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RESEARCH ARTICLE

Analysis of rs1048943 in the *CYP1A1* gene in laryngeal cancer - a case control study [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: The *CYP1A1* gene is essential in the metabolism of carcinogenic polycyclic aromatic hydrocarbons (PAHs). Numerous studies have investigated the links between the polymorphism Ile462Val rs1048943 A>G and its association with different cancers but the results have been variable. In this study, we studied rs1048943 in Sudanese patients diagnosed with laryngeal cancer.

Methods: This is a case-control study of 49 laryngeal cancer cases and 50 healthy controls. Restriction fragment length polymorphisms (RFLP) were used to analyse the fragment region of the *CYP1A1* gene that contains rs1048943.

Results: The average age of the patients was 51.2 years old and the male to female ratio was 1:1. Well differentiated squamous cell carcinoma constituted most of the histopathological diagnoses. Ile462Val rs1048943 A>G was not found in any of the cases but was found in heterozygous form in four controls ($p = 0.1331$, OR 0.1044, 95% CI, 0.0054-1.9924).

Conclusions: Our study did not show a significant association of *CYP1A1* Ile462Val rs1048943 A>G with laryngeal cancer. Future studies may need to test additional single nucleotide polymorphisms (SNPs) in laryngeal cancer tissue samples to identify the effect of those SNPs on laryngeal cancer.

Keywords

CYP1A1 gene, laryngeal cancer, rs1048943, squamous cell carcinoma. Upper Aero-digestive tract cancers.

Open Peer Review

Approval Status

	1	2
version 1		
05 Feb 2021	view	view

1. **Vijaylakshmi Jain** , Jawahar Lal Nehru Memorial Medical College, Raipur, India
2. **Tracie Cheng** , Griffith University, Queensland, Australia

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: **Abdalwahab M:** Formal Analysis, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Ellaithi M** : Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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Introduction

Several epidemiological studies have suggested that tobacco smoking and alcohol consumption increase the risk of developing laryngeal cancer^{1–6}. Certain genetic polymorphisms have been identified as genetic risk factors specifically for laryngeal squamous cell carcinoma (SCC)⁷. Cytochrome P450 (CYP) enzymes are the key enzymes in phase I of the metabolism of carcinogenic polycyclic aromatic hydrocarbons (PAHs)⁸. PAHs are carcinogens associated with laryngeal cancer. Different studies have pointed to the involvement of CYP in procarcinogen activation^{9–12}. The *CYP1A1* polymorphisms rs1048943 A>G and rs4646903 T>C have been the most frequently studied¹³. *CYP1A1* rs1048943 A>G is an amino acid change at codon 462. *CYP1A1* rs4646903 T>C is characterized by the T-to-C mutation at nucleotide 3801 in the 3'-flanking region of the gene¹⁴. Numerous studies have investigated the links between the polymorphisms rs1048943 and rs4646903 and the risk of laryngeal cancer, but the results appear to be inconsistent. The Ile462Val rs1048943 A>G polymorphism in the *CYP1A1* gene is located in exon 7 in the heme binding region and leads to a doubling of enzyme activity¹⁵. Heme plays a vital regulatory role in the cell¹⁶.

The objective of this study was to find out if there was an association between the rs1048943 polymorphism and laryngeal cancer in Sudanese patients. The hypothesis was that the polymorphism might play a biological role in laryngeal cancer progression specifically in Sudanese patients.

Methods

Study design and participants

This was a case-control study carried out during the years 2019–2020. It involved 49 patients diagnosed with different grades and types of laryngeal cancers and 50 healthy controls. DNA samples for all participants were recruited from the upper aero digestive tract cancer (UADT) biobank. This biobank contains biological materials and data from cancer patients as well as control healthy individuals. These samples were collected from newly diagnosed cases to the biobank during the years 2014–2016 from the Radiation and Isotope Centre Khartoum (RICK), Sudan. The controls group was collected from healthy individuals recruited from the UADT biobank during the same period. The biobank includes a large number of DNA samples from both cancer patients and healthy controls; however, our selection was restricted to the patients diagnosed with cancer of the larynx only and controls were matched to patients by age and sex. The sample size was based on the availability of laryngeal samples in the biobank and matching controls. The biobank had a total number of 49 laryngeal cancer cases, so all were included.

Sample collection and DNA extraction

DNA samples from blood were obtained from the UADT biobank. The DNA had been previously extracted using the *innuPREP mini Kit* from Analytik Jena. DNA quantification (ng/μL) was determined with a 5 μL DNA sample using the *NanoDrop 2000* (Cat. No. ND-2000, Thermo Fisher Scientific). The DNA samples were stored at -80°C.

Polymerase chain reaction method

The region that contains the changes of the nucleotides A>G which identifies rs1048943 was amplified by PCR using universal eubacterial primers to produce an amplicon of 226 bp. The primers were designed using *Primer 3*. Accordingly, the forward sequence was (CTCACCCCTGATAGGATAGT-GCTAT) and the reverse sequence was (TTTGGAAGTGCT-CAGCAGCAGCAGCAG). The reaction was performed in a total volume of 20 μL using the Maxime PCR premix kit i-startaq (Cat. No 25165): 0.3 μL of each primer, 14.7 μL DW and 5 μL of the DNA template. The PCR was performed in a Thermo cycler (*Technique TC-412*, UK) with an initial denaturation at 95°C for 2 minutes, followed by 35 denaturation cycles at 95°C for 30 seconds, primer annealing at 58°C for 30 seconds extension at 72°C for 30 seconds and a final extension at 72°C for 5 minutes. After that, gel electrophoresis was performed to detect the presence of the amplified DNA fragments in the PCR product. The gel contained agarose gel dissolved in a buffer to 1.5 % to which an ethidium bromide (0.5 μg/ml) was added. The size of the wild type band was 226 bp. The electrophoresis result was photographed with the *Syngene InGenius LHR2 gel imaging system* (Syngene, Woonsocket, RI, USA).

Restriction fragment length polymorphism (RFLP)

We used the *NEBcutter V2.0* web tool provided by New England Bio labs to select the restriction enzyme specific to the position of the polymorphism. Accordingly, the 2-base pair product of the *CYP1A1* gene was digested with the BsrDI restriction enzyme. The enzyme recognized a short specific sequence of the nucleotide base, then catalysed hydrolysis (cleavage of the chemical bond that binds the nucleotide) by adding water molecules. The digestions were performed in a total volume of 31 μL. The reaction mixture consisted of 10 μL of the PCR product, 1 μL of the restriction enzyme and 2 ml of 10x buffer, with the volume adjusted by 18 μL sterile DW. Electrophoresis was performed on the digested product with 1.5 (w/v) agarose and 0.5 μg/ml ethidium bromide, and photographs were taken with the Syngene InGenius LHR2 gel documentation system (Syngene, Woonsocket, RI, USA). The sizes of the bands after digestion were 150 bp and 76 bp.

Data analysis

Statistical analysis was performed using *Stata v11* software (Statacorp, College Station, Texas). Results were presented in frequencies for the grades and types of laryngeal cancer. Genotyping analysis was performed using the Chi-square test in order to estimate the association of the different alleles to laryngeal carcinomas. The statistical level of significance was set at p value 0.05.

Results

There were a total of 23 male and 26 female cases with a male to female ratio of 1:1¹⁷. Their average age was 51.2 years old (range 13–80 years). The control group contained 23 males and 27 females with a male to female ratio of 1:1.7. Their average age was 51.6 (range 19–80) (*Table 1*).

Histopathological classification showed that 27 patients (55.1%) were diagnosed with well differentiated SCC, 12 (24.7%) with moderate SCC, 5 (10.2%) with poorly differentiated SCC and 1 (2 %) with SCC without indication of the degree (Table 2). Other histopathological types (adenoid cystic carcinoma, diffuse large cell lymphoma, mucoepidermoid carcinoma, and laryngeal papillomatosis) had a sample of 1 (2%) participant each.

The genotypic distribution of rs1048943 A>G is shown in Table 3. The wild type homozygous genotype A/A was present in

all the cases (100%) yet not the heterozygous (AG) or homozygous mutant (GG) genotype (0%). Of the controls, four had the heterozygous allele AG, 46 had the homozygous wild type AA genotype, and none had the GG genotype ($p = 0.1331$, OR 0.1044 and 95% CI 0.0054 to 1.9924) (Table 3).

Discussion

Laryngeal cancer is one of the most predominant cancers of the upper respiratory tract¹⁸. It is rare in individuals under 60 years of age with three quarters of all diagnoses occurring in people over 60¹⁹. In the current study, the mean age of the

Table 1. Sample characteristics.

	Males	Females	Male: Female ratio	Age range (years)	Average (years)
Cases	23	26	1:1	13-80	51.2
Controls	23	27	1:1.7	19-80	51.6

Table 2. Histopathological diagnosis of the cases.

	Number of patients (%)
Squamous cell carcinoma (SCC)	
Well differentiated SCC	25 (50%)
Moderate differentiate SCC	12 (24%)
Poorly differentiated SCC	5 (10%)
Unknown differentiated SCC	5 (10%)
Other types of laryngeal cancer	
Adenoid cystic carcinoma	1 (2%)
Diffuse large cell lymphoma	1 (2%)
Mucoepidermoid carcinoma	1 (2%)
Laryngeal papillomatosis	1 (2%)

Table 3. The genotype distribution of rs1048943 in laryngeal and hypopharyngeal cancer patients and controls.

Genotype	Cases %	Controls %	P value	OR (95% CI)
rs1048943				
AA	50/50 (100)	46/50 (92%)	0.1331	0.1044 (0.0054 to 1.9924)
AG	0/50 (0)	4/50 (8%)		
GG	0/50 (0)	0/50 (0%)		

participants was 51.2 years which is a rare age of onset for laryngeal cancer; however, young patients are expected to have a better prognosis²⁰.

Worldwide, laryngeal cancer occurs more commonly in men than in women^{21,22}. In the Sudan cancer registry for the years 2009–2013, the ratio of men to women diagnosed with laryngeal was about 2.8:1. This contradicts the almost equal ratio we found in this study. This could be because all cases in this study were collected from Radiation and Isotope Centre of Khartoum (RICK). On the other hand, cancer registry cases were collected from numerous hospitals and pathology labs from all over Sudan.

In this study squamous cell carcinoma was the most common type of laryngeal cancer, which reflect the type of cells in the larynx. Well differentiated squamous cell carcinoma was the dominating grade, suggesting that the cancer was detected early.

The CYP enzymes, especially CYP1A1, play an important role in laryngeal cancers¹⁵ as well as in lung cancer²³, prostate cancer²⁴, colorectal cancer¹⁴ and renal cell carcinoma²⁵. Here, rs1048943 showed no association with laryngeal cancer ($p = 0.1331$). In view of the small sample size but with our high statistical power we deem these results to be reliable and accurate. We can conclude that rs1048943 might be a polymorphism found in different ethnicities but not directedly linked to laryngeal cancer in Sudanese patients. Hence, it is necessary to study *CYP1A1* using more advanced technology like next generation sequencing (NGS) to identify SNPs associated with laryngeal cancer.

Conclusion

To sum up, the present study did not find an association between rs1048943 and laryngeal cancer in Sudanese patients.

Data availability

DRYAD: rs1048943 in CYP1A1 gene in laryngeal cancer-Sudanese study. <https://doi.org/10.5061/dryad.j3tx95xcm17>.

This project contains the following underlying data:

- Sudan-larynx-SNP_analysis.xlsx (demographics and clinical information of participants).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Consent for publication

Written informed consent for publication of the participants’ details was obtained from the participants.

Ethical consideration

The project was approved by the National Research Ethical Review Committee Health Research Council, Republic of Sudan National Ministry of Health in 2014 (NO:UADT/6.2014). All samples are part of the UADT cancer biobank. A signed consent form was submitted by all study participants.

Authors contributions

Marwa Abdalwahab conducted the laboratory work and contributed in Manuscript writing. Dr. Mona Ellaithi was the principle investigator and writer of the manuscript.

Acknowledgements

The Establishment of the Upper Aerodigestive Tract (UADT) Biobank reported in this article was undertaken with the support of a Return Grant obtained within the framework of the International Agency for Research on Cancer (IARC) Research Training and Fellowship Programme (CRA No. FEL/13/03).

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Reviewer Report 19 April 2022

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Tracie Cheng

Griffith University, Queensland, Australia

Thank you to the authors for submitting this work.

1. The introduction requires more input regarding the current state of knowledge of polycyclic aromatic hydrocarbons (PAHs) and their carcinogenicity.
2. How do PAHs get metabolised from a procarcinogenic state to a carcinogenic molecule via CYP1A1?
3. What role does rs1048943 play if there is an SNP in the heme binding region?
4. The authors state numerous studies have investigated CYP1A1 SNPs and laryngeal cancer, could you please cite these studies?
5. On what basis in the literature do the authors make their hypothesis?

Unfortunately, the sample size is low in this study, though the authors did use every laryngeal cancer case available in the biobank. The authors hypothesise that rs1048943 contributes to the progression of laryngeal cancer in Sudanese patients, however, their results nullify this.

- Can the authors suggest why this SNP is not involved in laryngeal cancer?
- Interestingly, 4 healthy controls express heterozygous GA alleles, can this be explained?

Thank you again to the authors.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: CYP1A1 SNPs in colorectal cancer and molecular oncology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 06 April 2022

<https://doi.org/10.5256/f1000research.31289.r127855>

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Vijaylakshmi Jain

Jawahar Lal Nehru Memorial Medical College, Raipur, India

The study entitled '**Analysis of rs1048943 in the CYP1A1 gene in laryngeal cancer - a case-control study**' focuses on the association of rs1048943 gene polymorphism with laryngeal cancer in the Sudanese population.

One of the most common tumors of the upper respiratory system is laryngeal cancer. The CYP1A1 gene is required for carcinogenic polycyclic aromatic hydrocarbon metabolism (PAHs). Numerous studies have looked into the relationship between the polymorphism Ile462Val rs1048943 A>G and various malignancies, however, the results have been mixed. Men were more likely than women to get laryngeal cancer. From the year 2009 to 2013, the ratio of men to women was around 2.8:1, which contradicts the current study. The average age of participants was 51.2 yrs. The most common type of laryngeal cancer in this study was squamous cell carcinoma, which reflects the type of cells in the larynx. The most common grade was well-differentiated squamous cell carcinoma, indicating that the malignancy was discovered early. There was no link between rs1048943 and laryngeal carcinoma in this study ($p = 0.1331$). The study deduces that rs1048943 is

a polymorphism that is common in people of many ethnicities but is not connected to laryngeal cancer in Sudanese patients.

1) Is the work clearly and accurately presented and does it cite the current literature?

A- Partly, as some major papers were not mentioned here. Like, Ahmed S, Abdelrhman S, Taha M, Malik M, El Naseh W. Etiology and Clinical Presentation of Laryngeal Cancer in Sudanese Patients. *Inter J Otorhinolaryngology*. 2018;5(1):3.¹

2) Is the study design appropriate and is the work technically sound?

A- Partly, Smoking is the predominant factor of laryngeal cancer but it has not been associated or linked to the study anyhow. Laryngeal cancer is a multifactorial disease but no such correlations were made in the study.

5) Are all the source data underlying the results available to ensure full reproducibility?

A- Partly, there is mention of the figure but no figures were attached in the manuscript.

6) Are the conclusions drawn adequately supported by the results?

A- No, as already mentioned laryngeal cancer is a multifactorial disease where smoking predominates. But, the study only talks about the age, division of cancer, and genotyping which is insufficient to draw any major conclusion when talking about the Sudanese population. Some biochemical correlations and other factors can be correlated to make such bold decisions on a population.

Last but not the least, an increased sample size would be appropriate to conclude its association.

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular oncology, microbiology, virology, proteomic, bioinformatics, mycology;

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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