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Study of P53 Expression in Dysplasia and Squamous Cell Carcinoma of Upper Aerodigestive Tract: An Ominous Sign

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Abstract:
Background*: Oral squamous cell carcinoma (SCC) is one of the most common malignancy and is a major cause for morbidity and mortality in developing nations. The objective of the study was to assess p53 expression in a range of upper aerodigestive tract lesions to relate the results to the clinical outcome in patients with dysplastic lesions and SCC.*
Materials and Methods*: The expression of p53 was investigated by immunohistochemistry method in 50 histopathologically diagnosed patients which comprised of 15 dysplasia cases and 35 SCC cases of upper aerodigestive tract region. The expression of p53 was scored based on percentage of positive tumor cells and the staining intensity.*
Results*: The expression of p53 was detected in 60% of dysplastic lesions and 71% of SCCs. There was significant increase in staining intensity and percentage of tumor cells expressing p53 from dysplasia to different grades of SCC.*
Conclusion*: Present study concluded that combined histological analysis along with p53 immunoexpression can improve the evaluation of premalignant and malignant lesions for treatment.*
Key Words*: Dysplasia,Carcinoma,Immunohistochemistry,p53.*

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I. Introduction

Globally about five lakh new oral and pharyngeal cancers are diagnosed annually. Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy and is a major cause for morbidity and mortality in developing nations[1].

Cancer has a multifactorial aetiology and is a multistep process involving initiation, promotion and tumour progression. It arises by a complex process involving a series of genetic alterations which leads to cellular proliferation and differentiation[2].The risk of malignant transformation increases with increasing grades of dysplasia.

There can be significant improvement in the prognosis for oral cancer, if it can be detected at an early stage. So, the immunohistochemical studies to as" tumor markers", has received considerable attention[3]. Among these, p53 tumour suppressor gene (TP53) product, p53, deserves particular attention, not only because of its central role in genomic stability and cell cycle regulation, but also because its function is abrogated in most human cancers and, in the case of oral mucosa, also in preinvasive stages[4].

Apart from tumor cells, if adjacent keratinocytes express p53 positivity, it helps to assess the adequacy of surgical excision, because ,although, apparently totally excised, positive tumor cells at the margins are responsible for malignant transformation and local recurrency[5].

p53 mutations in UADT SCC are also associated with resistance to cisplatin based chemotherapy and radiation resulting in poor prognosis compared to SCC patients with wildtype p53 [6]. Thereby,it helps the clinician in selecting p53 targeted therapy in case of p53 positive tumors for better management.

Therefore, this study is taken up to assess the immunohistochemical expression of p53 in upper aerodigestive tract tumorogenesis and its degree of expression from dysplasia to malignancy,which is also important for prognostication.

II. Materials And Methods

This 2 year prospective study was performed on 50 histopathologically proven squamous (intraepithelial and invasive) lesions of the upper aerodigestive tract after obtaining the proper approval from ethical committee and consent obtained from the patient party during the study period (August'15-May'17).

The sample for this study was obtained from the archieves of the Department of Pathology,Hi Tech Medical College,Utkal University,Bhubaneswar,Odisha,India.

Total 50 histologically diagnosed cases,comprising 15 dysplastic lesions and 35 squamous cell carcinoma(SCC) were included in our study.Among dysplasia,6 were of grade I, 6 were grade II and 3 were grade III lesions. Among SCC ,20 were WD SCC,11 were MD SCC, and 4 were PD SCC..

From the paraffin-embedded blocks,two sections of 3µm were taken on poly-L-lysine –coated glass slides for immunohistochemical staining of p53 .The immunostaining was carried out using avidin biotin peroxidase technique.

Heat-mediated antigen retrieval was performed using citrate buffer in the microwave oven set at 540º for 30 minutes.Ready-to-use mouse IgG-1 anti p53 (DO-7) monoclonal antibody (BioGenex, Milmont drive, Fremont, California- 94538 USA) was used as primary antibody and was incubated for 60 min, followed by super enhancer for 25 min. For secondary antibody application, slides were incubated with polymer-horseradish-peroxidase reagent (antimouse and antirabbit IgG labeled with enzyme polymer in phosphate-buffered saline) for 30 min.3,3- diaminobenzidene (DAB) (DAB tetrahydrochloride) solution was used as the chromogen and counterstaining was done with Mayer's hematoxylin.

When a distinct brown staining was confined to the nuclei, p53 expression was considered positive.

The positive control was taken from previously diagnosed case of SCC.The negative control consisted of the replacement of the primary antibody for 1%bovine serum albumin,diluted in phosphate saline solution(TRIS).

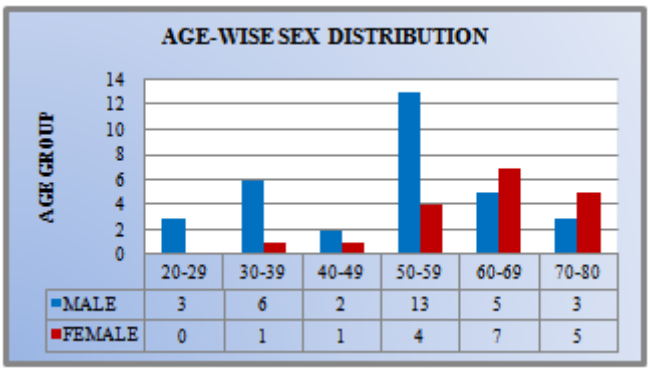
The percentage of positive cells was scored according to the method of Lehadh et al.[7]as follows: (+++) = strong staining (more than 50% stained); (++) = moderate staining (25 –50% stained; (+) = weak staining (5 – 25%); 0 =negative (less than 5% stained).

STATISTICAL ANALYSIS was made considering the clinical,histopathological and immunohistochemical data.Then transformed to a master chart by using Microsoft excel sheet,which was then subjected to statistical analysis using chi square test by using SPSS,version 20.Analysis was done in the form of percentage and represented as tables and figures where necessary.P value of ≤ 0.05 is considered as statistically significant.

III. Results

An observational clinical correlation study of 50 patients with UADT dysplasia and SCC were undertaken to study the immunohistochemistry for the detection of p53 status and correlation with histopathological grading of squamous lesions.

In the present study,minimum age of the patient involved was 25 years,and maximum age was 75 years.So,the mean age was 50 years.Maximum number of patients (34%) were in the age group of 50-59 years.Patients having dysplasia or carcinoma which are younger than 50 years were 26% (22% Male& 4% Female) and more than 50 years were74%(42% Male &32% Female),Dig 1.



Dig. 1- Bar diagram showing Age-Wise sex distribution in cases studied

In the present study,the lesions were commoner in males.There were 64% male and 36% female in the cases studied.

Sites involvements in cases studied is shown in table 1. Majority of the cases (56%) presented on tongue.

Table 1 : Sites involvements in cases studied

SITE	MALE(%)	FEMALE(%)	NO. OF PATIENTS (N=50)	%
TONGUE	15(30%)	13(26%)	28 (56%)	56%
BUCCAL MUCOSA	10(20%)	1(2%)	11 (22%)	22%

PHARYNX	4(8%)	2(4%)	6 (12%)	12%
LARYNX	3(6%)	2(4%)	5 (10%)	10%

(Chi Square=4.83 p value=0.18 Not Significant)

Clinically ,15 (30%) cases were diagnosed as dysplasia and 35 (70%) cases were diagnosed as SCC. 9 (60%) cases of dysplastic lesions expressed p53 in basal & suprabasal regions,and 6 (40%) cases didn't express p53.In SCC 25 (71.4%) cases expressed p53 in invasive cell nests, islands and cords of proliferative neoplastic epithelial cells; and 16 (32%) didn't express.

In our study,out of total 50 cases,34 cases were p53 positive and 16 cases were p53 negative.In mild dysplasia cases,2 out of 6 i.e. (33.3%)showed p53 +ve and both had 1+ staining intensity.4 (66.7%)cases were p53-ve. In moderate dysplasia cases,4 out of 6 i.e. (66.7%)were p53 +ve and 2 (33.3%)cases were p53-ve. P53 staining intensity was 2+ in 3 cases and 3+ in 1 case.In severe dysplasia cases,all 3 (100%) were p53 +ve and p53 staining intensity was 3+ in all the cases. In WD SCC cases,13 out of 20 i.e. 65% were p53 +ve and 7 i.e. 35% were p53 –ve. P53 staining intensity was 1+ in 7 cases,2+ in 5 cases and 3+ in 1 case.In MD SCC cases,9 out of 11 i.e. 81.8% were p53 +ve and 2 i.e. 18.2% were p53 –ve.p53 staining intensity was 2+ in 8 cases and 3+ in 1 case.In PD SCC cases,3 out of 4 i.e. 75% were p53 +ve and 1 i.e. 25% was p53-ve.All 3 cases showed 3+ p53 staining intensity. Frequency ,distribution & immunostaining intensity to p53 in different histologic zones of UADT dysplasia and SCC are shown in table 2.The association was highly significant(P = 0.001).

Table 2: Frequency, Distribution & Intensity of p53 immune reactive staining in UADT Lesions

Type of tumor	P53+ve	%	P53-ve	%	1+	%	2+	%	3+	%	No. of cases
Mild dysp.	2	33.3	4	66.7	2	33.3	-	-	-	-	6
Mod. Dysp.	4	66.7	2	33.3	-	-	3	50	1	16.6	6
Sev. Dysp.	3	100	0	-	-	-	-	-	3	100	3
WD SCC	13	65	7	35	7	35	5	25	1	5	20
MD SCC	9	81.8	2	18.2	-	-	8	72.7	1	9	11
PD SCC	3	75	1	25	-	-	-	-	3	75	4
Total	34		16		9		16		9		50

sensitivity=68% (chi square=44.959 p value=0.001 highly significant)

IV. Discussion

As oral squamous cell carcinoma is one of the major cause of morbidity and mortality in India and in many developing nations, constant research on its prognostic and predictive markers are going on for its timely and targeted management. As p53 is one of the early prognostic markers, we took to study this important prognostic marker and correlated with various histologic grades of dysplasia and OSCC lesions. This is prospective study included 50 cases of squamous cell lesions of UADT which came to our institution Hi Tech Medical College and Hospital during the study period.

In the present study ,mean age of study population was 50years ,which was similar to P. Baweja et al [8] ,but lower when compared to Deniz et al[9] and Ashraf et al[10].In our study, it was noted that the lesions were commoner in male than female.Similar observations were made by P Baweja et al[8] and Claudia et al[11] .But in the study done by Juan C et al[12] female involvement was more than male. In the present study,most of lesions were located in tongue and buccal mucosal regions. Similar observation was made by ,P Baweja et al[8] , Claudia et al[11] and Juan C et al [12].Incidence of tumors were highest in tongue in all these studies.

In the present study,p53 positivity was seen in 60% of the dysplastic lesions and negative in 40 %. Similar observation was noted in A Jain et al[13] study.P53 positivity percentage was lower when compared to S Kananet al[14] and Lehadh et al[7].

Not all mutations cause loss of normal function, and for p53 there is still uncertainty about the role of specific mutants in the biology of neoplasia (Prives C, HallPA. 1999) [15].

It has therefore been suggested that apart from the p53 loss-of-function mutants there are also some "gain-of-function" mutants, providing new or altered properties (Prives C,Hall PA.1999) [15].

Tumours with nonsense or frameshift mutations also result in the production of unstable, truncated proteins, which are also negative on immunohistochemistry.These types of mutations are estimated to account for less than 15% of human tumour mutations, depending on the cancer (Soussi T et al.)[16] . The present study data shows,the number of p53 positive cells increased gradually with progression of dysplastic lesion from mild to severe .Similar observation was noted in A Jain et al [13] and Sauter et al [17] study.

In the present study,p53 positivity was seen in 71.4% of the SCC cases and negative in 28.6 %. Similar observation was noted in P Baweja et al [8]study.P53 positivity percentage was slightly higher when compared to R Verma et al[18] and AMJ et al[19]. p53 couldn't be detected in 28.6% of SCC cases in our study,suggesting that p53 mutation is not essential for tumor transformation. The data in present study shows that there was

progressive increase in percentage of p53 positivity with progression of lesion from WD SCC to MD SCC.The maximum number of p53 positive cases were seen in MD SCC.Similar finding was seen in Juan et al [12] and P Bawejaet al[8] study.

Decrease in percentage of p53 +ve tumor cells, with progression of lesion from MD SCC to PD SCC could be due to presence of tumor cells with nonsense or frameshift mutations,resulting in unstable, truncated proteins which are p53 –ve [16],and indicates aggressive nature of p53-ve SCCs[20].

V. Conclusion

The immunoexpression of p53, increased with increasing grades of dysplasia indicating that they may be used as predictive markers in oral cancer development. Progressive increase in percentage of p53 positivity was noted with progression of lesion from WD SCC to MD SCC.Maximum number of p53 positivity were seen in MD SCC cases.

Decrease in percentage of p53 positivity was noted with progression of lesion from MD SCC to PD SCC,which could be due to presence of tumor cells with nonsense or frame shift mutations,resulting in unstable & truncated protein which are p53 –ve and indicates aggressive nature of p53-ve SCCs.As p53 –ve cases are more aggressive,their management will differ from p53+ve cases which require targeted therapy. Hence, immunohistochemical assessment of p53 should be incorporated as a routine investigation. This along with histopathological grading and staging will guide the clinicians to make correct choice of treatment protocols.

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