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Analysis of non small cell lung carcinoma according to 2015 WHO classification and assess its prognostic and therapeutic implication in our study population

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Abstract: *Lung cancer is the leading cause of cancer related mortality worldwide. The precise diagnosis and subclassification based on small biopsies have direct impact on patient management and prognosis. The 2015 WHO classification of lung Tumors has been published with important changes from the 2004 WHO classification. It includes use of immunohistochemistry, emphasis on genetic studies and new classification for small biopsies and cytology similar to that proposed in the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification.*

Objective: *To classify lung cancer based on 2015 WHO classification of lung tumour and to assess its therapeutic and prognostic implications in our study population.* ***Materials and methods:*** *A Retrospective analysis of small biopsy proven specimens of lung cancer were analysed based on Morphology, Special stains and IHC.* ***Results:*** *Based on morphology, NSCLC constitutes majority 82.5% of cases, among which SCC constitutes 59.2%, adenocarcinoma constitutes 25.82% and NSCLC-NOS constitutes 19.8%. With the use of special stains (Alcian blue/PAS) and the IHC markers (p40 and TTF 1), the percentage of NSCLC – NOS was minimised from 26% to 1.32%.* ***Conclusion:*** *The Classification based on WHO 2015 Classification of lung cancer is superior in terms of diagnostic, therapeutic and prognostic implications. Subtyping of NSCC-NOS using special stains and IHC markers is found extremely useful as diagnosis favouring adenocarcinoma have various therapeutic options.*

Keywords: *Non small cell lung carcinoma, Immunohistochemistry*

I. Introduction

Worldwide Lung carcinoma is the leading cause of cancer related mortality.It constitutes 12.5% of all newly detected cancers and 17.8% of cancer related deaths(1).

Around 68-72% of lung malignancies detected are not resectable at diagnosis, as most of these patients seek treatment with advanced disease. Now the primary method of diagnosing lung cancer are small biopsies and cytology. Until the past decade further classification of NSCLCs were not done, as it does not offer any survival advantage. But with several specific therapeutic options available today especially for patients with adenocarcinoma, further sub-classification of NSCLC based on small tissue biopsy and cytology using tumour markers and special stains have become necessary. (2)

WHO classification of lung cancer(2004) is based on resection specimens and primarily addressed only them. This classification did not propose any standardised criterias for small biopsies and cytology in lung cancer.(1)But the new 2015 WHO classification of Tumors of the lungs has been published with important changes from the 2004 WHO classification. It includes use of immunohistochemistry, emphasis on genetic studies and new classification for small biopsies and cytology similar to that proposed in the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification..(3)

Analysis of non small cell lung carcinoma according to 2015 WHO classification and assess its prognostic and therapeutic implication in our study population

This study is aimed at classifying the lung cancer with small biopsies using special stains and Immuno-histochemistry (IHC) markers according to 2015 WHO classification of lung and to analyse its therapeutic implications.

Inclusion criteria:

FOB biopsies and CT Guided biopsies from clinically and radiologically suspected cases of lung cancer.

Exclusion criteria:

1) Cases treated with prior Chemotherapy and radiotherapy.
2)Resected specimens.
3)Small cell carcinoma

Procedure methodology

This is a retrospective study based on small biopsy proven lung cancer conducted at department of pathology, Government Madras medical College and Hospital, during the period between March 2014 to February 2015.A total of 323 were small biopsies from lung (Transbronchial, Endobronchial, Ultrasound guided biopsies and Computed tomography guided core biopsies) were received during this period , of which 187 turned to be malignant.

Study Design: Retrospective study

Study Location: This was a tertiary care teaching hospital based study done in Department of Pathology, at madras medical college

Study Duration: 1 year

Sample size:151

Subjects & selection method: Detailed history of the cases regarding age, sex, site, tumor location, radiological findings, FOB findings, cytological findings were obtained for all the 187 cases.

Among these

NSCLCs	-	151 cases
Small cell lung carcinoma	-	13
Others	-	23

These 151 Non small cell lung carcinoma cases were reviewed and sub classified based on H&E morphology according to WHO classification criteria. Tumours were sub typed as **adenocarcinoma** if it showed features of gland formation and/ or mucin production**, squamous cell carcinoma** if it showed features of keratinazation or intercellular bridges , and **NSCLC-NOS** (undifferentiated non small cell carcinoma) if it lacked both glandular or squamous patterns. Out of these 151 cases, based on morphology seen in biopsy further segregation done as follows:

Group 1: Cases that could be subtyped by morphology alone as adenocarcinoma or squamous cell carcinoma (111 cases)

Group 2: Cases that are poorly differentiated non small cell lung carcinoma (NSCLC-NOS) (40 cases).

All 40 cases of poorly differentiated non small cell lung carcinomas (NSCLC-NOS) from group 2 were included for special stain (ALCIAN BLUE/PAS) and IHC study (TTF-1, p40).

Five –micron thick paraffin sections were cut and stained with combined mucin stain, TTF-1 and p40 and the tumours were sub-typed based on the algorithm followed by the WHO 2015 Classification.

IMMUNOHISTOCHEMICAL EVALUATION:

Antigen	Vendor	species	Dilution	Positive control
TTF- 1	BIOGENIX	Mouse	Ready to use	lung Adenoca
P40	BIOGENIX	Mouse	Ready to use	Lung sq cell ca

INTERPRETATION AND SCORING SYSTEM:

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization(nuclear), percentage of cells stained and intensity of reaction.

In this study, for evaluation of TTF1 and p40 proteins, greater than 10% expression of the tumour marker within tumour cells is considered as positive. Cases with no focal areas of positive staining and with less than 10% staining are considered negative.

Statistical analysis:

Immunohistochemical analysis was done in paraffin embedded tisse samples using the statistical packagefor social science software version 15.5 which consisted computing the frequency counts and percentages for qualitative variables and mean for quantitative variables.P value of 0.005 was taken as cut-off point to determine statistically significant results.

II. Result

Of the 151 non small cell carcinoma of lung (NSCLCs) was classified according to WHO classification into squamous cell carcinoma, adenocarcinoma, poorly differentiated non small cell lung carcinoma (which lack squamous or adeno differentiation). Among this SCC of lung were the most common type which accounts for 72 cases (59.2%), adenocarcinoma constituted 39 cases (25.82%), NSCLC-NOS were 40 cases(26.49%)

Table no: 1 Subtyping of NSCLC-NOS was done based on the algorithm given by 2015 WHO classification of lung tumours such as the tumours which are positive for TTF-1 and or mucin positive as NSCC favouring adenocarcinoma and those tumours which are positive for p40 are termed as NSCC favouring squamous cell carcinoma. If both TTF-1/ mucin stains and p40 are positive then the possibility of adenosquamous carcinoma should be considered. But if both TTF-1/mucin and p40 are negative and fails to show any squamous or glandular morphology, the diagnosis still remains as NSCC-NOS.(Table:1)

TABLE:1SUBTYPING OF NSCLC BASED ON MUCIN STAIN AND IHC MARKERS

Markers	ADC	SCC	ADENOSQ	NSCLC-NOS
AB/PAS	+	-	+/-	-
TTF-1	+	-	+	-
P40	-	+	+	-

TABLE :2 TTF-1 was expressed in 50% of the adenocarcinoma and p40 was positive in 42.5% of squamous cell carcinoma. Both markers were positive in 1 case and it was considered as adenosquamous carcinoma.5 % of the tumours were negative for both markers and were considered as NSCC –NOS.

Table no 2 : Subtyping of NSCLC based on IHC markers.

TTF-1/mucin stain		P40Subtypes	Number ofcases	Percentage
+-	ADC	20	50%	
--	SCC	17	42.5%	
+	+ADENOSQ	1	2.5%	
--	NSCC –NOS	2	5%	

Chart:1

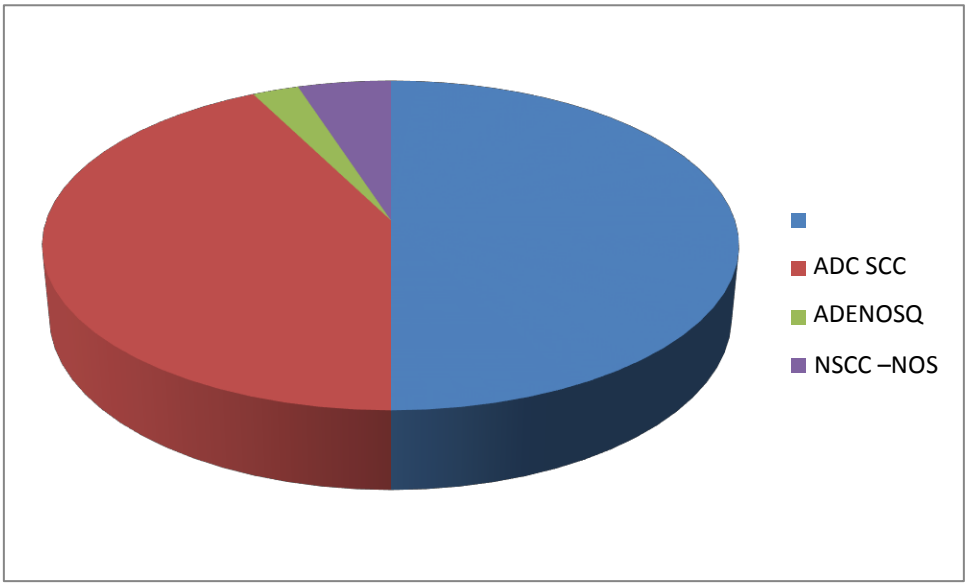


Table : 3 According to 2015 WHO classification ,squamous cell carcinoma were the commonest subtype accounting for 58.2% of cases.According to 2004 WHO classification ,NSCLC –NOS accounts for 26%. But in this study with the use of mucin stain, TTF-1 and p40 markers ,its percentage minimized into 5% because of subcategorization.TTF-1 and mucin stain positive cases were subtyped as favouring adenocarcinoma and p40 positive cases were subtyped as favouring squamous cell carcinoma. 5% of cases were negative for both TTF-1 and p40 , so it was still typed as NSCC-NOS category

Table no: 3: Subtypes of Non small cell lung carcinoma based on 2015 WHO classification

Subtype	Number ofcases	percentage
Adenocarcinoma	39	25.82%
NSCCfavor adenocarcinoma	20	13.24%
Squamouscell ca	72	47.68%
NSCC-favorsq cellca	17	11.25%
NSCC-favor adenosqca	1	0.66%
NSCC-NOS	2	1.32

Chart:2

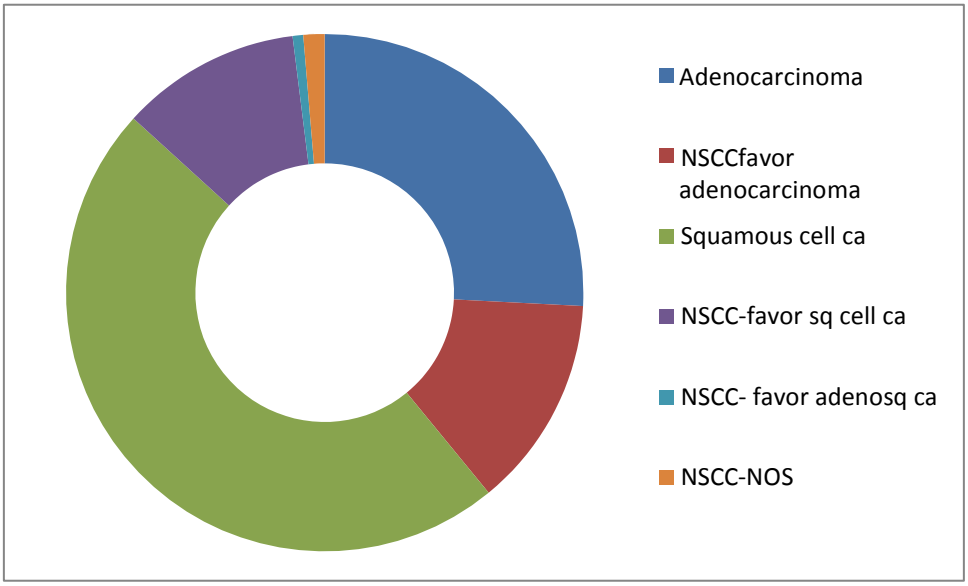


Table no: 4 According to 2015 WHO classification of lung tuomors ,with the use of AB/PAS stain , TTF-1 and p40 ,40 cases of non small cell lung carcinoma –not otherwise specified type were subtyped into favouring adenocarcinoma which constitutes 50% (n=20),favouring squamous cell carcinoma which constitutes 42.5%(n=17) of cases ,possible adenosquamous carcinoma were 2.5% (n=1) and unsubtyped were 5% (n=2)

Table no:4subtyping of NSCLC-not otherwise specified

2004WHOclassification	2015WHOclassification
NSCLC–NOS(40cases)	Favoringadenocarcinoma 20(50%)
	Favouringsquamouscell carcinoma17(42.5%)
	Favouringadenosquamous 1(2.5%)
	NSCLC-NOS-2(5%)

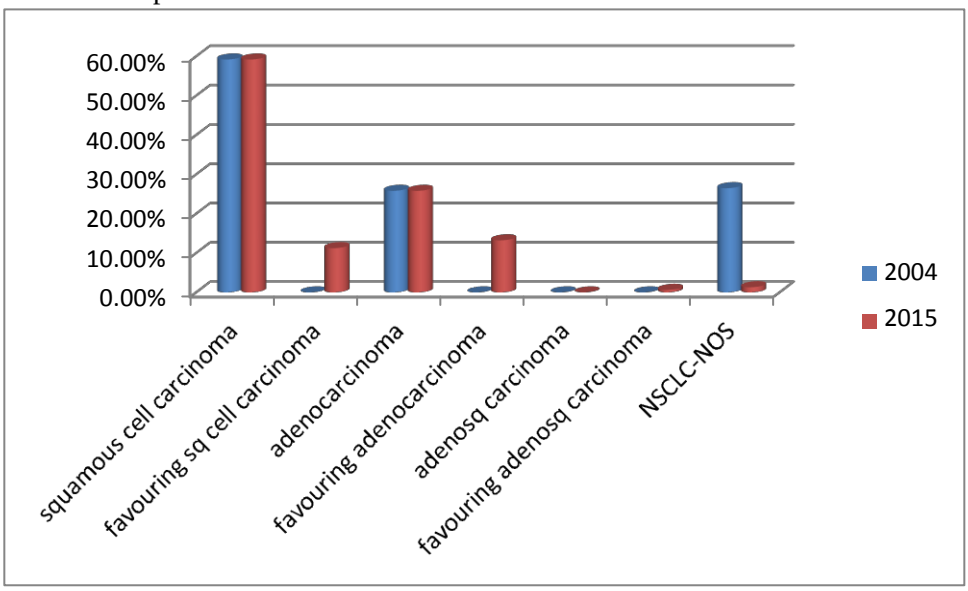
Table no: 5 Based on this study,on comparing 2004 WHO classification and 2015 WHO classification , squamous cell carcinoma was the commonest type identified.Based on 2004 WHO classification ,NSCLC-NOS category constituted 26% of total but according to new 2015 WHO classification with the use of TTF-1 and p40 not otherwise specified category was minimized to 1.32% . In new classification ,it was further

subtyped into favoring adenocarcinoma,favoring squamous cell carcinoma and favoring adenosquamous cell carcinoma

Table:5 Comparision of 2004 WHO classification and 2015 WHO classification

2004 WHO classification	2015 WHO classification
Squamous cell carcinoma- 59.2%	Squamous cell carcinoma – 59.2%
Not included	Favoring squamous cell carcinoma with use of IHC and special stain – 11.25%
Adenocarcinoma – 25.82%	Adenocarcinoma -25.82%
Not included	Favoring adenocarcinoma with use of IHC and special stain – 13.24%
Adenosquamous carcinoma -0	Adenosquamous carcinoma -0
Not included	Favoring adenosquamous carcinoma with use of IHC and special stain -0.65%
NSCLC-NOS - 26.49%	NSCC-NOS -1.32%

Chart: 5 Comparision of 2004 WHO classification and 2015 WHO classification



III Discussion

Lung cancer is the leading cause of cancer and cancer related mortality around the world and most of the chemotherapeutic drugs used today lack adequate efficacy and specificity.

In India ,lung cancer has prepondarance to males constituting 10.9% all cases of cancer and 13% of all cancer related mortality. Mostly lung cancer affects people in 4th to 6th decade. Even with the recent advances available in treatment, the prognosis of the lung cancer still remains very poor.

85% of all lung cancers are non small cell lung carcinomas and adenocarcinoma is the commonest histologic type seen. Until recently, the histological subclassification of NSCLCs had no clinically or therapeutic value[4].But now with the availability of targeted therapy and its differential

activity and adverse effects based on the type of the tumour, precise sub typing of non small cell lung carcinoma has become mandatory as it has direct impact on treatment and prognosis.

Whereever possible on small biopsy,the NSCLC has to be further subclassified into more accurate subtypes such as squamous cell carcinoma and adenocarcinoma,as it serves several purposes like

1.EGFR mutations should be tested in adenocarcinoma ,adenosquamous carcinoma and NSCLC-NOS .Because EGFR TK inhibitors is the primary treatment available for adenocarcinoma of lung.[5]

2.Those with adenocarcinoma histology or NSCLC-NOS showed better outcome with pemetrexed . Gemcitabine has shown improved outcome in patients with SCC. [6,7]

3.When Bevacizumab is used in patients with SCC of lung, life threatening haemorrhage can occur.[8]

Most of lung cancers diagnosed presents in advanced stage and resection rates were as low as 10 to 15 %. So only few cases will undergo complete histological examination. As the 2004 WHO classification , is based on resected specimens, the application of this classification has limited value.But currently with more therapeutic options available, the diagnosis based on small biopsy and cytology becomes crucial.

The recent 2015 WHO classification based on IASLC/ATS/ERS 2011 has included this criteria of small biopsy and cytology for diagnosis of lung carcinoma.

151 cases of Non small cell lung cancer diagnosed at our institute were selected for this study. Out of them 111 cases were morphologically subtyped and 40 cases were classified as NSCLC-NOS. These NOS specified cases were further sub classified using special stain (AB/PAS) and tumour markers (TTF-1 for adenocarcinoma and p40 for SCC). Based on these all the cases are classified according to the new 2015 WHO classification for the lung cancer. These results were compared with 2004 WHO classification.

This study showed that SCC of lung are the most common subtype which constitutes 59.2% followed by adenocarcinoma (25.82%) . This correlates with study done by Delik Erman et al**(10)**which says 60% of cases were squamous cell carcinoma and 30% were adenocarcinoma.

Squamous cell carcinoma was diagnosed histologically based on the presence of intercellular bridges and keratin formation**(11)**.(Figure :1) I n our study ,all the cases of squamous cell carcinoma showed intercellular bridges and keratinisation. The diagnosis of adenocarcinoma was made on the basis of glandular structure and mucin production.(figure :2,3,4&5)

Morphologic diagnosis always forms the basis of diagnosis and is further supplemented by Special stains and Immuno histochemical markers. In this study, the panel of AB/PAS, TTF-1 and p40 were used in 40 cases of poorly differentiated NSCLCs. Alcian blue-PAS positive in (n=16) 40% of cases.TTF-1 was positive in (n=20) 50% of cases and p40 was positive in(n=17) 42.5% 0f cases. Both TTF-1 and p40 were positive in (n=1) 2.5% of cases. Both TTF-1 and p40 were negative in (n=2) 5% of cases.

According to the study by Stenhouse G et al**(16)**,TTF-1 as a marker for adenocarcinoma was found to have specificity of 97-100%, but the sensitivity rate was low at 54-75%..Kaufmann et al**(12)**,concluded that 75% of nonmucinous adenocarcinomas demonstrated TTF-1 positivity. whereas mucinous adenocarcinoma showed positivity only in 10%. Therefore the combined usage of TTF-1 and mucin stain AB/PAS has more significant value in the diagnostic panel.

In our study one case of poorly differentiated adenocarcinoma was negative for TTF-1 stain , but, on the other hand it showed positivity for AB/PAS stain . Hence in correlation with Kauffman et al **(12)**, owing to the positivity of mucin stain in this study it is diagnosed as favouring adenocarcinoma.

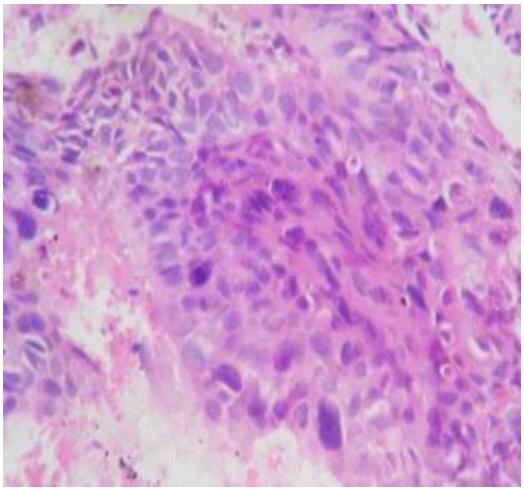


FIGURE:1 Squamous cell carcinoma 400 X

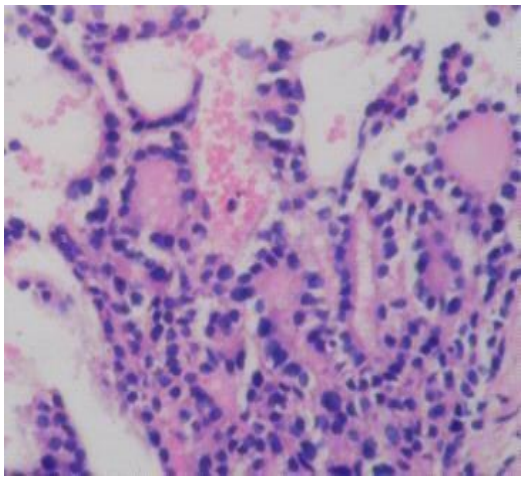


FIGURE :2 Adenoca with acinar pattern 400 X

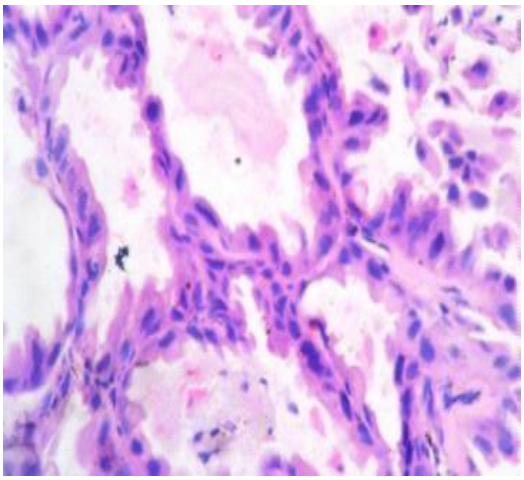


FIGURE:3 Adenoca with lepidic pattern 400x

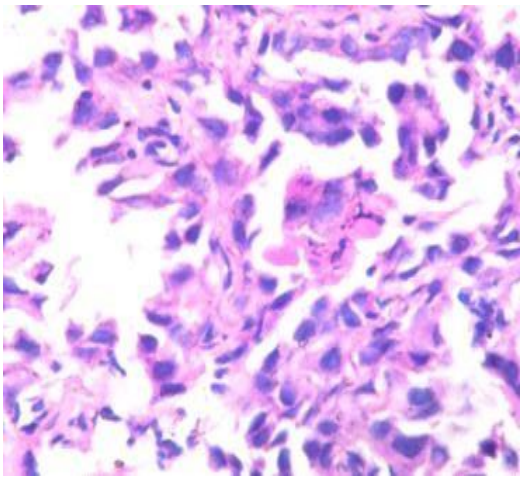


FIGURE:4 Adenoca with papillary pattern 400 x

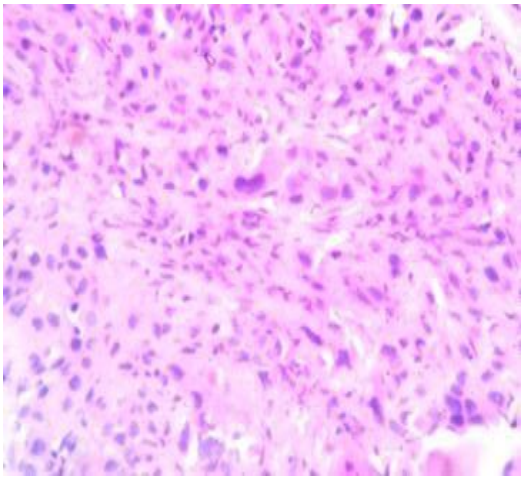


FIGURE: 5 Adenoca with solid pattern 400 x

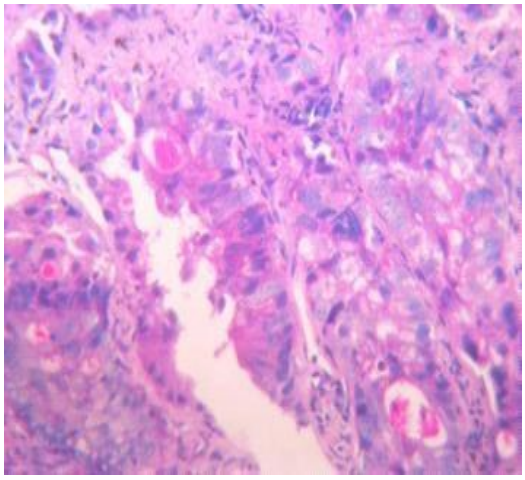


FIGURE :6 Neutral mucin positive adenoca400x

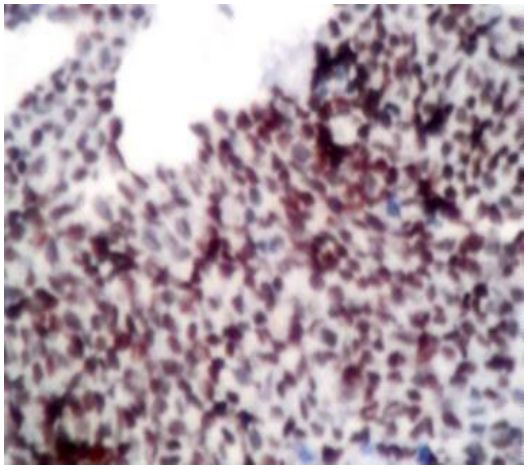


FIGURE:7 Strong nuclear positivity of TTF-1 favouring adenoca 400 X

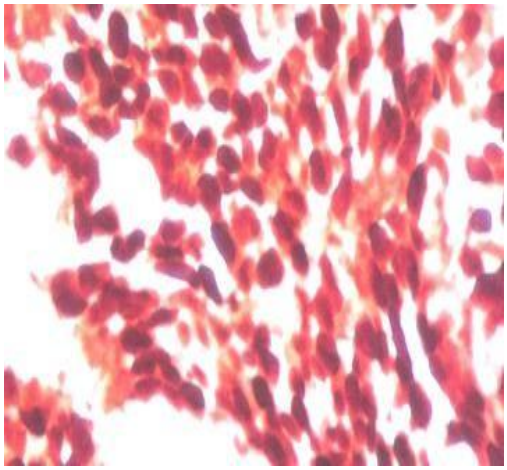


FIGURE : 8 strong nuclear positivity favoring in squamous cell carcinoma 400X

According to Wei zhao et al**.,** [(13)]p40 is considered to be the best marker in differentiating SCC and adenocarcinoma. p40 has sensitivity of 100%,and specificity of 100%. Present study is in accordance with this view. In the recent study by *Bishop et al* , in terms of sensitivity for SCC ,it was found to be equal for the two markers p40 and p63, but in terms of specificity p40 proved to be superior. Another study by Pelosi G et al,[(14)]also strongly recommend p40 as a reliable and more superior marker for SCC.

Hence the use of p40 in conjunction with TTF-1 was justified to be a promising diagnostic tool in the routine practise for subtyping of NSCLC.

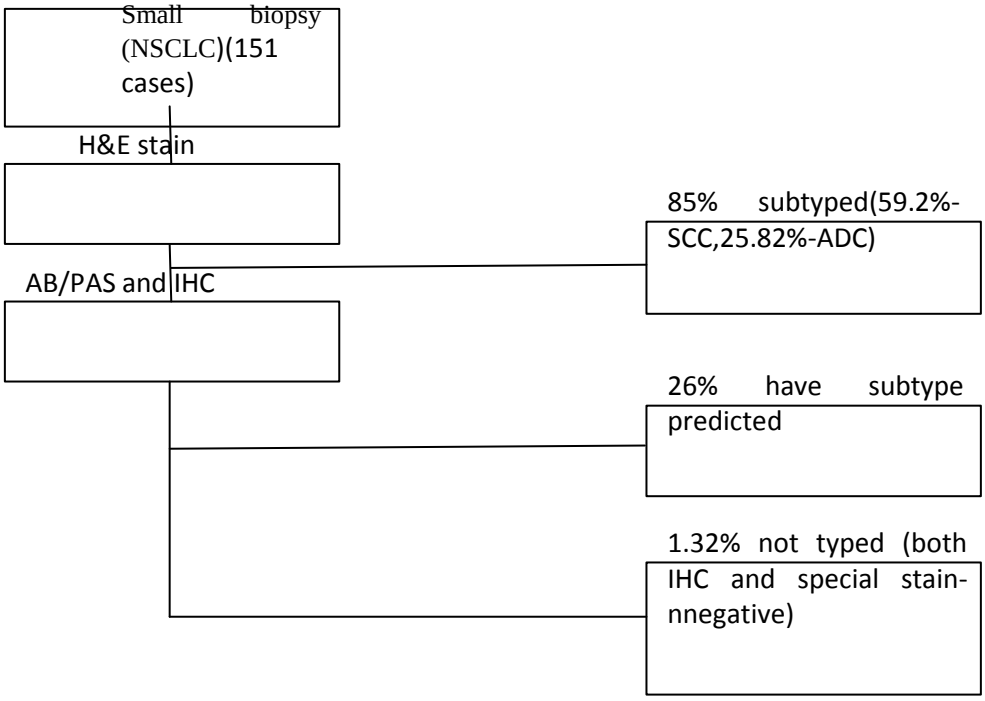
Giuseppe Pelosi et al[14], also emphasized the application of two marker panel which comprises of p40 and TTF-1 on small biopsy or cell block samples was considered more effective and reliable in correctly subtyping a vast majority of lung cancers. Similar findings were recorded in yet another study by "Brown and colleagues et al"[15] who concluded that the use of TTF-1/p40 cocktail on small biopsy for differentiating lung SCC and ADC was very effective. The added benefit is that we can employ this cocktail on a single slide.

In this study, among the 151 cases of NSCLCs, 40 cases belonged to not otherwise specified (NOS) constituting 26% of the total. But with the use of Mucin stains, and IHC markers TTF-1 and p40 , it was minimised to 1.32%

Also in this study, when intrepreted according to the new classififcation, among these 40 cases of undifferentiated non small cell carcinoma (NOS) group, were subtyped using special stains and IHC markers into

NSCC Favouring adenocarcinoma 20 cases (50 %),
NSCC Favouring squamous cell carcinoma 17cases (36.66%),
NSCC Favouring adenosquamous carcinoma was 1 case(3.33%)and
Unsubtyped were 5%(n=2).

Diagnostic algorithm for classification on NSCLC in small biopsy:



IV.Conclusion

According to this study we conclude that our role does not end in classifying lung malignancies as SCLC and NSCLC but has to be subtyped using special stains and IHC markers based on 2015 WHO classification. Special stains like Alcian blue-PAS and IHC markers like TTF-1 and P40 are found to be extremely efficient and useful markers. Furthermore ,in this era of targeted therapy, basic Histopathological diagnosis per se is not enough but further histological subtyping as well as molecular testing are of paramount importance, as these are the key factors determining the treatment options. Thus the surgical pathologist can be considered as the sole guardian of these small biopsy specimens-The very precious yet limited resource from which one needs to maximise the diagnostic yield.

Bibilography

1.DollR,PetoR(1981).Thecausesofcancer:quantitativeestimatesofavoidablerisksofcancerintheUnitedStatestoday.JNatl CancerInst66:1191-1308
2.TravisWD,collyTV,CorrinB,etal.HistologicalTypingoflungandpleuralTumors.3rded.Berlin,Germany:Springer;1999
3.TravisWD,BrambillaE,BurkeAP,MarxA,NicholsonAG(2015)WHOClassificationoftumorsofthelung,pleura,thymusand heart.IARCPress,Lyon,France
4..BurnettRA,SwansonBeckJ,HowatsonSR,etal.Observervariabilityinhistopathologicalreportingofmalignantbronchialbiopsy specimens.Jclinpathol1994;47:711-713
5..KwakEL,BangYJ,CamidgeDRetal(2010)Anaplasticlymphomakinaseinhibitioninnon-small-celllungcancer.NEnglJMed 363:1693–1703
6.ScagliottiGV,ParikhP,vonPawelJetal(2008)PhaseIIIstudycomparingcisplatinplusgemcitabinewithcisplatinplus pemetrexedinchemotherapy-naivepatientswithadvanced-stagenon-small-celllungcancer.JClinOncol26:3543–355116.
7..DeMarinisF,PereiraJR,FossellaFetal(2008)LungCancerSymptomScaleoutcomesinrelationtostandardefficacymeasures: ananalysisofthephaseIIIstudyofpemetrexedversusdocetaxelinadvancednon-smallcelllungcancer.JThoracOncol3:30–36
8.SandlerA,GrayR,PerryMCetal(2006)Paclitaxel-carboplatinaloneorwithbevacizumabfornon-smallcelllungcancer.NEngl JMed355:2542–2550
9..NavadaS,LaiP,SchwartzAG,KalemkerianGP.Temporaltrendsinsmallcelllungcancer:analysisofthenationalSurveillance EpidemiologyandEnd-Results(SEER)database[abstract7082]JClinOncol.2006;24(18S)suppl:384S.
10.DilekErman,FigenAtalay,ARetrospectiveEvaluationof571lungcancerpatients.TurkishRespiratoryJournal,2003:4(2):67-69
11.SuprunH,PedioG,RuttnerJR,Thediagnosticreliabilityofcytologictypinginprimarylungcarcinomawithareviewofthe literature.ActaCytol1980:24:494-500
12..KaufmannandDietel,Expressionofthyroidtranscriptionfactor-1inpulmonaryandextrapulmonarysmallcellcarcinomasand otherneuroendocrinecarcinomasofvariousprimarysites.Histopathology,Volume 36,Issue 5,pages415–420,May2000
13.WeiZhao,HuiWangetal,ΔNp63,CK5/6,TTF-1andnapsinA,areliablepaneltosubtypenon-smallcelllungcancerinbiopsy specimens.IntJClinExp.Pathol,2004;7(7),4247-4253
14.Giuseppepelosietal,ΔNp63(p40)andthyroidtranscriptionfactor-1immunoreactivityonsmallbiopsiesorcellblocksfortyping non-smallcelllungcancer:anoveltwo-hit,sparing-materialapproach.Journal ofthoracic oncology: official publication of theInternational Association for the Study of Lung Cancer(ImpactFactor:4.55).11/2011;7(2):281-90.
15.Brown AFTissue-preservingantibodycocktailstodifferentiateprimarysquamouscellcarcinoma,adenocarcinoma,andsmallcell carcinomaoflung.Arch Pathol Lab Med.2013Sep;137(9):1274-81.
16.KaufmannandDietel,Expressionofthyroidtranscriptionfactor-1inpulmonaryandextrapulmonarysmallcellcarcinomasand otherneuroendocrinecarcinomasofvariousprimarysites.Histopathology,Volume 36,Issue 5,pages415–420,May2000