Journal of Pharmaceutical Research International

33(63B): 381-390, 2021; Article no.JPRI.74357
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Genetic Alterations in CDH (Cadherin) Family of Genes and their Putative Association with Head and Neck Squamous Cell Carcinoma

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i63B35668

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/74357

Original Research Article

Received 20 October 2021
Accepted 27 December 2021
Published 29 December 2021

ABSTRACT

Background: Head and neck squamous cell carcinoma is considered to be a common type of human cancer. It is highly invasive with limited therapeutic options outside surgery, chemotherapy and radiation. Cadherin family genes comprises a family with more than 110 members which are calcium dependent transmembrane proteins which helps in cellular adhesion, signaling etc. There exist different functions of Cadherin genes in malignant cells. Uncontrolled mesenchymal- epithelial transition is seen in malignant situations where the cadherins serve as tumour suppressor genes but gets down regulated drastically to create tumour initiation and invasions. This study aims to study the association between genetic alterations in the cadherin family of genes and HNSCC patients.
Materials and Methods: The datas were collected from a promising database, cBioportal and the demographic details of the selected individuals(HNSCC patients) were tabulated and noted. The genetic variations in the genes were analysed using GnomAD analysis, oncoprint analysis and their various genetic alterations,frequency were tabulated and represented figuratively.

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Results and Discussion: On analysing the various genetic alterations discovered in cadherin family genes of HNSCC patients, CDH2 gene showed maximum genetic alteration(%). The different genetic alterations documented were splicing, deep deletion, amplification, etc. the oncoprint data analysis shows the frequency of the types of different genetic alteration in the five genes considered, their combination with any other genetic alteration seen in the other genes along with their frequency. The graphical representation of Kaplan meier analysis, showing over expression of CDH2 genes helps understand the survival rate of HNSCC patients. These analysis and tabulated documents, figures prove the association between genetic alterations in the cadherin family of genes and HNSCC.

Conclusion: There exists a significant association between HNSCC and genetic alterations seen in the cadherin family of genes.

Keywords: HNSCC; genetic alterations; cadherin family; analysis; association, novel mutations; innovative technology.

1. INTRODUCTION

Head and neck squamous cell carcinoma is considered to be a common type of human cancer. It is highly invasive with limited therapeutic options besides surgery, chemotherapy and radiation. The important risk factors are tobacco use, alcohol consumption and certain types of human papilloma viruses. Head and neck squamous cell carcinoma holds cancer in various locations, anatomy. Lip and oral cancer is the 15th most prevalent cancer in the world, with an incidence rate of 36.5 percent over the past three decades. Next comes larynx cancer which holds the 20th position as common cancer and has increased by 23.1% in recent decades. There are a number of risk factors that contribute to the development of these cancers, the most significant of which are cigarettes, alcohol, and HPV. However, non-essential causes such as betel nut chewing, impaired oral hygiene, HIV infections, genetic polymorphism, and others have raised our concern about its occurrence rates [1]. Studying its molecular genetics has brought many pathways involved to our notice. There are various tumour suppressor gene pathways involved in tumour initiation, progression and invasions. Concentrating more on this HNSCC genetics helps us understand its pathology to improvise the treatment methods [2]. Oral carcinoma caused by papillomavirus has its own route and prognosis. It's also worth noting that the prevalence of this form of oral carcinoma has grown. HPV-DNA positivity and other biomarkers such as p16 negativity are associated with a positive prognosis, whereas HPV DNA negative HPV induced carcinoma has the worst outcome [3]. Studies were performed to further understand the pathogenesis of HNSCC by examining different biomarkers correlated with the HNSCC population, such as potentially immune correlative biomarkers (PD-L1, PD-L2, interferons), and it was discovered that PD-L1 and IFN-gamma are associated, and using these markers on screening helps improve techniques for predicting responses [4]. These results help us understand the importance of studying the molecular and genetic analysis of HNSCC for further prognosis.

Various insights of different markers and molecular mechanisms are investigated in order to study the workings of the central nervous system, a complex, diverse, and well-organized system. The analysis of diverse superfamily gene groups reveals various pathways [5]. Cadherin comprises a family with more than 110 members which are calcium dependent transmembrane proteins which helps in cellular adhesion, signaling etc. [5,6]. Cadherin express cell to cell molecular adhesion with specific binding capacity. It has helped in spinal morphogenesis and transmembrane function [7]. These genes are also expressed in synapses and transmembranes for diverse functions like transduction, signalling, adhesion, etc. Cadherin genes have a number of roles in cancerous cells. In malignant conditions, unregulated mesenchymal-epithelial transformation happens when cadherins, which usually act as tumour suppressor genes, are dramatically downregulated, resulting in tumour initiation and invasion. There are frequent mutation accumulations found in different genes in these conditions which alters their function from preventing tumour progression [8]. The Cadherin gene also plays a major role in different psychiatric disorders due to its functions in CNS like ADHD, BD, Etc. The alterations and mutations found in these illnesses helps us understand the prognosis [9].

There are experiments that describe genetic abnormalities and mutations in various genes and gene families that serve as biomarkers, assisting in the early detection of various diseases and the development of more precise methods for improved outcomes. This tumour suppressor gene plays a role in cancer invasion and metastasis. Studies were done on meta analysing the association between CDH1 and HNSCC where promotor CDH1 promoter methylation has been significantly increased in these conditions affirming the association between HNSCC and cadherin gene [10]. This study aims to study the genetic alterations in the Cadherin family of genes and its putative association with head and neck squamous cell carcinoma. Our team has extensive knowledge and research experience that has translated into evidence based research with microbes [11–18], in metabolic disorders [23–30].

2. MATERIALS AND METHODS

The study was done under retrospective study design using the database, Bioportal which collects patients details in different cohorts. The database comprises different mutated and amplified genes along with TCGA, HNSCC cases were selected and tumor samples collected. The sequencing, alteration data along with demographic details were provided for the genes enlisted. The issues regarding these genes were submitted and analysed by cBioportal data and the samples used for further analysis. Oncoprint contains details on gene variations, types, protein coding, gene abnormalities, etc. this helps understand genotype, phenotype of diseases, novel variations. Table 1 represents the demographic details of the individuals (HNSCC patients) selected for the present study [31-33].

2.1 gnomAD Data Analysis

gnomAD dataset contains unrelated collections of wide spectrum individuals derived from large scale sequencing projects. Based on the consent, consortium, exome qualities the collected data and the documented of these dataset is compared and analysed for better understanding and accuracy [34].

2.2 Gene Expression and Survival Analysis

The expression of the gene presenting with highest frequency of gene alteration in HNSCC was analysed using the UALCAN (http://ualcan.path.uab.edu/cgi-bin/TCGA-survival) database. Survival curve analysis based on the tumor grade and expression profile was performed to demonstrate the putative role of Cadherin family of genes with HNSCC. Combined survival effect analysis of gene expression and other clinical parameters such as race, gender, tumor grade, cancer subtypes were assessed using log-rank test that generated a p value which was further used to indicate statistical significance of survival correlation between groups [35].

3. RESULTS

In the present study, cBioportal and TCGA was selected as the primary database, 528 HNSCC patients were selected with a gender ratio of 386(M):142(F) with diagnosis age between 19 to 90years. Among the selected individuals, 515 patients were smokers, 352 patients consumed alcohol,165 patients reported no history of alcohol consumption whereas information regarding the others were unknown, not available. The individuals of this selected group were grouped based on their histologic grading of neoplasm where 311 were categorised as grade 2 followed by 63-grade 1, 125- grade 3, 7-grade 4 and others, the details were unavailable. The selected individuals' data was collected and used for further analysis.

The oncoprint data was derived and tabulated focusing the cadherin family genes-*CDH1/CDH2/CDH3/CDH4/CDH5*) to study the genetic alterations experienced. Among the 5genes enrolled to study the association, CDH2 gene tends to have the maximum percentage of genetic alterations (4%) compared to others. The protein encoded, cytogenetic loci has also been mentioned. Splicing as a genetic variation is explicitly noted in CDH, gene variation as N814=R224H with an acute frequency of 0.05 and considered to be novel. All the possible types of genetic variations are documented for the 5 genes and compared to the gnomAD datas to categorise it as novel (encountered for the first time, not documented in any database compared before) or already documented before, if yes, the reference number for that variation has also been mentioned. Terminal mutations have been noticed in the genetic variation of *CDH3* gene at two places (E384, E177) which acts as a stop codon and prevents protein coding further. Genetic variations such as amplification, deep deletions were noticed and documented in all the

five genes enlisted. On comparing the five genes focused, CDH3 genes tend to have more genetic variations like amplification, deep deletion, terminal mutations, etc when compared to others.

Fig. 1 represents the oncoprint data of genetic alterations in the cadherin family of genes. *CDH2* genes tend to be more frequent in undergoing amplification and deep deletion. Missense mutations have also been encountered which seems to be common in *CDH2* and *CDH3* genes. The individuals with *CDH2* gene amplification have also experienced in-frame mutations/ missense mutations. There are also cases with missense mutation in both CDH1 and *CDH2* genes of HNSCC patients. There is also a patient reported with *CDH2* genes deep deletion along with missense mutation in *CDH3* gene. These results help us understand the various combinations of genetic variation seen in different genes of the same cadherin family in association with HNSCC patients.

Table 1. Demographic details of patients analyzed in the present study (as obtained from the cBioportal site)

Gender	Male (n = 386) Female (n = 142)
Mutation count	6-3181
Diagnosis age	19-90 years
Smoking status	Smokers: 515 Data not available: 12 Unknown: 1
Alcohol history	Yes – 352 No – 165 Data not available: 11
Neoplasm Histologic grade	Grade 1: 63 Grade 2: 311 Grade 3: 125 Grade 4: 7 Grade GX: 18 Data not available: 4
Race category	White: 452 African: 48 Asian: 11 American Indian or Alaska native: 2 Data not available: 15

Table 2. Gene alterations in type I classical cadherin gene family as assessed from the Oncoprint data

Gene	Protein coded	Cytogenetic loci	% of genetic alterations	Gene alterations	Variant allele frequency	gnomAD frequency
CDH1	Cadherin 1	16q22.1	1.8	Amplification		
				Deep deletion		
				N814=	0.05	Novel
				R224H	0.12	rs201511530
				V392_I393del	0.20	Novel
				D569E	0.26	rs876660905
				L15del	0.33	Novel
				D687H	0.14	Novel
				N558K	0.04	Novel
CDH2	Cadherin 2	18q12.1	4	Amplification		
				Deep deletion		
				R561Q	0.38	Novel
				S877R	0.14	Novel

Gene	Protein coded	Cytogenetic loci	% of genetic alterations	Gene alterations	Variant allele frequency	gnomAD frequency
				T445S	0.31	Novel
				G174V	0.20	Novel
				R421K	0.32	Novel
				E278Q	0.29	Novel
CDH3	Cadherin 3	16q22.1	2.2	Amplification		
				Deep deletion		
				E384*	0.27	Novel
				Q130E	0.21	Novel
				G721D	0.10	rs1242336678
				D805G	0.23	Novel
				E177*	0.17	Novel
				E811D	0.11	Novel
				P4S	0.45	Novel
				G706S	0.70	rs1284406451
CDH4	Cadherin 4	20q13.33	1.6	Amplification		
				A852S	0.16	Novel
				N388S	0.27	Novel
				D892E	0.26	Novel
				D262H	0.23	Novel
				D387N	0.10	Novel
				D908E	0.33	Novel
CDH15	Cadherin 15	16q24.3	1.6	Amplification		
				Deep deletion		
				D778N	0.16	Novel
				Q693H	0.71	Novel
				R280C	0.09	rs764686809
				V216L	0.32	Novel

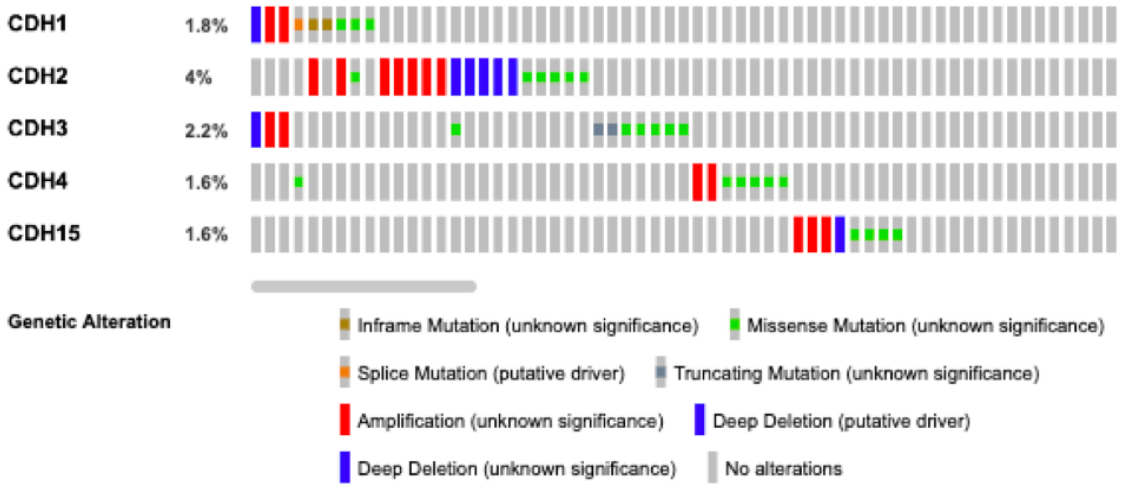

Fig. 1. Oncoprint data showing alterations in the type I classical cadherin family

Fig. 2 depicts the comparison of gene expression of *CDH2* gene between the samples of normal individuals and HNSCC patients. There has been significant elevation noted in gene expression of *CDH2* in HNSCC patients than the normal individuals with p value, $p=1.17X10^{-10}$, $p<0.05$. This proves that there is a significant over expression of *CDH2* in HNSCC patient samples. Fig. 3 depicts the Kaplan Meier analysis for the survival rate on effect of *CDH2* gene expression between high expression females and medium/ low expression males. This illustrates that high expression females have a shorter survival time than low/middle expression levels in males.

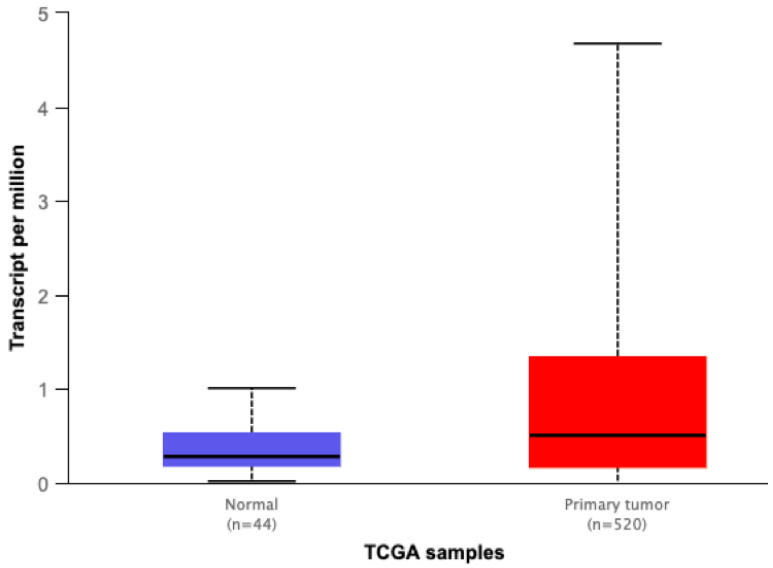

Fig. 2. Box-Whisker plot showing relative expression profile of *CDH2* gene (Normal vs primary tumor). The X axis denotes the TCGA samples (blue bar indicates normal and red bar indicates primary tumor) and Y axis denotes the transcripts per million values. The comparison of gene expression patterns between normal vs primary tumor was significant ($p = 1.17 X 10^{-10}$). A p value less than 0.05 was considered to be significant

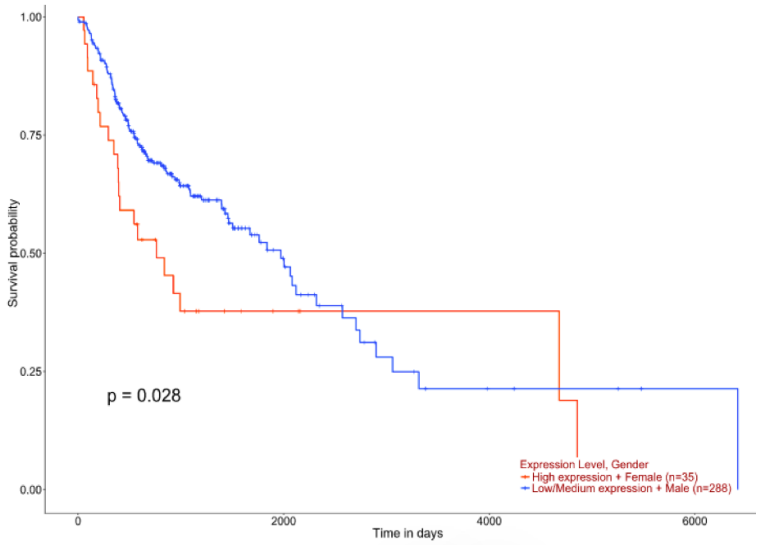

Fig. 3. Kaplan Meier plot showing the effect of *CDH2* expression level and gender on HNSCC patients. The x-axis represents the time in days and the y-axis represents the survival probability. The blue line indicates low expression of *CDH2* in males and the red line indicates high expression in females. A significant difference in the level of gene expression between the two groups was observed (p=0.028); p<0.05- significant

4. DISCUSSION

The significance of head and neck squamous cell carcinoma has been addressed in this study. Their global rankings as the most prevalent cancer, as well as their prevalence, occurrence rate, and survival rate, have all been addressed previously. The various risk factors which cause the incidence of HNSCC among different geographic locations have been enlisted in the order of their most and least incidence rates. The molecular pathways associated with this type of carcinoma should be studied which helps understand its pathogenesis. HNSCC is a

malignancy that can occur in a variety of locations and has a complicated pathogenesis involving a variety of biomarkers, mutations in normal cell constituents, and several molecular pathways. Understanding the pathogenesis and prognosis of the disease reliably includes research into the various aspects of molecules involved, different biomarkers, and their roles in normal and malignant conditions.It helps to gain knowledge about disease. Many genes are involved in pathogenesis and prognosis of HNSCC. Cadherins play an important role in tissue consistency and dysfunction. Cadherin-catenin complex which is needed for intact function on destabilisation results in tumor progression. E cadherin can also express as tumor suppressor gene which is involved in various oncogenic signalling pathways like MAPK, Ras, Rac1 signalling, hippo signalling, etc. any abnormalities in normal cadherin function may result in malignancy- tumor adhesion, invasion, aggressiveness [36]. The genetic modifications associated with this gene have been based as a result of the role of both HNSCC and its cadherin family gene function in the pathogenesis of this disease and focus.

There is various research previously done which focuses on the association of genetic alterations of different genes with different types of cancer. Study was done investigating the prevalence of *CDH*, hypermethylation among HNSCC primary tumor patients and their survival compared with other conditions. It has been found out that there was significant prevalence of hypermethylation of *CDH1* along with low smoking history. HPV16 infection driven HNSCC has better survival rate than other tumors noticed [37]. Study was done to review cadherin genes as potential prognostic factors in HNSCC on data collected from promising different databases like medline, embase, etc. it was found that E-Cadherin gene expression results in better overall survival and disease free survival in this malignancy [38]. There are studies which prove that E-cadherin expression is inversely correlated with tumor dedifferentiation and lymph node metastasis helps us understand the association of down regulated expression of E-cadherin with the prognosis of HNSCC in individuals [39,40]. Kevin et al, 2006 focused on correlating the expression of E-Cadherin, beta-catenin with survival rate and invasion in HNSCC, it was found that low expression E- cadherin in tumors has significant association with decreased overall survival, disease free survival, vascular invasion in HNSCC patients [41].

On reviewing various previous articles related to the cadherin family of genes and its association with head and neck squamous cell carcinoma, it helps us understand and support our findings in this present study. The graphs depict the elevated expression of *CDH2* gene in HNSCC condition. The different genetic alterations, it's frequency, different combinations has been tabulated and analysed to view the diverse outcomes and wide spectrum of genetic variations possible with the five genes (cadherin family) focused in this study. The previous study helps enhance the certainty of the survival seen in HNSCC patients with middle/low expression malesans high expression females of the *CDH2* gene of the cadherin family. This study primarily focuses on the genetic variations, outcome frequency, survival and gene expression to understand the importance of cadherin family genes as potential prognostic markers for further analysis, treatment options for HNSCC patients in the future world. Several in silico studies on numerous genes have been reported already [42-46].

The present study seems to have certain limitations like limited sample size(HNSCC patients) analysed, few number of analysis like gnomAD, oncoprint analysis were done to study the genetic alterations, the overexpression of CDH2 were compared only between hugh expression females and low/middle expression males. In future studies, these limitations should be considered and minimized. Further studies should focus more on various family of genes for the putative association of their genetic alterations with HNSCC patients in order to include thesegenes as various prognostic markers useful in understanding the prognosis, pathogenesis of the disease and helps targeting the treatment options.

5. CONCLUSION

This study primarily focuses on the genetic alterations of cadherin gpfamily of genes associated with head and neck squamous cell carcinoma discussing the different aspects and their role in pathogenesis and prognosis factors as early biomarker with better survival and putative significant association with head and neck squamous cell carcinoma.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

We thank Saveetha Dental College and Hospitals for providing us the support to conduct the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/74357