



**ORIGINAL RESEARCH PAPER**

**Pulmonary Medicine**

**ENDOBONCHIAL LUNG BIOPSIES, WHAT MATTERS? TYPE OF BIOPSY FORCEPS, NUMBERS OR SIZE OF TISSUE?**

**KEY WORDS:** Biopsy, Bronchoscopy, Cancer, Forceps

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**ABSTRACT**

Lung cancer is one of the commonest cancers and cause of cancer related deaths all over the world. Forceps biopsy is the most commonly used technique by bronchoscopists to sample the airway lesions which are endoscopically visible. In central airway lesions, forceps biopsy yield sensitivity of around 74% to 80%. The objective of our study was to find out the impact of size and number of biopsy tissue on the overall yield of endobronchial biopsy. This was a cross sectional comparative study. 50 patients with endobronchially visible lesions on bronchoscopy underwent biopsy using either cup or alligator forceps randomly in equal numbers. The number of biopsies, average size of the tissues obtained, complications and diagnostic yield were compared between the two types of biopsy forceps. The correlation between yield and number of biopsies/size of biopsies were also compared between the two forceps group. The diagnostic yield was 84% in alligator forceps and 88% in cup forceps group. The mean size of tissue in alligator and cup forceps was 3.72±0.74mm and 2.80±0.82mm respectively. Overall, four or more biopsies were required to obtain a yield of more than 90%. There was a positive correlation between the number of biopsies and the diagnostic yield (r=0.48, p<0.001). However the correlation between the size of biopsies and the diagnostic yield was not statistically significant (r=0.18, p=0.2). To conclude, the diagnostic yield in both alligator and cup forceps was similar. Larger tissue samples were obtained with alligator forceps. The diagnostic yield correlated with number of biopsies, but not with the size of the sample.

**INTRODUCTION**

Lung cancer is one of the commonest cancers and cause of cancer related deaths all over the world.<sup>1</sup> It accounts for 13 per cent of all new cancer cases and 19 per cent of cancer related deaths worldwide<sup>2</sup>. Bronchoscopy plays a pivotal role in evaluating lung masses and nodules, including those suspicious of malignancy. Whenever there is a lesion visible at bronchoscopy, Endobronchial biopsy is the most frequently used technique for obtaining a diagnosis. The yield of endobronchial biopsy depends on location, lesion accessibility, operator experience and tumour type as well. In literature, the overall yield for forceps biopsy is around 74-85%<sup>3</sup> whereas for exophytic tumours in central airways, yield is >90%. There had been many studies on conventional transbronchial biopsy comparing cup and alligator forceps based on yield and its correlation with size and number of biopsies. But there is scarcity of literature regarding the significance of size and number of biopsies for definitive diagnosis of endobronchial lesions or mucosal nodules.

**OBJECTIVES OF THE STUDY**

1. To study the differences in diagnostic yield in endobronchial lesions with cup forceps and alligator forceps.
2. To study the impact of size and number of biopsy tissue on the overall yield of endobronchial biopsy.

**MATERIALS AND METHODS**

This is a single centre cross sectional comparative study conducted in the Department of Respiratory Medicine, J L N Medical College, Ajmer after approval from ethical committee. 50 patients were enrolled randomly in two groups

(alligator or cup biopsies) after informed consent from February 2018 to January 2019.

**INCLUSION CRITERIA:** All the patients with endobronchially visible lesions, who otherwise did not have contraindication for bronchoscopy or biopsies and had already given consent for inclusion in the study.

**EXCLUSION CRITERIA:**

- 1) Unfit for bronchoscopy,
- 2) Relative and absolute contraindications for bronchoscopy,
- 3) Refused to sign consent form,
- 4) Only mucosal lesion,
- 5) Those with abnormal bleeding profile.
- 6) HIV(+)<sup>ve</sup> patients,
- 7) Those on anticoagulants

Patients with endobronchially visible lesions on bronchoscopy underwent biopsies using either cup or alligator forceps on alternate case basis.

**Tissue size criteria:** Biopsy tissue was classified as small, medium and large based on a fixed criteria<sup>4</sup>.

- Small tissue** - If the sample did not fill the cup of forceps
- Medium tissue** - If the sample filled the cup of forceps
- Large tissue** - If the sample was larger than the cup of forceps.

The average size of the tissues obtained, number of biopsies, complications and diagnostic yield were compared between alligator forceps group and cup forceps group. The correlation between yield and number of biopsies/size of

biopsies were also compared between the two forceps group. The size of the tissues obtained were measured using millimetre scale.

**STATISTICAL ANALYSIS**

The Statistical software namely SPSS 22.0, and R environment ver.3.2.2 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc. Descriptive and inferential statistical analysis has been carried out in the present study. Student t test ( two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Leven’s test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between the two groups, Non-parametric setting for Qualitative data analysis. Fisher Exact test was used when samples were very small.

**RESULTS**

**Table I: Demographic data of patients studied**

Age (yrs)	Alligator Forceps		Cup Forceps	
	Male	Female	Male	Female
31-40	0	1	0	0
41-50	5	0	4	0
51-60	7	1	5	0
61-70	9	1	8	1
>70	0	1	7	0
Total	21	4	24	1

The mean age of the patients in alligator forceps group and cup forceps group was 58.20±10.60 and 64.88±11.32 years respectively. Most of the study subjects were males. 84% of patients in alligator forceps group and 96% of patients in cup forceps group were males which was not statistically significant. (table I)

**Table II: Diagnostic Yield Among Cup And Alligator Forceps**

Diagnosis	Alligator forceps (n=25)	Cup forceps (n=25)	Total (n=50)
No diagnosis	4(16%)	3(12%)	7(14%)
Confirmed diagnosis	21(84.0%)	22(88.0%)	43(86.0%)
Squamous cell carcinoma	5(24%)	11(50%)	16(74%)
Small cell carcinoma	10(47%)	4(18%)	14(65%)
Adenocarcinoma	5(24%)	6(27%)	11(51%)
Poorly differentiated carcinoma	1(5%)	1(5%)	2(10%)

(P value=1.000)

The diagnostic yield was 84% in alligator forceps and 88% in cup forceps group. The biopsies were inconclusive in 16% and 12% of alligator and cup forceps respectively (Table II).

**Table III: Average Size (mm) of Biopsy tissues In Two Groups Of Patients Studied**

Average Size (mm)	Alligator forceps	Cup forceps	Total
2-2.9	1(4%)	10(40%)	11(22%)
3-3.9	8(32%)	11(44%)	19(38%)
4-4.9	13(52%)	3(12%)	16(32%)
5 or more	3(12%)	1(4%)	4(8%)
Total	25(100%)	25(100%)	50(100%)
Mean ± SD	3.72±0.74	2.80±0.82	3.26±0.90

(P value=<0.001)

Alligator forceps yielded a relatively larger sized biopsies as compared to cup forceps. 24 (96%) of the biopsies with

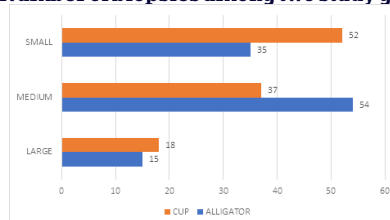
alligator forceps were more than 3 mm in size while only 15 (60%) of the biopsies with cup forceps were more than 3mm in size. The mean size of tissue in alligator and cup forceps was 3.72±0.74mm and 2.80±0.82mm respectively. The difference was statistically significant (Table III).

**Table IV: Average Size of Biopsy tissues and its Correlation with Incidence Of Cancer Among Patients Studied**

Average Size(mm)	Diagnosis		Total	P value
	Inconclusive	Confirmed diagnosis		
Alligator forceps				
2-2.9	0(0%)	1(100%)	1(100%)	0.169
3-3.9	3(37.5%)	5(62.5%)	8(100%)	
4-4.9	1(7.6%)	12(92.3%)	13(100%)	
5 or more	0(0%)	3(100%)	3(100%)	
Total	4(16%)	21(84%)	25(100%)	
Mean ± SD	3.25±0.50	3.81±0.75	3.72±0.74	
Cup Forceps				
2-2.9	2(20%)	8(80%)	10(100%)	0.301
3-3.9	1(9.09%)	10(90.9%)	11(100%)	
4-4.9	0(0%)	3(100%)	3(100%)	
5 or more	0(0%)	1(100%)	1(100%)	
Total	3(12%)	22(88%)	25(100%)	
Mean ± SD	2.33±0.58	2.86±0.83	2.80±0.82	

Average tissue size of (3-3.9)mm was present in 8 cases of alligator forceps group, of which 5(62.5%) had confirmed diagnosis, and 11 cases of cup forceps group, of which 10(90.9%) had confirmed diagnosis. 4-4.9 mm average size tissue was present in 13 cases of alligator forceps group, of which 12 (92.3%) had confirmed diagnosis, and 3 cases of cup forceps group, of which all (100%) had confirmed diagnosis. 2.5mm was the minimum size of biopsy tissue obtained with alligator forceps, while 2 mm was the minimum size of biopsy tissue obtained with cup forceps. The difference was not statistically significant (Table IV).

**Figure I: Number of biopsies among two study groups**



Out of a total 104 biopsy tissues obtained with alligator forceps, 35(33.7%) were small, 54(51.9%) were medium and 15(14.4%) were large. In case of cup forceps, out of total 107 tissues, 52(48.6%) were small, 37(34.6%) were medium and 18(16.8%) were large (Figure I).

**Table V: Number Of Biopsies And Their Relation To Diagnostic yield**

No of biopsies	Diagnosis		Total	P value
	Inconclusive	Confirmed diagnosis		
Alligator forceps				
1	0(0%)	0(0%)	0(0%)	0.009
2	0(0%)	0(0%)	0(0%)	
3	3(75%)	1(25%)	4(100%)	

4	1(7.2%)	13(92.8%)	14(56%)	0.226
5	0(0%)	7(100%)	7(100%)	
Total	4(16%)	21(84%)	25(100%)	
<b>Cup Forceps</b>				
1	0(0%)	0(0%)	0(0%)	0.226
2	0(0%)	0(0%)	0(0%)	
3	1(33.3%)	2(66.6%)	3(100%)	
4	2(16.7%)	10(83.3%)	12(100%)	
5	0(0%)	10(100%)	10(100%)	
Total	3(12%)	22(88%)	25(100%)	

In the alligator forceps arm, in cases where only 3 samples were taken, 25% were only conclusive for a confirmed diagnosis. In contrast where 4 samples were taken 92.8% were conclusive and 100% conclusive where 5 samples were taken. The difference in yield was statistically significant (p value=0.009).

In the cup forceps arm, in cases where 3 samples were taken the results were conclusive in 66.6% of cases, where 4 samples were taken, it was conclusive in 83.3% and 100% conclusive where 5 samples were taken. These differences in yield were not statistically significant. Overall, it was observed that at least 5 tissues were required in both the groups to obtain 100% diagnosis (Table V).

**Table VI: Complications in two groups of patients studied**

Complications	Alligator forceps	Cup forceps	Total
None	24(96%)	22(88%)	46(92%)
Bleeding	1(4%)	3(12%)	4(8%)
Total	25(100%)	25(100%)	50(100%)

Bleeding was seen only in 4% cases of alligator forceps and 12% cases of cup forceps. The difference was not statistically significant. The bleeding observed was only mild, which was controlled by self or required cold saline instillation. None of the patient in both the groups had major bleed (Table VI).

**Table VII: Correlation Between Yield And Number Of Biopsies / Size Of Biopsies Irrespective Of Forceps**

	Point biserial correlation	P value
Number of biopsies	0.48	<0.001
Average size of biopsies	0.18	0.2

**Table VIII: Correlation Between Yield And Number Of Biopsies/Size Of Biopsies (Alligator Forceps)**

	Point biserial correlation	P value
Number of biopsies	0.59	0.002
Average size of biopsies	0.28	0.17

**Table IX: Correlation Between Yield And Number Of Biopsies/Size Of Biopsies (Cup Forceps)**

	Point biserial correlation	P value
Number of biopsies	0.34	0.09
Average size of biopsies	0.22	0.3

There was a positive correlation between the number of biopsies with the diagnostic yield in alligator (correlation= 0.48, p < 0.001) and cup forceps (correlation= 0.34, p=0.09). The positive correlation between the size of biopsies and the diagnostic yield was not statistically significant in both alligator (correlation=0.28, p=0.17) and cup forceps (correlation= 0.22, p=0.3) groups (Tables VII, VIII).

**DISCUSSION**

Correlation between numbers of biopsies and size of biopsies has been widely studied for transbronchial lung biopsies in interstitial lung diseases, also comparing the role of type of biopsy forceps. This is one of the first study which have attempted to find out the correlation between diagnostic yield and number and or size of biopsies in endobronchial lesions. Flexible optic bronchoscopy was done for these patients and biopsy of the endobronchial lesions were taken using either cup forceps or alligator forceps which was randomly allotted.

The mean age of the patients in alligator forceps group and cup forceps group was 58.20±10.60 and 64.88±11.32 respectively. The overall mean age was 61.54±11.37 years. Majority of the patients in both groups were above 50 years. 84% of patients in alligator forceps group were males whereas 96% of patients in cup forceps group were males. Combining both groups, 90% were males and 10% females. The mean size of tissue in alligator and cup forceps was 3.72±0.74mm and 2.80±0.82mm respectively. The difference in size of biopsy was statistically significant with a p value less than 0.001. *Sehgal et al*<sup>7</sup> had conducted study in 150 patients with sarcoidosis in which transbronchial lung biopsy was performed with alligator and cup forceps. The size of biopsy sample was smaller in the cup forceps group (2.3 ± 0.11 mm) when compared to alligator forceps (2.7 ± 0.06 mm). However the difference was not statistically significant (p=0.5). In the study by *Almadani et al*<sup>8</sup>, comparing different types of forceps in transbronchial biopsy of 80 patients, the average size of biopsy sample obtained through alligator forceps was 1.98 mm compared to 1.83 mm in the cup forceps arm. Thus we conclude that biopsies are relatively larger in size for endobronchial lesions as compare to TBLB.

Minimum of 3 biopsies were taken in all patients of the study. In 16% of cases belonging to alligator forceps and 12% in the cup forceps arm, only 3 samples were taken. 52% cases of alligator forceps group and 48% cases of cup forceps had 4 biopsies each whereas 32% of alligator forceps group and 40% of cup forceps group had 5 biopsies each. Number of biopsies taken largely depended upon the patient cooperation, cough during the procedure, visibility because of bleed and sufficiency of the tissues obtained.

In the alligator forceps arm, 1 small sample was obtained in 56%, 2 small samples in 24% of the cases, and 3 small samples in 12% of the cases. In 8% of the cases no small sample was obtained. Among patients who underwent biopsy with cup forceps; 1 small sample was obtained in 16%, 2 small samples in 42% and 3 small samples in 18%. Overall, 9(36%) patients biopsied with alligator forceps yield 2 or more number of small tissue as compared to 21(84%) cases in cup forceps group.

In the alligator forceps group, in the patients whom 2-2.9 mm tissues were obtained 100% were diagnostic, 3-3.9 mm tissue samples yielded diagnosis in 62.5%, 4-4.9 mm tissue samples yielded 92.3% and 100% when the tissue biopsy size was >5 mm. However, there was no statistical significance between the sample size and yield in the alligator forceps arm (p= 0.169).

In the cup forceps arm, 80% of the 2-2.9 mm and 90.9% of the 3-3.9 mm tissue samples were diagnostic. 100% of both 4-

4.9mm and >5 mm tissue samples were diagnostic However, this again did not amount to statistical significance in total yield among them( $p=0.301$ ).

We also compared the diagnostic yield and the number of biopsies taken in both groups. In the alligator forceps arm, in cases where only 3 samples were taken , 25% were only conclusive, where 4 samples were taken 92.8% were conclusive and 100 % conclusive where 5 samples were taken. The difference was statistically significant ( $p=0.009$ ). The low yield with three samples in this group could be explained based on small tissues obtained in majority of these patients.

In the cup forceps arm, in cases where 3 samples were taken the results were conclusive in 66.6% of cases, where 4 samples were taken, it was conclusive in 83.3% and 100 % conclusive where 5 samples were taken. The results were, however, not statistically significant( $p=0.226$ ). Overall, four or more biopsies were required to obtain a yield of more than 90%. In a study by **Gellert et al<sup>vi</sup>** , unequivocal histological evidence of carcinoma was obtained in 78.6% of the 215 patients with visible tumours. When only one biopsy specimen was taken evidence of carcinoma was obtained in 65.2% of cases. They observed that at least five biopsy specimens were required to provide a greater than 90% probability of obtaining at least one positive sample.

Complications from bronchoscopy are rare. Bleeding, pneumothorax, respiratory distress are some of the complications associated with the procedure. In our study, bleeding was seen only in 4% cases of alligator forceps and 12% cases of cup forceps( $p=0.609$ ).The difference in bleeding rate among two forceps group may be because of better crushing effect of tissues by alligator forceps. We compared our study with the study by **Jabbardarjani et al<sup>viii</sup>** . Of a total of 88 transbronchial lung biopsy samples taken by the alligator forceps, 59 had no bleeding (67%), 28 had mild bleeding (31.8%), and 1 had significant bleeding (1.1%). Of a total of 88 samples obtained by the cup forceps, 55 had no bleeding (62.5%), 28 had mild bleeding (31.8%), and 5 had significant bleeding (5.7%). The difference was not statistically significant( $p=0.246$ ). In the study by **Sehgal et al<sup>v</sup>** , there was moderate to severe bleeding in 15.5 % of patients who underwent biopsy (transbronchial) with cup forceps compared to 8.6 % in the alligator forceps arm ( $p= 0.32$ ). In the **Almadani et al<sup>e</sup>** study, in patients who underwent biopsy with alligator foreps,7 patients (17.5%) had significant bleeding versus 3 patients (7.5%) in the cupped forceps group. Again the differences, in terms of severity of bleeding was probably because of the type of biopsy, i.e endobronchial versus transbronchial.

We also studied the correlation between the number and size of tissue samples with the diagnostic yield using point biserial correlation. There was a positive correlation between the number of tissue samples with the diagnostic yield in alligator (correlation= 0.48,  $p < 0.001$ ) as well as cup forceps (correlation= 0.34,  $p=0.09$ ).

However the correlation in the cup forceps group was not statistically significant. The positive correlation between the size of biopsies and the diagnostic yield was not statistically significant in both alligator (correlation=0.28,  $p=0.17$ ) and cup forceps (correlation= 0.22,  $p=0.3$ ) groups. We also looked at the correlation between the number and size of biopsies with the diagnostic yield after combining both the alligator and the cup forceps group. There was a positive correlation between the number of biopsies and the diagnostic yield ( $r= 0.48$ ,  $p < 0.001$ ). However the correlation between the size of biopsies and the diagnostic yield was not statistically significant ( $r=0.18$ ,  $p= 0.2$ ) . In a study by **Popovich et al<sup>ix</sup>** , it was seen that 1 biopsy will prove more than 90% diagnostic

accuracy for central visible carcinomas, however the diagnostic yield increases if more biopsies are taken especially in peripheral carcinomas, where as many as 10 samples may be required. **Shure et al<sup>x</sup>** also concluded that with good visualization, two biopsy specimens of a mass lesion provide a very good diagnostic yield and three provide an excellent yield.

## CONCLUSION

We conclude that, the use of alligator forceps yielded bigger tissue samples than cup forceps. However, the diagnostic yield was similar in both groups irrespective of the size of tissues. The incidence of bleeding was higher in the cup forceps group which was mild in nature and was immediately controlled with cold saline. No other complications were reported. There is positive correlation between the number of biopsies and the diagnostic yield in the alligator forceps subgroup and both groups combined.

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