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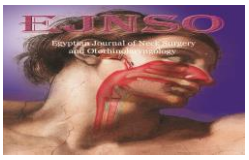

Polymorphism in a tumour suppressor (TP53) gene (G215C) and risk of squamous cell carcinoma of the larynx

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Abstract:

Laryngeal cancers are equivalent to one-third of head and neck cancers and are considered an important source of morbidity and mortality. Early-stage disease is highly curable with either surgical or radiation monotherapy, whereas late-stage disease has a worse outcome. The p53 protein is situated in the cell nuclei and play role in cell cycle checkpoint regulation, apoptosis, DNA repair, and the regulated repairing process of the damaged DNA caused by chemicals, radiation, and ultraviolet rays. If this process is arrested due to any cause, the p 53 transmits a signal to trigger cell apoptosis and prevents the cell from replication and hence tumor development. About 14 SNPs have been identified in the wild-type TP53 gene, which could change the function of the p53 protein. One of the most common SNPs of the TP53 gene is TP53c215C>G(Pro72Arg) (rs1042522), located in the proline-rich domain of p53, which is important in normal p53 function. Studies show that the arginine (Arg) variant is able to induce apoptosis faster and more efficiently than proline (Pro), while the Pro variant is better for inducing cycle arrest. It has been reported that Arg72Pro SNP in the TP53 gene can increase the risk of cancers.

Keywords: Cancer larynx; Genetic polymorphism; TP53; Larynx

Introduction

Laryngeal carcinoma is equivalent to one-third of head and neck cancers and is considered an important factor in cancer incidence and mortality. Monotherapy either surgical or radiation is highly curable in the early stage of the disease but not for the late-stage disease which has a bad prognosis. [1]

How malignant diseases progress is not yet clearly understood. Elderly men are more susceptible to laryngeal carcinoma.[2-3] Smoking and alcohol were reported as important predisposing factors in developing laryngeal cancer. [3]

Genetic and epigenetic factors also play a role in laryngeal carcinoma incidence as they change oncogenes expression, and enzymes involved in carcinogen metabolism by changing their encoding gene and tumor suppressor genes. [3-6]

The p53 protein is found in the cell nuclei and play role in cell cycle checkpoint regulation, apoptosis, DNA repair, and the regulated repairing

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process of the damaged DNA caused by chemicals, radiation, and ultraviolet rays. If this process is arrested due to any cause, the p 53 sends signals to stimulate cell death and stops the cell from replication and so tumor development. Tumor protein (TP53) is located on chromosome 17 (17 p13.1) acting as a tumor suppressor gene and encodes tumor protein P53.[7]

Single nucleotide polymorphisms (SNPs) destroy the function of the protein encoded by genes and hence have an important role in carcinogenesis. SNPs are a low-risk factor in carcinoma, but in combination with other risk factors like smoking and alcohol, can increase the risk of carcinoma incidence. [8-10]

The commonest kind of DNA change is single nucleotide polymorphisms (SNPs) which occur with single nucleotide substitution to another. [11]

TP53 gene wild type has multiple SNPs and may reach 14 types, which could change the function of the p53 protein. [12]

One of the most common SNPs of the TP53 gene is TP53c215C>G(Pro72Arg) (rs1042522), located in the proline-rich domain of p53, which is important in normal p53 function. [13] Some reports show that the arginine (Arg) variant can start cell death faster than proline (Pro), while the Pro variant is more in inducing cycle arrest.[14] It has been reported that Arg72Pro SNP in the TP53 gene can increase ease risk of carcinoma.

This study aims to detect Polymorphism in the tumor suppressor (TP53) gene (G215C) and squamous cell carcinoma of the larynx risk.

Patients and methods:

This study was done in the ENT department, faculty of medicine, South Vally and Assiut University, and the genetic department, faculty of agriculture, Assiut university during the period from November 2021 to May 2022.

One hundred patient was enrolled in this study. They were divided into 2 equal groups, Group I: cancer larynx patient, diagnosed by direct endoscopy, biopsy, and histopathological examination and CT larynx and neck for confirmation of diagnosis and detection of site, size, the extension of the tumor and presence or absence of lymph node. Non-squamous cell carcinoma or previously irradiated cancer larynx patient was excluded. Group II: Clinical normal larynx. Both groups were subjected to a collection of blood samples for genetic study for DNA polymorphism.

The TP53 gene genotypes frequencies is studied in relation to the site, extension and histopathological grading of tumor and absence or presence of LN and the p value significant with the site and tumor extension but not to histopathological grading or to presence or absence of lymph nodes.

Samples

1. DNA was isolated from both Blood samples and then stored until usage at -20ºC.
2. The 259-bp fragments containing the SNP were amplified using specific primers 5’-AATGGATGATTTGATGCTGTCCC -3’ and 5’-CGTGCAAGTCACAGACTTGGC -3’
3. AATGGATGATTTGATGCTGTC CCCGGACGATATTGAACAATGG TTCACTGAAGACCCAGGTCCAG ATGAAGCTCCCAGAATGCCAG AGGCTGCTCCCCSCGTGGCCCC TGCACCAGCAGCTCCTACACCG GCGGCCCCTGCACCAGCCCCCT CCTGGCCCCTGTCATCTTCTGTC CCTTCCCAGAAAACCTACCAGG

GCAGCTACGGTTTCCGTCTGGG CTTCTTGCATTCTGGGACAGCC AAGTCTGTGACTTGCACG.

4. PCR reactions were done in a reaction containing water, 2x master mix (Thermo Scientific), 30 pmol of each primer, and 100 ng of DNA template.
5. The thermal cycler was programmed as follows: 5 min at 94 °C for initial denaturation, 35 cycles of 30 s at 94°C (denaturation), 30 s at 58°C (annealing), 1 min at 72°C (extension) and terminal extension at for 5 min at 72°C.
6. The PCR product was purified by PCR purification kits (Thermo Fisher)
7. Then digested by 1 U of BSTUI restriction enzyme at 37°C overnight followed by electrophoresis in 1.5% agarose gel.
8. PCR products containing a G nucleotide at the polymorphic site will be digested into two fragments, 160 bp, and 99 bp, while those with C will not because of the absence of an SCRFI recognition site.

MW	CC	CG	GG
259	+	+	
160		+	+
99		+	+

Statistical analysis:

Data entry and data analysis were done using SPSS version 22 (Statistical Package for Social Science). Data were presented as number, percentage, mean, and standard deviation. The Chi-square test was used to compare qualitative variables. Independent samples t-test was used to compare quantitative variables between groups. P-value considered statistically significant when $P < 0.05$.

Results

Males represented 75 and 25 were females of the study size.

In **group I**: cancer larynx the mean age was 64.36 ± 9.96 with a male-to-female frequency of 90.0% to 10.0% (table 1). **Group II**: Clinical normal larynx in which the mean age was 62.08 ± 10.07 with male to the female frequency of 60.0% to 40.0% (table 1).

Table (1): Personal data of the studied groups:

Personal data	Group I (n= 50)		Group II (n= 50)		P-value
	No.	%	No.	%	
Sex:					
Male	45	90.0%	30	60.0%	0.001*
Female	5	10.0%	20	40.0%	
Age: (years)					
Mean ± SD	64.36 ± 9.96		62.08 ± 10.07		0.258
Range	45.0-80.0		44.0-96.0		

The characteristic of cancer larynx patients is shown in (table 2).

Table 2: characteristic of cancer larynx patients:

	No. (n= 50)	%
Site of lesion:		
Transglottic	22	44.0%
Supraglottic	15	30.0%
Glottic	13	26.0%
Tumor extension:		
T1	14	28.0%
T2	20	40.0%
T3	7	14.0%
T4	9	18.0%
Histological differential:		
G1	9	18.0%
G2	21	42.0%
G3	20	40.0%
LN:		
Negative	21	42.0%
Positive	29	58.0%

The TP53 gene genotype frequencies in **group I** were 17 (34%), 22(44%), and 11(22%) for GG, GC, and CC respectively (Figure 1), and in **group II** were 31(62%), 15(30%) and 4(8%) for GG, GC and CC respectively. The p-value is significant between each genotype in both groups with the highest frequency for cancer larynx being GC genotype (table 3).

The relation between TP53 gene genotypes and the tumor characteristics was as follow the genotype for the transglottic lesion were 9.1%, 40.9% and 50% ,the supraglottic lesion 60%,40% and 0% and the glottic lesion were 46,2% , 53,8% and 0% for GG,GC and CC respectively and for T1 were78.6%,21.4% and 0.0%, for T2 were 10.0%, 65.0% and 25.0%, for T3 were 0.0%, 42.9% and 57.1% and for T4 were 44.4%, 33.3% and 22.2% for GG,GC and CC respectively (table 4).

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Table (3): Genotype

Genotype	Group I (n= 50)		Group II (n= 50)		P-value
	No.	%	No.	%	
GG Pro/Pro	17	34.0%	31	62.0%	0.013*
GC Pro/Arg	22	44.0%	15	30.0%	
CC Arg/Arg	11	22.0%	4	8.0%	

Table (4): Relation between genotype and clinical data of cancer larynx:

	Genotype						P-value
	GG		GC		CC		
	No.	%	No.	%	No.	%	
Site of lesion:							
Transglottic	2	9.1%	9	40.9%	11	50.0%	
Supraglottic	9	60.0%	6	40.0%	0	0.0%	0.000*
Glottic	6	46.2%	7	53.8%	0	0.0%	
Tumor extension:							
T1	11	78.6%	3	21.4%	0	0.0%	
T2	2	10.0%	13	65.0%	5	25.0%	0.000*
T3	0	0.0%	3	42.9%	4	57.1%	
T4	4	44.4%	3	33.3%	2	22.2%	
Histological differential:							
G1	6	66.7%	3	33.3%	0	0.0%	
G2	3	14.3%	11	52.4%	7	33.3%	0.054
G3	8	40.0%	8	40.0%	4	20.0%	
LN:							
Negative	8	38.1%	9	42.9%	4	19.0%	0.847
Positive	9	31.0%	13	44.8%	7	24.1%	

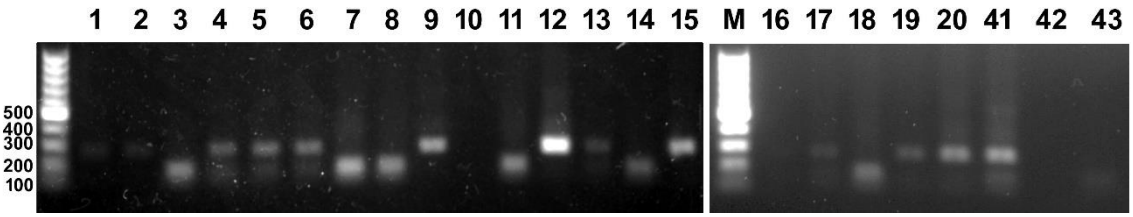

Figure (1): Example of the TP53 gene genotype frequencies

Discussion :

The commonest laryngeal malignancy is Squamous cell carcinoma.[15]The laryngeal carcinoma prognosis is typically better if early diagnosis is. As it is multifactorial, in carcinogenesis It is mandatory to detect key genes such as p53 that can lead to uncontrolled cell division and other mechanisms.[16]

The TP53 protein has an important role in apoptosis, cell cycle regulation, DNA repair, maintenance of genomic integrity, and apoptosis.[17]

One of the TP53 gene single nucleotide polymorphisms occurs at codon 72 where proline substitution for arginine (Arg72Pro). [18-19]

In this study cancer larynx incidence was found more with the pro alle either heterozygous GC (44%) or homozygous GG (34%) than the worldwide type arg alle CC (22%) and this agrees with Zemleduch et al who reported in their study on the polish patient that TP53Pro allele increase the risk of laryngeal carcinoma. Also, Gottschlich et al. found that the Pro72Pro TP53 genotype occurred with a lower median age. but disagree with, Sourvinos et al. reporting that the Arg72Arg TP53 genotype may increase the incidence of laryngeal carcinoma and with Escalante et al how reported that C alle have an increased risk of laryngeal carcinoma.[20-23]

Saleem et al in their study on oral squamous cell carcinoma that the homozygous change GG was more frequent (48.07%) than heterozygous GC (43.46%) or homozygous CC (8.47%) in the affected patient.[24]

These differences in the impact of the Arg72Pro TP53 gene SNP on laryngeal and another carcinoma can be attributed to genetic heterogeneity. Different environmental factors, including diet, smoking, alcohol, and diet combined with genetic factors can also affect the Arg72Pro polymorphism's influence on laryngeal carcinoma.[6]

The Arg72 allele in homozygotes was reported to exhibit a 15-fold more cell death-inducing ability than the Pro72 allele. But the TP53 Pro variant results in more cells arresting in the G1 cell cycle phase than the TP53Arg protein variant.[13, 25]

The TP53 Proprotein variant may induce less apoptosis due to its decreased mitochondrial allocation, which would result in decreased availability of the TP53 protein for interaction with the pro-apoptotic BAK protein. [13, 26]

Conclusion:

The pro alle either heterozygous GC or homozygous GG increases the risk of squamous cell laryngeal carcinoma more than the worldwide type Arg alle CC.

Conflict of interest: There is no conflict of interest***.***

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