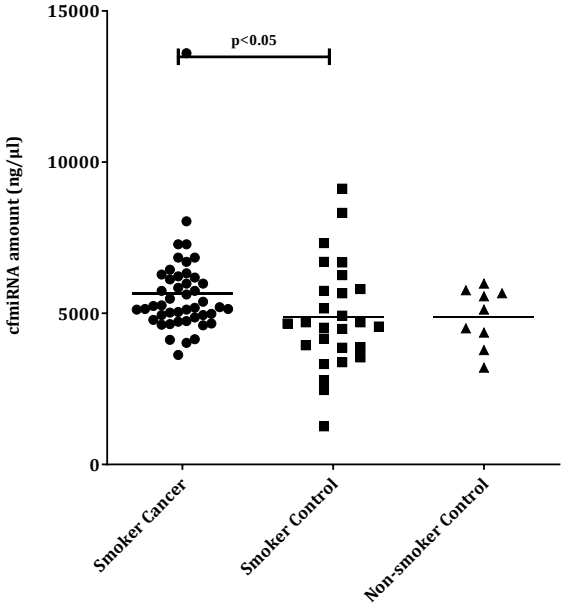

Fig. 6. the amount of cfmiRNA among the smoker cancer with smoker control and non-smokers control individuals

In this study, the effects of several factors were investigated by applying multiple regression analysis to the evaluated parameters. Firstly, non-normal distribution data were determined by the Kolmogorov Smirnov test, and then LG10 transformation was applied to the nucleic acid amount for normalization cfDNA, cfRNA, and cfmiRNA were selected as the dependent variable in separate models.

The models were adjusted for several factors including age, smoking habits, and cancer/healthy. Where cfDNA and cfRNA were as a dependent variable in separate models, no variable was found to be significant in the model. On the other hand, in the case of cfRNA as a dependent variable, we found that cancer or health is the significant factor that affects the cfmiRNA amount in plasma (R^2=0.09, p=0.01) which indicates the significant differences of cfmiRNA amount in multivariable model.

We set up general linear model which includes catercorical variable (tumor stages, taking radiotherapy, tumor sites, seconder diseases, and urban or rural living condition) and continius varible (age). With cfmiRNA as the dependent variables, we found that only tumor stages is the major factor that affected the cfmiRNA amount in the circulation.

When cfRNA is dependent variable, we found that seconder diseases can alter the level of RNA in circulation (Table 3). In case of DNA dependence, we did not find any variable which altered the level of DNA significantly.

Table 1. Correlation coefficients between the amounts of nucleic acid obtained in individuals with LC.

	cfmiRNA	cfDNA	cfRNA
cfmiRNA	1.00		
cfDNA	0.78**	1.00	
cfRNA	0.39**	0.39**	1.00

**p < 0.01; (cfmiRNA: circulating free microRNA, cfDNA: circulating free DNA, cfRNA: circulating free RNA, LC: larynx cancer)

Table 2. Correlation coefficients between the amounts of nucleic acid obtained in healthy individuals

	cfmiRNA	cfDNA	cfRNA
cfmiRNA	1.00		
cfDNA	0.36*	1.00	
cfRNA	0.31*	0.19	1.00

*p<0.05

Table 3. General Linear Model

S.O.V.	Dependent Variable: cfmiRNA	Dependent Variable: cfRNA
	Sig.	Sig.
Age	0.535	0.273
Tumor stages (T1, T2, and T3)*	0.019	0.982
Taking radiotherapy (Yes/No)	0.290	0.967
Tumor sites (Glottik, Supraglottic)	0.935	0.957
Additional diseases	0.205	0.042
Rural/urban life	0.253	0.677

*We excluded T4 due to lower number of patients

4. Discussion

In this study, we evaluated the levels of circulating cell-free nucleic acid amount in the plasma samples of the laryngeal carcinoma patients and healthy control. Our results revealed a consistently altered level of cfmiRNA and cfRNA when compared to the laryngeal carcinoma patients and healthy control. The amount of cfDNA is generally higher in patients with different types of cancer. Although the presence of nucleic acids was determined in the 1940s [19], the importance of them has recently been shown for some types of cancer and some chronic diseases. A higher level of cfDNA was reported in pancreatic cancer patients compared with healthy individuals [20]. Subsequent studies also supported such findings [21]. Higher plasma cfDNA amount increased in ovarian cancer and thus it was suggested as a new biomarker for diagnosis and prognostic applications of such patients [22]. Similarly, it has been demonstrated that circulating free DNA can be used as a marker of breast tumor [23]. The amount of free circulating tumor-derived DNA in the serum can be used to diagnose head and neck squamous cell carcinoma [24]. In the present study, the mean amount of cfDNA obtained in the cancer group was 1137.9 ng/µl and 1216.2 ng/µl for the HC group. In our study, no difference was found in terms of cfDNA when comparing LC and healthy individuals. Since there has been no study on LC, we could not compare our results with different populations.

Although the release mechanism of RNA into bodily fluids is similar to DNA [25], the roles of cfRNA have been relatively investigated compared to studies with cfDNA. Nasopharyngeal carcinoma has been associated with cfRNA and thus, cfRNA is suggested as a biomarker for the diagnosis [26]. In another study, the function of circular RNA was investigated in laryngeal squamous cell carcinoma tissues [27]. They showed that circular RNAs are irregular in these tissues and hsa_circRNA_100855 and hsa_circRNA_104912 may be potential biomarkers for LC [27]. In the present study, we found a lower amount of cfRNA in laryngeal carcinoma patients. In our study, the average cfRNA amount in the cancer group was 6221.7 ng/µl, whereas this value was 7064.1 ng/µl for the HC group. In the same study (25), it was reported that the amount of circRNA decreases as the tumor stage increases or the patients with advenced clinical stage. In our study, we found that among the patient groups, only the amount of cfRNA of patients with comorbidities was affected compared to other patients, and this was statistically significant. When we looked at the patients in the T3 group alone, we found that the amount of cfRNA was lower compared to T2 and T1s, but we found that this was not statistically significant since the number of advanced stage patients was small.

miRNA has been shown to play important roles in the stages of cancer formation [28] which indicates the importance of miRNAs for the diagnosis and treatment of cancer disease. It has been reported that 52.5% of miRNA genes were in cancer-related genomic regions or in fragile regions [29]. Abnormal miRNA expression was found to be a common feature in different types of cancer [30]. An increase in some types of miRNAs has been observed in lung cancer [31]. In breast cancer, the expression of miR-21 was increased in tumor samples, and irregularity was observed in other miRNAs [32]. The expression of many miRNAs has been shown to have higher potential as biomarkers as compared to mRNAs for predicting prognosis of colorectal cancer and for the diagnosis of specific stages [33]. miR-196a may be an important biomarker in diagnosing and treating LC, but further studies are needed [34]. In another study, miRNA-196a was compared between laryngeal dysplasia and early-stage laryngeal cancers, and it was stated that it could be used in diagnostic and therapeutic follow-up since expression was observed to increase significantly in early stage cancers (33).

In our study, the amount of cfmiRNA was 5646.5 ng/µl in the cancer group and 5028.3 ng/µl in the HC group. The amount of cfmiRNA obtained in the cancer group was found significantly to be higher than the HC group. This shows that our results are compatible with previous studies. In addition, in our study, it was observed that as the tumor stage increased, the amount of cfmiRNA increased and this was statistically

significant. This supports the literature, too. This provided evidence that cfmiRNA can be used in the diagnosis and treatment of laryngeal cancer.

In our study, all patients were smokers. The HC group was divided into two groups as a smoker and a non-smoker. After a comparison of ccfDNA and ccfRNA levels, no significant difference was found between smokers laryngeal carcinoma patients and smoker-healthy control. However, more miRNA was detected in the plasma of smokers with cancer than in the HC group. Especially in terms of miRNA, the smoking habit seems a factor that affects the amount of circulating miRNA.

It was reported that glottic cancer was common in males, while supraglottic cancer was common in females [35]. In healthy individuals, the amount of cfDNA was found to be different between genders. The amount of cfDNA of the male was found to be higher than that of women [36]. In our study, extracellular DNA and RNA were found to be different in males. It was found to be less in males with cancer. On the other hand, cfmiRNA obtained in males with cancer was found to be significantly higher than in HC females. Although it is difficult to evaluate the results of our study since cancer patients were completely male, there are some predictions that there may be differences in terms of gender as a general tendency. In this context, more studies are needed in larger and sexually balanced populations. In our study, a positive and significant relationship was found between cfmiRNA and DNA, cfmiRNA and cfRNA. On the other hand, a positive and significant correlation was also found between cfDNA and cfRNA. These results in the patient group suggest that these three molecules can be released into the blood by common mechanisms such as apoptosis and/or necrosis [25].

5. Conclusion

In summary, according to the results of our study, cfmiRNA and cfRNA have the potential to become biomarkers for LC. However, this study was conducted with a limited patient population and there were no non-smokers in this group. Further work in large patient and HC group populations is required to evaluate the suitability of the circulating cell-free nucleic acid for investigating different biomarkers of LC. Furthermore, subsequent studies are recommended to investigate genomic, epigenomic and epitranscriptomic alterations in circulating cell-free DNA and RNA in circular blood.

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

There were several limitations to our study. Firstly, the number of patients is not higher in our study. Secondly, cancer patients are only males which was an obstacle for the evaluation of gender. Thirdly, we could not measure apoptosis and/or necrosis that enable us to elucidate the mechanisms by which circulating nucleic acids are mainly released. Lastly, we could not measure DNAse and RNAse enzyme in all samples which might affect the amount of the circulating nucleic acids. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publication

All authors read and approved the final manuscript for publication.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

Authors' contributions

Conceptualization: Ozge Caglar Cil.
Data curation: Basak Yavuz.
Formal analysis: Akin Cayir.
Investigation: All authors.
Methodology: Basak Yavuz.
Project administration: Zahra Tavassoli.
Resources: All authors.
Supervision: Ozge Caglar Cil.

Validation: Basak Yavuz.
Visualization: Akin Cayir.
Writing–original draft: Ozge Caglar Cil.
Writing–reviewing & editing: All authors.

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References

1. Gupta B, Johnson NW, Kumar N (2016) Global epidemiology of head and neck cancers: a continuing challenge. Oncology 91 (1): 13-23. doi: https://doi.org/10.1159/000446117
2. Falco M, Tammaro C, Takeuchi T, Cossu AM, Scafuro G, Zappavigna S, Itro A, Addeo R, Scrima M, Lombardi A (2022) Overview on molecular biomarkers for laryngeal cancer: looking for new answers to an old problem. Cancers 14 (7): 1716. doi: https://www.mdpi.com/2072-6694/14/7/1716#
3. Salhab HA, Fares MY, Khachfe HH, Khachfe HM (2019) Epidemiological Study of Lung Cancer Incidence in Lebanon. Medicina 55 (6): 217. doi: https://doi.org/10.3390/medicina55060217
4. Pera E, Moreno A, Galindo L (1986) Prognostic factors in laryngeal carcinoma: a multifactorial study of 416 cases. Cancer 58 (4): 928-934. doi: https://doi.org/10.1002/1097-0142(19860815)58:4%3C928::AID-CNCR2820580421%3E3.0.CO;2-J
5. Mayeux R (2004) Biomarkers: potential uses and limitations. NeuroRx 1: 182-188. doi: https://doi.org/10.1602/neurorx.1.2.182
6. Fernández-Lázaro D, García Hernández JL, García AC, Córdova Martínez A, Mielgo-Ayuso J, Cruz-Hernández JJ (2020) Liquid biopsy as novel tool in precision medicine: Origins, properties, identification and clinical perspective of cancer's biomarkers. Diagnostics 10 (4): 215. doi: https://doi.org/10.3390/diagnostics10040215
7. Sargeant AM, Pandiri AR, Funk K, Nolte T, Keane K (2023) Carcinogenicity Assessment. In: Haschek and Rousseaux's Handbook of Toxicologic Pathology, Volume 2: Safety Assessment Environmental Toxicologic Pathology. Elsevier, pp 125-172. doi:https://doi.org/10.1016/B978-0-12-821047-5.00006-3
8. Mattox AK, Douville C, Wang Y, Popoli M, Ptak J, Silliman N, Dobbyn L, Schaefer J, Lu S, Pearlman AH (2023) The origin of highly elevated cell-free DNA in healthy individuals and patients with pancreatic, colorectal, lung, or ovarian cancer. Cancer discovery 13 (10): 2166-2179. doi: https://doi.org/10.1158/2159-8290.CD-21-1252
9. Cabus L, Lagarde J, Curado J, Lizano E, Perez-Boza J (2022) Current challenges and best practices for cell-free long RNA biomarker discovery. Biomarker research 10 (1): 62. doi: https://doi.org/10.1186/s40364-022-00409-w
10. Ray SK, Mukherjee S (2022) Cell Free DNA as an Evolving Liquid Biopsy Biomarker for Initial Diagnosis and Therapeutic Nursing in Cancer- An Evolving Aspect in Medical Biotechnology. Curr Pharm Biotechnol 23 (1): 112-122. doi: https://doi.org/10.2174/1389201021666201211102710
11. Zhang L, Liu X, Wang C, Li X, Tian G, Wang W (2023) Cell-Free RNA Sequencing from Biofluid Samples. Methods in molecular biology (Clifton, NJ) 2695: 9-25. doi: https://doi.org/10.1007/978-1-0716-3346-5_2
12. Pallante P, Visone R, Croce CM, Fusco A (2010) Deregulation of microRNA expression in follicular cell-derived human thyroid carcinomas. Endocrine-related cancer 17 (1): F91-F104. doi: https://doi.org/10.1677/ERC-09-0217
13. Salajegheh A, Vosgha H, Rahman AM, Amin M, Smith RA, Lam AK-Y (2015) Modulatory role of miR-205 in. Endocrinology 55: 183-196. doi: http://dx.doi.org/10.1530/JME-15-0182
14. Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. cell 75 (5): 843-854. doi: https://doi.org/10.1016/0092-8674(93)90529-Y

15. Yates LA, Norbury CJ, Gilbert RJ (2013) The long and short of microRNA. Cell 153 (3): 516-519. doi: https://doi.org/10.1016/j.cell.2013.04.003
16. Møller HG, Rasmussen AP, Andersen HH, Johnsen KB, Henriksen M, Duroux M (2013) A systematic review of microRNA in glioblastoma multiforme: micro-modulators in the mesenchymal mode of migration and invasion. Molecular neurobiology 47: 131-144. doi: https://doi.org/10.1007/s12035-012-8349-7
17. Hayes J, Peruzzi PP, Lawler S (2014) MicroRNAs in cancer: biomarkers, functions and therapy. Trends in molecular medicine 20 (8): 460-469. doi: https://doi.org/10.1016/j.molmed.2014.06.005
18. Wang J, Chen J, Sen S (2016) MicroRNA as biomarkers and diagnostics. Journal of cellular physiology 231 (1): 25-30. doi: https://doi.org/10.1002/jcp.25056
19. Cirmena G, Dameri M, Ravera F, Fregatti P, Ballestrero A, Zoppoli G (2021) Assessment of circulating nucleic acids in cancer: from current status to future perspectives and potential clinical applications. Cancers 13 (14): 3460. doi: https://doi.org/10.3390/cancers13143460
20. Brancaccio M, Natale F, Falco G, Angrisano T (2019) Cell-free DNA methylation: the new frontiers of pancreatic cancer biomarkers' discovery. Genes 11 (1): 14. doi: https://doi.org/10.3390/genes11010014
21. Zhong XY, Ladewig A, Schmid S, Wight E, Hahn S, Holzgreve W (2007) Elevated level of cell-free plasma DNA is associated with breast cancer. Archives of gynecology and obstetrics 276: 327-331. doi: https://doi.org/10.1007/s00404-007-0345-1
22. Kamat AA, Bischoff FZ, Dang D, Baldwin M, Han LY, Lin YG, Merritt WM, Landen CN, Lu C, Gershenson DM (2006) Circulating cell-free DNA: a novel biomarker for response to therapy in ovarian carcinoma. Cancer biology & therapy 5 (10): 1369-1374. doi: https://doi.org/10.4161/cbt.5.10.3240
23. Umetani N, Giuliano AE, Hiramatsu SH, Amersi F, Nakagawa T, Martino S, Hoon DS (2006) Prediction of breast tumor progression by integrity of free circulating DNA in serum. Journal of clinical oncology 24 (26): 4270-4276. doi: https://doi.org/10.1200/JCO.2006.05.9493
24. Nunes DN, Kowalski LP, Simpson AJ (2001) Circulating tumor-derived DNA may permit the early diagnosis of head and neck squamous cell carcinomas. International journal of cancer 92 (2): 214-219. doi: https://doi.org/10.1002/1097-0215(200102)9999:9999%3C::AID-IJC1176%3E3.0.CO;2-C
25. Gahan PB (2016) Circulating nucleic acids in early diagnosis, prognosis and treatment monitoring, vol 5. Springer. doi:https://doi.org/10.1007/978-94-017-9168-7
26. Wong BC, Chan KA, Chan AT, Leung S-F, Chan LY, Chow KC, Lo YD (2006) Reduced plasma RNA integrity in nasopharyngeal carcinoma patients. Clinical cancer research 12 (8): 2512-2516. doi: https://doi.org/10.1158/1078-0432.CCR-05-2572
27. Xuan L, Qu L, Zhou H, Wang P, Yu H, Wu T, Wang X, Li Q, Tian L, Liu M (2016) Circular RNA: a novel biomarker for progressive laryngeal cancer. American journal of translational research 8 (2): 932. doi: https://pubmed.ncbi.nlm.nih.gov/27158380
28. Hussen BM, Hidayat HJ, Salihi A, Sabir DK, Taheri M, Ghafouri-Fard S (2021) MicroRNA: A signature for cancer progression. Biomedicine & Pharmacotherapy 138: 111528. doi: https://doi.org/10.1016/j.biopha.2021.111528
29. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proceedings of the National Academy of Sciences 101 (9): 2999-3004. doi: https://doi.org/10.1073/pnas.0307323101
30. Naeini MM, Ardekani AM (2009) Noncoding RNAs and cancer. Avicenna journal of medical biotechnology 1 (2): 55-70. doi:

https://pubmed.ncbi.nlm.nih.gov/23407615
31. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nature reviews cancer 6 (11): 857-866. doi: https://doi.org/10.1038/nrc1997
32. Sempere LF, Christensen M, Silahtaroglu A, Bak M, Heath CV, Schwartz G, Wells W, Kauppinen S, Cole CN (2007) Altered MicroRNA expression confined to specific epithelial cell subpopulations in breast cancer. Cancer research 67 (24): 11612-11620. doi: https://doi.org/10.1158/0008-5472.CAN-07-5019
33. Faber C, Kirchner T, Hlubek F (2009) The impact of microRNAs on colorectal cancer. Virchows Archiv 454: 359-367. doi: https://doi.org/10.1007/s00428-009-0751-9
34. Saito K, Inagaki K, Kamimoto T, Ito Y, Sugita T, Nakajo S, Hirasawa A, Iwamaru A, Ishikura T, Hanaoka H (2013) MicroRNA-196a is a putative diagnostic biomarker and therapeutic target for laryngeal cancer. PloS one 8 (8): e71480. doi: https://doi.org/10.1371/journal.pone.0071480
35. Kokoska MS, Piccirillo JF, Haughey BH (1995) Gender differences in cancer of the larynx. Annals of Otology, Rhinology & Laryngology 104 (6): 419-424. doi: https://doi.org/10.1177/000348949510400601
36. Meddeb R, Dache ZAA, Thezenas S, Otandault A, Tanos R, Pastor B, Sanchez C, Azzi J, Tousch G, Azan S (2019) Quantifying circulating cell-free DNA in humans. Scientific reports 9 (1): 5220. doi: https://doi.org/10.1038/s41598-019-41593-4

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