# Results

Results

This study was performed on 40 lung tissue lesions, all of them are malignant tumors, and 10 cases of non-neoplastic lung diseases were taken as a control.

Histopathological results

Among the 40 examined cases of NSCLCss, there were 15 cases (37.5%) of squamous cell carcinoma (**Sq.C.C**), 9 (22.5%) Adenocarcinoma (**AC**), and 16 (40%) undifferentiated large cell carcinoma (**UDLCC**).
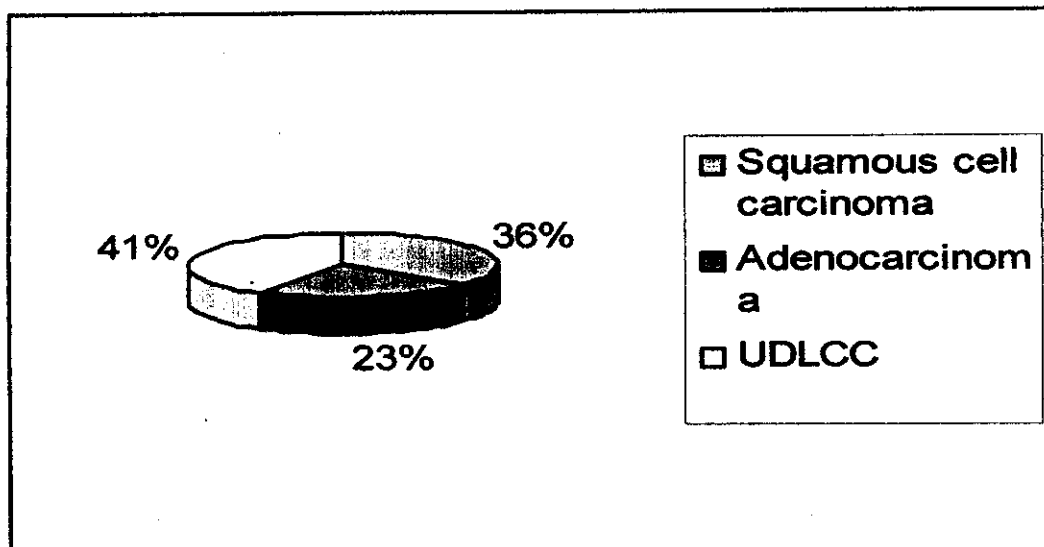
Fig (4): Histopathological diagnosis of all examined cases.

Table (4): Relation of histopathological types of NSCLCs to histopathological garde of NSCLCs.

Histopathological type	No. of cases	Histopathological grade							
		WD		MD		PD		UD	
		No	%	No	%	No	%	No	%
Sq.C.C	15	0	0%	7	46.6%	8	53.4%	0	0%
Adenocarcinoma	9	3	33.3%	4	44.5%	2	22.2%	0	0%
UDLCC	16	0	0%	0	0%	0	0%	16	100%
Total	40	3	7.5%	11	27.5%	10	25%	16	40%

Clinical results

The age of the patients ranged from 38 year up to 90 year with a mean age 55.6 year.

Out of 40 cases of NSCLCs, 12 cases (30%) were females and 28 cases (70%) were males. The male to female ratio was 7:3.

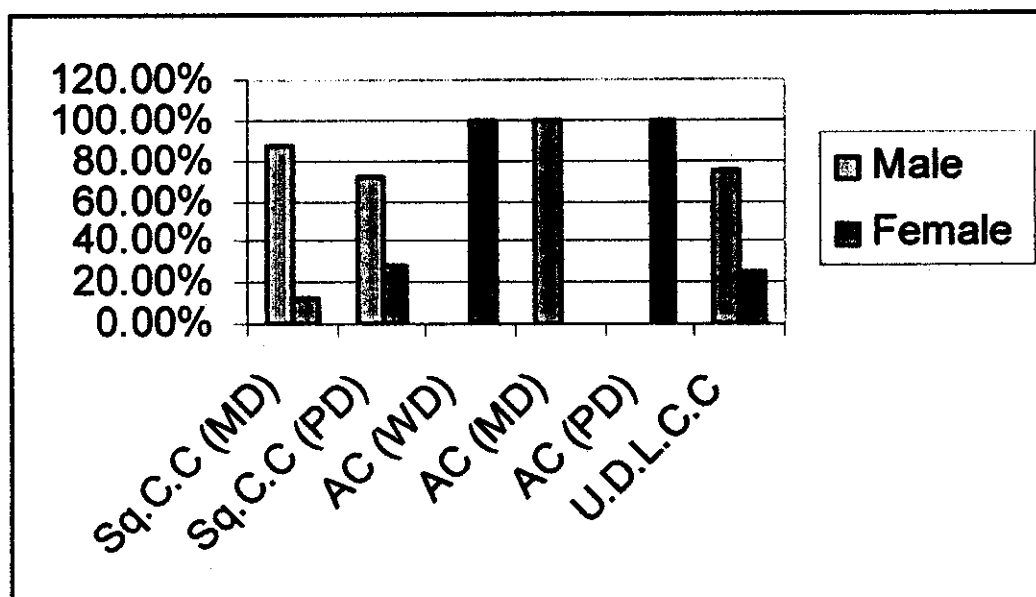**Table (5): Relation of histopathological types and grades of NSCLCs to age of patients.**

Histopathological type	No. of cases	Age in years					
		31-40	41-50	51-60	61-70	71-80	81-90
Sq.C.C	15	3	4	3	4	0	1
MD	8	1	3	1	2	0	1
PD	7	2	1	2	2	0	0
Adenocarcinoma	9	0	4	3	1	1	0
WD	3	0	3	0	0	0	0
MD	4	0	1	2	1	0	0
PD	2	0	0	1	0	1	0
UDLCC	16	1	6	2	5	2	0
Total	40	4	14	8	10	3	1

N.B: There was insignificant positive correlation between histopathological types of NSCLCs and age of patients (p>0.05).

N.B: There was insignificant positive correlation between histopathological grades of NSCLCs and age of patients (p>0.05)

Table (6): Relation of histopathological types and grades of NSCLCs to sex of patients.

Histopathological type	No. of cases	Sex of patients			
		Male		Female	
		No.	%	No.	%
Sq.C.C	15	12	80%	3	20%
MD	8	7	87.5%	1	12.5%
PD	7	5	71.4%	2	28.6%
Adenocarcinoma	9	4	44.5%	5	55.5%
WD	3	0	0%	3	100%
MD	4	4	100%	0	0%
PD	2	0	0%	2	100%
UDLCC	16	12	75%	4	25%
Total	40	28	70%	12	30%

Graph (1): Relation of histopathological types and grades of NSCLCs to sex of patients.

N.B: There was insignificant correlation between histopathological types of NSCLCs and sex of patients (p>0.05).

Correlation between histopathological types and grades of NSCLCs to size of these tumors:

Table (7): **Relation of histopathological types and grades of NSCLCs to their size:**

| Hitopathological type	No. of cases	Size of tumors							
		T1		T2		T3		T4	
		No.	%	No.	%	No.	%	No.	%
Sq.C.C	15	8	53.3%	2	13.4%	4	26.7%	1	6.6%
MD	8	5	62.5%	2	25%	1	12.5%	0	0%
PD	7	3	42.9%	0	0%	3	42.9%	1	14.2%
Adenocarcinoma	9	4	44.4%	4	44.4%	0	0%	1	11.2%
WD	3	2	66.7%	1	33.3%	0	0%	0	0%
MD	4	2	50%	2	50%	0	0%	0	0%
PD	2	0	0%	1	50%	0	0%	1	50%
U.D.L.C.C	16	3	18.7%	5	31.3%	4	25%	4	25%
Total	40	15	37.5%	11	27.5%	8	20%	6	15%

N.B: According to (Mountain et al, 1999):

T1:- Tumor $\leq$ 3 cm in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e. not in the main bronchus).

T2:- Tumor with any of the following features of size or extent:

- \> 3 cm in greatest dimension

- involves main bronchus, $\geq$ 2 cm distal to carina

- invades the visceral pleura

- associated with atelectasis or obstructive pneumonitis that extends to the hilar region but not involve the entire lung

T3:- Tumor of any size that directly invades any of the following: chest wall (including superior sulcus tumors), diaphragm, mediastinal pleura, parietal pericardium; or tumor in the main bronchus < 2 cm distal to carina, but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung.

T4:- tumor of any size that invades the following: mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina; or tumor with a malignant pleural or pericardial effusion, or with satellite tumor nodule (s) within the ipsilateral primary tumor lobe of the lung.
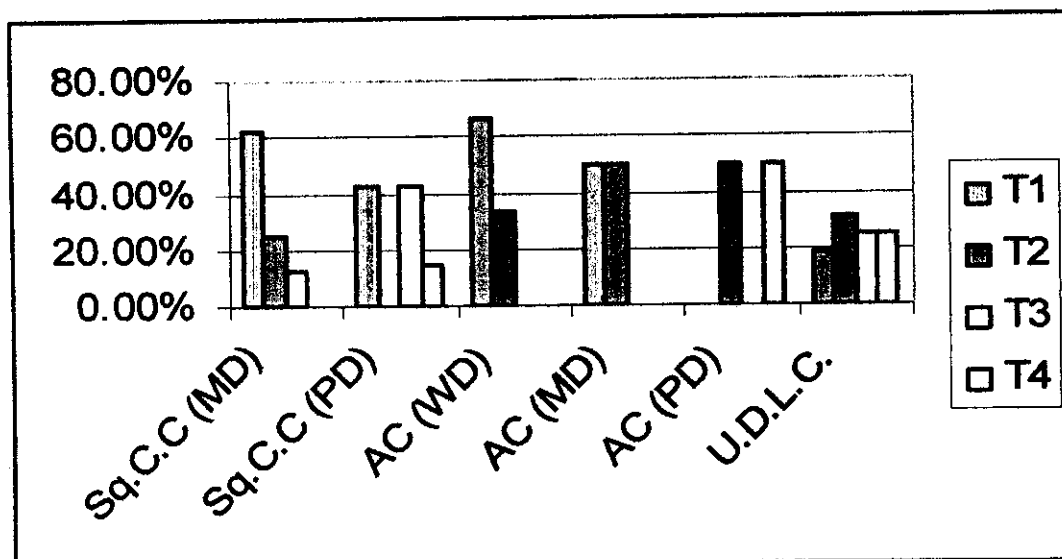
Graph (2): Relation of histopathological types and grades of NSCLCs to their size.

N.B: There was insignificant positive correlation between histopathological types of NSCLCs and the size of them (p>0.05).

N.B: There was significant positive correlation between histopathological grads of NSCLCs and the size of them (p<0.05).

Correlation between histopathological types and grades of NSCLCs to state of LNs metastasis:

Table (8): Relation of histopathological type of NSCLCs to state of LNs metastasis.

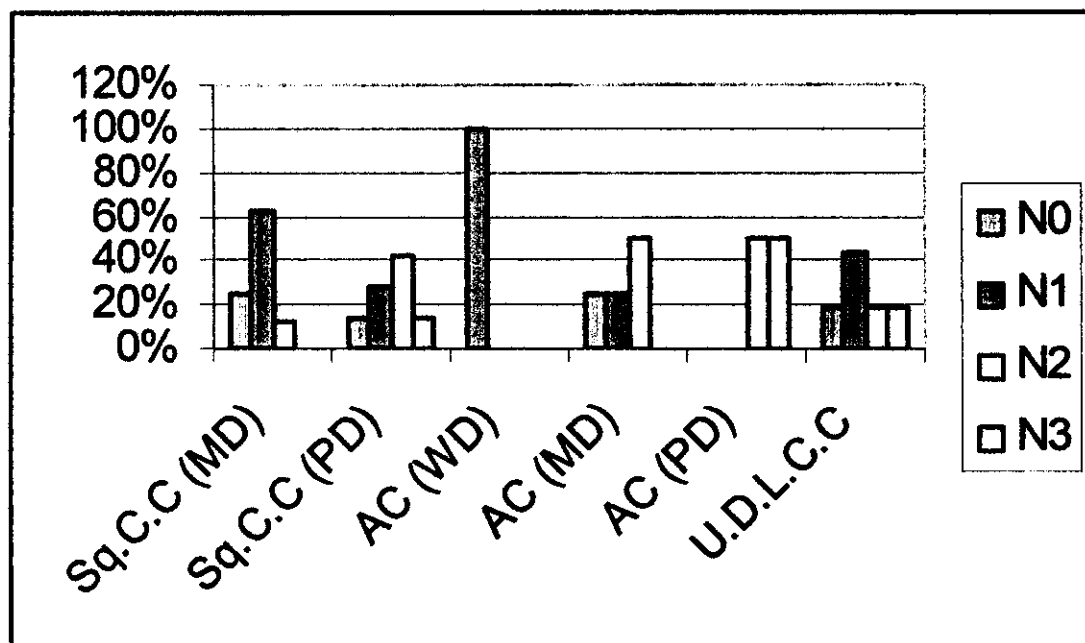| Hitopathological type	No. of cases	State of LNs metastasis							
		N0		N1		N2		N3	
		No.	%	No.	%	No.	%	No.	%
Sq.C.C	15	3	20%	7	46.6%	4	26.6%	1	6.8%
MD	8	2	25%	5	62.5%	1	12.5%	0	0%
PD	7	1	14.3%	2	28.6%	3	42.8%	1	14.3%
Adenocarcinoma	9	4	44.4%	1	11.2%	3	33.3%	1	11.1%
WD	3	3	100%	0	0%	0	0%	0	0%
MD	4	1	25%	1	25%	2	50%	0	0%
PD	2	0	0%	0	0%	1	50%	1	50%
U.D.L.C.C	16	3	18.7%	7	43.9%	3	18.7%	3	18.7%
Total	40	10	25%	15	37.5%%	10	25%	5	12.5%

N.B: According to (Mountain et al, 1999):
N0: No lymph node involvement.
N1:- Metastasis to ipsilateral peribronchial and/ or ipsilateral hilar lymph nodes, and intrapulmonary nodes involved by direct extension of the primary tumor
N2:- Metastasis to ipsilateral mediastinal and/ or subcarinal lymph node(s)
N3:- Metastasis to contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

Graph (3): Relation of histopathological types of NSCLCs to state of LNs metastasis.

N.B: There was insignificant positive correlation between histopathological types of NSCLCs and state of LNs metastasis (p>0.05).

N.B: There was insignificant positive correlation between histopathological grads of NSCLCs and state of LNs metastasis (p>0.05).

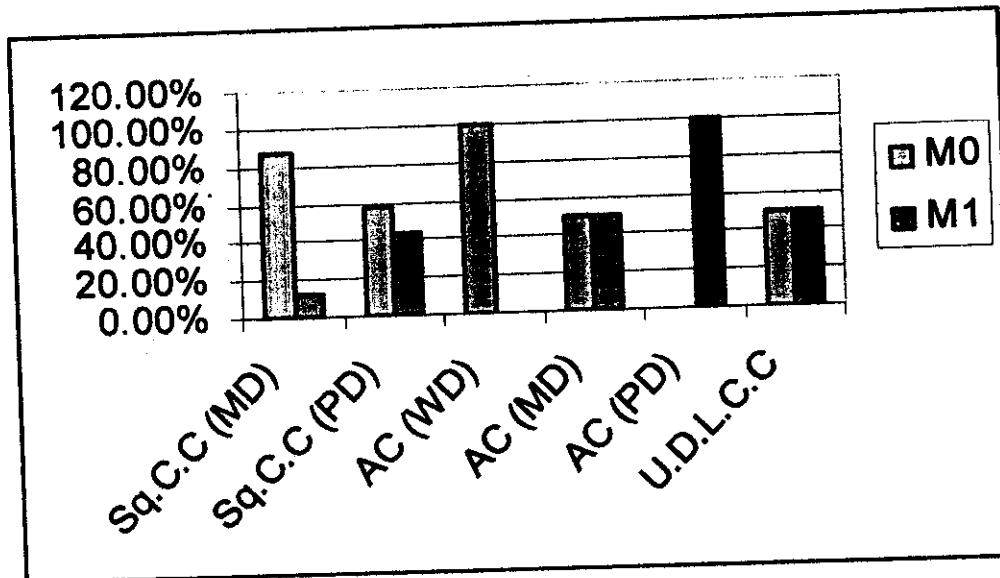# Correlation between histopathological types and grades of NSCLCs to state of distant metastasis:

Table (9): Relation of histopathological types and grades of NSCLCs to state of distant metastasis.

Histopathological type	No. of cases	State of distant metastasis			
		M0		M1	
		No.	%	No.	%
Sq.C.C	15	11	73.3%	4	26.7%
MD	8	7	87.5%	1	12.5%
PD	7	4	57.1%	3	42.9%
Adenocarcinoma	9	5	55.5%	4	44.5%
WD	3	3	100%	0	0%
MD	4	2	50%	2	50%
PD	2	0	0%	2	100%
UDLCC	16	8	50%	8	50%
Total	40	24	60%	16	40%

N.B: According to (Mountain et al, 1999):

M0:- No distant metastasis

M1:- Distant metastasis present

Graph (4): Relation of histopathological types and grades of NSCLCs, to state of distant metastasis.
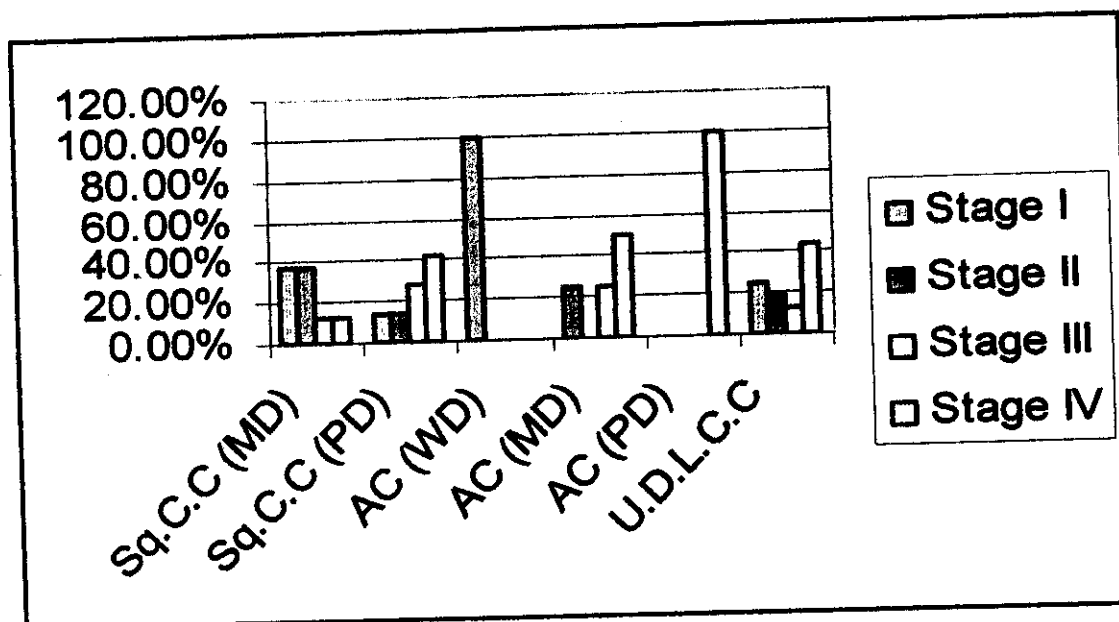
N.B: There was insignificant positive correlation between histopathological types of NSCLCs and state of distant metastasis (p>0.05) as liability to distant metastasis not more in a specific type of NSCLCs.

N.B: There was insignificant positive correlation between histopathological grads of NSCLCs and state of distant metastasis (p>0.05).

Correlation between histopathological types and grades of NSCLCs to the stage (according to TNM staging system).

Table (10): Relation of histopathological types, grades of NSCLCss, to the stage of these tumors.

Hitopathological type	No. of cases	Stage of tumors							
		Stage I		Stage II		Stage III		Stage IV	
		No.	%	No.	%	No.	%	No.	%
Sq.C.C	15	4	26.6%	4	26.7%	3	20%	4	26.7%
MD	8	3	37.5%	3	37.5%	1	12.5%	1	12.5%
PD	7	1	14.3%	1	14.3%	2	28.6%	3	42.8%
Adenocarcinoma	9	4	44.5%	0	0%	1	11%	4	44.5%
WD	3	3	100%	0	0%	0	0%	0	0%
MD	4	1	25%	0	0%	1	25%	2	50%
PD	2	0	0%	0	0%	0	0%	2	100%
U.D.L.C.C	16	4	25%	3	18.8%	2	12.5%	7	43.7%
Total	40	12	30%	8	20%	5	12.5%	15	37.5%

Graph (5): Relation of histopathological types and grades of NSCLCss, to the stage of such tumors.

N.B: There was insignificant positive correlation between histopathological types and stage of NSCLCs ($p>0.05$).

N.B: There was significant positive correlation between histopathological grades of NSCLCs and stage of tumors ($p < 0.05$) as the higher the grade of NSCLCs the more advanced stage of such tumors.

Immunohistochemical staining results for

1-CD34

CD34 expression in control group:

The immunohistochemical staining of CD34 revealed positive staining of the 10 control cases. It appeared as a brown membranous staining of blood vessels and endothelial cells. Six cases (60%) were hypervascular, while the other 4 cases (40%) were hypovascular.

CD34 expression in non small cell lung cancer:

Immunohistochemical staining of CD34 appeared as a brown membranous staining of blood vessels and endothelial cells. 18 cases (10 cases of Sq.C.C, 3 cases of adenocarcinoma, and 5 cases of UDLCC) were hypovascular; however the other 22 cases (5 cases of Sq.C.C, 6 cases of adenocarcinoma, and 11 cases of UDLCC) were hypervascular.

Relation between histopathological types, grades of NSCLCs, to extent of CD34 expression:

Table (11): Relation of histopathological types and grades of NSCLCs to microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression.

| Histopathological type	No. of cases	Extent of CD34 expression			
		Hypovascular		Hypervascular	
		No.	%	No.	%
Control groups	10	4	40%	6	60%
Sq.C.C	15	10	66.7%	5	33.3%
MD	8	6	75%	2	25%
PD	7	4	57.1%	3	42.9%
Adenocarcinoma	9	3	33.3%	6	66.7%
WD	3	2	66.7%	1	33.3%
MD	4	1	25%	3	75%
PD	2	0	0%	2	100%
UDLCC	16	5	31.3%	11	68.7%
Total	50	22	44%	28	56%
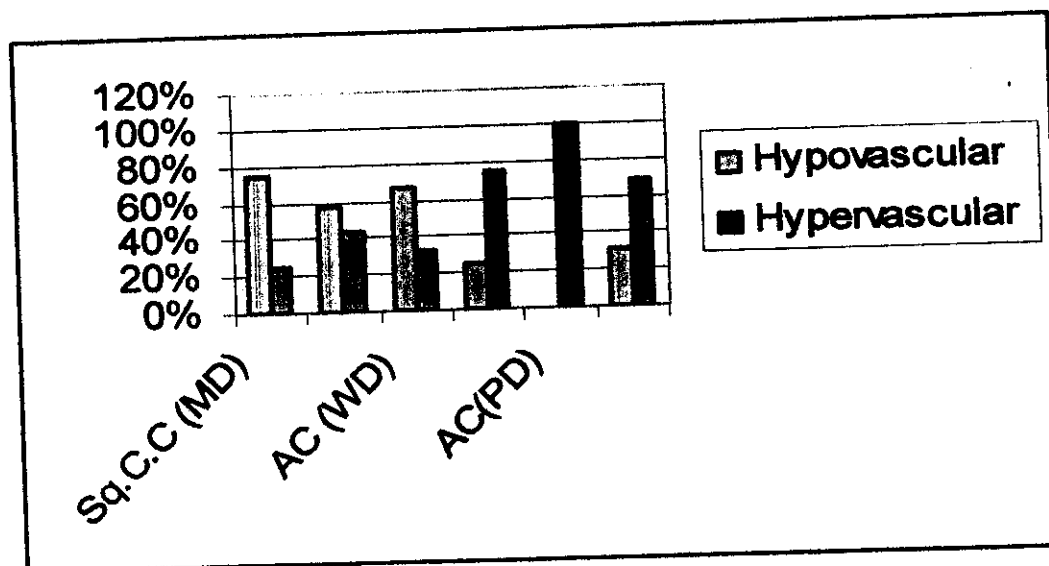
Graph (6): Relation of histopathological types and grades of NSCLCs to microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression.

N.B: There was insignificant positive correlation between histopathological types of NSCLCs and extent of CD34 expression (p>0.05) as there is no great difference in extent of CD34 expression between different histopathological types of NSCLCs.

Table (12): Relation of microvessel density (MVD) (angiogenesis) assessed_by extent   of CD34 expression and grade of differentiation of NSCLCs:

| Tumor grade	No. of cases	Hypovascular		Hypervascular	
		No.	%	No.	%
WD. Tumors	3	2	66.7%	1	33.3%
MD tumors	12	7	58.3%	5	41.7%
PD tumors	9	4	44.5%	5	55.5 %
UD tumors	16	5	31.3%	11	68.7%
Total	40	18	45%	22	55%
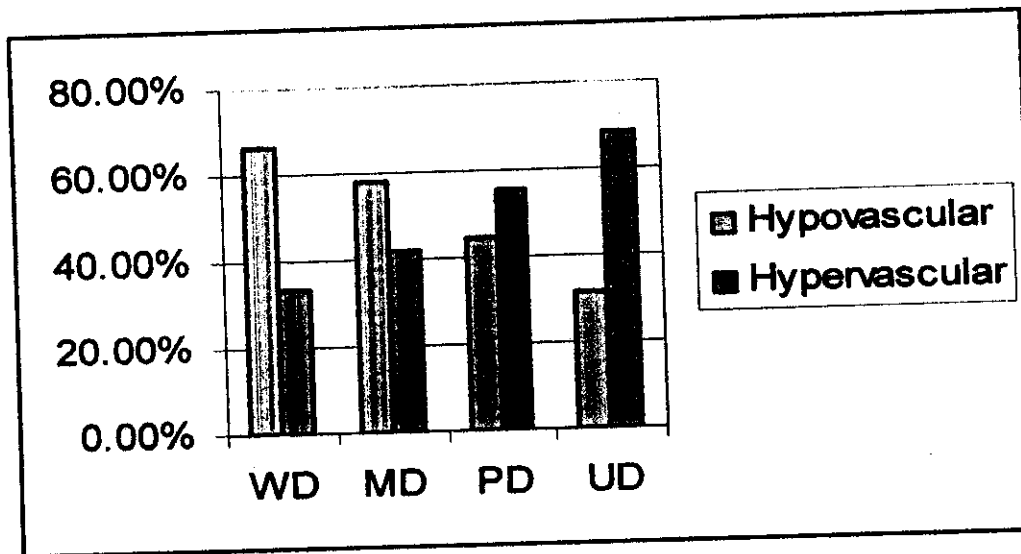
Graph (7): Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and grade of differentiation of NSCLCs.

N.B: Although there was insignificant positive correlation between histopathological grades of NSCLCs and extent of CD34 expression (p>0.05), but from the table and the graph, it seems that the poorer the grade of differentiation of these tumor the higher the extent of expression of CD34.

Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and size of NSCLCs:

Table (13): Relation between microvessel density (MVD) (angiogenesis) assessed by extent   of CD34 expression and size of NSCLCs:

Size of tumor	No of cases	% of cases	Extent of CD34 expression			
			Hypovascular		Hypervascular	
			No	%	No	%
T1	15	37.5%	11	73.3%	4	26.7%
T2	11	27.5%	5	45.5%	6	54.5%
T3	8	20%	2	25%	6	75%
T4	6	15%	0	0%	6	100%
Total	40	100%	18	45%	22	55%
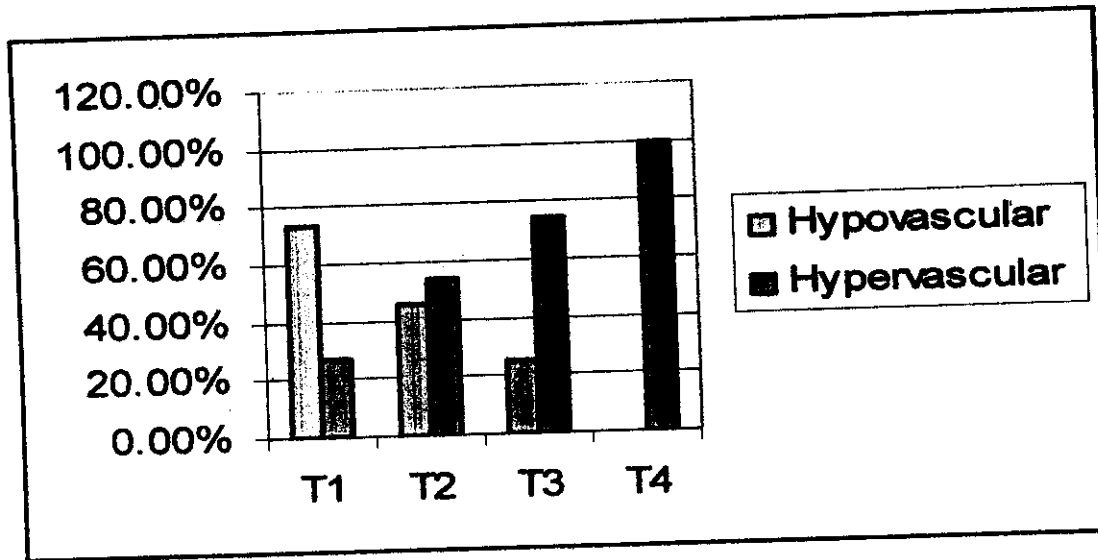
Graph (8): Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and size of NSCLCs.

N.B: Statistically, There was a significant positive correlation between size of NSCLCs and extent of CD34 expression (p<0.05).
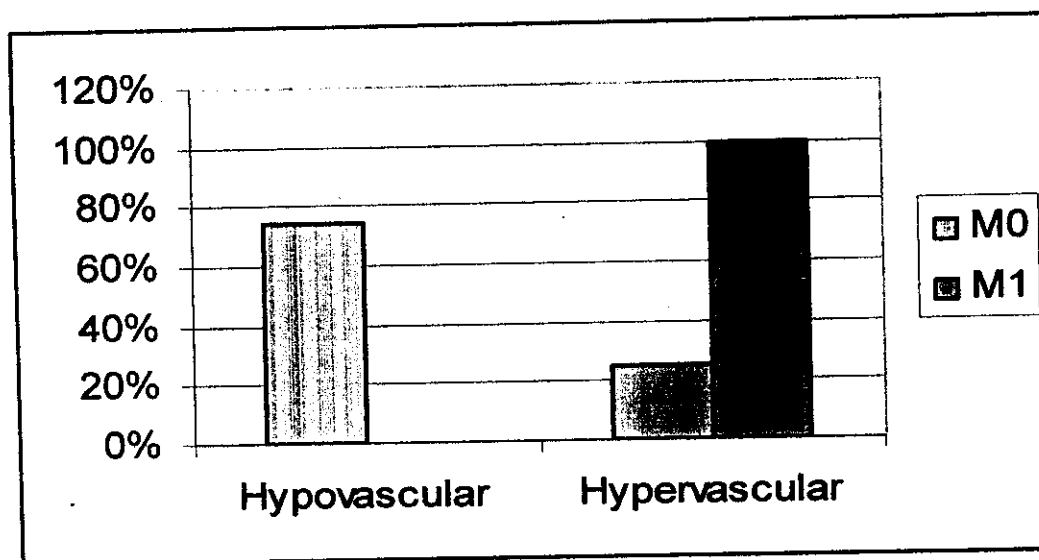
Relation between histopathological types and state of LNs metastasis, to microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression:

Table (14): Relation of microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and state of LNs metastasis:

| State of LNs metastasis	No. of cases	Hypovascular		Hypervascular	
		No.	%	No.	%
N0	10	6	60%	4	40%
N1	15	8	53.3%	7	46.7%
N2	10	4	40%	6	60%
N3	5	0	0%	5	100%
Total	40	18	45%	22	55%
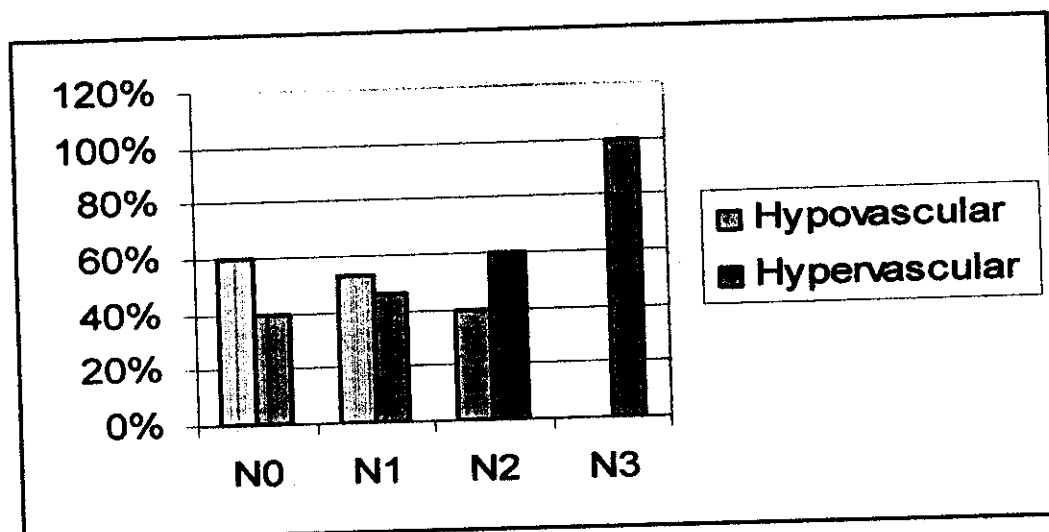
Graph (9): Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and state of lymph nodes metastasis.

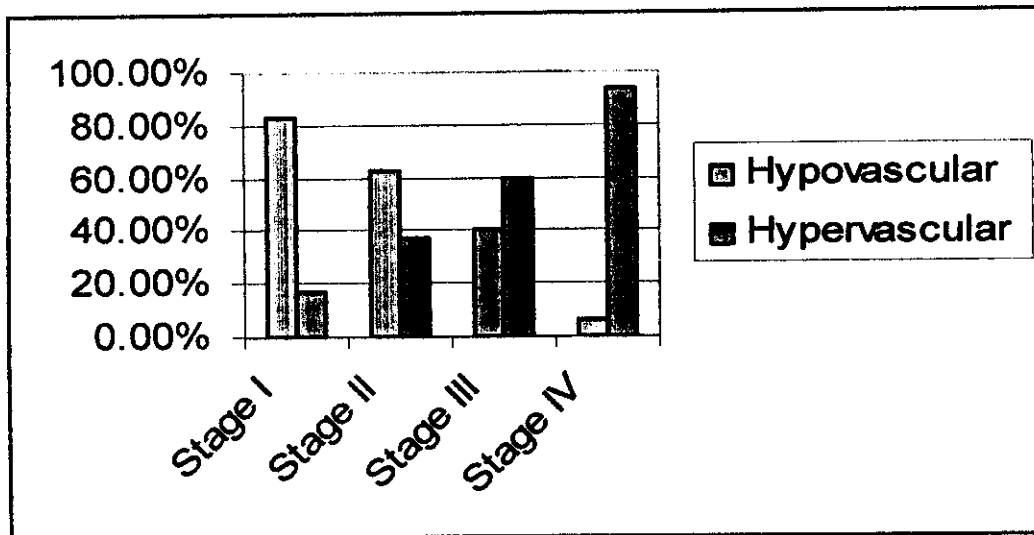N.B: There was insignificant positive correlation between state of LNs metastasis and extent of CD34 expression (p>0.05).

Table (15): Relation of histopathological types and state of LNs metastasis to microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression.

Histopathological type	No. of cases	Extent of CD34 expression			
		Hypovascular		Hypervascular	
		No.	%	No.	%
Sq.C.C	15	10	66.7%	5	33.3%
N0	3	2	66.7%	1	33.3%
N1	7	6	85.5%	1	14.5%
N2	4	2	50%	2	50%
N3	1	0	0%	1	100%
Adenocarcinoma	9	3	33.3%	6	66.7%
N0	4	2	50%	2	50%
N1	1	0	0%	1	100%
N2	3	1	33.3%	2	66.7%
N3	1	0	0%	1	100%
UDLCC	16	5	31.3%	11	68.7%
N0	3	2	66.7%	1	33.3%
N1	7	2	28.7%	5	71.3%
N2	3	1	33.3%	2	66.7%
N3	3	0	0%	3	100%
Total	40	18	45%	22	55%

Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and state of distant metastasis.

Table (16): Relation between extent of expression of CD34 and state of distant metastasis and microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression:

Distant metastases	No. of cases	Hypovascular		Hypervascular	
		No.	%	No.	%
M0	24	18	75%	6	25%
M1	16	0	0%	16	100%
Total	40	18	45%	22	55%

Graph (10): Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and state of distant metastases.

Table (17): Relation of histopathological type, state of distant metastasis to microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression.

Histopathological type	No. of cases	Extent of CD34 expression			
		Hypovascular		Hypervascular	
		No.	%	No.	%
Sq.C.C	15	10	66.7%	5	33.3%
M0	11	10	90.9%	1	9.1%
M1	4	0	0%	4	100%
Adenocarcinoma	9	3	33.3%	6	66.7%
M0	5	3	60%	2	40%
M1	4	0	0%	4	100%
UDLCC	16	5	31.3%	11	68.7%
M0	8	5	62.5%	3	37.5%
M1	8	0	0%	8	100%
Total	40	18	45%	22	55%
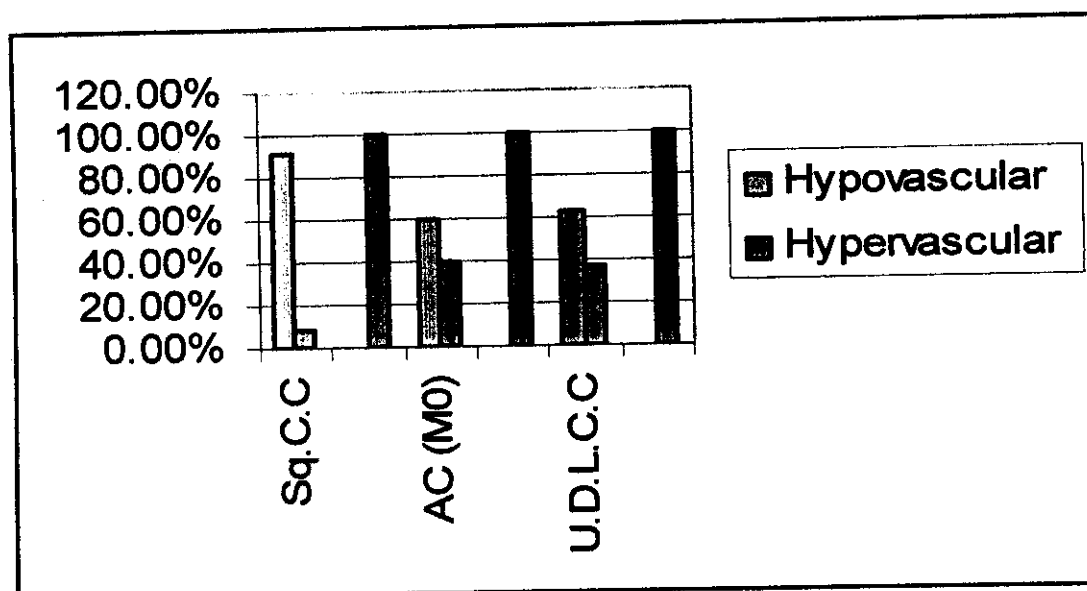
Graph (10): Relation of histopathological types and state of distant metastasis to microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression.

N.B: There was significant positive correlation between state of distant metastasis and extent of CD34 expression (p<0.05). This means that tumors with distant metastases showed higher extent of CD34 expression.

Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and stage of NSCLCs:

Table (18): Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and stage of NSCLCs.

Tumor stage	No. of cases	Hypovascular		Hypervascular	
		No.	%	No.	%
Stage I	12	10	83.4%	2	16.6%
Stage II	8	5	62.5%	3	37.5%
Stage III	5	2	40%	3	60%
Stage IV	15	1	6.6%	14	93.4%
Total	40	18	45%	22	55%

Graph (12): Relation between microvessel density (MVD) (angiogenesis) assessed by extent of CD34 expression and stage of NSCLCs.

N.B: Statistically, there was a significant positive correlation between

extent of CD34 expression and stage of NSCLCs

(P value < 0.05) i.e; the advanced the stage of tumors, the higher

the extent of CD34 expression.

Table (19): Relation between microvessel density (MVD) (angiogenesis) assessed by extent   of CD34 expression and clinico-pathological parameters:

Clinico-pathological parameter	Extent of CD34 expression			
	Hypovascular		Hypervascular	
	No.	%	No.	%
1)Type of tumor				
Sq.C.C	10	66.7%	5	33.3%
Adenocarcinoma	3	33.3%	6	66.7%
U.D.L.C.C	5	31.3%	11	68.7%
2)Grade of tumors				
WD	2	66.7%	1	33.3%
MD	7	58.3%	5	41.7%
PD	4	44.5%	5	55.5%
UD	5	31.3%	11	68.7%
3)State of LNs metastasis				
N0	6	60%	4	40%
N1	8	53.3%	7	46.7%
N2	4	40%	6	60%
N3	0	0%	5	100%
4)State of distant metastasis				
M0	18	75%	6	25%
M1	0	0%	16	100%
5)Stage of tumors				
Stage I	10	83.4%	2	16.6%
Stage II	5	62.5%	3	37.5%
Stage III	2	40%	3	60%
Stage IV	1	6.6%	14	93.4%

2-MMP-2

MMP-2 expression in control cases:

The immunohistochemical staining for MMP-2 revealed negative staining of normal bronchial epithelium of the 10 control cases, but positive staining of stromal fibroblasts and endothelial cells.

MMP-2 expression in non small cell lung cancer cases:

The immunoreaction products of MMP-2 were fine granular and localized in the cytoplasm of tumor cells, also positive staining of stromal fibroblasts and endothelial cells was detected.

Relation between extent of MMP-2 expression and histological type, grade of NSCLCs:

Table (20): Relation between extent of MMP-2 expression and histopathological types, grades of NSCLCs:

Type of tumor	No. of cases	Extent of MMP-2 expression							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
Sq.C.C	15	4	26.7%	6	40%	2	13.3%	3	20%
MD	8	3	37.5%	3	37.5%	2	25%	0	0%
PD	7	1	14.6%	3	42.8%	0	0%	3	42.8%
Adenocarcinoma	9	2	22.2%	2	22.2%	4	44.5%	1	11.1%
WD	3	1	33.3%	1	33.3%	1	33.4%	0	0%
MD	4	1	25%	1	25%	2	50%	0	0%
PD	2	0	0%	0	0%	1	50%	1	50%
U.D.L.C.C	16	3	18.8%	3	18.8%	7	43.6%	3	18.8%
Total	40	9	22.5%	11	27.5%	13	32.5%	7	17.5%
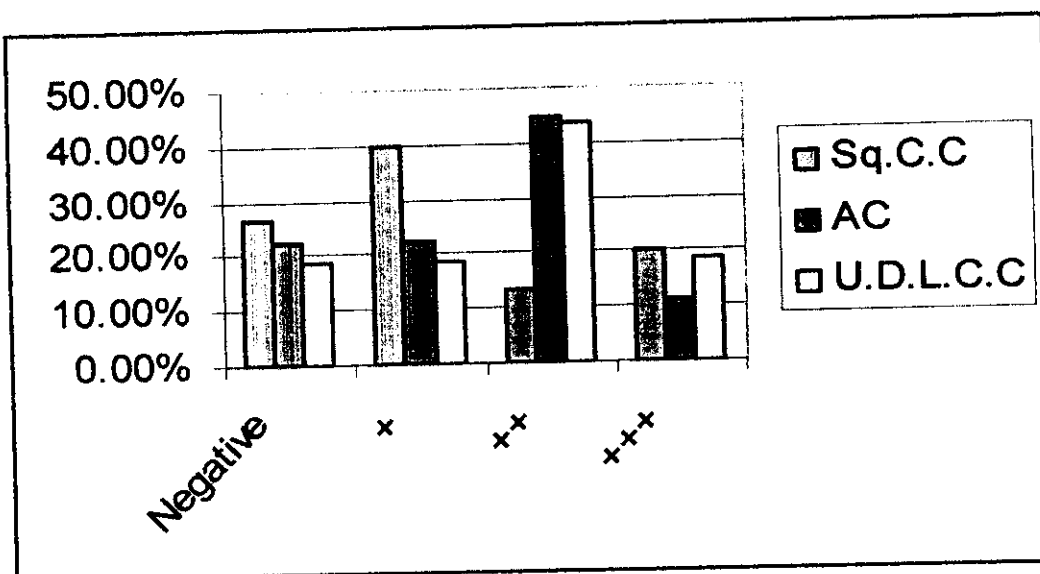
Graph (13): Relation between extent of MMP-2 expression and histopathological types of NSCLCs:

N.B: Statistically, there is insignificant positive correlation between extent of MMP-2 expression and histopathological types of NSCLCs (P value > 0.05) i.e. there is no great difference in extent of expression of MMP-2 between different types of NSCLCs.
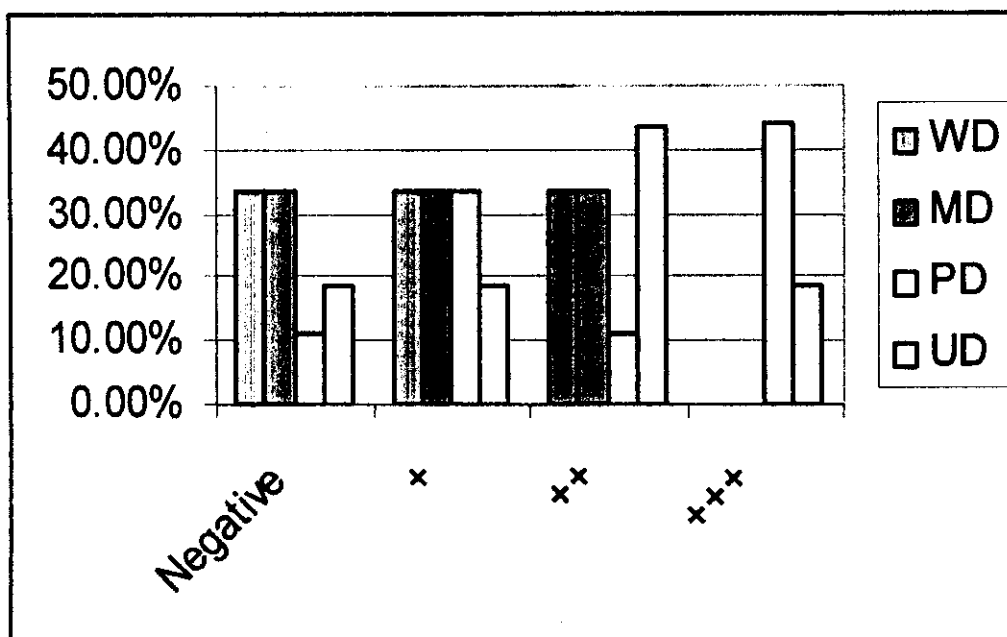
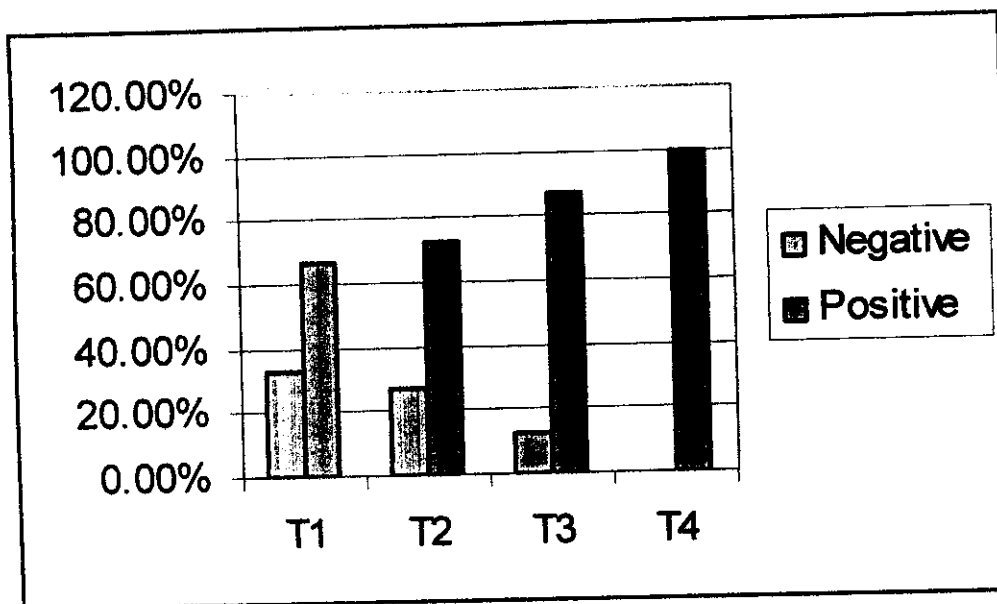**Graph (14): Relation between extent of MMP-2 expression and histopathological grades of NSCLCs.**
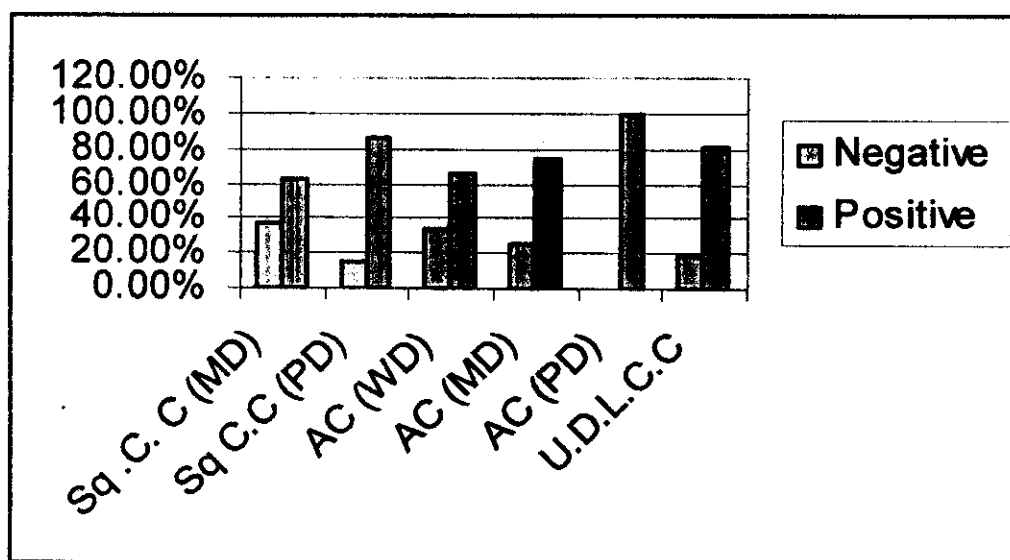
Graph (15): Relation between MMP-2 expression and histopathological types and grades of NSCLCs:

N.B: Statistically, there is insignificant positive correlation between extent of MMP-2 expression and grades of NSCLCs (P value > 0.05).
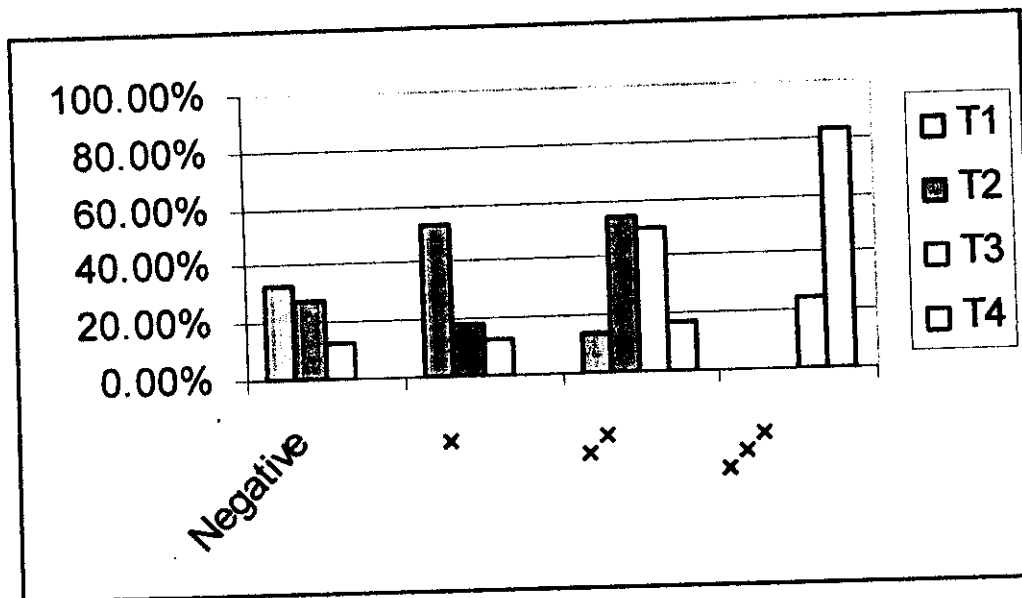
Graph (16): Relation of extent of MMP-2 expression to size of NSCLCs:

Graph (17): Relation of MMP-2 expression to size of NSCLCs:

N.B: Statistically, There was a significant positive correlation between size of NSCLCs and extent of MMP-2 expression (p<0.05).

Relation of extent of MMP-2 expression to state of lymph nodes metastases:

Table (22): Relation between extent of expression of MMP-2, and state of lymph nodes metastases:

State of LNs metastases	No.of cases	Extent of MMP-2 expression							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
N0	10	4	40%	4	40%	2	20%	0	0
N1	15	2	13.3%	6	40%	6	40%	1	6.7%
N2	10	3	30%	1	10%	4	40%	2	20%
N3	5	0	0	0	0	1	20%	4	80%
Total	40	9	22.2%	11	27.5%	13	32.5%	7	17.5%
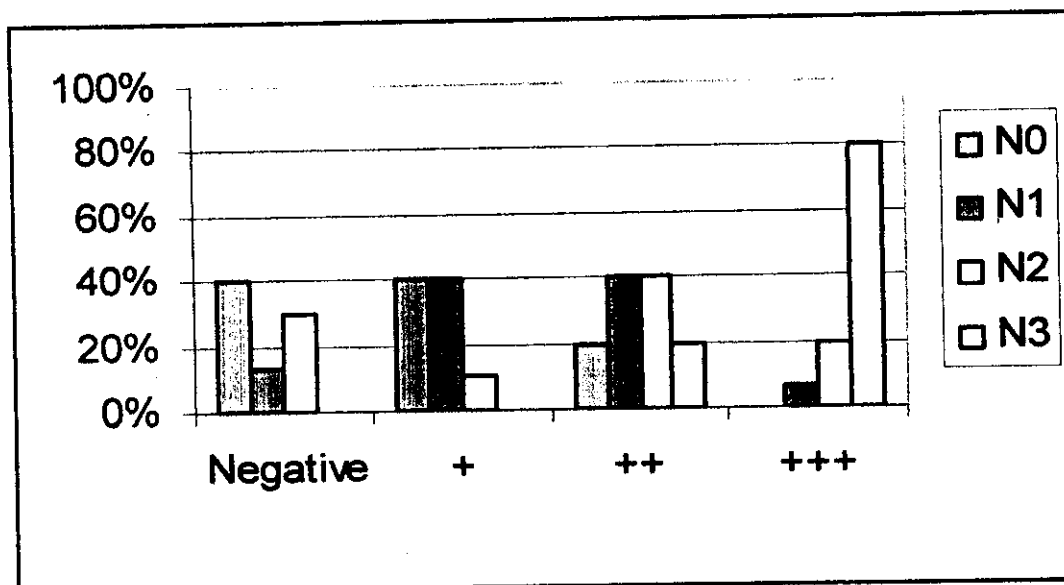
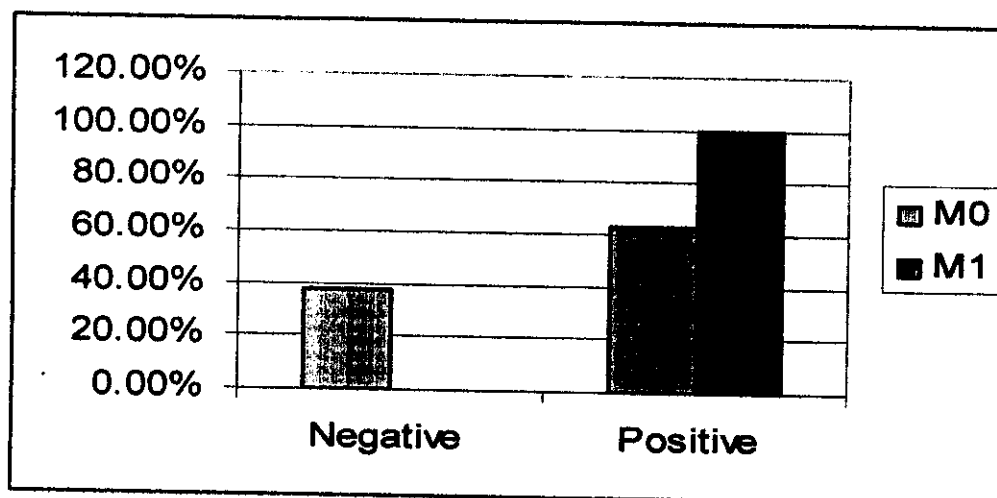**Graph (18): Relation between extent of MMP-2 expression and state of LNs metastasis.**
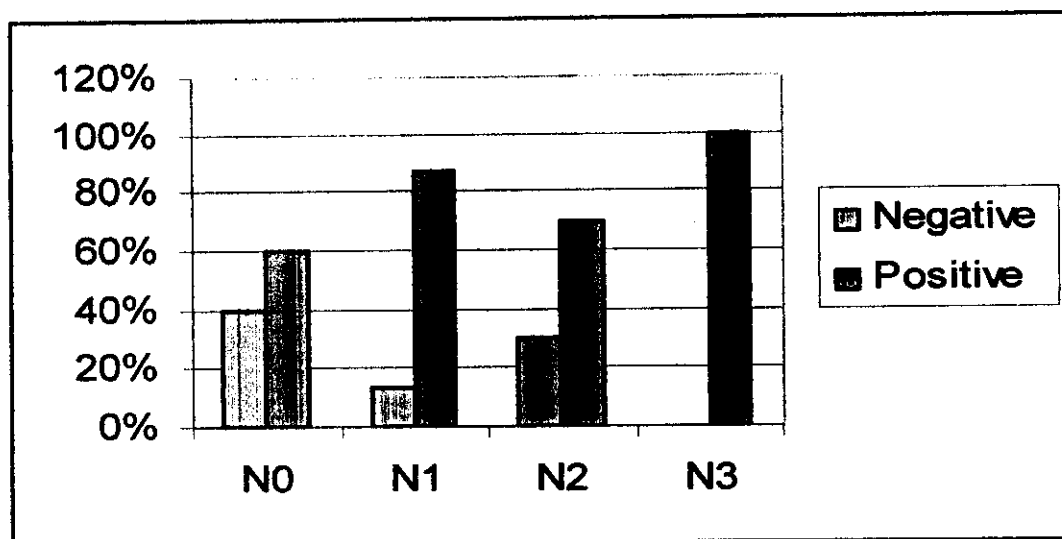
Graph (19): Relation between MMP-2 expression and state of LNs metastasis.

N.B: There is insignificant positive correlation between extent of MMP-2 expression and state of LNs metastasis (p>0.05).

Table (23): Relation between histopathological types and state of lymph nodes metastasis to extent of expression of MMP-2:

Histopathological type	No.of cases	Extent of MMP-2 expression							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
SqC.C	15	4	26.7%	6	40%	2	13.3%	3	20%
N0	3	2	66.7%	0	0%	1	33.3%	0	0%
N1	7	1	14.3%	5	71.4%	1	14.3%	0	0%
N2	4	1	25%	1	25%	0	0%	2	50%
N3	1	0	0%	0	0%	0	0%	1	100%
Adenocarcinoma	9	2	22.2%	2	22.2%	4	44.5%	1	11.1%
N0	4	1	25%	2	50%	1	25%	0	0%
N1	1	0	0%	0	0%	1	100%	0	0%
·N2	3	1	33.3%	0	0%	2	66.7%	0	0%
N3	1	0	0%	0	0%	0	0%	1	100%
UDLCC	16	3	18.8%	3	18.8%	7	43.6%	3	18.8%
N0	3	1	33.3%	2	66.7%	0	0%	0	0%
N1	7	1	14.3%	1	14.2%	4	57.3%	1	14.2%
N2	3	1	33.3%	0	0%	2	66.7%	0	0%
N3	3	0	0%	0	0%	1	33.3%	2	66.7%
Total	40	9	22.5%	11	27.5%	13	32.5%	7	17.5%

Relation of extent of expression of MMP-2 to state of distant metastasis:

Table (24): Relation of extent of expression of MMP-2 and state of distant metastasis:

| State of distant metastases	No. of cases	Extent of MMP-2 expression							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
M0	24	9	37.5%	11	45.8%	4	16.7%	0	0
M1	16	0	0%	0	0%	9	56.3%	7	43.7%
Total	40	9	22.5%	11	27.5%	13	32.5%	7	17.5%
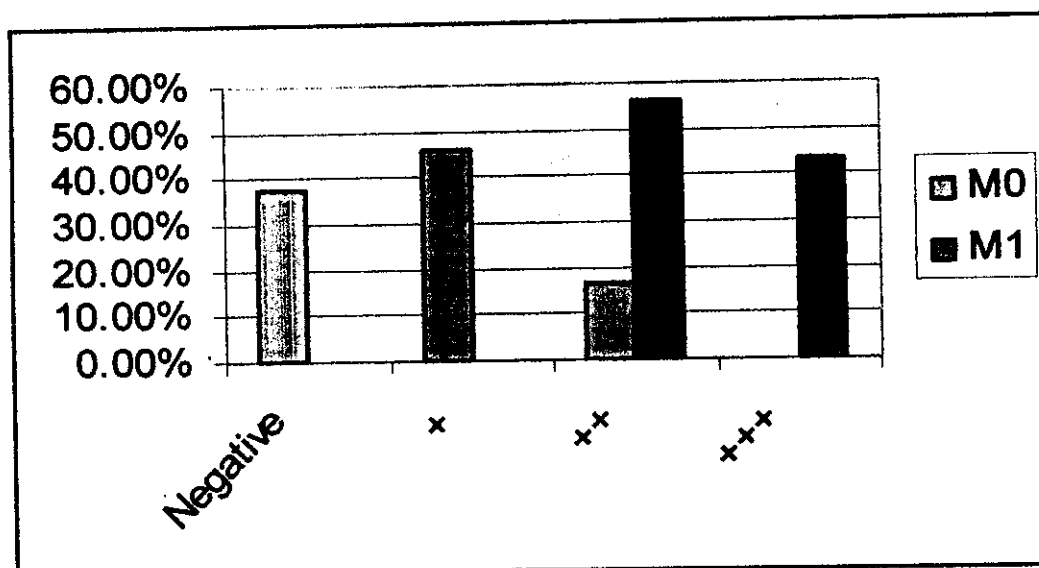
Graph (20): Relation between extent of MMP-2 expression and state of distant metastasis.

N.B: There is a significant positive correlation between extent of MMP-2 expression and state of distant metastasis (P value< 0.05).

Table (25): Relation of extent of expression of MMP-2 and state of distant metastasis:

Histopathological type	No. of cases	Extent of MMP-2 expression							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
Sq.C.C	15	4	26.7%	6	40%	2	13.3%	3	20%
M0	11	4	36.4%	6	54.5%	1	9.1%	0	0%
M1	4	0	0%	0	0%	1	25%	3	75%
Adenocarcinoma	9	2	22.2%	2	22.2%	4	44.5%	1	11.1%
M0	5	2	40%	2	40%	1	20%	0	0%
M1	4	0	0%	0	0%	3	75%	1	25%
UDLCC	16	3	18.8%	3	18.8%	7	43.6%	3	18.8%
M0	8	3	37.5%	3	37.5%	2	25%	0	0%
M1	8	0	0%	0	0%	5	62.5%	3	37.5%
Total	40	9	22.5%	11	27.5%	13	32.5%	7	17.5%

Graph (21): Relation between MMP-2 expression and state of distant metastasis.

Table (26): Relation of extent of expression of MMP-2 and stage of NSCLCs:

Stage of tumor	No. of cases	Extent of expression of MMP-2							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
Stage I	12	6	50%	4	33.3%	2	16.7%	0	0%
Stage II	8	0	0%	6	75%	2	25%	0	0%
Stage III	5	3	60%	1	20%	1	20%	0	0%
Stage IV	15	0	0	0	0%	8	53.3%	7	46.7%
Total	40	9	22.5%	11	27.5%	13	32.5%	7	17.5%
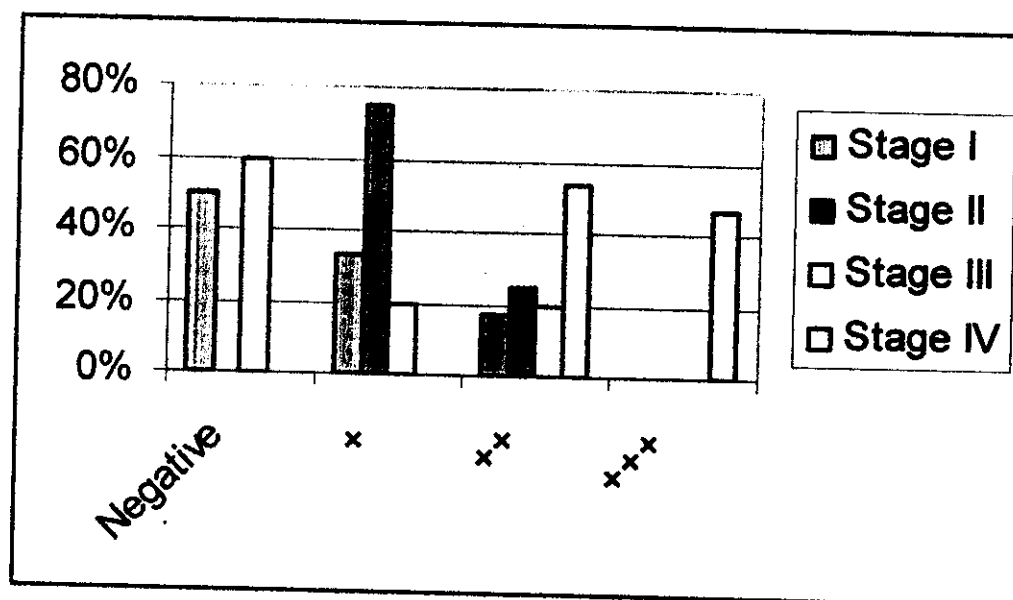
Graph (22): Relation of extent of expression of MMP-2 and stage of NSCLCs.
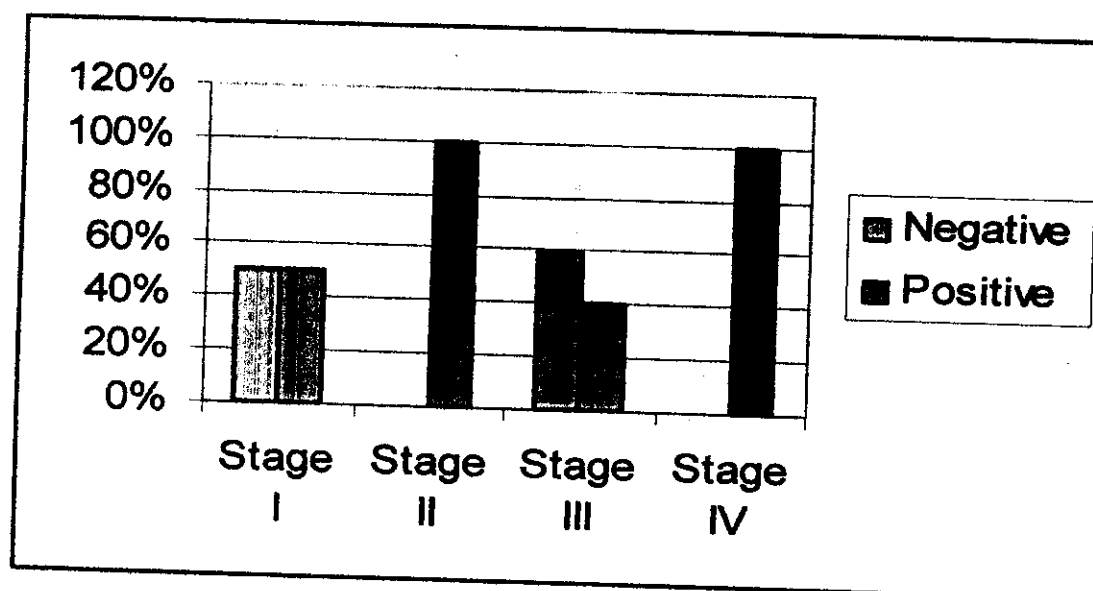
Graph (23): Relation between expression of MMP-2 and stage of NSCLCs.

N.B: There is a significant positive correlation between extent of MMP-2 expression and stage of NSCLCs (P value < 0.05) i.e. the more advanced the stage of NSCLCs, the higher the extent of expression of MMP-2.

Table (27): Relation of extent of expression of MMP-2 and stage of NSCLCs:

Histopathological type	No. of cases	Extent of expression of MMP-2							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
Sq.C.C	15	4	26.6%	6	40%	2	13.4%	3	20%
Stage I	4	3	75%	0	0%	1	25%	0	0%
Stage II	5	0	0%	5	100%	0	0%	0	0%
Stage III	2	1	50%	1	50%	0	0%	0	0%
Stage IV	4	0	0%	0	0%	1	25%	3	75%
Adenocarcinoma	9	2	22.2%	2	22.2%	4	44.4%	1	11.2%
Stage I	4	1	25%	2	50%	1	25%	0	0%
Stage II	0	0	0%	0	0%	0	0%	0	0%
Stage III	1	1	100%	0	0%	0	0%	0	0%
Stage IV	4	0	0%	0	0%	3	75%	1	25%
UDLCC	16	3	18.7%	3	18.7%	7	43.8%	3	18.8%
Stage I	4	2	50%	2	50%	0	0%	0	0%
Stage II	3	0	0%	1	33.3%	2	66.7%	0	0%
Stage III	2	1	50%	0	0%	1	50%	0	0%
Stage IV	7	0	0%	0	0%	4	57.1%	3	42.9%
Total	40	9	22.5%	11	27.5%	13	32.5%	7	17.5%

Relation between extent of expression of MMP-2 and microvessel density (MVD) (angiogenesis) indicated by extent of CD34 expression.

Table (28): Relation between microvessel density (MVD) (angiogenesis) indicated by extent of CD34 expression to extent of MMP-2 expression

Extent of expression of CD34	No.of cases	Extent of MMP-2 expression							
		-ve		+1		+2		+3	
		No.	%	No.	%	No.	%	No.	%
Hypovascular	18	9	50%	8	44.4%	1	5.6%	0	0%
Hypervascular	22	0	0%	3	13.7%	12	54.6%	7	31.7%
Total	40	9	22.5%	11	27.5%	13	32.5%	7	17.5%
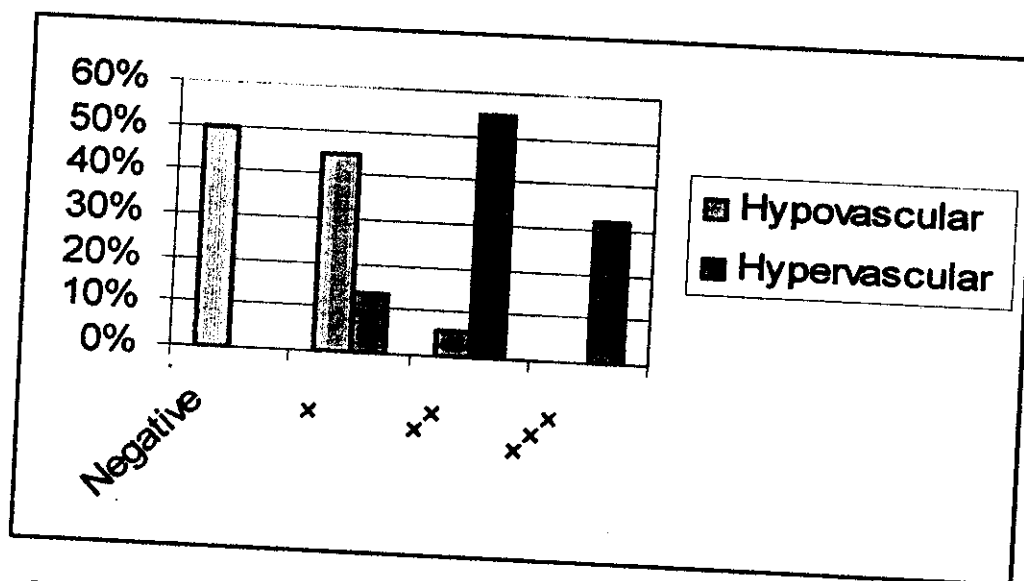
Graph (24): Relation between extent of MMP-2 expression and microvessel density (MVD) (angiogenesis) indicated by extent of CD34 expression.

N.B: There is a significant positive correlation between extent of CD34 expression and extent of MMP-2 expression (P value< 0.05) i.e. hyper vascular tumors showed higher extent of MMP-2 expression.

Table (29): Relation between extent of MMP-2 expression and different clinico-pathological parameters.

Clinicopathological parameter	Extent of MMP-2 expression							
	-ve		+1		+2		+3	
	No.	%	No.	%	No.	%	No.	%
1)Type of tumor								
Sq.C.C	4	26.7%	6	40%	2	13.3%	3	20%
Adenocarcinoma	2	22.2%	2	22.2%	4	44.4%	1	11.2%
U.D.L.C.C	3	18.8%	3	18.8%	7	43.6%	3	18.8%
2)Grade of tumor								
WD	1	33.3%	1	33.3%	1	33.3%	0	0%
MD	4	33.3%	4	33.3%	4	33.3%	0	0%
PD	1	11.2%	3	33.3%	1	11.2%	4	44.3%
UD	3	18.8%	3	18.8%	7	43.6%	3	18.8%
3)State of LNs metastasis								
N0	4	40%	4	40%	2	20%	0	0%
N1	2	13.3%	6	40%	6	40%	1	6.7%
N2	3	30%	1	10%	4	40%	2	20%
N3	0	0	0	0%	1	20%	4	80%
4)State of distant metastasis								
M0	9	37.5%	11	45.8%	4	16.7%	0	0%
M1	0	0%	0	0%	9	56.3%	7	43.7%
5)Stage of tumors								
Stage I	6	50%	4	33.3%	2	16.7%	0	0%
Stage II	0	0%	6	75%	2	25%	0	0%
Stage III	3	60%	1	20%	1	20%	0	0%
Stage IV	0	0%	0	0%	8	53.3%	7	46.7%

Results of AgNORs staining:

control group:

The AgNORs appeared as small, rounded, dense dots of uniform size and shape. Not more than 2 dots/nucleus could be noted in normal lung tissue cases with a range from 1.2 up to 1.8 dots/nucleus per case (mean 1.5).

Malignant group:

The nuclei of malignant cells contained larger number of AgNORs (up to 9), which showed more tendency to be pleomorphic both in shape and size, with some tendency to clumping. Some clusters are formed of small, dense irregular dots arranged at periphery of nucleoli. Additional, smaller distinct irregular dots were also seen dispersed in the nucleoplasm and not restricted to nucleoli. Table (30) summarizes the results of AgNORs examinations in different NSCLCs. These results are also demonstrated graphically in graph (26).

Table (30): AgNORs results in control and NSCLCs:

Histopathological type	No.	AgNORs properties				
		Range	Mean	Shape	Size	Distribution
Control	10	1.2-1.8	1.5	Uniform, rounded, regular, dense dots.	Uniform, small.	Present in the nucleoplasm.
Malignant tumors Sq.C.C Adenocarcinoma U.D.L.C.C	40 15 9 16	4.7-9.3 6.3-8.4 4.7-8.3 6.5-9.3	7.2 7.4 6.5 8	Pleomorphic, irregular dense dots, some tendency to clumping.	Pleomorphic, but generally small.	Arranged at the periphery of the nucleoli, other dots are dispersed in the nucleoplasm.
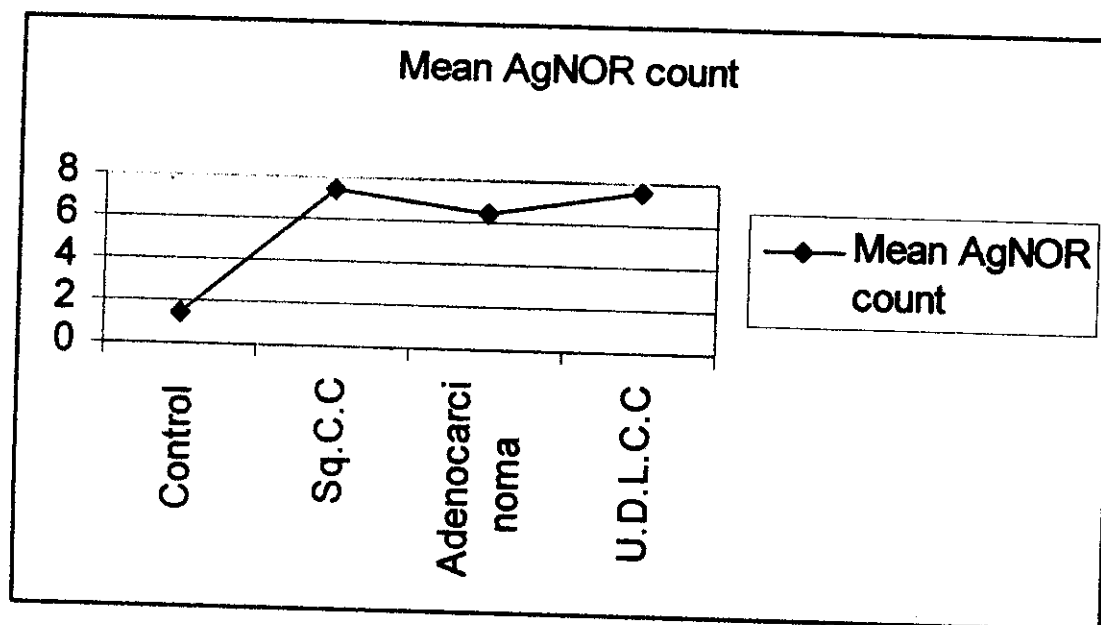
Graph (26): Mean AgNORs values in control and malignant cases.

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Correlation between AgNORs count/cell and grade of NSCLCs:

The AgNORs count/cell and tumor grade of lung carcinoma is illustrated in table (30) and graph (26).

The mean AgNORs count of MD Sq.C.C was (7 dots/nucleus), in comparison to the mean AgNORs count of PD Sq.C.C (7.85 dots/nucleus).

The mean AgNORs count of WD Adenocarcinoma was 5.1 dots/ nucleus, in comparison to the mean AgNORs count of MD Adenocarcinoma (6.7 dots/nucleus), and the mean AgNORs count of cases of PD Adenocarcinoma (6.8 dots/ nucleus).

The mean AgNORs counts of U.D.L.C.C was 8 dots/nucleus.

The mean AgNORs count/cell increased gradually with progression and decreased differentiation of NSCLCs. There is a significant +ve correlation between mean AgNORs count/cell and tumor grade (P < 0.05), and the AgNORs could differentiate between low grade and high grade tumors.

Table (31): AgNORs counts in different grades of NSCLCs.

Grade of tumor	No. of cases	AgNORs	
		Range	Mean
WD	3	4.9-6.1	5.1
MD	12	4.7-8.3	6.9
PD	9	5.8-8.4	7.6
UD	16	6.5-9.3	8
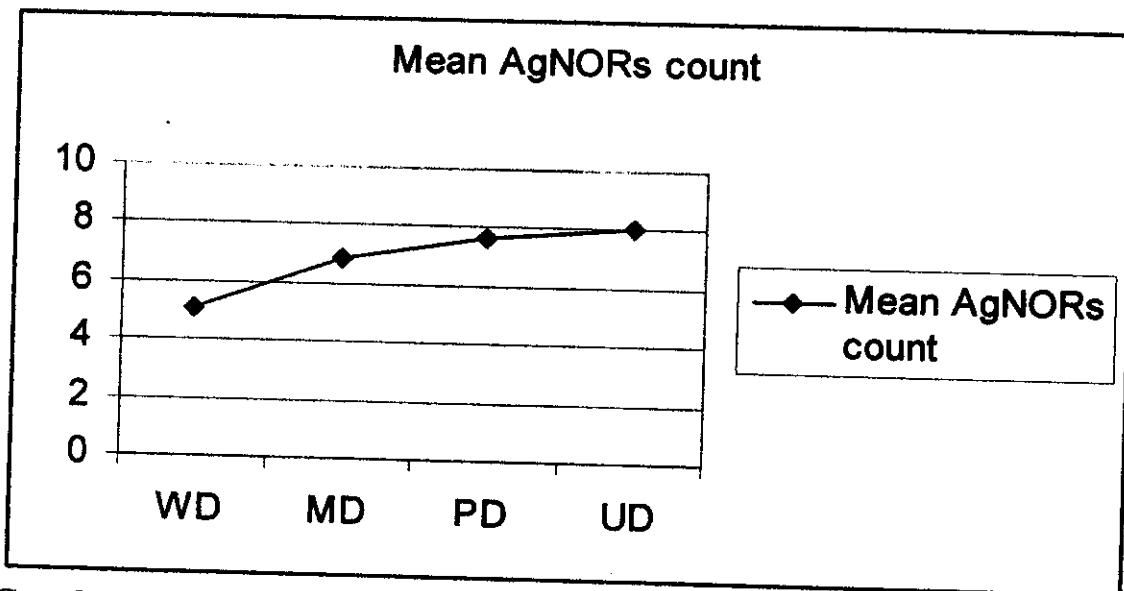
Graph (27): Relation between mean AgNORs count/cell and different grades of NSCLCs.

N.B: A significant positive correlation was found between mean AgNORs count and grade of NSCLCs ($P < 0.05$).

<u>Correlation between mean AgNORs count and state of LN metastases:</u>

Table (32): Correlation between mean AgNORs count and state of lymph nodes metastasis:

State of LNs metastases	No. of cases	AgNORs	
		Range	Mean
N0	10	4.9-8.7	7.31
N1	17	5.8-9.3	7.32
N2	9	4.7-9.1	7.56
N3	4	6.4-7.3	7.57
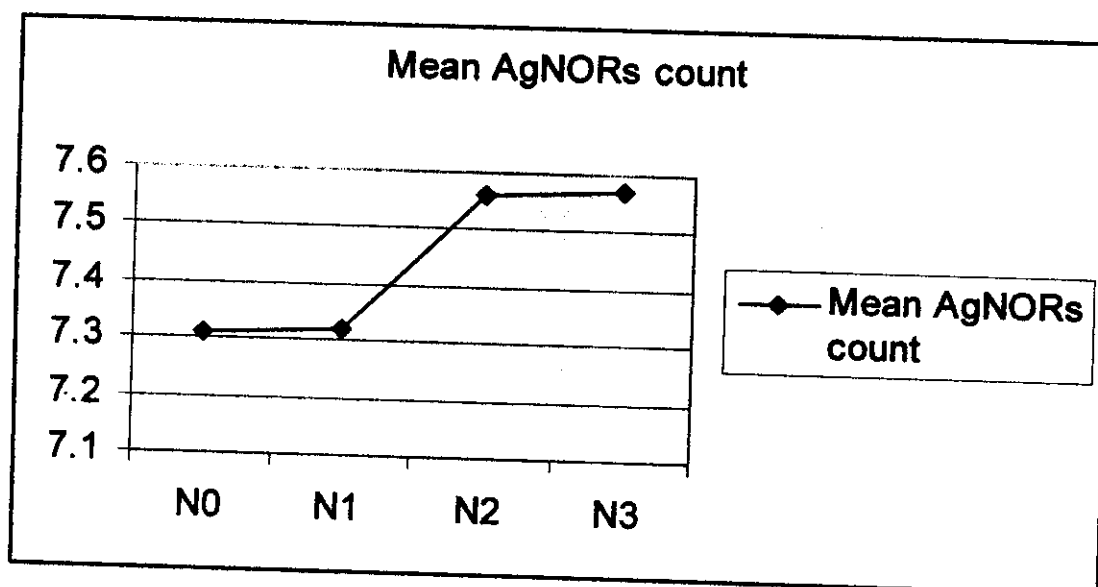
Mean AgNORs count

Graph (28): Mean AgNORs count/nucleus in relation to state of LNs metastases.

N.B: There is insignificant positive correlation between mean AgNORs count/nucleus and state of LNs metastasis (p > 0.05).

Correlation between mean AgNORs count/nucleus and stage of the NSCLCs:

Table (33): AgNORs counts in different stages of NSCLCs:

Stage of tumor	No. of cases	AgNOR	
		Range	Mean
Stage I	12	4.9- 8.3	7.1
Stage II	8	6.7- 8.4	7.5
Stage III	5	4.7- 8.4	7
Stage IV	15	5.8- 9.3	7.4
Total	40	4.7- 9.3	7.2
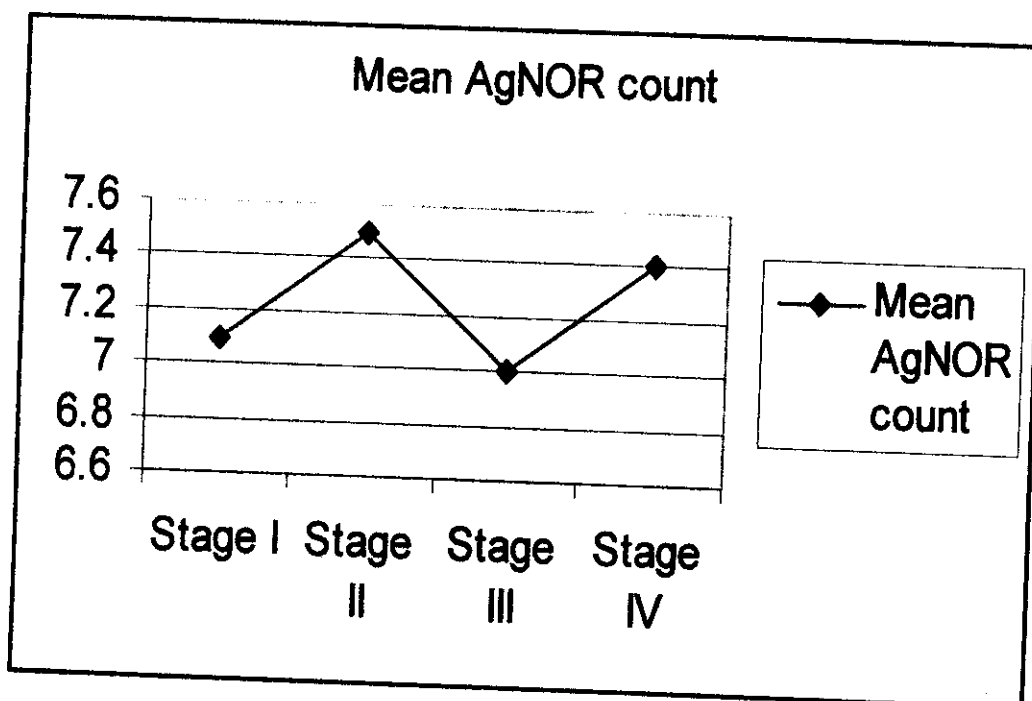
Graph (29): Mean AgNORs count/nucleus in relation to stage of NSCLCs.

N.B: There is insignificant positive correlation between mean AgNORs count/nucleus and stage of NSCLCs ($p > 0.05$).

Correlation between AgNORs count/nucleus and extent of CD34 expression:

Table (34): Correlation between extent of CD34 expression and AgNORs count/nucleus in NSCLCs.

Extent of CD34 expression	No. of cases	AgNOR	
		Range	Mean
Hypovascular	18	4.7-8.4	7
Hypervascular	22	5.8-9.3	7.52
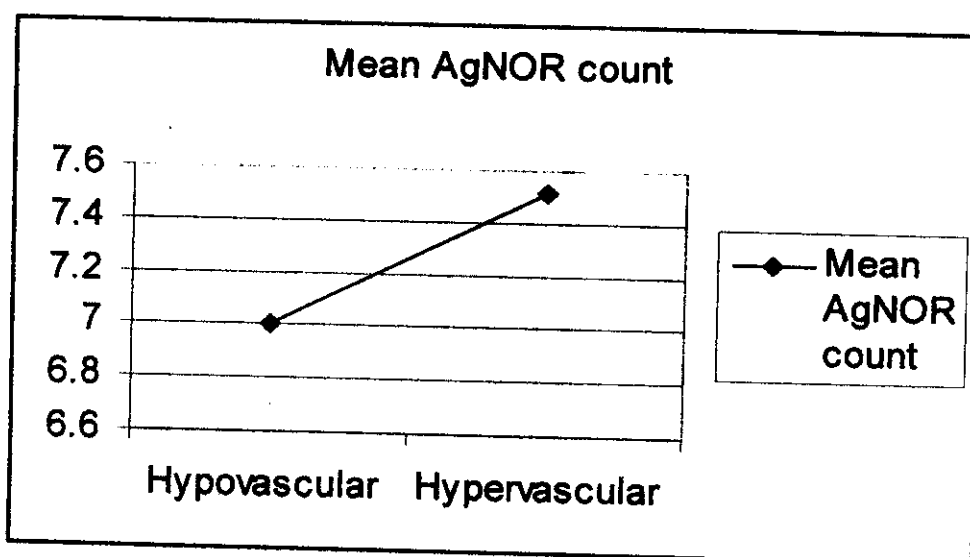
Graph (30): Correlation between extent of CD34 expression and AgNORs count/nucleus in NSCLCs.

N.B: There is insignificant positive correlation between extent of expression of CD34 and mean AgNORs count/nucleus ($P > 0.05$).

<u>Correlation between extent of expression of MMP-2 and mean AgNORs count in NSCLCs:</u>

Table (35): Correlation between extent of MMP-2 expression and mean AgNORs count in NSCLCs.

Extent of MMP-2 expression	No. of cases	AgNORs	
		Range	Mean
-ve	9	4.7-7.9	6.7
+1	11	4.9-8.7	7.6
+2	13	6.1-9.3	7.5
+3	7	5.8-8.	7.3
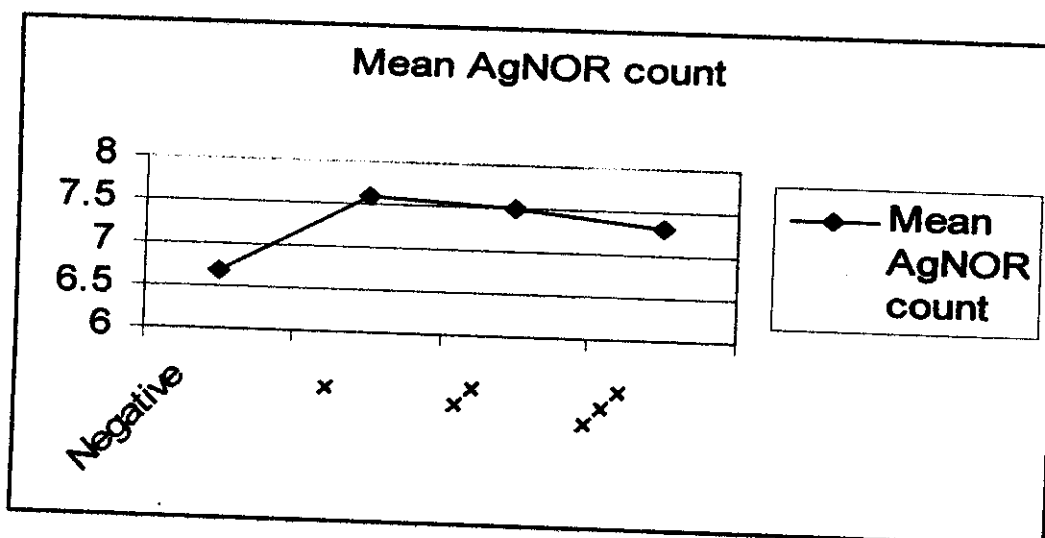
Mean AgNOR count

Graph (31): Correlation between extent of MMP-2 expression and mean AgNORs count in NSCLCs.

N.B: There is insignificant correlation between extent of MMP-2 expression and mean AgNORs count/nucleus in NSCLCs (P > 0.05).
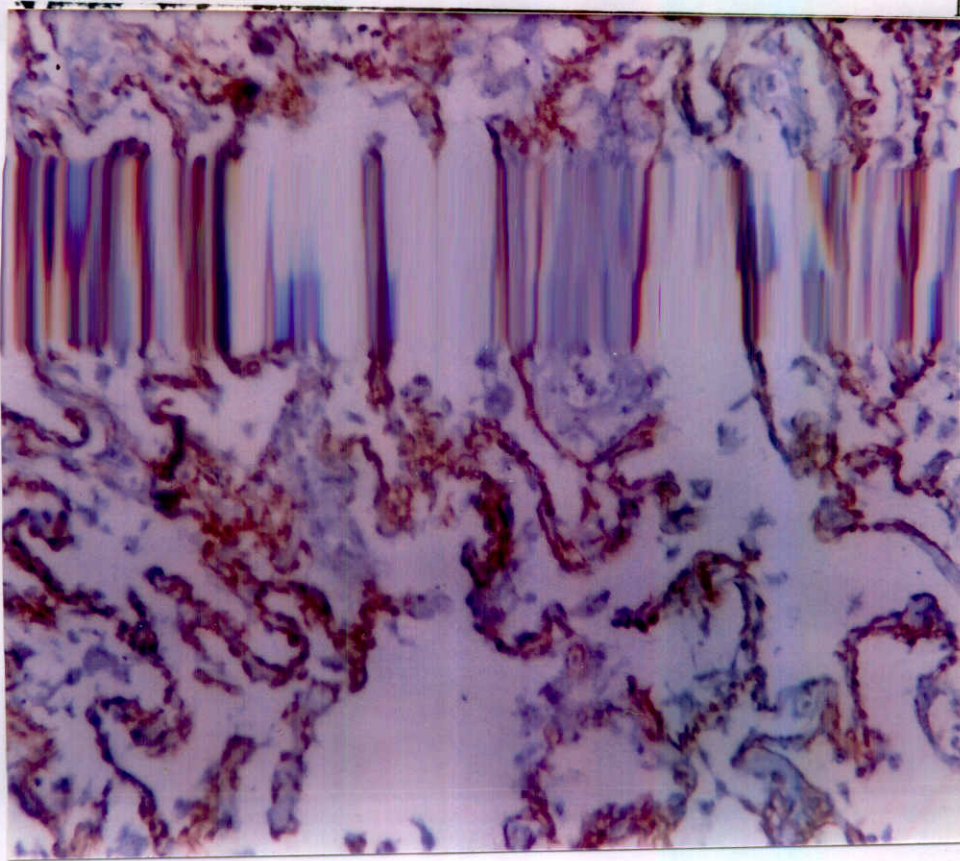
Fig (5): A case of emphysema that was taken as a control showing
immunostaining for CD34 that appears as brownish
membranous staining of (>30) newly formed blood vessels.
Streptavidin Biotin-DAB x 200.
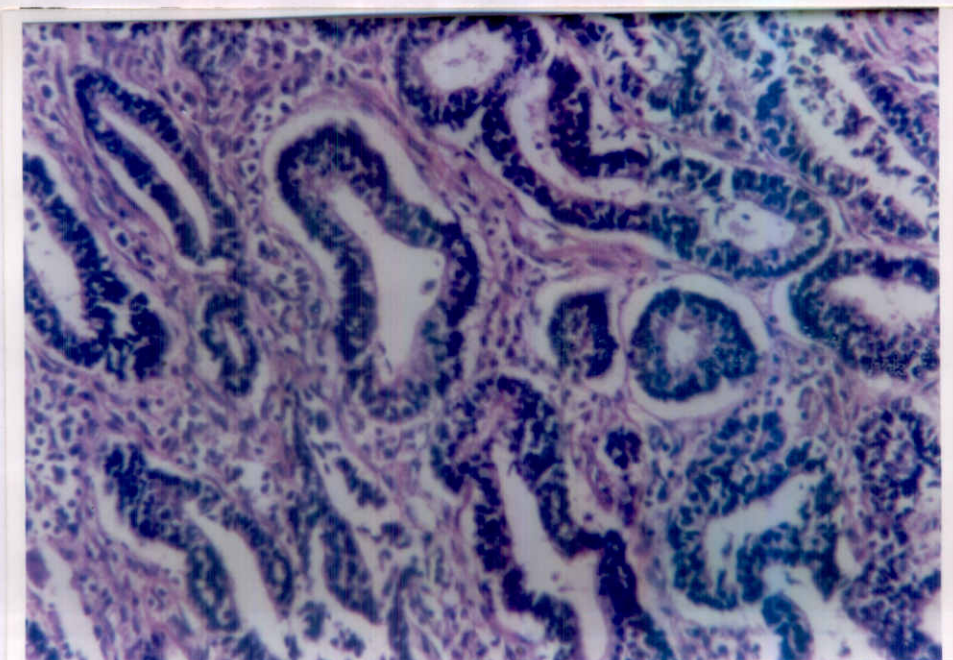
Fig (6): Well-differentiated Adenocarcinoma formed of well formed
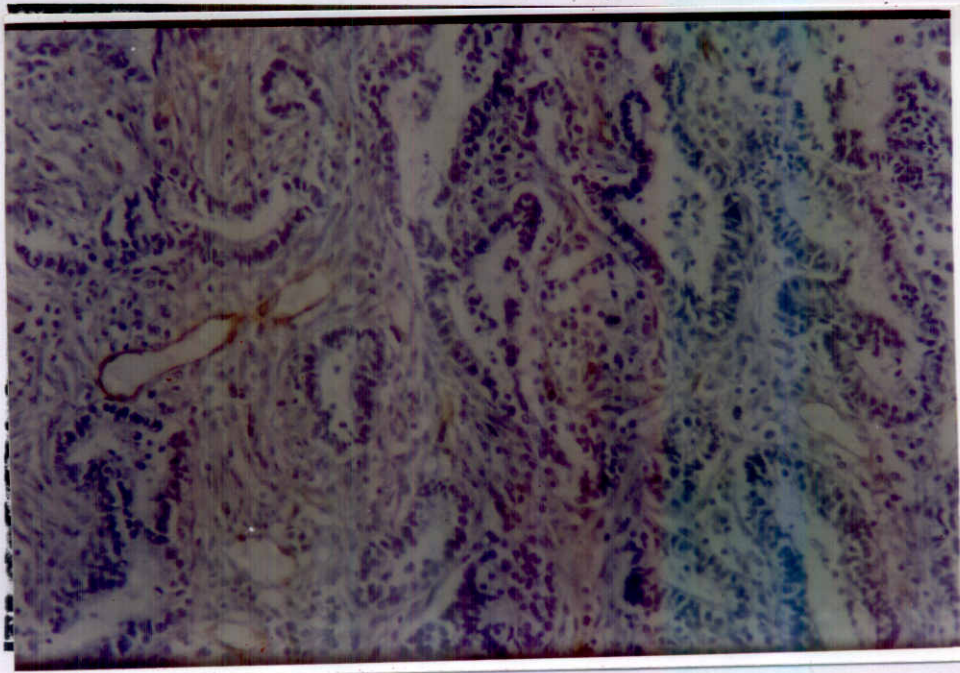
Fig (7): Well-differentiated Adenocarcinoma showing immuno staining for CD34 that appears as brownish membranous staining of (<30) newly formed blood vessels. Streptavidin Biotin-DAB x 200.
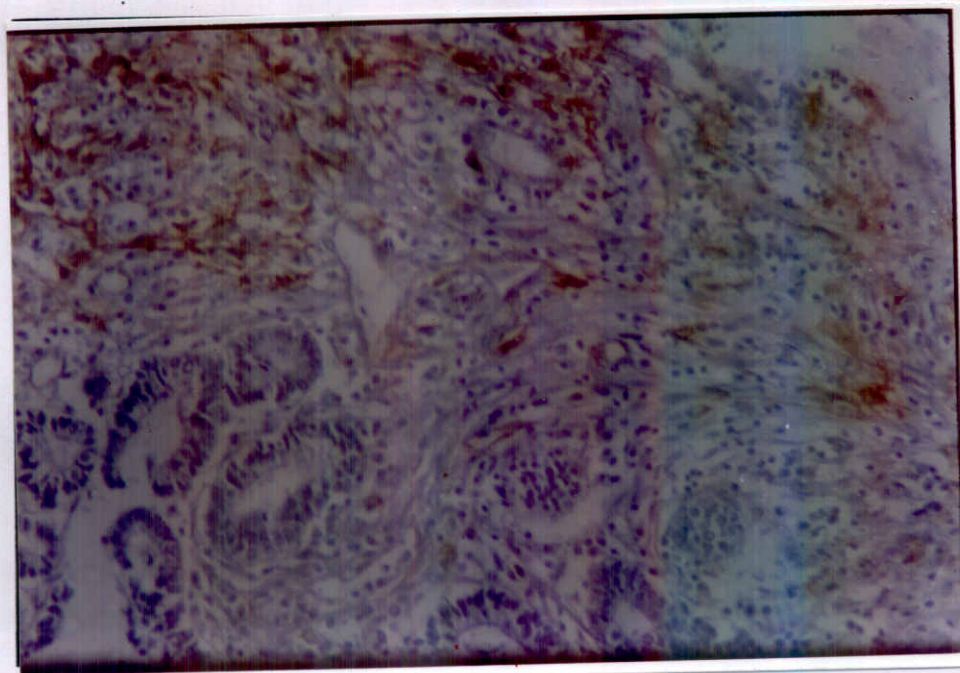
Fig (8): Well-differentiated Adenocarcinoma showing immuno staining for CD34 that appears as brownish membranous staining of (>30) newly formed blood vessels. Streptavidin Biotin-DAB x 200.
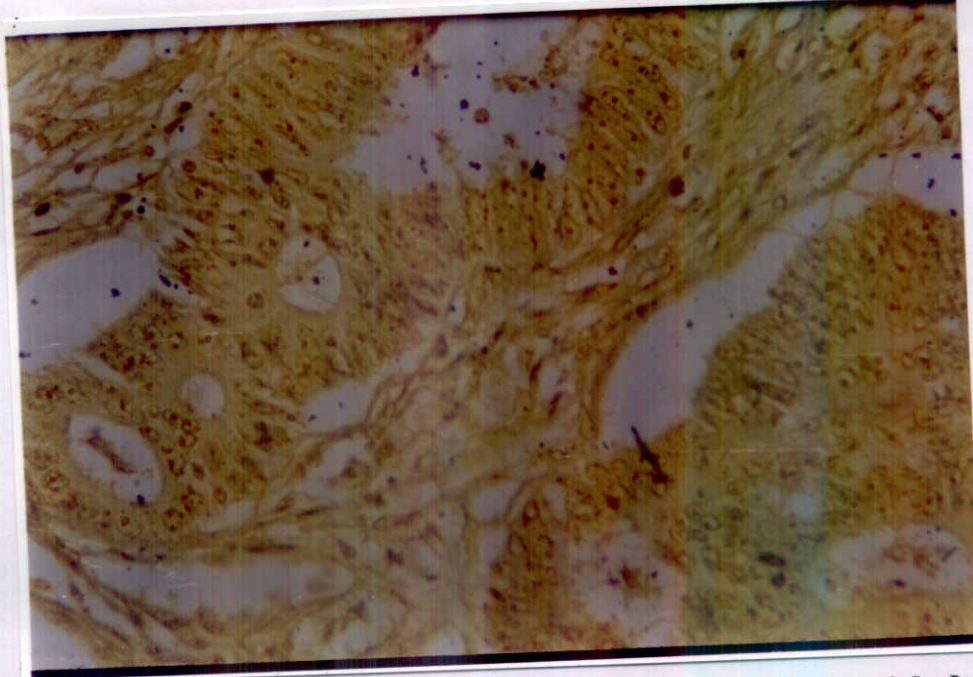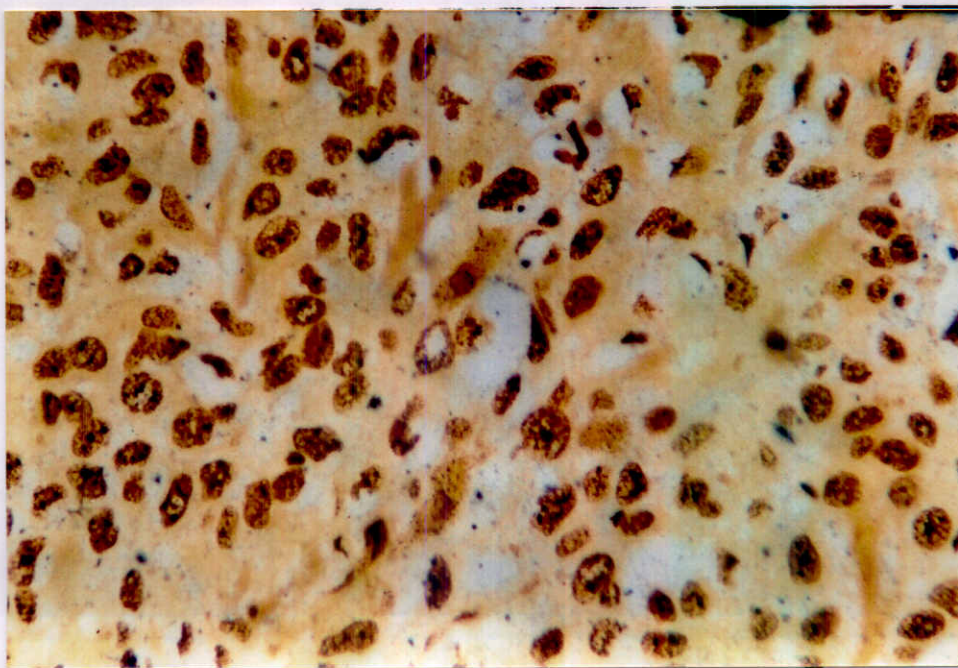
Fig (9): Well-differentiated Adenocarcinoma showing brownish dots
variable in size and shape with mean count 5 dots/nucleus.Silver
colloidal stain for AgNORs x400.
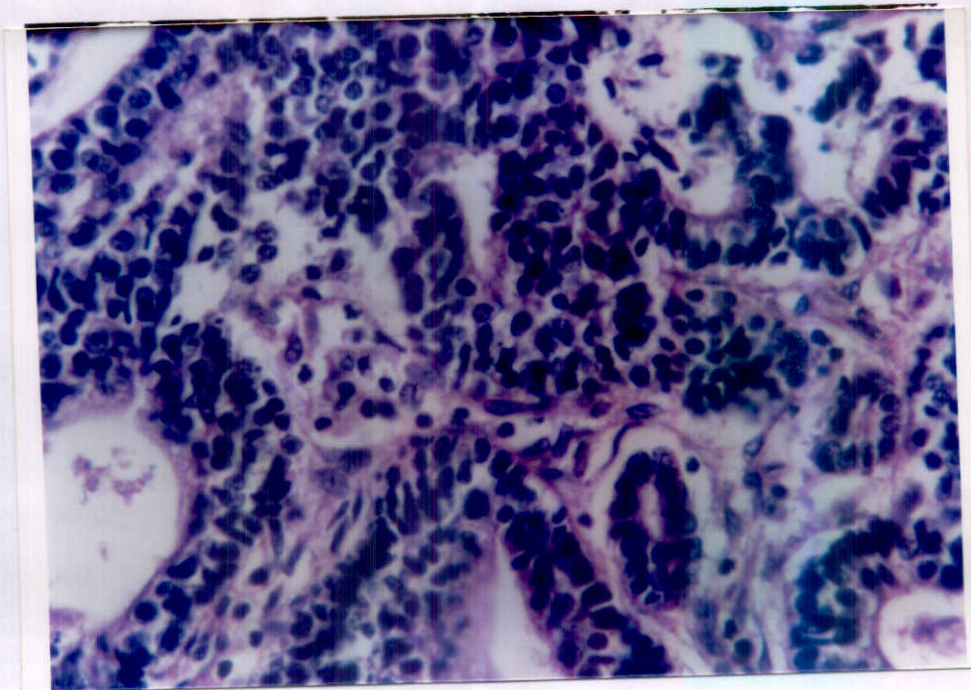
Fig (10): Moderate-differentiated adenocarcinoma formed of
alternating areas of acini and solid sheets of malignant cells.
H&E x 400.
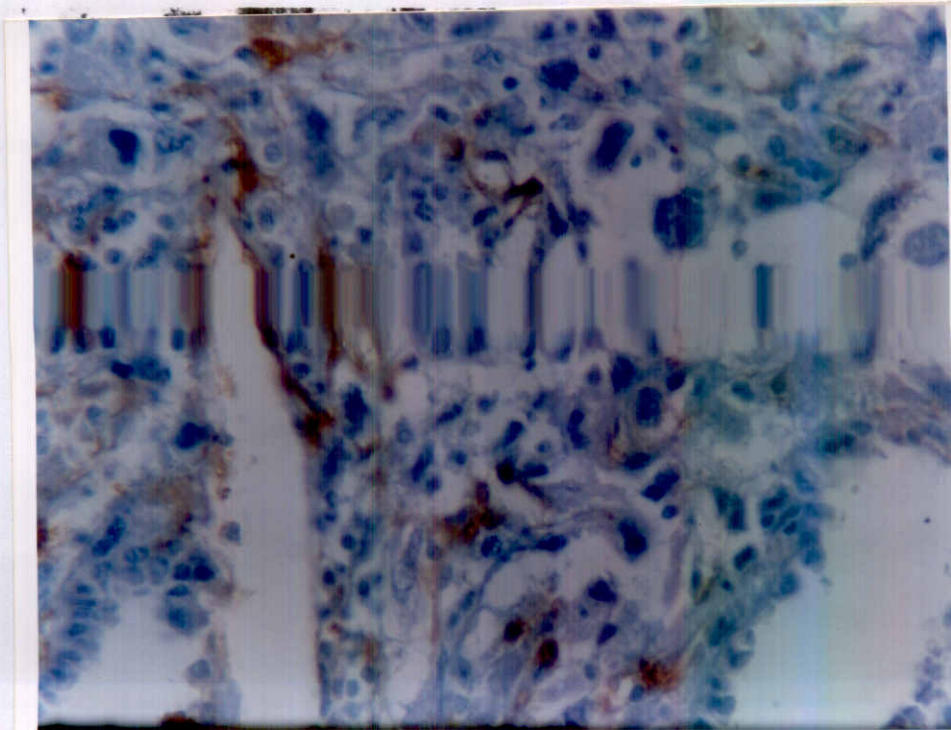
Fig (11): Moderate--differentiated Adenocarcinoma showing immuno
staining for CD34 that appears as brownish membranous
staining of (<30) newly formed blood vessels. Streptavidin
Biotin-DAB x 400.
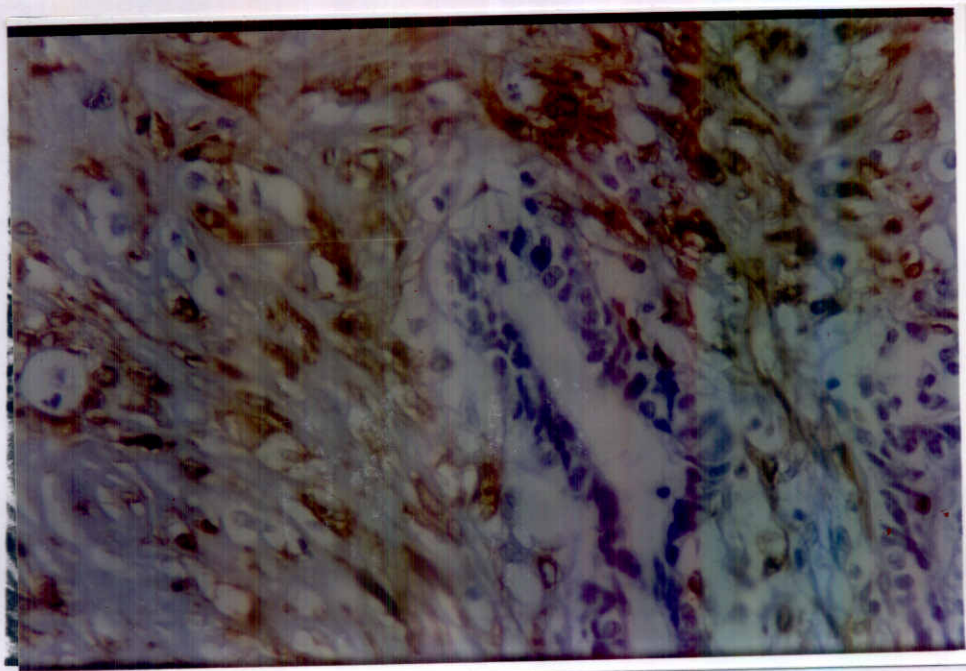
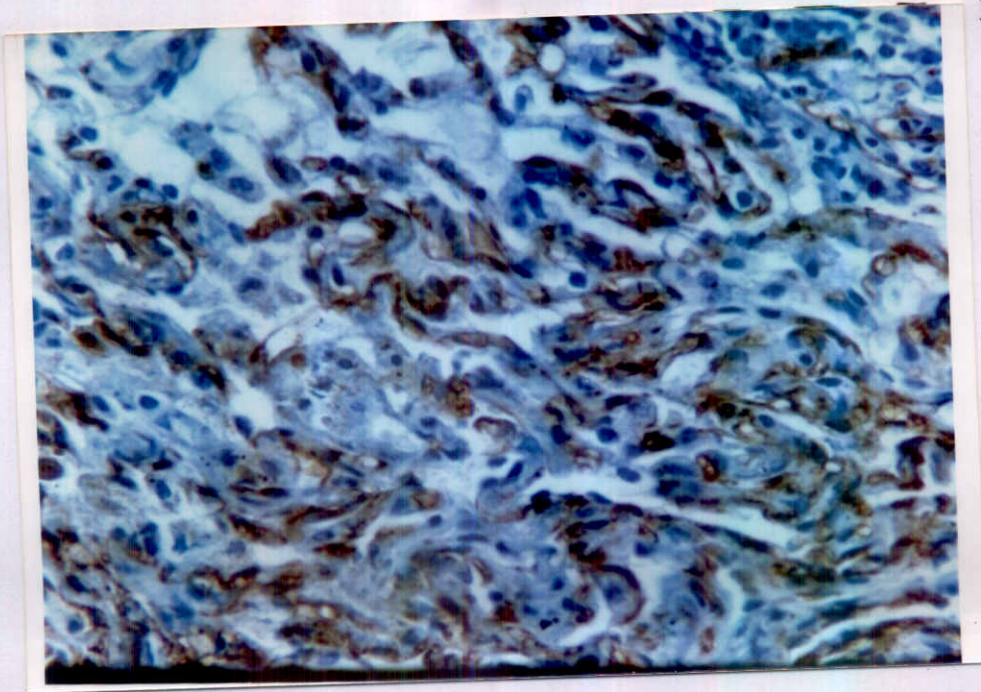Fig (12): Moderate- differentiated Adenocarcinoma showing immuno
staining for CD34 that appears as brownish membranous
staining of (>30) newly formed blood vessels. Streptavidin
Biotin- DAB x 400.
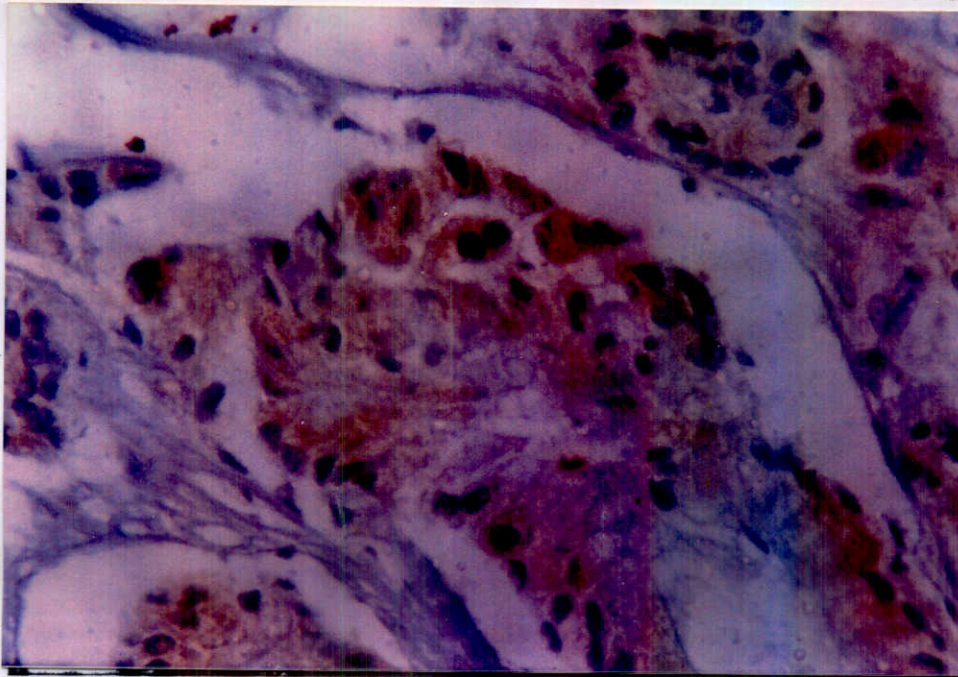
Fig (13): Moderate - differentiated bronchioloalveolar carcinoma
showing immuno staining for MMP-2 that appears as brownish
granular cytoplasmic staining of (>40%) of malignant cells.
Streptavidin Biotin-DAB x 400.
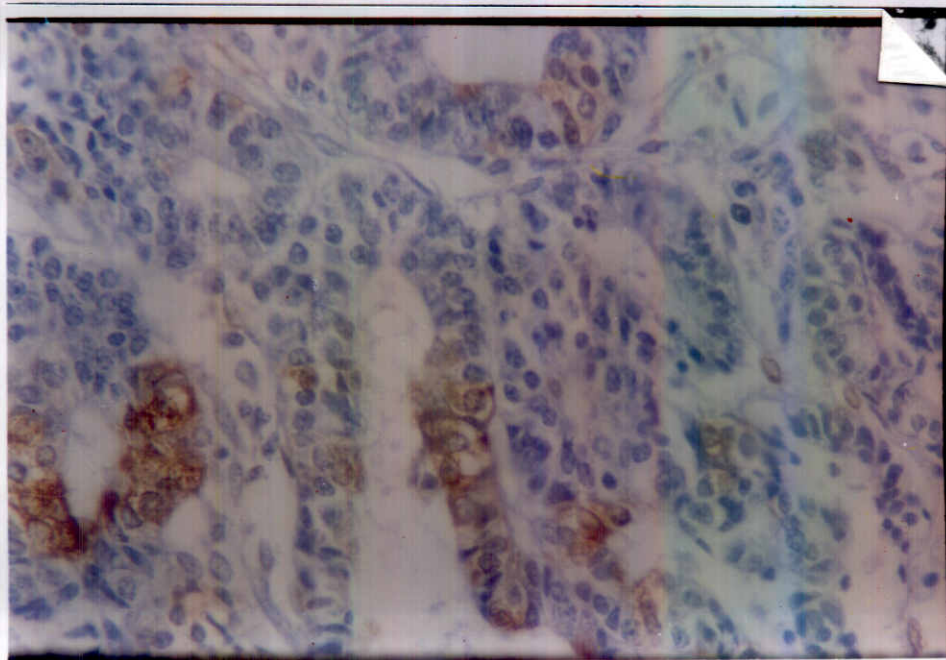
Fig (14): Moderate - differentiated Adenocarcinoma showing immuno
staining for MMP-2 that appears as brownish granular
cytoplasmic staining of (>40%) of malignant cells. Streptavidin
Biotin-DAB x 400.
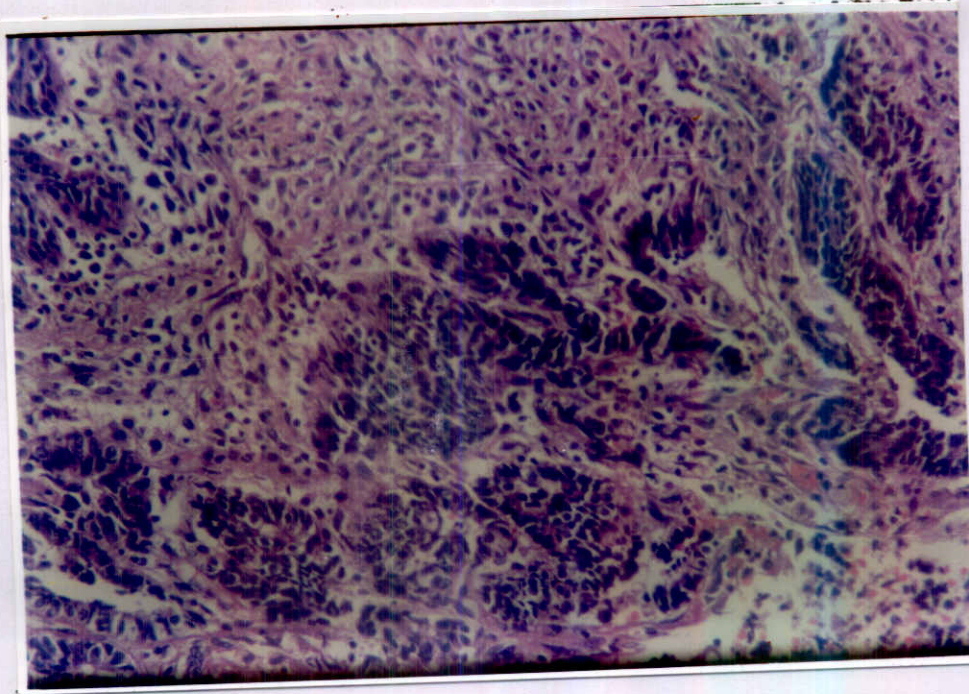
Fig (17): Poorly-differentiated Adenocarcinoma formed of solid sheets of malignant cells. H&E x 400. (200)
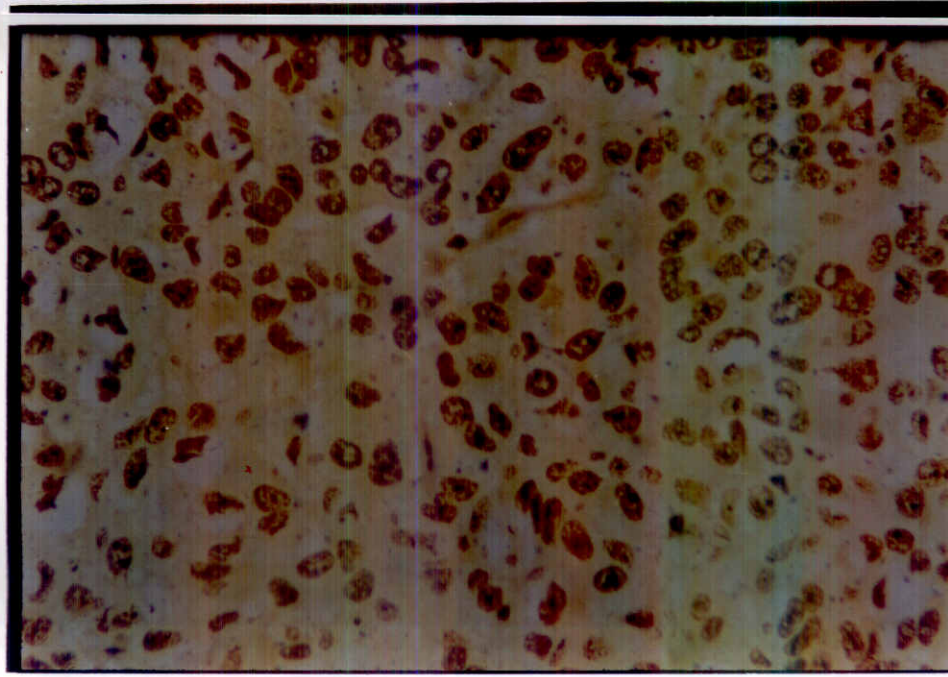

Fig (18): Poorly -differentiated Adenocarcinoma showing brownish dots variable in size and shape with mean count 6.8 dots/ nucleus. Silver colloidal stain for AgNORs x400.
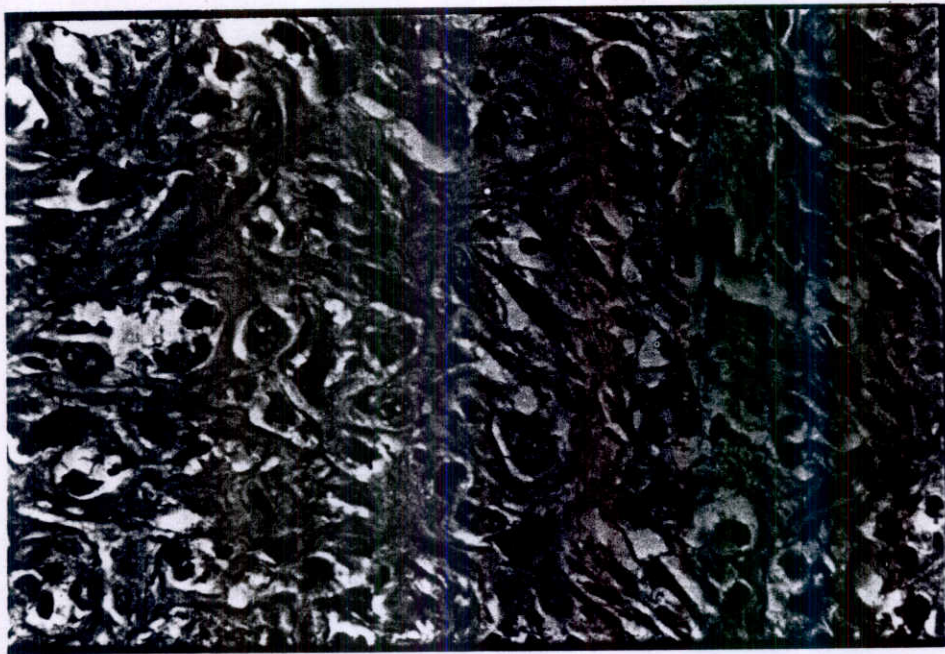
Fig (19): Undifferentiated large cell carcinoma formed of large
anaplastic cells with marked loss of polarity. H&E x 400.
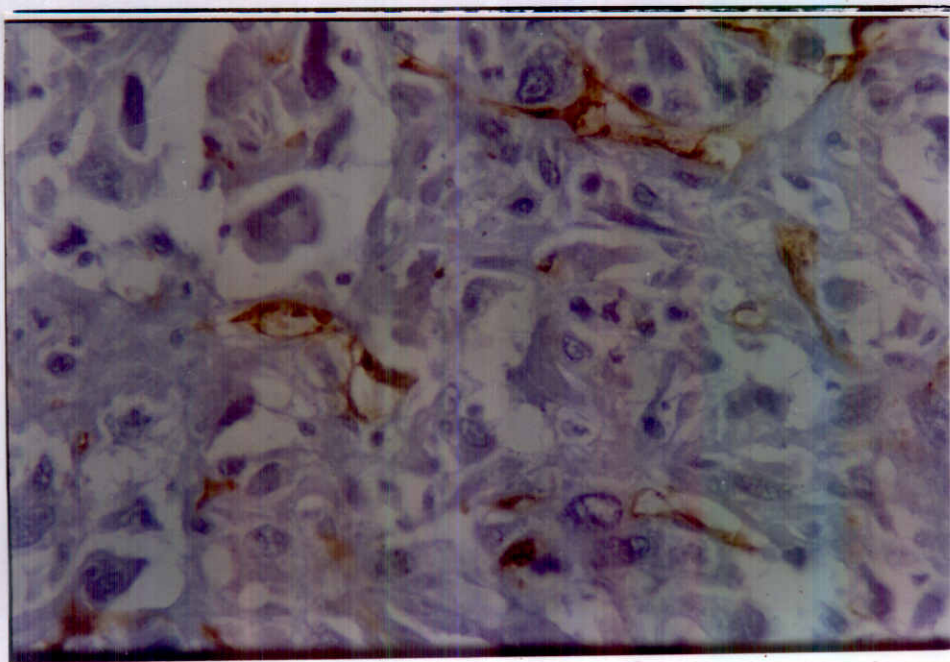
Fig (20): UDLCC showing immuno staining for CD34 that appears as
brownish membranous staining of (<30) newly formed blood
vessels. Streptavidin Biotin-DAB x 400.

Fig (21): UDLCC showing immuno staining for CD34 that appears as brownish membranous staining of (>30) newly formed blood vessels. Streptavidin Biotin-DAB x 400. 200
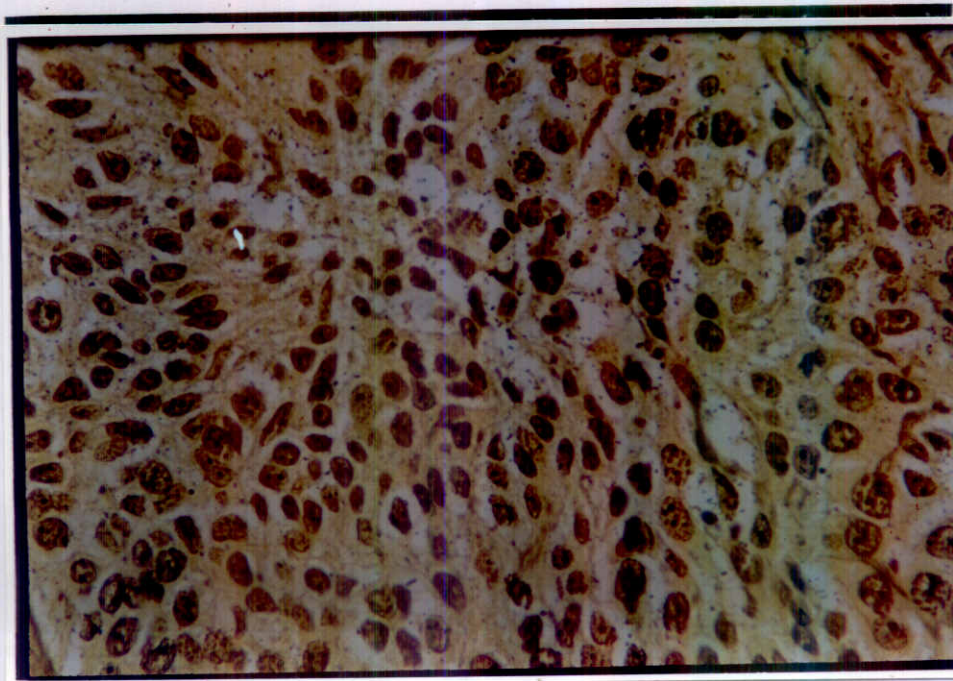
Fig (22): UDLCC showing brownish dots variable in size and shape with mean count 8 dots/ nucleus. Silver colloidal stain for AgNORs x400.

Fig (23): UDLCC showing brownish dots variable in size and shape with mean count 8 dots/ nucleus. Silver colloidal stain for AgNORs x400.
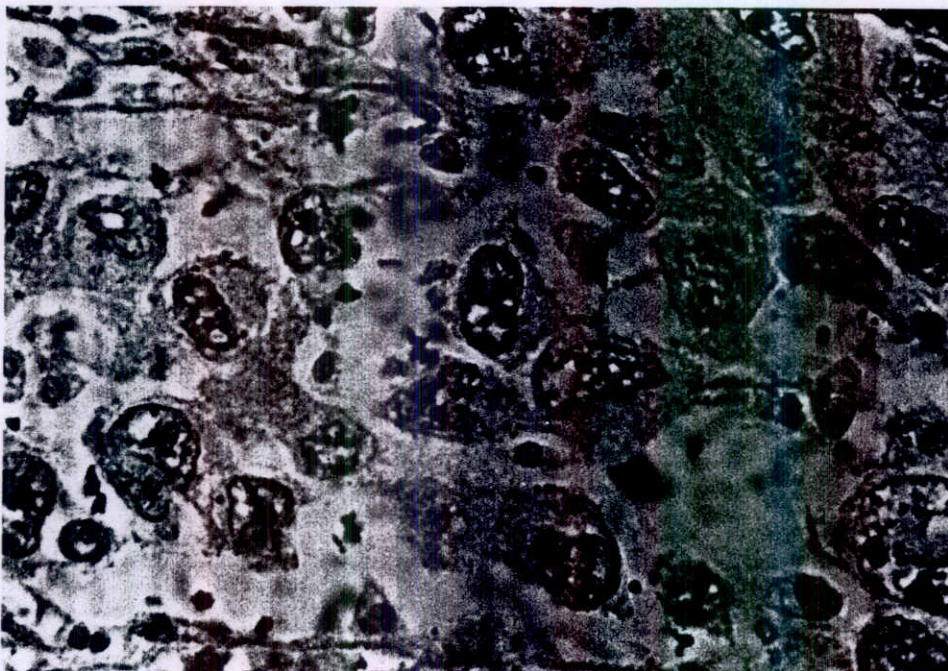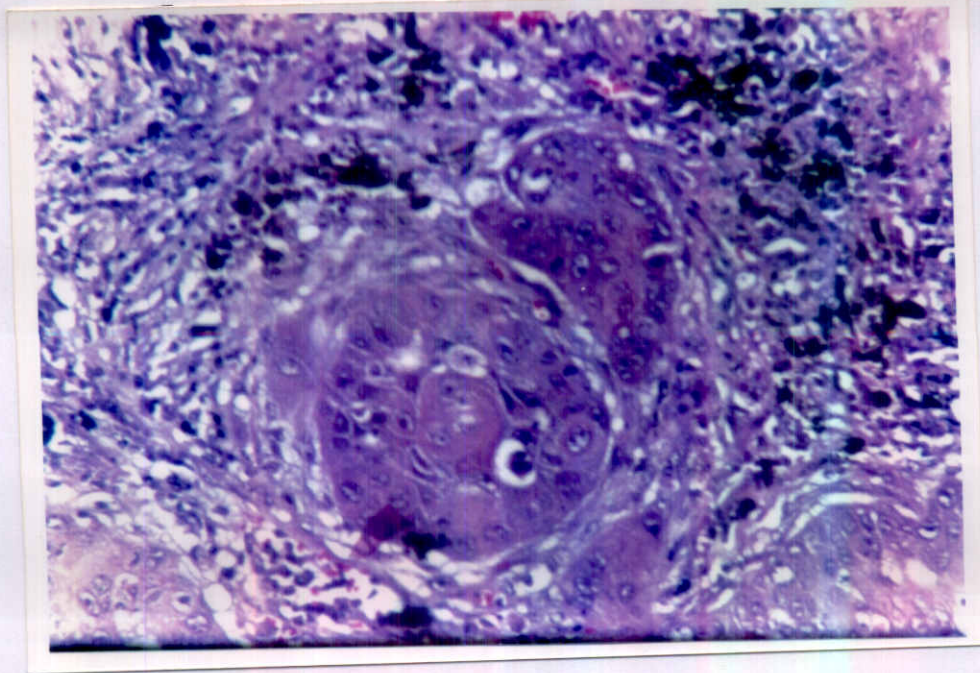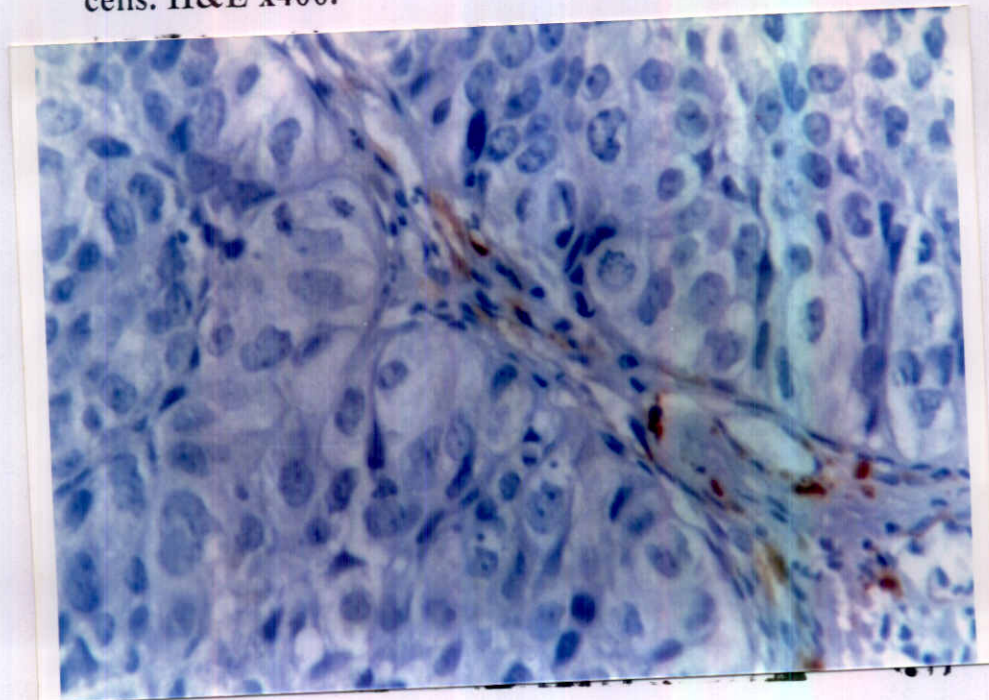

Fig (24): UDLCC showing brownish dots variable in size and shape with mean count 8 dots/ nucleus. Silver colloidal stain for AgNORs x1000.

Fig (25): Moderate- differentiated squamous cell carcinoma showing occasional cell nests with solid areas of malignant squamous cells. H&E x400.

Fig (26): Moderate- differentiated squamous cell carcinoma showing immuno staining for CD34 that appears as brownish membranous staining of (<30) newly formed blood vessels. Streptavidin Biotin-DAB x 400.
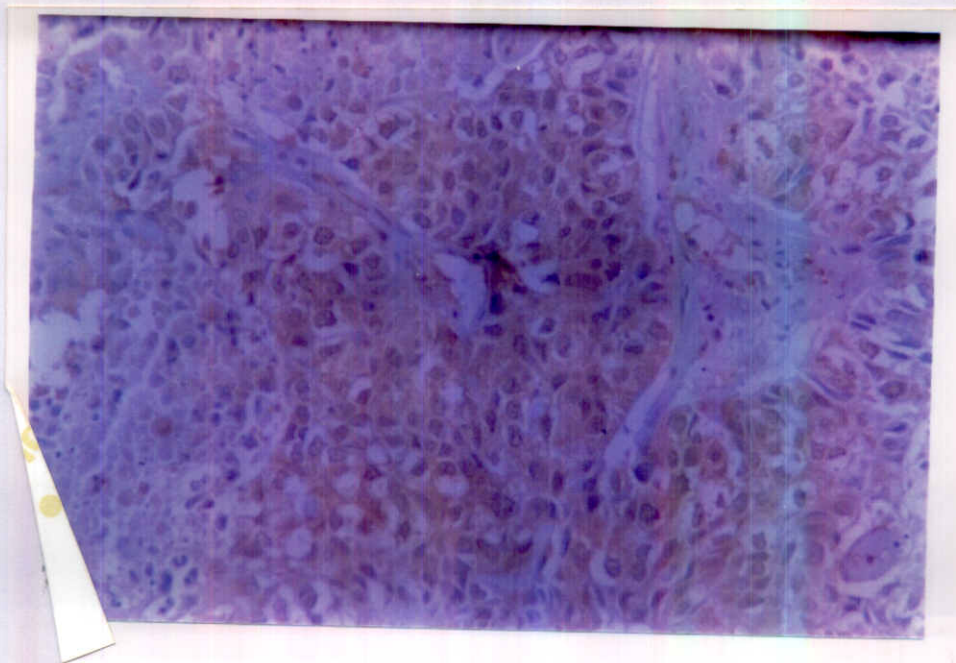
Fig (27): Moderate- differentiated squamous cell carcinoma showing immuno staining for MMP-2 that appears as brownish granular cytoplasmic staining of (>40%) of malignant cells. Streptavidin Biotin-DAB x 200.
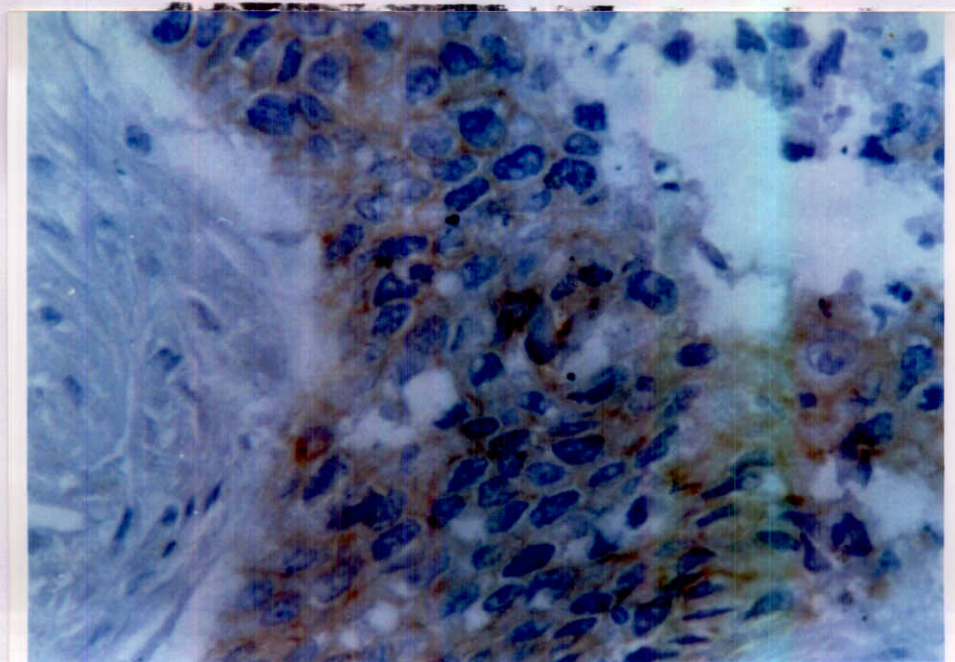

Fig (28): Moderate- differentiated squamous cell carcinoma showing immuno staining for MMP-2 that appears as brownish granular cytoplasmic staining of (>40%) of malignant cells. Streptavidin Biotin-DAB x 400.