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Original Research Article

Utility of immunohistochemistry with CK7 and CK20 in metastatic carcinoma

Shraddha Shankarrao Mular[1]*, **N. S Mani**[1], **Reena Bharadwaj**[1]

[1] *Dept. of Pathology, Bharati Vidyapeeth Medical College, Pune, Maharashtra, India*

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ABSTRACT

Background: Carcinoma of unknown primary origin (CUP) is a diverse group of cancers that is defined by the presence of metastatic disease with no identified primary tumor at initial presentation. Simple epithelium of the various organs is the most common source of the cancer. These carcinomas usually metastasize to lymph nodes and various other organs. Diagnosis of metastatic carcinoma of unknown origin can be challenging and also critical for clinical and therapeutic decisions. Immunohistochemistry (IHC) using CK7 and CK20 is an essential ancillary tool for the identification and the differential diagnosis of carcinomas of epithelial origin especially of undetermined primary.
Aim and Objective: In this study we have aimed to evaluate the utility of immunohistochemistry by using CK7 and CK20 antibodies to identify the primary site in metastatic carcinoma.
Materials and Methods: This study included 70 histopathological cases with metastatic carcinoma received in Pathology laboratory in Bharati hospital over a period of two year. Metastatic carcinoma confirmed by histopathology examination and IHC using CK7 and CK20 was performed.
Results: Total 70 cases examined for utility of CK7 and CK20 in metastatic carcinoma in various organs. Most common specimen was liver with metastatic carcinoma. Algorithmic IHC examination of CK7 and CK20 was done which showed CK7+CK20- pattern was the common pattern followed by CK7-CK20+. All cases of metastatic carcinoma lung, breast and thyroid followed CK7+CK20- pattern, 90% cases of colon carcinoma followed CK7-CK20+ pattern. However, Ovary, pancreas and gallbladder showed CK7+CK20- and CK7+CK20 pattern of expression. CK7-CK20- pattern was seen in cervical carcinoma, renal cases.
Conclusion: The study suggested that the combination of CK7 and CK20 was helpful in suggesting the likely primary site of malignancy.

1. Introduction

Carcinoma of unknown primary origin (CUP) is a diverse group of cancers that is defined by the presence of metastatic disease with no identified primary tumor at initial presentation.[1] It accounts for 2 to 5% of all cancers, and it is the fourth most common cause of cancer death. The determination of the primary site in metastatic carcinomas can be challenging but is critical for clinical and therapeutic decisions.[2] Simple epithelia of the different organs are the source of the majority of cancers. Usually, these carcinomas spread to nearby lymph nodes as well as organs such as the liver, brain, lung, and bone. These epithelium of malignant tissue expresses specific cytokeratin, which are intermediate filaments of the cytoskeleton of cells.[3] Immunohistochemistry (IHC) markers are useful to define tumor lineage and are important for guidance together with the patient's presentation and imaging studies to select the best therapy.

Many epithelia show expression of CK7, such as the bile duct, urothelium, and glandular and columnar epithelium of

* *Corresponding author*.
E-mail address: shra.m20@gmail.com (S. S. Mular).

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the lung, breast and cervix. However, most gastrointestinal (GI) epithelia, hepatocytes, squamous epithelium, and tubules of the kidney do not have CK7. On the other hand, CK20 is expressed relatively in the GI epithelium, urothelium, and epidermal Merkel cells.[4,5] Combination of CK7 and CK20 expression in metastatic carcinoma can be useful in determining the origin of the primary tumor.[6] The diagnostic algorithm as used by Wang et al. and various studies, uses patterns of IHC staining for cytokeratins (CK) 7 and 20 given in Table 1 below.[3,5]

Immunohistochemistry (IHC) using CK7 and CK20 is an essential ancillary tool for the identification and the differential diagnosis of carcinomas of epithelial origin, especially of undetermined primary.[7] Though a battery of more specific markers is now becoming available, their application increases the cost to laboratories and patients. CK7 and CK20 continue to be recommended in view of the ease of application, clarity of IHC results and low costs. In view of the above, the present study was conducted to determine the continuing utility of CK7 and CK20 IHC in predicting the primary site of malignancy at this tertiary care center.

2. Materials and Methods

The present study was carried out in the Pathology Department, Bharati Vidyapeeth Deemed to be University Medical College, Hospital and Research Centre, Pune, India. Seventy histopathology specimens with metastatic carcinoma diagnosed histopathologically over a period of two years (from 2021 to 2023) were included in this study. Fresh slides were prepared from formalin fixed and the paraffin embedded blocks and stained for hematoxylin-eosin stain using standard protocols and histopathological confirmation of the diagnoses was carried out. Details of the patients were retrieved from the test requisition forms in the histopathology record section. Immunohistochemistry using CK7 (Clone: OV-7L 12/30) and CK20 (Clone: KS 20.8) antibodies was performed on sections from paraffin embedded blocks after antigen retrieval. IHC was carried out by the standard peroxidase-antiperoxidase technique. Immunostaining was assessed semi quantitatively. All cases were analyzed for positivity in IHC with CK7 and CK20 and correlated with site and with respect to the algorithms in use for 4 groups: both cytokeratin 7 and 20 positive (CK7+/20+), only cytokeratin 7 positive (CK7+/20-), only cytokeratin 20 positive (CK7-/20+), and both cytokeratin7 and 20 negative (CK7-/20-).

3. Results

70 cases were studied, out of which 45 cases were of unknown primary origin and 25 cases were of known primary origin. Among these 70 cases, metastatic carcinoma was observed in 51.4% (36) of women and 48.6% (34) of men, resulting in a female: male ratio of 1.05:1. The most common age group of presentation was 51 to 60 years and constituted 27.1% (19) of the cases and mean age of the patients was 57.7 years. In the present study, various types of tissues, biopsies, and fluids were examined histopathologically with metastatic carcinoma diagnosed in these specimens, Liver biopsy samples accounted for the highest proportion at 28.5% (20), followed by bone tissues at 14.2% (10), lymph nodes and omental tissue each of 10% (7). Six Pleural fluids (8.5%) were received for cell block preparation. Metastases in lung, ovary, peritoneum and pleura found in each 4.2% (3) cases were also examined. We have also found metastasis of carcinoma in specimens like biopsies from iliac fossa mass, retroperitoneum, psoas tissue, necrotic tissue, cerebellum, oesophagus and the prostate.

The histopathological analysis of 70 cases of metastatic carcinoma revealed adenocarcinoma as the predominant type, accounting for 77.1% (54) cases, followed by undifferentiated carcinoma in 15.7% (11) cases. Squamous cell carcinoma was seen in 4.3% (3), and metastasis of follicular carcinoma of the thyroid was observed in 2.9% (2) cases and frequency of histopathology morphology mentioned in Table 2. The predominant pattern was found to be CK7+ CK20 - which accounted for 51.4% (36) of the cases, followed by CK7- CK20 + seen in 18.6% (13) of the cases. Pattern of CK7+ CK20+ was seen in 15.7% (11) cases and CK7- CK20- was seen in 14.3% (10) of the cases and frequency is mentioned in Table 3.

According to the CK7 and CK20 staining patterns, the primary was suggested, which was confirmed with further investigations and special tests wherever possible. Out of the 70 cases, in 62 cases we were able to find out primary by using CK7 and CK20, while in 8 cases, it was not possible to determine the primary site by any means. In these lung and colon carcinomas were the most frequent carcinomas to present with metastasis, with primary accounting for 14.28% each of the total 70 cases.

The CK7+CK20- pattern of expression was most seen in all cases of metastatic carcinoma of lung (10/10), breast (4/4) and thyroid (3/3), which is followed by ovary (6/9). This pattern was also shown by cases of metastatic carcinoma of pancreatic (4/9), gastric (1/3) and gallbladder origin (2/4). One case of a mesothelioma was CK7 positive only. Figures 1, 2 and 3 shows metastatic adenocarcinoma of lung in cervical lymph node, which has shown CK7+CK20- pattern of expression.

The CK7-CK20+ pattern was seen in cases of colon origin carcinoma (9/10) followed by pancreas (2/9) and ovary (1/9). Figures 4, 5 and 6 shows metastatic adenocarcinoma of colon in lung, which shown a CK7-CK20+ pattern of expression. The CK7+CK20+ pattern was seen in the metastatic carcinoma from colon (1/10), ovaries (2/9), pancreas (3/9), gallbladder (1/4) and stomach (2/3).

The CK7-CK20- pattern was seen in 2 cases each of renal, squamous cell carcinoma, cervical carcinoma and prostate metastatic carcinoma. This was also seen in one case each of the lip and gallbladder metastatic carcinoma. Figures 7 and 8 shows metastatic adenocarcinoma of prostate in bone tissue, which shown a CK7-CK20- pattern of expression. Frequency of final primary malignancy with CK7 and CK20 pattern is mentioned in Table 4.

Out of 62 cases in which the final primary was suggested, 91.9% of cases followed the CK7 CK20 immunohistochemistry pattern of expression. Only 08.06% of cases did not follow the pattern of IHC staining.

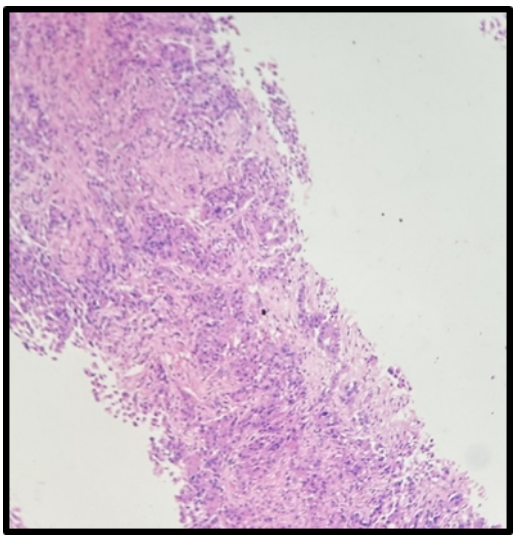

Figure 1: H&E 100X, Metastatic adenocarcinoma of lung in cervical lymph node

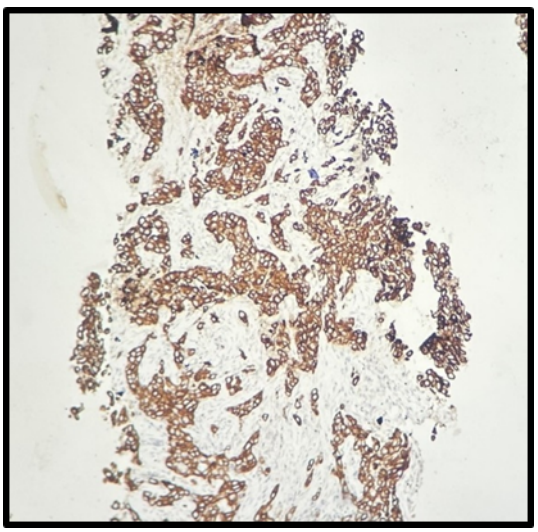

Figure 2: CK7 IHC marker positive

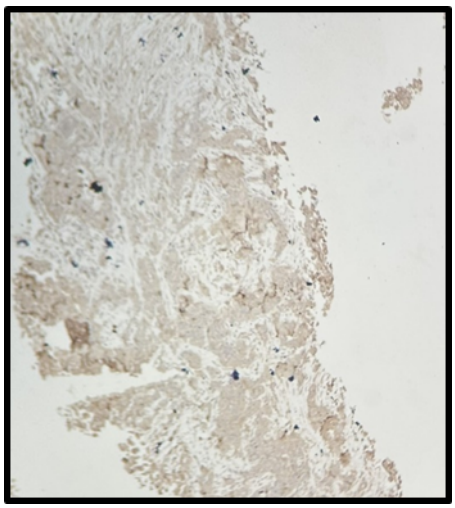

Figure 3: CK20 IHC marker negative

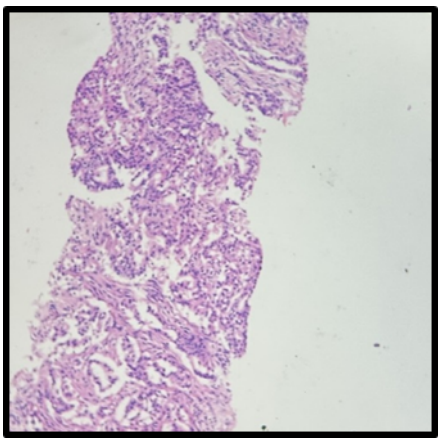

Figure 4: H&E 100X, Metastatic adenocarcinoma of colon in lung

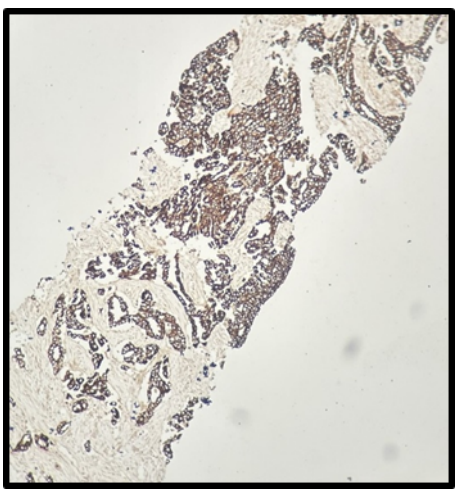

Figure 5: CK20 IHC marker positive

Table 1: Algorithm for primary site in staining pattern with CK7 and CK20[3,5,8]

CK7+ CK20-	CK7+ CK20+	CK7- CK20+	CK7- CK20-
Lung carcinoma Endometrial carcinoma Breast carcinoma Thyroid carcinoma Pancreatic carcionoma Cholangiocarcinoma Ovarian carcinoma Gastric carcinoma Mesothelioma	Urothelial carcinoma Cholangiocarcinoma Pancreatic carcionoma Gastric carcinoma Ovarian carcinoma	Colorectal carcinoma Gastric carcinoma Merkel cell carcinoma	Hepatocellular carcinoma Carcinoma prostate Squamous cell carcinoma Renal cell carcinoma

Table 2: Frequency of histopathological differentiation

Histopathological differentiation	Frequency	Percentage(%)
Adenocarcinoma	54	77.1%
Follicular carcinoma of thyroid	02	2.9%
Squamous cell carcinoma	03	4.3%
Undifferentiated carcinoma	11	15.7%
Total	70	100%

Table 3: Frequency immunohistochemistry pattern

IHC pattern	Frequency	Percentage (%)
CK7+ CK20-	36	51.4%
CK7 - CK20+	13	18.6%
CK7+ CK20+	11	15.7%
CK7- CK20-	10	14.3%
Total	70	100%

Table 4: Summary of final primary in metastatic carcinoma with CK7 and CK20 pattern

Final primary	Frequency	CK7+ CK20-	CK7+ CK20+	CK20+ CK7-	CK7- CK20-	Percentage (%)
Lung	10	10	0	0	0	14.28%
Colon and rectum	10	00	1	9	0	14.28%
Ovary	09	6	2	1	0	12.85%
Pancreas	09	4	3	2	0	12.85%
Breast	04	4	0	0	0	05.71%
Gall bladder	04	2	1	0	1	05.71%
Stomach	03	1	2	0	0	04.28%
Thyroid	03	3	0	0	0	04.28%
Renal	02	00	0	0	2	02.85%
Squamous cell carcinoma	02	0	0	0	2	02.85%
Cervix	02	0	0	0	2	02.85%
Prostate	02	0	0	0	2	02.85%
Lip	01	0	0	0	1	01.42%
Mesothelioma	01	1	0	0	0	01.42%
No primary found	08	5	2	1	0	11.42%
Total	70	36	11	13	10	100%

4. Discussion

The present study was undertaken to determine the utility of immunohistochemistry by using CK7 and CK20 antibodies to identify the primary site in metastatic carcinomas. The findings highlight the critical role of immunohistochemical markers, which will help in diagnosing and managing cancers of unknown primary origin (CUP), guiding targeted therapeutic approaches.

Eiman O.R. Omar, et al.[9] found a high incidence of 20.6% in the age group of 60-69 years, with a slightly higher prevalence among males (51.77%) compared to females (48.23%) and Royal College of Pathologists - Cancer datasets, where the median age was 60 years with a male-to-female ratio of 53% to 47%.[10] In our study, most cases were observed in the age group of 51 to 60 years, comprising 27.1% of the total. The mean age was 57.7 years. As

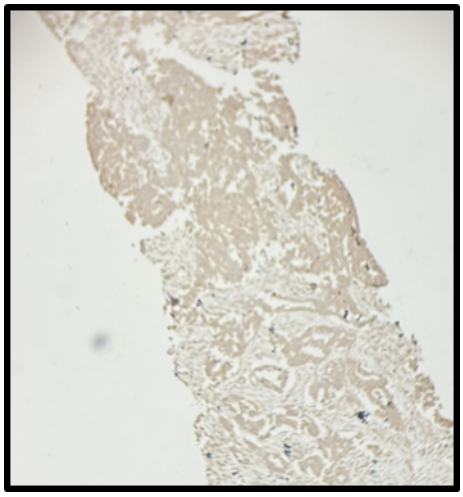

Figure 6: CK7 IHC marker negative

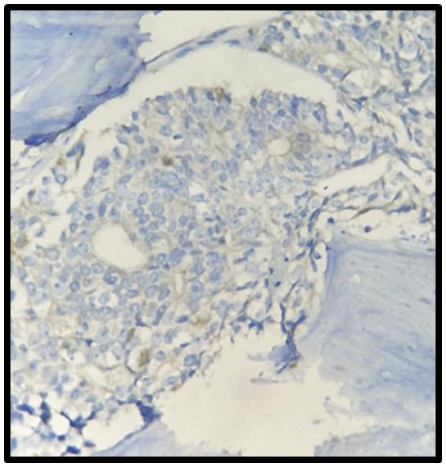

Figure 7: CK7 IHC marker negative in metastatic adenocarcinoma of prostate in bone tissue

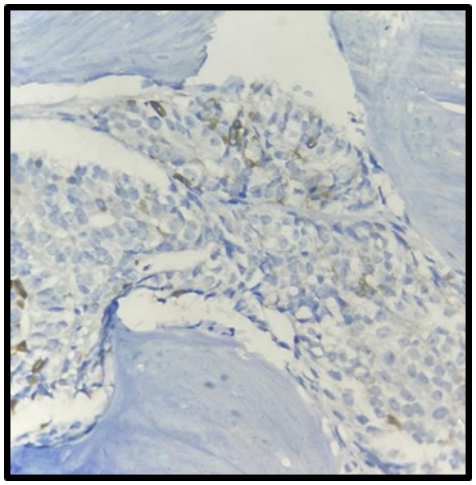

Figure 8: CK20 IHC marker negative in metastatic adenocarcinoma of prostate in bone tissue

compared to these studies, we had a slightly greater female predominance (51.4%) than male (48.6%). Eiman O.R. Omar et al. identified lymph nodes (41.84%) and liver (12.77%) as the most frequent sites of presentation.[9] But in the present study, liver biopsy was the most common tissue found with metastasis accounting for 28.5% (20), followed by bone tissues at 14.2% (10) and lymph nodes at 10% (7). Also, omental tissue (10%), pleural fluids (8.5%) for cell block and metastases in lung, ovary, peritoneum and pleura were found in 4.2% cases each. Histomorphology is the most important feature for a pathologic differential diagnosis of metastatic carcinoma. In our study, the histopathological analysis of metastatic carcinoma revealed adenocarcinoma as the predominant type, accounting for 77.1% of cases. This is consistent with findings from various studies and review articles, which consistently report adenocarcinoma as the most common histopathological entity in cases of metastatic carcinoma.[1,5,9,11,12] Our findings also align closely with those of Eiman O.R. Omar et al., who similarly identified adenocarcinoma (75.89%) as the leading histological type followed by undifferentiated neoplasms (14.18%), squamous cell carcinoma (7.09%) and carcinoma with neuroendocrine differentiation (2.84%) in their study cohort.[9] Following adenocarcinoma, our study found undifferentiated carcinoma (15.7%) to be the next most frequent type, followed by squamous cell carcinoma (4.3%) and follicular carcinoma of the thyroid (2.9%) as mentioned in (Table 2).

Immunohistochemistry (IHC) is auxiliary histochemical technique and crucial for tumor diagnosis. Immunohistochemical studies facilitate the identification of metastatic carcinoma by origins and its subtypes. In identifying malignancies various immunohistochemical markers are used and cytokeratins most commonly. These are intermediate filament protein in cells and exhibit specific patterns of expression that are associated with the type of epithelium. CK7 and CK20 are commonly used markers for diagnosis of metastatic carcinoma.[13] The diagnostic method combines joint IHC staining for cytokeratins CK7 and 20 to provide a rough differential diagnosis. D. Dum et al.[12] and Eiman O.R. Omar et al.[9] CK7 positive, CK20 negative pattern to be the most common pattern in CUP of this study. Our study also revealed similar finding showing predominant pattern as CK7+ and CK20 – which accounted for 51.4% of the cases. The next frequent pattern was CK7- CK20 + which was seen in 18.6% of the cases, as mentioned Table 3.

Similar to findings of Chu et al,[3] D. Dum et al.,[12] Eiman O.R Omar, et al[9] we found in present study the CK7 expression alone was found to be highly sensitive in 100% lung, breast and thyroid cancers. Conversely, CK20 expression alone demonstrated a high sensitivity in 90% colorectal adenocarcinomas. Only 10% case of colon cancer showed expression of both CK7 and CK20 which did not

correlate. A study by D. Dum et al. [24] showed that 6.6% colonic cancers showed expression of both CK7 and CK20 (CK7+CK20+). Tot T et. Al[14] found that mesothelioma expressed only CK7 positivity. Similarly, we also found one of the cases of Mesothelioma with CK7 positivity only which was confirmed with Calretinin.

Of the 9 cases of ovarian origin 66.7% cases showed CK7+CK20- pattern, 22.2% cases showed CK7+CK20+ pattern and 11.1% case showed CK7-CK20+ pattern expression. These findings are similar to those of T.S. Loy et al.[15]

Janick Selves et al[5] and Bayrak et al showed a[16] CK7+/CK20- pattern to be of gastrointestinal origin from Pancreas, gall bladder and colon, small intestine. A diffuse or focal CK7+/CK20+ profile pattern was seen in lung (both mucinous and intestinal subtypes), pancreas, GIT (gastric, oesophageal, small intestinal), ovarian (mucinous subtype) and bladder. Similarly, we demonstrated CK7+ CK20- pattern in 44.4% pancreas, 50% gallbladder and 33.3% stomach metastatic carcinomas. CK7+CK20+ pattern was seen in 33.3% pancreas, 25% gallbladder and 66.7% stomach carcinomas. Two cases of pancreatic carcinomas showed CK-CK20+ pattern of expression and one case of gallbladder showed CK7-CK20- pattern of expression.

CK7-CK20- was seen in prostate, cervical, renal and squamous cell carcinoma cases. Frequency of final primary with CK7 and CK20 pattern is mentioned in Table 4. Among 70 cases studied primary cancer sites were identified in 62 cases, 91.9% of cases correlated with CK7 and CK20 profiles. Overall, CK7 and CK20 immunohistochemical markers continue to offer significant utility in identifying the primary origin of metastatic carcinoma, with distinct sensitivity patterns across different cancer types.

5. Conclusion

The findings in this study indicate, immunohistochemical examination of CK7 and CK20 continues to be useful for predicting the probable primary site in patients who have metastatic carcinoma at different sites. The CK7+/CK20- pattern was highly sensitive of carcinomas of lung, breast and thyroid origin. Maximum sensitivity was also observed with CK7-CK20+ pattern in staining in carcinoma of colon. Variations in sensitivity among different cancers, such as ovary, gallbladder and pancreatic cancer, emphasize the varied applications of CK IHC in clinical settings. This straightforward algorithmic method applying histomorphology and IHC using the algorithm with CK7 and CK20 can create a baseline to stratify the potential source of primary in a setting with limited resources. To determine the primary site and enable proper therapy, practitioners should prioritize simple procedures rather than aggressive diagnostic workups, which are typically not cost-effective. Patients with metastatic carcinomatous deposits in different tissues with unknown primary will benefit from additional research using markers specific to their organs or tumors, which will help identify the source of primary malignancy.

Limitation of the study are the small sample size and smaller variety of tumor types. It is envisaged that a larger study with greater variety would establish the firm base of utility of CK7 and CK20 in a resource constrained environment and of benefit to those who may not be able to afford large and costly panels of IHC.

6. Source of Funding

This study was funded by Institutional funding of the Bharati Vidyapeeth Medical College, Pune, Maharashtra.

7. Conflict of Interest

Nil.

8. Ethical Approval

This study was done after taking approval from Ethical committee of the Institution with Ref: BVDUMC/IEC/110.

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Author's biography

Shraddha Shankarrao Mular, PG Student https://orcid.org/0000-0003-0039-4225

N. S Mani, Professor https://orcid.org/0000-0003-3952-9908

Reena Bharadwaj, Professor https://orcid.org/0009-0004-0762-5166

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