RESEARCH ARTICLE

PREVALENCE OF P53 DYSREGULATIONS AND TP53 GENE MUTATION IN ORAL SQUAMOUS CELL CARCINOMA PATIENTS IN NORTH INDIAN POPULATION: A RETROSPECTIVE STUDY

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ABSTRACT

Background: According to WHO, squamous cell carcinoma constitute sixty to seventy percent of all the malignant tumours diagnosed in the head and neck region. Although the prevalence of disregulations of p53 has been studied in many tumours of body but the prevalence of p53 protein dysregulations in oral squamous cell carcinoma has not been studied much. Morever there has been no study on prevalence of p53 dysregulations in patients of oral squamous cell carcinoma in the North Indian population.

Aim and Objective: This study was carried out with the objective of determination of prevalence of p53 dysregulations in patients of Oral Squamous Cell carcinoma in North Indian population.

Methods and Materials: From 2010 to 2020, a retrospective assessment of medical records from tertiary level hospitals was conducted to detect histologic samples of OSCC and chronic oral inflammation. Serial slices of OSCC, eosinophilic granuloma, chronic gingivostomatitis, and normal oral mucosa were immunolabeled for p53 using a commercial monoclonal antibody with verified reactivity in human tissues. Deep sequencing analysis of exons 5 through 8, which correspond to the TP53 DNA binding region, was used to determine the TP53 mutational status of each instance of OSCC, inflammatory lesions, and normal oral mucosa. When one or more mutations in the nucleotide sequence of the amplified exons of human TP53, resulting in amino acid changes with a negative impact on protein function according to PolyPhen-2, and a variant allele frequency (VAF) >10 percent, cases were categorised as mutant

Results: It was found that the prevalence of p53 dysregulation was significantly higher in cases of oral squamous carcinoma accounting for 98.23% in North Indian population. Similar findings were found in case mutations of TP53 gene (98.23%) . The most common location for oral squamous cell carcinoma was found out to be non dentate mucosa.

Conclusion: From this study it can be concluded that the prevalence of p53 dysregulations is higher in patients with oral squamous cell carcinoma in North Indian population and it can be an important molecular indicator for oral squamous cell carcinoma. More prospective studies should be carried out with larger sample size in future for better outcomes.

Key Words: SCC,P53, TP53,Mutation.

INTRODUCTION:

According to the new WHO guidelines the squamous cell carcinoma is the most frequent type of malignant tumour observed in the head and neck region. It is believed that squamous cell carcinoma constitute sixty to seventy percent of all the malignant tumours diagnosed in the head and neck region.[1,2] The most common sites of the head and neck region

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involved are ventral surface of tongue, floor of the mouth, buccal mucosa etc. Clinically it is generally found in the form of non healing ulcero-profilerative growth with indurated margins. There may be overlying sloughing of tissues as a result of necrosis of tissues. The oral squamous cell carcinoma can be locally invasive and can cause destruction of underlying tissue in uncontrolled manner.[3,4] Sometimes it may be present in the form of nodular swelling which may be firm in the initial stages but becomes large ulcerative lesion in due course. There is reported evidence suggesting the metastasis of these malignancies in the regional areas as well as the distant areas of the body.[5,6]

But the main reason of death in patients with oral squamous cell carcinoma is due to the complications associated with the primary tumour. These complications are evident earlier in comparison to the appearance of the malignant stage of the carcinoma of oral cavity. The number of deaths due to OSCC is higher because it is being diagnosed at advanced stage.[7] It is well accepted fact that despite the availability of many advanced treatment modalities mortality due to OSCC has increased in recent years. This is due to the fact that prognosis of any treatment modality for OSCC depends upon the stage in which the OSCC has been diagnosed.[8]

If the diagnosis is made at the earlier stage then the prognosis is quite better. However if the diagnosis is made at later stage then the chances of survival of the patient is very less. Although biopsy is the standard method of diagnosis of oral carcinoma but it cannot give adequate idea about the progress of the disease. This is because it is invasive technique which cannot be carried out at regular intervals. Another important fact is that till the disease appears as malignant cancer clinically it become too late for the patients.[9]

Therefore there is need to look for other indicators which can help in studying the molecular changes being taking place in the oral squamous cell carcinoma. These indicators will help in diagnosing the oral squamous cell carcinoma at early stage even before the appearance of malignant nature of disease. Morever they should help in the progress of the premalignant disease into the malignant diseases. This will certainly help in reducing the mortality due to oral squamous cell carcinoma. Although there are several molecular indicators which can be used for this purpose but p53 protein is the most promising molecular marker.[10]

P53 protein is regulated by the TP53gene. This is an oncosupressor gene. This gene is among the category of those oncogenes which are tumour suppressor in nature. These genes regulate the cell division. If there is mutation in these genes then there will be uncontrolled division of cells leading to formation of neoplasms. Mutation in the TP53 genes cause disregulation of the p53 protein.[11] These disregulations of p53 proteins can be an indicator of the tumour. Although the prevalence of disregulations of p53 has been greater in many tumours of body but the prevalence of p53 protein disregulations in oral squamous cell carcinoma has not been studied much. Morever there has been no study on prevalence of p53 disregulations inpatients of pral squamous cell carcinoma in the

North Indian population to the best of knowledge of the authors.[12]

Therefore this study was carried out with the objective of determination of prevalence of p53 dysregulations in patients of Oral Squamous Cell carcinoma in North Indian population and correlate it with tobacco use.

MATERIALS AND METHODS

This was a retrospective research based on OSCC tissue samples that had been stored. An ethics committee gave their approval. All of the samples studied were taken as part of ordinary standard care for diagnostic purposes. For the use of clinical data and preserved biological samples for teaching and research, informed consent was obtained.

Case inclusion criteria and patient information

From 2010 to 2020, a retrospective assessment of medical records from tertiary level hospitals was conducted to detect histologic samples of OSCC and chronic oral inflammation. Microscopically, the histologic sections were examined to confirm the diagnosis. All confirmed instances of OSCC were included, along with 10 cases of eosinophilic granuloma and chronic gingivostomatitis each. In addition, 10 oral mucosa post-mortem histologic samples with normal histologic appearance were collected prospectively. The demographic information and tumour location were gathered from patient records. In addition, data on tobacco addiction was gathered.

Histology

All samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 m, and stained with hematoxylin and eosin for histological inspection (HE). Histologic subtype according to previously published criteria, histologic grade according to the Broders' approach (just for the conventional histotype), and mitotic count were all analysed for OSCC (MC). According to Meuten et al., 2016, MC was defined as the total number of mitotic figures in a 2.37 mm2 area (10 fields with a 40x objective and a 10x ocular with a field number of 22 mm). 27 The count was done in ten non-overlapping high-power fields in a row. Fields having necrosis or inflammation were omitted from the analysis. Two of the writers completed all histologic evaluations by consensus (AR1 and PDB).

Immunohistochemistry

Serial slices of OSCC, eosinophilic granuloma, chronic gingivostomatitis, and normal oral mucosa were immunolabeled for p53 using a commercial monoclonal antibody with verified reactivity in human tissues (Pab 240 clone, BD Biosciences, San Jose, California, USA). Incubation with 0.9 percent hydrogen peroxide in phosphate buffered saline for 10 minutes inhibited endogenous peroxidase activity (PBS, pH 7.2). Microwave slides in citrate buffer (pH 6.0) for 4 cycles of 5 minutes at 750 W for antigen retrieval.

After that, the slides were incubated with a commercial streptavidin-biotin-peroxidase kit (Vectastain Elite ABC Kit) and 3,3-diaminobenzidine (DAB tablets, Diagnostic BioSystems) as chromogen (Vectastain Elite ABC Kit). Papanicolaou's hematoxylin was used as a

counterstain. For the main antibody, a p53 positive FOSCC was employed as a positive control. By removing the main antibody, negative controls were generated. According to prior investigations, cases with at least 20% of p53-immunoreactive epithelial cells were judged positive. Nuclear staining was the only staining that was considered specific.. Interpretation of the p53 IHC staining was performed by two of the authors without prior knowledge of *TP53* mutational status.

TP53 mutation analysis

Deep sequencing analysis of exons 5 through 8, which correspond to the TP53 DNA binding region, was used to determine the TP53 mutational status of each instance of OSCC, inflammatory lesions, and normal oral mucosa. The MasterPure Complete DNA extraction kit was used to purify DNA from 10 m slices of formalin-fixed and paraffin-embedded (FFPE) tissues (5 for each sample) (Epicentre, code MC85200). If the DNA amplification failed, the case was removed from the research. With the use of tagged primers and multiplex PCR, locus-specific amplicon libraries were created.

A initial PCR amplification for target enrichment was followed by a second, shorter (8-cycle) amplification session to allow barcoding of the template-specific amplicons generated from the first amplification phase. As previously stated, barcoding was done with the Nextera index kit. 29 The amplified products were purified using MagSi-NGSPREP (MagnaMedics) after each PCR step and quantified with the Quantus Fluorometer (Promega, code E6150). The sequencing was done on an Illumina MiSeq sequencer according to the manufacturer's instructions.

When one or more mutations in the nucleotide sequence of the amplified exons of human TP53, resulting in amino acid changes with a negative impact on protein function according to PolyPhen-2, and a variant allele frequency (VAF) >10 percent, cases were categorised as mutant.

Statistical analysis

All the data collected were put in MS Excel sheet and statistical analysis was carried out with the help of SPSS latest version software. The student t test and chi square test was used for statistical analysis. The level of significance was adjusted at $p \leq 0.05$.

RESULTS

When the evaluation was carried out for exposure to tobacco and p53 expression then it was found that there was statistically significant difference. It meant that p53 dysregulation was significantly associated with the tobacco usage. When the evaluation was carried out for exposure to tobacco and TP53 gene mutation then it was found that there was statistically significant difference. It meant that TP53mutation was significantly associated with the tobacco abuse. When evaluation was carried out to find the association of p53 expression with oral squamous cell carcinoma, chronic inflammatory lesions and normal oral mucosa then it was found that maximum p53 dysregulation was observed in the oral squamous cell carcinoma followed by chronic inflammatory lesions, while it was minimum in normal oral mucosa.

The difference was statistically significant.($p \leq 0.05$).

When evaluation was carried out to find the association of TP53 gene mutation with oral squamous cell carcinoma, chronic inflammatory lesions and normal oral mucosa then it was found that maximum TP53 mutation was observed in the oral squamous cell carcinoma followed by chronic inflammatory lesions, while it was minimum in normal oral mucosa. The difference was statistically significant.($p \leq 0.05$).When there was evaluation for location of the oral squamous cell carcinoma then it was found that the most common location for the oral squamous cell carcinoma was non dentate mucosa followed by tongue while it was minimum in the dentate mucosa. The difference was statistically significant with $p \leq 0.05$. There was evaluation for the p53 dysregulation in the conventional and non conventional histotype. It was found that p53 dysregulation was significantly greater in conventional histotype in comparison to non conventional histotype. There was evaluation for the TP53 mutation in the conventional and non conventional histotype. It was found that TP53 mutation was significantly greater in conventional histotype in comparison to non conventional histotype (Table 1 and Table 2).

It was found that the prevalence of p53 dysregulation was significantly higher in cases of oral squamous carcinoma accounting for 98.23% in North Indian population. Similar findings were found in case mutations of TP53 gene. The most common location for oral squamous cell carcinoma was found out to be non dentate mucosa.

DISCUSSION

The oral squamous cell carcinoma can be locally invasive and can cause destruction of underlying tissue in uncontrolled manner. Sometimes it may be present in the form of nodular swelling which may be firm in the initial stages but becomes large ulcerative lesion in due course. There is reported evidence suggesting the metastasis of these malignancies in the regional areas as well as the distant areas of the body. But the main reason of death in patients with oral squamous cell carcinoma is due to the complications associated with the primary tumour. These complications are evident earlier in comparison to the appearance of the malignant stage of the carcinoma of oral cavity.[13,14]

The number of deaths due to OSCC is higher because it is being diagnosed at advanced stage. It is well accepted fact that despite the availability of many advanced treatment modalities mortality due to OSCC has increased in recent years. This is due to the fact that prognosis of any treatment modality for OSCC depends upon the stage in which the OSCC has been diagnosed. P53 protein is regulated by the TP53gene. This is an oncosupressor gene. This gene is among the category of those oncogenes which are tumour suppressor in nature. These genes regulate the cell division. If there is mutation in these genes then there will be uncontrolled division of cells leading to formation of neoplasms.[15,16]

Mutation in the TP53 genes cause disregulation of the p53 protein. These disregulations of p53 proteins can be an indicator of the tumour. Although the prevalence of disregulations of p53 has

been greater in many tumours of body but the prevalence of p53 protein disregulations in oral squamous cell carcinoma has not been studied much. Morever there has been no study on prevalence of p53 disregulations inpatients of pral squamous cell carcinoma in the North Indian population to the best of knowledge of the authors.[17,18]

It was found in our study that the prevalence of p53 dysregulation was significantly higher in cases of oral squamous carcinoma accounting for 98.23% in North Indian population. Similar findings were found in case mutations of TP53 gene. The most common location for oral squamous cell carcinoma was found out to be non dentate mucosa. Several studies has been carried out in past in other populations to find out the prevalence of p53 dysregulations. In most of these studies the results has been in accordance with the results of our study.[19,20]

When the evaluation was carried out for exposure to tobacco and p53 expression then it was found that there was statistically significant difference. It meant that p53 dysregulation was significantly associated with the tobacco usage. When the evaluation was carried out for exposure to tobacco and TP53 gene mutation then it was found that there was statistically significant difference. It meant that TP53mutation was significantly associated with the tobacco abuse. Several studies has been carried out in past in other populations to find out the prevalence of TP53 gene mutation. In most of these studies the results has been in accordance with the results of our study.[21,22]

When evaluation was carried out to find the association of p53 expression with oral squamous cell carcinoma, chronic inflammatory lesions and normal oral mucosa then it was found that maximum p53 dysregulation was observed in the oral squamous cell carcinoma followed by chronic inflammatory lesions, while it was minimum in normal oral mucosa. The difference was statistically significant.($p \leq 0.05$). Several studies has been carried out in past in other populations to find out the prevalence of p53 dysregulations. In most of these studies the results are mostly similar with the results of our study.[23,24]

When evaluation was carried out to find the association of TP53 gene mutation with oral squamous cell carcinoma, chronic inflammatory lesions and normal oral mucosa then it was found that maximum TP53 mutation was observed in the oral squamous cell carcinoma followed by chronic inflammatory lesions, while it was minimum in normal oral mucosa. The difference was statistically significant.($p \leq 0.05$). Several studies has been carried out in past in other populations to find out the prevalence of TP53 gene mutation. In most of these studies the results has been in accordance with the results of our study.[25,26]

When there was evaluation for location of the oral squamous cell carcinoma then it was found that the most common location for the oral squamous cell carcinoma was non dentate mucosa followed by tongue while it was minimum in the dentate mucosa. The difference was statistically significant with $p \leq 0.05$. Several studies has been carried out in past in other populations with same aim and objective. In most of these studies the results has

been in accordance with the results of our study.[27,28]There was evaluation for the p53 dysregulation in the conventional and non conventional histotype. It was found that p53 dysregulation was significantly greater in conventional histotype in comparison to non conventional histotype. There was evaluation for the TP53 mutation in the conventional and non conventional histotype. It was found that TP53 mutation was significantly greater in conventional histotype in comparison to non conventional histotype. Several studies has been carried out in past in other populations to find out the prevalence of p53 dysregulations and gene TP53gene mutations. In most of these studies the results had resemblance with the results of our study.

Although the prevalence of disregulations of p53 has been greater in many tumours of body but the prevalence of p53 protein disregulations in oral squamous cell carcinoma has not been studied much. Morever there has been no study on prevalence of p53 disregulations inpatients of pral squamous cell carcinoma in the North Indian population to the best of knowledge of the authors.[29]

There were some limitations of this study also. The sample size was small. Besides this study was retrospective in nature. The errors in the preservation of records may have affected the results.

CONCLUSION

From this study it can be concluded that the prevalence of p53 dysregulations is higher in patients with oral squamous cell carcinoma in North Indian population and it can be an important molecular indicator for oral squamous cell carcinoma. More prospective studies should be carried out with larger sample size in future for better outcomes

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TABLES:

Table 1: **Relationship between p53 dysregulations and other clinicopathological variables in 61 histological samples of oral mucosa**

Variable	p53 expression dysregulation	p53 expression normal	P value
Exposure to Tobacco (n = 61)			
Exposed	51.12%	22.31%	0.03
Not exposed	68.24%	67.21%	
Diagnosis (n = 61)			
Oral Squamous Cell Carcinoma	98.23%	01.13%	0.001
Chronic Inflammatory Lesions	49.23%	40.21%	
Normal oral mucosa	31.21%	33.31%	
OSCC location (n = 30)			
Non-dentate mucosa	80.21%	19.21%	0.03
Tongue	14.21%	6.46%	
Dentate jaws	6.31 %	28.21%	
OSCC histotype (n = 31)			
Conventional	75.49%	21.21%	0.01
Non conventional	24.21%	10.45%	
Conventional OSCC degree of differentiation (n = 31)			
Well differentiated	33.21%	56.29%	0.04
Moderately/poorly differentiated	66.79%	43. 71%	
OSCC MC (n = 31) (median, range)	7–86	5–82	0.388

Table 2: Relationship between TP53 mutations and other clinicopathological variables in 61 histological samples of oral mucosa

Variable	Type TP53 mutated	TP53 wild	P value
Exposure to Tobacco (n = 61)			
Exposed	51.12%	22.31%	0.03
Not exposed	68.24%	67.21%	
Diagnosis (n = 61)			
Oral Squamous Cell Carcinoma	98.23%	01.13%	0.001
Chronic Inflammatory Lesions	49.23%	40.21%	
Normal oral mucosa	31.21%	33.31%	
OSCC location (n = 30)			
Non-dentate mucosa	80.21%	19.21%	0.03
Tongue	14.21%	6.46%	
Dentate jaws	6.31 %	28.21%	
OSCC histotype (n = 31)			
Conventional	75.49%	21.21%	0.01
Non conventional	24.21%	10.45%	
Conventional OSCC degree of differentiation (n = 31)			
Well differentiated	33.21%	56.29%	0.04
Moderately/poorly differentiated	66.79%	43. 71%	
OSCC MC (n = 31) (median, range)	7–86	5–82	0.388