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Computational approach towards Identification of Functional Mutations in Il-6 Cytokine Family and It's Putative Association with Head and Neck Squamous Cell Carcinoma

Research Article

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Abstract

Objectives: The present study aimed to identify alterations in the IL-6 family to derive a putative association with HNSCC (head and neck squamous cell carcinoma).
Subjects and Methods: Computational approaches have been used to find the functional mutation in the IL-6 family. The cBioportal database served as the primary source of data for analyzing the mutations. The TCGA (The Cancer Gene Atlas, Firehose legacy) dataset encompassing 504 samples of HNSCC patients were included in the study.
Results: Alterations were observed in CLCF1 (7%), CNTF (2.4%), CTF 1(0.2%), IL-6 (2.2%), IL-11 (0.8%), LTF (1%), OSM (0.6%) genes. Further, we found that three out of four mutations exhibited deleterious consequences, and one out of four missense variants exhibited neutral effect upon substitution of glycine with amino acid serine.
Conclusion: Experimental validation is warranted to draw a strong association between the gene alterations and disease phenotype.
Dental implication: Identification of genetic alterations in crucial genes would aid in the selection of genetic markers with strong association with HNSCC.

Keywords: HNSCC; In Silico; IL-6; Mutations; Polymorphisms; Biomarkers.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a biologically diverse and genomically heterogeneous disease that arises from the squamous cell carcinoma lining of the upper aerodigestive tract including lip and oral cavity coma nasal cavity, paranasal sinuses, nasopharynx oropharynx larynx and hypopharynx [1-2]. Worldwide, HNSCC for more than 650000 cases and 340000 deaths annually [3]. In the United States itself, head and neck cancer accounts for 3% of malignancy with almost 53000 American developing head and neck cancer annually and 10800 equal then comes the disease [4]. In Europe, the numbers are even higher. In 2012, new cases accounted for 250000 and 63500 deaths. In Indian scenario, head and neck cancer accounts for 30% of all cancers. HNSCC is a multifactorial disease but usually caused due to tobacco and tobacco related products. The main risk factors associated with head and neck squamous cell carcinoma are environmental and lifestyle factors, culture of chewing tobacco, alcohol consumption and smoking. Recent epidemiological studies have associated human papillomavirus with head and neck carcinoma [5]. Interleukin 6 is a pleiotropic which plays an important role in a number of cellular processes including proliferation, survival differentiation, migration and invasion and is located on chromosome 7. IL-6 is a multifunctional cytokine with both anti-inflammatory and proinflammatory properties. IL-6 regulates progression and tumor metastasis by modulating tumor angiogenesis and tumor lymphangiogenesis studies have proved that IL-6 levels are exponentially upgraded in cancer patients. According to the find-

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ings made by researchers, it has been discovered that IL-6 can lead to cancer initiation and progression [6].

Materials and Methods

The cBioportal database hosts several datasets on HNSCC of which the TCGA-Firehose Legacy was selected for the present study. The demographic details of the patients in the dataset is given in table 1. A total of 504 whole genome sequences with mutation and copy number variants information from a total of 528 HNSCC samples were analysed to identify the mutations in the genes of the IL-6 pathway.

Sample data set

Bioportal is an exhaustive collection of molecular profiling information from cancer tissues and cell lines [7, 8], The database is easy to use/user friendly and host genetic epigenetic and proteomic information of the registered cases. The sample data includes the sequence information of 528 HNSCC patients which is used for the study.

Oncoprint data analysis

A single query for mutation analysis was initiated by selecting the OSCC cases from cBioportal database. The case data set included 528 sequenced tumors out of which 504 were analyzed for mutations in genes associated with IL-6 signaling pathway. The gene cluster included CLCF1, CNTF, CTF1, CTF2P, IL-6, IL-11, LIF, OSM. The set of genes were user defined and entered into the query.

Protein network interactions

The interactions of protein encoded by the gene with the highest frequency of mutation was assessed by submitting the query protein in the STRING v10.5 pipeline (https://string-db.org/cgi/input.pl?sessionId=TLDBRo1NeAXZ&input_page_show_search=on) [9].

gnomAD analysis

The genome aggregation database (gnomAD) is an exhaustive collection of data spanning 125, 748 exome sequences and 15,708 whole genome sequences from unrelated individuals sequenced and deposited as part of various disease-specific or population genetic studies. This data source was used to verify whether the variants identified in the present study are reported elsewhere in the other populations. The search could also provide an insight about the minor allele frequency of the variants in the population by which nature of the variants can be ascertained (Version 4, 2020) (Table 2) [10].

Protein stability analysis

The I-Mutant server [11] was used for prediction of protein stability changes upon single nucleotide mutations leading to change in the amino acid being encoded by the triplet codon. The server uses either protein sequence or structure to predict stabilization and destabilization of protein structure in the majority of cases. The prediction was based on running the query with protein sequence downloaded in the FASTA format from the public do-

Table 1. Demographic details of patients analyzed in the present study (as obtained from the cBioportal site - TCGA - Firehose legacy dataset).

Gender	Male (n = 386)
	Female (n = 142)
Mutation count	Jun-81
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. Type and frequency of gene alteration observed in the genes encoding proteins of the IL-6 pathway in HNSCC patients.

Gene	Protein	Cytogenetic location	Type of Mutation (%)	Frequency	Amino Acid Change
CLCF1	Cardiotrophin Like Cytokine Factor 1	11q13.2	Gene amplification	7	-
			Deep deletion		
CNTF	Ciliary Neurotrophic Factor	11q12.1	Gene amplification	2.4	-
CTF1	Cardiotrophin 1	16p11.2	Gene amplification	0.2	-
CTF2P	Cardiotrophin 2, Pseudogene	16p11.2	-	0	-
IL6	Interleukin 6	7p15.3	Gene amplification	2.2	
			G10S (Novel)		Glycine - serine
			S104C (Novel)		Serine - Cysteine
					Serine - Cysteine
IL-11	Interleukin 11	19q13.42	Gene amplification	0.8	-
LIF	Leukemia Inhibitory Factor	22q12.2	Gene amplification	1	
			K181N (Novel)		Lysine-Asparagine
			S157I		Serine-Isoleucine
OSM	Oncostatin M	22q12.2	Gene amplification	0.6	-

Table 3. Consequences of mutation on protein stability and associated pathogenicity as predicted by the IMutant suite and PROVEAN tools.

Gene	Alteration	I-Mutant prediction	I-Mutant Score	PROVEAN prediction	PROVEAN Score
IL-6	G10S	Decreased stability	-0.86	Neutral	0.701
	S104C	Decreased stability	-1.12	Deleterious	-2.55
LIF	K181N	Increased stability	-0.39	Deleterious	-4.24
	S157I	Decreased stability	-1.01	Deleterious	-3.23

main (https://www.ncbi.nlm.nih.gov/protein/). Upon substitution with the variant amino acid the stability changes were further assessed using the free energy stability change (ΔΔG) value. A value less than 0 and greater than 0 implies decrease and increase in protein stability respectively (Table 3).

Pathogenicity analysis

PROVEAN (Protein Variation Effect Analyzer) [12] predicts the impact on the biological function of a protein upon substitution with a amino acid (Table 2). The present analysis employs a user defined query of missense variants entered along with the reference sequence obtained from the NCBI database with a default cut-off value of -2.5. The results returned scores based on amino acid substitutions and classified them as either neutral or deleterious depending on the PROVEAN scores. A score less than -2.5 or greater than -2.5 was considered to be deleterious and neutral respectively (Table 3).

Results

Data was collected using computational methods. Demographic details of the patients in the TCGA dataset if given in Table 1. The CLCF-1 gene has the majority of amplifications (7%). CNTF 1 has 1% amplification whereas CNTF accounted for 2.4% amplification. Other members of the IL-6 gene families were computed for functional mutations. CT2P observed no alteration whatsoever. Most of the gene families showed amplification. IL1 (0.8%), IL-6 (2.2%), IL-6 and LIF is also one gene family where mis-sense mutation along with gene amplification was observed (1%) (Table 2; Figure 1 and 2). The consequence of mutations on the proteins encoded by IL-6 and LIF genes were recorded (Table 3). The protein protein interaction network for the gene which demonstrated highest frequency of mutation was shown in figure 3.

Discussion

Several different studies were carried out in our institutions pertaining to the genetic and pathological analysis of oral cancer [13, 14]. A correlation was charted between both the ethnicities taking age, habits and the gradation of neoplasm into consideration. Grading of HNSCC was done according to WHO classification. The classification [15] is as follows:

Grade I (G1): Well differentiated (low grade)

Figure 1. Oncoprint data demonstrating the alterations in IL-6 gene family. Red bars - represents gene amplification; blue bars - represents deep deletions; light green spots - represents missense mutations of unknown significance; grey bars - represent no alterations.

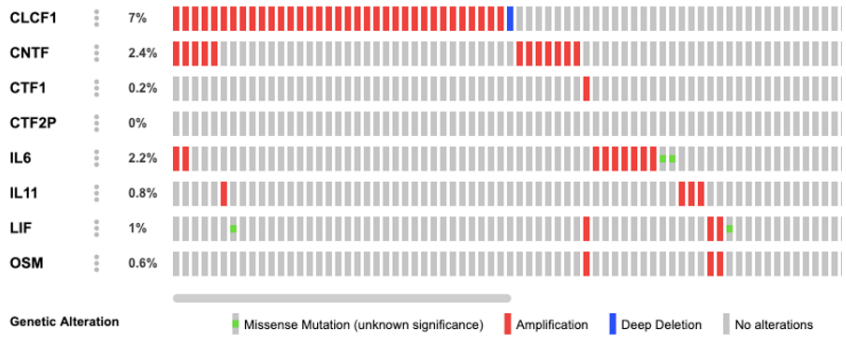


Figure 2. Functional non-synonymous mutation observed in (a) IL-6 gene and (b) LIF gene.

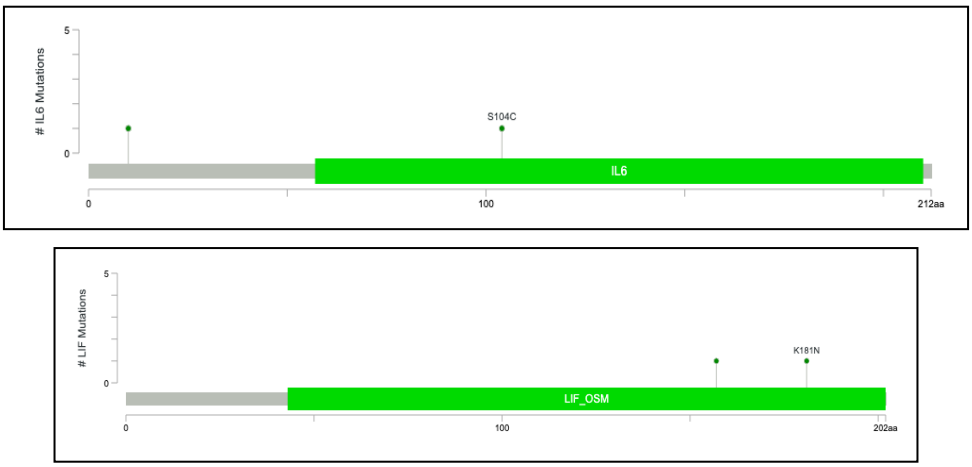
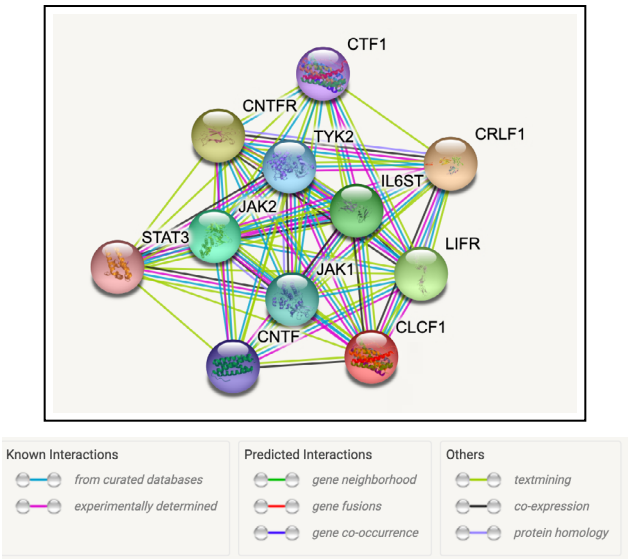


Figure 3. Protein interaction network of CLCF1 gene which presented with the highest frequency of gene alteration.



Grade II (G2): Moderately differentiated (intermediate grade)
Grade III (G3): Poorly differentiated or anaplastic (high grade)
Grade IV (G4): Undifferentiated (high grade)
Grade x (Gx): Grade cannot be assessed (undetermined grade).

Various computational approaches interpreted that several gene alterations have been identified in the IL-6 family, most of which are gene amplifications [16]. IL-6 and LIF genes show presence of non-synonymous mutations in OSCC patients. The mutations were found to be novel as assessed by the gnomAD database. Among the four missense mutations three were found to decrease the stability of the protein. Except for the G10S substitution in IL-6 protein, other amino acid substitutions were found to be deleterious. (Table 3) The highest frequency of alterations was found in CLCF1 (Cardiotrophin like cytokine factor 1) gene [17]. The association of IL-6 with clinical parameters and oncological outcomes in head and neck cancer has been studied greatly since the last 20 years [18]. Several studies have shown that elevated levels of IL-6 family genes have shown either increased mutation or gene alterations. Previous studies have found out that IL-6 levels

in serum is responsible for OSCC [19-22]. Most of the IL-6 family genes demonstrated either increased mutation or amplification. In previous studies it has been stated that IL-6 signalling is associated with progression and treatment resistance of OSCC [24]. Although certain studies reported negative association of IL-6 with OSCC [24] certain other studies have shown positive association with IL-6 gene which was shown to promote cancer metastasis by inducing epithelial mesenchymal transition via the JAK- STAT3-SNAIL signalling pathway [23].

In a previous study conducted, the prevalence of HNSCC in Hispanic population was 7.5%. Factors like alcohol consumption, age, sex and socioeconomic factors are responsible for OSCC [25]. In a previous study correlations were made on polymorphism of alleles of IL-6 with regards to tobacco and alcohol consumption [24, 26]. No significant association was found in GC allele, but CC allele showed significant association of OSCC if a person intakes alcohol and tobacco regularly (Singh et al., 2015). Similar results were procured in a study where patients consumed alcohol and suffered from breast cancer [27-30]. Contrary to the previous studies, a study was conducted where a positive association was found between gene polymorphism in IL-6 with OSCC [28]. Various studies have been conducted where a positive association was detected in which polymorphisms have led to progression of oral cancer, breast cancer, prostate cancer and colorectal cancer [31, 32]. Even though in the current study a disparity is observed between the ethnicities, it is previously proven that HNSCC has its definite path of propagation and that ethnicity is not a predisposing factor for HNSCC initiation as well as progression [33, 34]. Several studies using computational approach have been carried out to identify potential markers in crucial genes implicated in tumorigenesis [35-40]. Hence such approach holds promising while handling exhaustive collection of data to be probed in to select a few markers of clinical significance.

Conclusion

All the genes of the IL-6 family have undergone alterations precisely gene amplifications and missense mutations. Further experimental studies are required to arrive at an association between IL-6 gene and OSCC in south Indian population.

References

[1]. Noble DJ, Jefferies SJ. Squamous cell carcinoma of the oral cavity, oropharynx and upper oesophagus. Medicine. 2019; 47: 269–74.

[2]. Gupta V, Ramani P. Histologic and immunohistochemical evaluation of mirror image biopsies in oral squamous cell carcinoma. J Oral Biol Craniofac Res. 2016; 6(3):194-197. PMID: 27761383.

[3]. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424. PMID: 30207593.

[4]. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020; 70(1): 7-30. PMID: 31912902.

[5]. Day AT, Dahlstrom KR, Lee R, Karam-Hage M, Sturgis EM. Impact of a tobacco treatment program on abstinence and survival rates among current smokers with head and neck squamous cell carcinoma. Head Neck. 2020; 42(9): 2440-2452. PMID: 32476217.

[6]. St John MA, Li Y, Zhou X, Denny P, Ho CM, Wenyuan Shi, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2004; 130(8): 929-35. PMID: 15313862.

[7]. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Bülent Arman Aksoy, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012; 2(5): 401–4. PMID: 22588877.

[8]. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, S Onur Sumer, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013; 6(269): l1. PMID: 23550210.

[9]. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Jaime Huerta-Cepas, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019; 47(D1): D607-D613. PMID: 30476243.

[10]. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Qingbo Wang, et al. Genome Aggregation Database Consortium, Neale BM, Daly MJ, MacArthur DG. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020 May; 581(7809): 434-443. PMID: 32461654.

[11]. Capriotti E, Fariselli P, Casadio R. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res. 2005; 33: W306-10. PMID: 15980478.

[12]. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. 2015; 31(16): 2745-7. PMID: 25851949.

[13]. Jayaraj G, Sherlin HJ, Ramani P, Premkumar P, Natesan A. Stromal myofibroblasts in oral squamous cell carcinoma and potentially malignant disorders. Indian J Cancer. 2015; 52(1): 87-92. PMID: 26837985.

[14]. Jayaraj G, Sherlin HJ, Ramani P, Premkumar P, Anuja N. Cytomegalovirus and Mucoepidermoid carcinoma: A possible causal relationship? A pilot study. J Oral Maxillofac Pathol. 2015; 19(3): 319-24. PMID: 26980959.

[15]. Akhter M, Hossain S, Rahman QB, Molla MR. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. J Oral Maxillofac Pathol. 2011; 15(2): 168-76. PMID: 22529575.

[16]. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells. Hum Exp Toxicol. 2019; 38(6): 694-702. PMID: 30924378.

[17]. Hannah.R, Pratibha R, Herald.JS, Gheena R, Abilasha R, Jayaraj G, et al. Awareness about the use, Ethics and Scope of Dental Photography among Undergraduate Dental Students Dentist Behind the lens. Research J. Pharm. and Tech. 2018; 11(3): 1012-1016.

[18]. Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer Treat Rev. 2012; 38(7): 904-10. PMID: 22651903.

[19]. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med. 2019; 48(4): 299-306. PMID: 30714209.

[20]. Sridharan G, Ramani P, Patankar S. Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Cancer Res Ther. 2017; 13(3): 556-561. PMID: 28862226.

[21]. Jinno T, Kawano S, Maruse Y, Matsubara R, Goto Y, Taiki Sakamoto, et al. Increased expression of interleukin-6 predicts poor response to chemoradiotherapy and unfavorable prognosis in oral squamous cell carcinoma. Oncol Rep. 2015; 33(5): 2161-8. PMID: 25761055.

[22]. Thangaraj SV, Shyamsundar V, Krishnamurthy A, Ramani P, Ganesan K, Muthulakshmi Muthuswami, et al. Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations. PLoS One. 2016; 11(6): e0156582. PMID: 27280700.

[23]. Yadav A, Kumar B, Datta J, Teknos TN, Kumar P. IL-6 promotes head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the JAK-STAT3-SNAIL signaling pathway. Mol Cancer Res. 2011; 9(12): 1658-67. PMID: 21976712.

[24]. Singh PK, Chandra G, Bogra J, Gupta R, Kumar V, Jain A, et al. Association of interleukin-6 genetic polymorphisms with risk of OSCC in Indian population. Meta Gene. 2015; 4: 142-51. PMID: 26005639.

[25]. Schrank TP, Han Y, Weiss H, Resto VA. Case-matching analysis of head and neck squamous cell carcinoma in racial and ethnic minorities in the United States--possible role for human papillomavirus in survival disparities. Head Neck. 2011; 33(1): 45-53. PMID: 20848419.

[26]. Ko YC, Huang YL, Lee CH, Chen MJ, Lin LM, Tsai CC. Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. J Oral Pathol Med. 1995; 24(10): 450-3. PMID: 8600280.

[27]. Balasubramanian SP, Azmy IA, Higham SE, Wilson AG, Cross SS, Cox A, et al. Interleukin gene polymorphisms and breast cancer: a case control study and systematic literature review. BMC Cancer. 2006; 6: 188. PMID: 16842617.

[28]. Vairaktaris E, Yiannopoulos A, Vylliotis A, Yapijakis C, Derka S, S Vassiliou, et al. Strong association of interleukin-6 -174 G>C promoter polymorphism with increased risk of oral cancer. Int J Biol Markers. 2006; 21(4):246-50. PMID: 17177164.

[29]. Wang YF, Chang SY, Tai SK, Li WY, Wang LS. Clinical significance of inter-

leukin-6 and interleukin-6 receptor expressions in oral squamous cell carcinoma. Head Neck. 2002; 24(9): 850-8. PMID: 12211049.

[30]. Lagmay JP, London WB, Gross TG, Termuhlen A, Sullivan N, Amy Axel, et al. Prognostic significance of interleukin-6 single nucleotide polymorphism genotypes in neuroblastoma: rs1800795 (promoter) and rs8192284 (receptor). Clin Cancer Res. 2009; 15(16): 5234-9. PMID: 19671870.

[31]. Landi S, Gemignani F, Bottari F, Gioia-Patricola L, Guino E, María Cambray, et al. Polymorphisms within inflammatory genes and colorectal cancer. J Negat Results Biomed. 2006; 5: 15. PMID: 17062130.

[32]. Mandal S, Abebe F, Chaudhary J. -174G/C polymorphism in the interleukin-6 promoter is differently associated with prostate cancer incidence depending on race. Genet Mol Res. 2014; 13(1):139-51. PMID: 24446297.

[33]. Harris GJ, Clark GM, Von Hoff DD. Hispanic patients with head and neck cancer do not have a worse prognosis than Anglo-American patients. Cancer. 1992; 69(4): 1003-7. PMID: 1735067.

[34]. Swathy. S, Gheena. S, Sri Varsha. L. Prevalence of pulp stones in patients with history of cardiac diseases. Research J Pharm and Tech. 2015; 8(12): 1625-1628.

[35]. Jayaseelan VP, Arumugam P. Virtual screening of mutations in antioxidant genes and its putative association with HNSCC: An in silico approach. Mutat Res. 2020 Jun 9; 821: 111710. PMID: 32593872.

[36]. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. Am J Cancer Res. 2020; 10(8): 2546-2554. PMID: 32905518.

[37]. Dhivyadharshini J, Girija ASS, Paramasivam A, Priyadharsini JV. Decoding the Genetic Alterations in PRAME Gene Family and Its Association with Head and Neck Squamous Cell Carcinoma. Journal of Pharmaceutical Research International. 2020: 32(20): 93-102.

[38]. Nirubama K, Girija ASS, Paramasivam A, Priyadharsini JV. Genetic Aberrations in Caspase Family of Genes and Their Possible Association with HNSCC. Journal of Pharmaceutical Research International. 2020: 32(15): 83-95.

[39]. Sivarajan M, Girija ASS, Paramasivam A, Priyadharsini JV. Computational Approach to Identify Mutations in Genes of Notch Signaling Pathway and Its Association with OSCC. Journal of Pharmaceutical Research International. 2020; 32(20): 84-92.

[40]. Akshayaa L, Girija ASS, Paramasivam A, Priyadharsini JV. Deciphering the Genetic Aberrations in DNA Damage Response Genes and Their Possible Association with HNSCC. Journal of Pharmaceutical Research International, 2020; 32(15): 156-169.