EVALUATION OF EARLY LYMPH NODE METASTASIS IN SQUAMOUS CELL CARCINOMA OF THE PENIS WITH THE AID OF p53 IMMUNOMARKER.

ABSTRACT:

1. **Background:** Use of p53 as a prognostic marker to detect early lymph node metastasis in carcinoma of penis so as to avoid unnecessary lymphadenectomy.
2. **Aim:** To detect early metastasis in amputed specimens of penis, by using p53 immuno marker, and correlation with the histopathological variables and clinical stage.
3. **Materials and Methods:** Retrospective study of 49 cases of partial /total amputation with lymphadenectomy of penile cancers were divided into metastatic and non metastatic groups on H&E sections in which 16/49 had metastasis Histological slides from all cases were processed with immunohistochemical technique using anti-p53 antibodies. The p53 tumor density was calculated in 100 cells with 20% cut off and was graded as positive when more than 20% of the tumor cells with positive nuclear staining. All the pathological and clinical variables were calculated by Chi square test.
4. **Results:** 13 /49 cases showed increased p53 tumor density, pvalue of 0.05 was considerd significant in our study. P53 did not correlate statistically with histopathological and clinical variables like phimosis, BXO, clinical node status, tumor type, tumor grade, depth of invasion, LVI. P53 had low positive predictive

value. Therefore in our study p53 cannot predict the early lymph node metastasis because of low positive predictive value, even though they are sensitive.

Conclusions: P53 Immunomarker had low specificity and low positive predictive value, which suggest that it is not able to predict lymph node metastasis even though it is sensitive and therefore they are not helpful to decide for prophylactic lymphadenectomy.

Key words: SCC, Lymphatic , Lymphovascular invasion (LVI), p53.

INTRODUCTION

Squamous cell carcinoma of the penis is common in developing countries like India and its incidence is similar to that of cervical cancer. It forms 20% of all malignancies in Asian countries. the mean age at presentation is 60 years; however, in areas of high incidence, penile carcinoma presents at an earlier age. In India, the age of presentation can be as low as 35 years[1-2]. Penile cancers with involvement of lymph nodes have a poor prognosis and is the most important predictor of outcome for survival in penile carcinoma. However clinical estimation of the lymph node status in these patients is still a major problem, since 20-25% of clinically node negative patients can have occult nodal metastasis[3-4]. The traditional elective lymphadenectomy accounts for overtreatment in up to 80% of patients and is associated with high morbidity (30-90%) [4-5]. By using p53 Martin et al and Zhu et al showed that there is increased risk of lymph node metastasis with increased p53 expression in the tumor and these patients have poor

outcome.[5, 8, 9]. Thus, here we attempt to predict early metastasis in these tumors, so that major surgery can be avoided and thereby prevent morbidity in a subset of patients.

MATERIALS AND METHODS:

The data and histologic slides of a 49 cases of carcinoma of penis done by partial/Total amputation of penis from year 2006-2014 were retrived from the archives of Christian Medical College, Vellore. The study was retrospective and archival stained and mounted slides and formalin fixed paraffin embedded tissue blocks were retrieved. Clinical details were obtained from clinical work station and the initial pathological diagnosis was reviewed from Pathology work station using PACS (Picture archival and communication System). The samples had been fixed overnight in 10% buffered formalin and they were embedded in paraffin wax using conventional methods. Haematoxylin and eosin stained slides of all cases of carcinoma of penis were reviewed. 3-4 micron sections were taken from the paraffin embedded blocks for performing immunohistochemistry p53 by using the standard DAKO protocols. In the current investigation, we initially reviewed 57 diagnosed cases of SCC of penis that underwent partial or total amputation with lymphadenectomy from a period of 8 & 1/2 years (January 2006 to September 2014), out of which 8 cases were excluded as paraffin blocks were not available or adequate for immunohistochemical study. The remaining 49 cases were divided into those with metastatic nodes and those with no nodal metastasis. Accordingly there were 16 cases that had pathological lymph node metastasis and 33 cases that did not have metastasis. All available clinical parameters and pathological variables were included in the study so

as to compare their relation with these prognostic marker. Cases that were excluded were "slide review only" cases, amputation specimens referred from other hospitals without lymph node status or clinical details, patients who underwent wedge biopsy only or amputation in our center but without lymph node dissection, and blocks without complete epithelium.

Following variables were studied:

- Age of the patient.
- Presence of phimosis.
- Balanitis xerotica obliterans
- Circumcision.
- Clinical stage.
- Gross tumor size
- Depth of invasion.
- Histological type
- Histological grade
- Infiltration of corpus cavernous/spongiosum
- Urethral infiltration
- Lympho vascular invasion on H&E(LVI)
- Density of p53 in the tumor.
- Lymphatic density assessment.

- Pathological involvement of superficial and deep inguinal and pelvic lymph nodes.

Histological grade was classified as per the Broder's system as G1 (well), G2 (Moderate) and G3 (poorly) differentiated.

Quantification of Tumor density for p53:

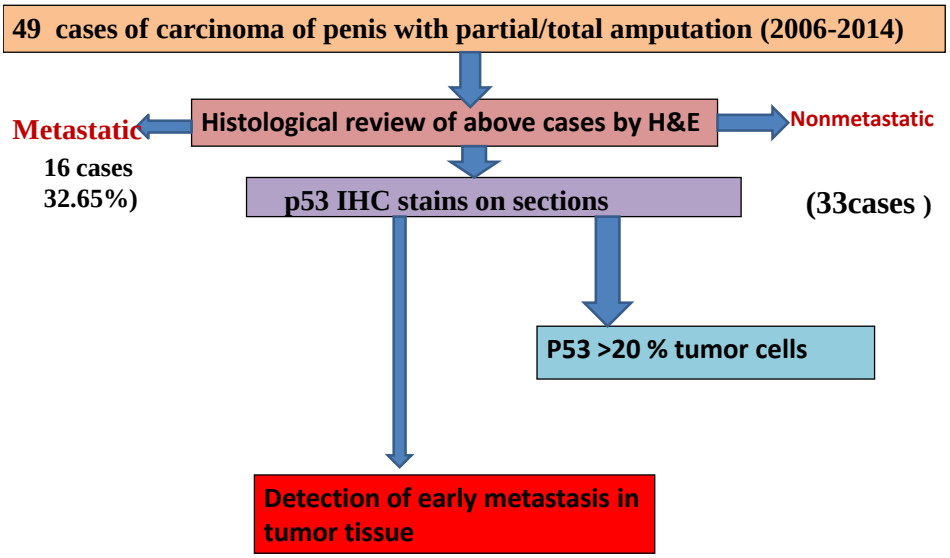
p53 density was calculated in 100 tumor cells under low power field (10x) and expressed in per cent. p53 was graded as positive when at least 20% of the tumor cells showed nuclear staining.

Immunohistochemistry Method:

P53 immunostains was carried out using automation by Ventanna Bench Mark XT autostainer, using DAKO reagents and the same guidelines were used for both markers. Paraffin embedded tissue sections were cut at 4μ thickness and floated in poly L-Lysine coated slides and incubated overnight at 37˚C. These slides were then treated with 4% milk solution for 10 minutes to eliminate the hydrophobic effect and give positive charge to the slides. Then the slide labels were bar coded and the labeled slides were loaded in Ventanna Benchmark XT autostainer (a fully automated immunostainer).

Specific protocols were selected according to the marker. A standard protocol was used for most of the markers with minimal variation in certain cases.

Figure: 1 Algorithm of assessment of p53 in Carcinoma of penis



Statistical Analysis

- Data was analysed using a statistical software STATA version 13.1.
- Descriptive statistics for continuous data were expressed as mean with S.D or median.
- Categorical data was expressed as frequencies and percentages.

- Association of p53 with clinical stage, histological grade and lymphovascular invasion was calculated using chi square test.
- P value of <0.05 was considered significant.

- The following formula was used to calculate the sample size.

  N=4xpxq/d2

  P=sensitivity/specificity.

  Q=1-P d=precision-10%

Results

A total of 49 SCCs of penis with lymphadenectomy were included in our study from January 2006 to September 2014 from the department of General pathology, Christian Medical College, Vellore.

Mean age of presentation in the study was 53 with a range of 35-81 years.

3(6.12%) patients had balanitis xerotica obliterans, 10(20.41%) had phimosis, 3(6.12%) patients had undergone circumcision. Majority (43/49) of our patients underwent partial amputation while only 6 patients had total amputation.

The most common site of tumor was the glans (44/54-89.80%) and majority of them presented with ulcerating lesion. The remaining tumors were located in the prepuce 3(6.12%) and coronal sulcus 2 (4.08 %.).

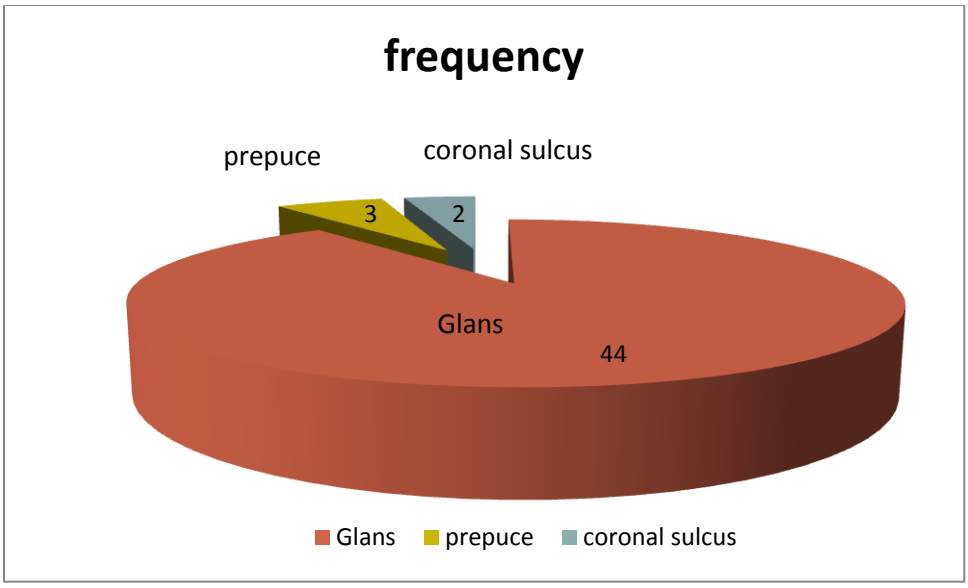


Figure 1: Distribution of tumour site in study cohort

Common histological type of the tumour was conventional squamous cell carcinoma.

Most of the cases 30 (61.22%) were of grade 1 histology, 15(30.61%) of grade 2 and 4(8.16%) were of grade 3 histology.

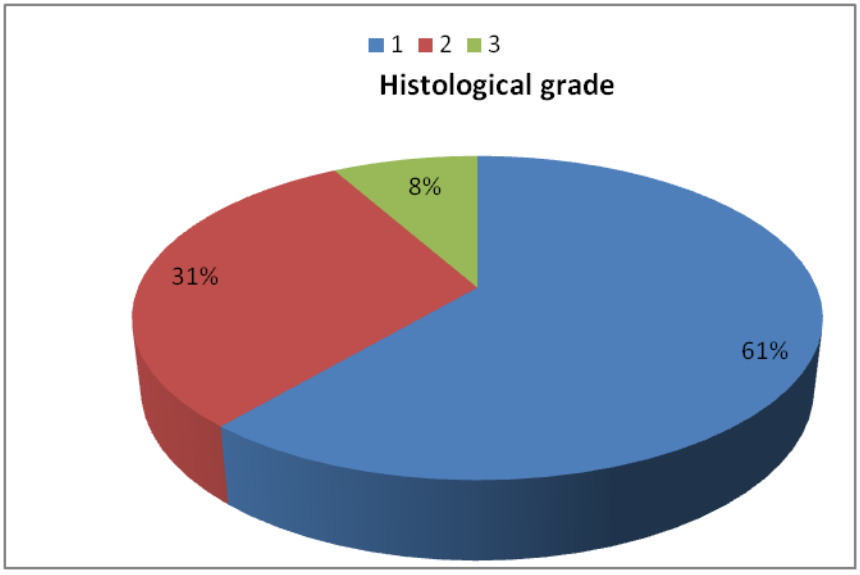


Fig 2: Distribution of histological grade

17/49(34.69) cases showed lymphovascular invasion.

35(71.43%) showed invasion into corpus cavernosum and/or spongiosum.

Mean depth of invasion was 0.9mm, maximum depth of 3cm and minimum depth of 0.1mm.

Pathologically nodal metastasis was found in 16 of the total 49 cases (32.65%). Of these 13/49(26.53%) cases showed metastasis in the right superficial inguinal lymph node groups, 9/49(18.37%) in left superficial inguinal lymph node group, 4/49(8.16%) in the right deep inguinal lymph nodes, 2/49(4.08%) in the left deep inguinal lymph nodes, and 3 (6.12%) right pelvic lymph node.

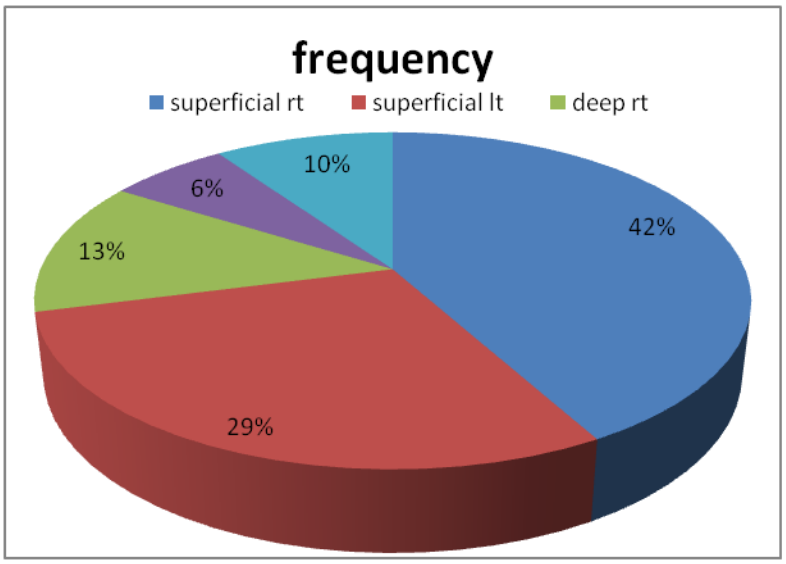


Figure 3: Distribution of pathological node positive status.

Clinical node status was N0 in 32/49(65.30%), N1 in 6/49(12.24%) cases, N2 in 1/49(2.04%), N3 in 3/49 (6.12%) and Nx 7(14.29%).

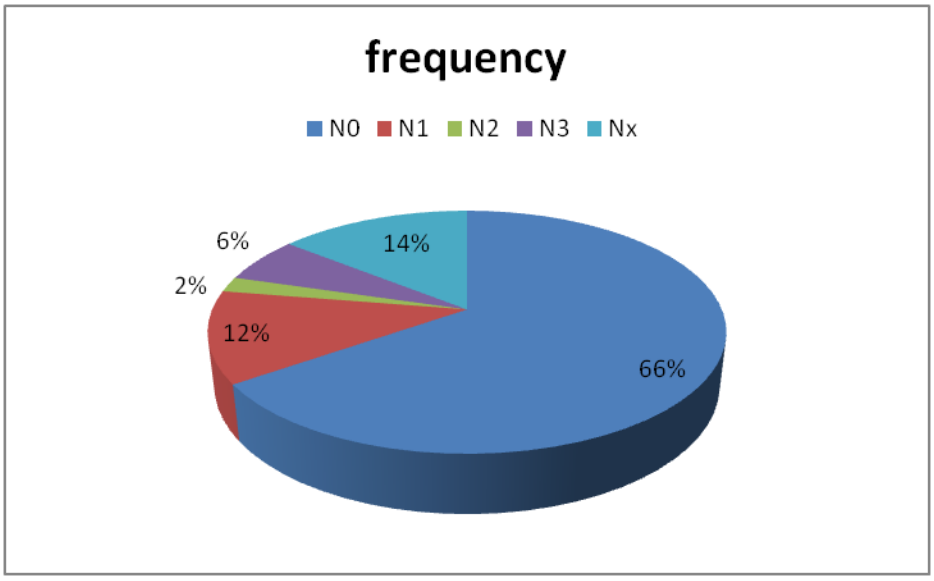


Fig 4: Distribution of clinical node.

Commonest pathological stage noted was pT2 23/49(46.94%), followed by T1 in 14/49(28.57) & pT3 in 12/49(24.49).

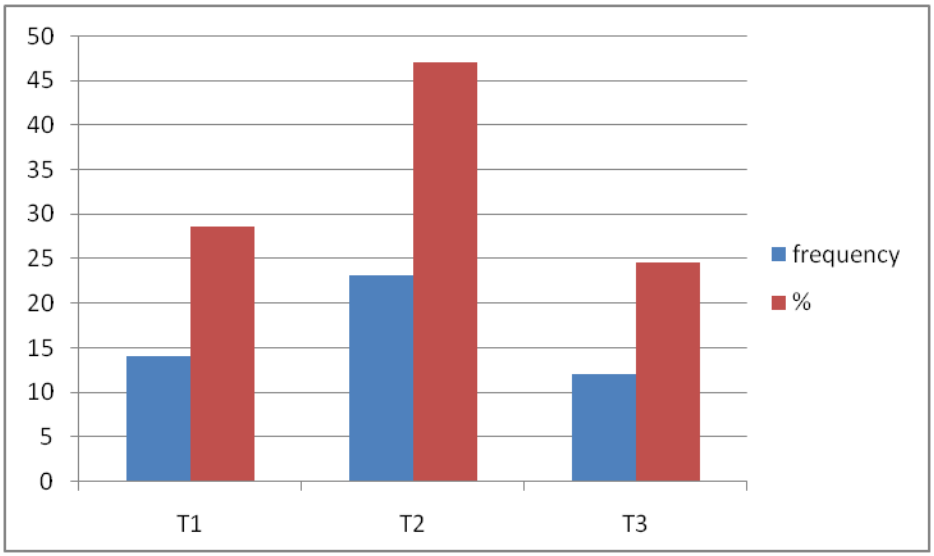


Fig 5: Distribution of T stage.

Table: 1 for p53 cases above and below the cut off.

P53cut off	Frequency	%
<20	13	26.53
>20	36	73.47
Total	49	100.00

Assessment of p53 density showed that 36 of the total 49 cases had a high (> 20 %) p53 density while the remaining cases had less than 20 % tumor density in the tumor cells (73.47%).

Table: 2 Depicts Lymph node status in relation with p53

	P53 positive	P53 negative	Total lymph nodes
Metastatic	13	3	16
Nonmetastatic	23	10	33
Total cases	36	13	49

The above table shows that of the 36 cases that had high p53 density, only 13 cases showed nodal metastasis and 23case did not show nodal metastasis.

This result shows that p53 has 81.3% sensitivity and specificity of 30.3% respectively, thus giving a positive predictive value of 36.1% (CI 20.8%-53.8%) and negative predictive value of 76.9% (CI 46.2%-95.0%). Overall p53 does not show statistical significant correlation with nodal metastasis.

The following variables do not show statistically significant correlation with p53 and are therefore independent factors for prognosis.

Age (p0.4), BXO (p 0.7), phimosis (p 0.7), circumscision (p 0.7), Node status (p0.4), histological grade (p 0.3), depth of invasion (p 0.9), pathological lymph node status (p0.3) and lymph vascular invasion (p0.7).

It is seen that the only statistically significant correlation with p53 tumor density was the T stage with value of 0.04. .Hence involvement of corpus is statistically significant with (p 0.01).

All 49 cases had tumor free skin, corporal and urethral margins (100%)**.**

On immunohistochemistry 36 patients had increased p53 density (>20 cut off) in the amputated specimens, of which 23 cases had negative nodes. Only 13 cases showed nodal metastasis and therefore in these cases p53 was early predictor of metastasis.

Lymph node	p53		Total
Positive	11	5	16
Negative	19	14	33
Total	30	19	49

Discussion

The most important prognostic factor in squamous cell carcinoma of penis is lymph node metastasis. European Association of Urology guidelines stratify patients into 3 risk groups: low (Tis, pTaG1-G2, pT1G1), intermediate (pT1 G2), and high (pT2/T3 G2/ G3)[5]. The therapy of choice is node dissection for patients with clinically palpable inguinal lymph nodes and for those with unfavorable histopthological characteristics such as basaloid, sarcomatoid patterns of growth and increased depth of invasion. In the current study we have tried to analyse the role of p53 as predictive factor for penile carcinoma to better define the strategies for treatment of penile cancer. We evaluated the association of p53 with the clinical and histopthological variables related to potential lymph node metastasis .

The expression of p53 has been shown to have prognostic importance in head and neck SCC by Lopes et al. Our study is based on a recent original article by Lopes, for p53 expression in predicting early lymph node metastasis . To date, there has not been any reported study regarding the usefulness of the markers in penile carcinoma from India. Being a referral centre many cases of penile carcinoma are diagnosed every year in our hospital. The study was carried out on 49 patients and the aim was to assess the correlation of the above markers with age, clinical stage of the disease, histological type, histological grade, depth of invasion, common nodes involved, and association of precancerous lesions like phimosis and BXO and status of margins. This was done by calculating the tumor density by p53 marker. They were evaluated by using the cut off value for this marker by Lopes et al. The sensitivity and specificity, positive predictive value, and P value for each marker were calculated. The mean age of presentation in this population of study was 53 with a range of (35 -81) in total of 49 cases and this is similar to the study by WHO.

10(20.41%) cases presented with phimosis and 3 (6.12%) with BXO, which was confirmed on histopthological slides during the initial diagnosis. These variables did not have statically significant correlation with the above tumor markers. Most of the patients had undergone partial amputation, only six underwent total amputation.

Similar sampling was seen in other studies[25-27,] Of these 49 patients, histopthological evaluation showed 16cases with lymph node metastasis, and the most common lymph node group involved was right superficial 13/49(26.53%) group of lymph nodes.

Clinically however the commonest presentation was N0 stage that formed 29 (59.18%) and these findings are similar to the study by Lopes et al. Our analysis showed that majority of our patients (23/49) presented in pathological stage pT2 followed by pT1 and this was similar to studies by Minardi et al and Lopes. group[24, 25, 27.] However some other authors have noted pT3 and pT4 as the common stage[25.]. . The commonest site of involvement was glans penis (44/49), which was similar to findings in other studies[25-28].

The predominant histological type in our study was conventional squamous cell carcinoma which was also similar to other studies [25-27]. Most common histological grade of the tumor was well differentiated SCC followed by moderately differentiated and few cases (5%) were poorly differentiated which is parallel to Lopes et al study. A recent study states that higher grade and more aggressive tumors are prone to disseminate, early even without lymph angiogenesis, while the more differentiated tumors need denser lymphatic network in the form of increased LVD for their metastatic spread[51]

All 49 cases had tumor free skin, soft tissue and urethral resection margins. Lymphovascular invasion was seen in all the 16 cases that histologically had metastatic lymph nodes. It was stated by Lopes et al that there is strong association of this variable with increased chances of metastasis[25]

Histopthological stage was only parameter which was statistically significant in p53 study. Evaluation of p53 in our study showed that there were 36 cases with more than 20% p53 tumor density and 13 cases with less than 20% p53 density. Out of 16 positive lymph node cases, 13 cases showed high density.

However 23/33 negative lymph nodes also showed increased tumor density for lymph node metastasis. Present study showed that increased p53 tumor density had increased LVI with increased nodal metastasis. . Analysis showed that only the histological stage statistically correlated with p53 which is directly proportional to the lymph node metastasis.

This was similar to the study done by Martins et al and Zhu et al. In our study majority were at T2 stage, and this stage the marker was sensitive but yet was not a predictor of early metastasis as the statistical positive predictive value was low. This marker statistically did not correlate with other multivariate variables. Relationship of each marker with other variables calculated by Chi square test showed that, age (p0.4), BXO (p 0.7), phimosis(p 0.7), circumscision (p 0.7) , Node status (p 0.4), histological grade (p 0.3), depth of invasion (p 0.9), pathological lymph node status (p 0.3) and lymph vascular invasion (p 0.7) did not statistically correlate with p53. Therefore they are independent marker of prognosis. Only T stage with pvalue of 0.04 is statistically correlated with p53 .Hence involvement of corpus is statistically significant with (p 0.01). This marker has sensitivity of 81.3% and specificity of 30.3%.

Conclusion:

- Total 49 cases of partial total/ amputation of penis were studied.
- The sensitivity of p53 to detect early metastasis in SCC of penis was 81.3% and specificity was 30.3%.
- Therefore p53 is very sensitive to detect even mild increased tumor density in SCC of penis at 20 % cut off.
- Among the different clinicopathological variables assessed, p53 correlated only with the pathological stage of the disease.
- The positive predictive value for p53 to predict metastasis was only 36.1 %(CI 20.8% -53.8%) and negative predictive was 76.9% (CI 46.2%-95.0%) .

To conclude, this is the only study done in India by using the p53 marker to detect early metastasis in partial /total amputed specimens in SCC of penis. p53 is extensively studied in different carcinomas at different sites. In our study this marker had low specificity and low positive predictive value, which suggest that it is not able to predict lymph node metastasis even though it is sensitive and therefore it cannot be helpful to decide for prophylactic lymphadenectomy. Specificity could have been achieved, if the sample size was large and if the cut off for p53 was kept at 5%.

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ABBREVATIONS:

SCC: Squamous cell carcinoma.

BXO: Balanitis xerotica obliterans

NOS: Not otherwise specified.

hr HPV: High risk Human Papilloma Virus