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Correlation of Grading, Staging and Tumor Characteristics with Micro vascular Density by CD34 and CD 105 in Colorectal Carcinoma

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Abstract: *Colorectal carcinoma is one of the commonest cause of death worldwide. Folkman first proposed that tumor angiogenesis could serve as a potential target for anticancer therapy. Sustained angiogenesis has been the one of the hallmarks of malignancy. Angiogenesis has been studied using immunohistochemical (IHC) staining of microvessels with markers like* CD 31, CD34, CD105, Factor VIII related antigen, PECAM -1 and vWF *then by calculating the Micro vessel density (MVD) and other morphometrical parameters like vessel caliber(VC),micro vessel cross-sectional area(VCSA) and % Total vessel area (%TVA). The objective of the study is two fold first to compare intratumoral microvessels density and extratumoral microvessels density, second to find out any significant positive correlation between intratumoral microvessel density with grading and staging by using CD34 and CD105.*

Results: *Intratumoral MVD was significantly more as compared to extratumoral MVD in our study with p value <0.001 by unpaired 't' test . Intra tumoral MVD, VC, and %TVA does not differ among different grades and stages of tumor. Intratumoral MVD and %TVA were increased uniformly compared to extratumoral MVD and %TVA irrespective of TNM staging, grading, lymphnode metastasis, perineural and lymphangioinvasion. As neo angiogenic vessels are marked by CD105, CD 105 appear to be a better marker than CD 34 for studying neo angiogenesis. Further interventional studies were needed to determine definite role of CD 105 as a target molecule for anti angiogenic therapy similar to bevacizumab*

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I. Introduction

Colorectal cancer (CRC), incidence is increasing in incidence and one of the most common cancers causing death worldwide[1], is treated with surgery, and patients with stage III (Dukes' C) and a subset of stage II CRC (Dukes' B2) are treated with adjuvant therapies. Still the loco regional recurrence after curative resection remains a problem. The main prognostic factors in CRC are lymph node involvement, size of the tumor and stage of disease [2].

Folkman first proposed that tumor angiogenesis could serve as a potential target for anticancer therapy [3]. A tumor cannot grow beyond 1 to 2mm even if it possesses all the genetic aberrations required for malignant transformation. Angiogenesis is essential facet of malignancy. Neovascularization has a dual effect it not only provides nutrition to the tumor cells, the endothelial cells also release growth factors like insulin like growth factor(IGF) and platelet derived growth factors(PDGF) which helps in growth of the tumor. Since tumor growth and metabolism of the tumor depends on the quantum of blood circulating through the microvessels, which in turn depends on the microvessel cross sectional area, estimation of mean caliber, cross sectional area and caliber distribution of the microvessels of CRC, and their correlation with tumor grade may have a prognostic bearing in CRC.

Angiogenesis is studied by calculating the microvessel density (MVD) i.e. number of vessels per unit area or per field. Intratumoral MVD is the gold standard method for study of angiogenesis. MVD, as a surrogate marker of tumoral angiogenesis, has been proposed to identify patients at high risk of recurrence. It was first developed by Weidner et al in 1991 using immunohistochemical (IHC) staining of microvessels. MVD can be estimated by staining endothelial cells of blood vessels by various IHC markers like CD 31, CD34, CD105, Factor VIII related antigen, PECAM -1 and vWF. RS Saad et al concluded that CD105 as a more sensitive and specific marker for tumor angiogenesis than any other commonly used pan endothelial marker such as CD31 and CD34 from their study[4]. Weidner and Tenderenda et al carefully counted the microvessels (per 200x

field), and graded MVD (1 to 4+), in the most active areas of neovascularization concluded that MVD may be an independent predictor of metastasis[5].

II. Aims and Objective

The objective of the study is two fold first to compare intratumoral microvessels density and extratumoral microvessels density, second to evaluate any significant positive correlation between intratumoral microvessel density with grading and staging by using CD34 and CD105. Intratumoral MVD was significantly more as compared to extratumoral MVD in our study. Intra tumoral MVD, VC, and %TVA does not differ among different grades and stages of tumor. This will improve our current knowledge of the neoangiogenesis in colorectal carcinoma. It will endeavor to establish the definitive role of CD 105 and CD34 expression in colorectal carcinoma.

III. Material And Methods

This is a retrospective study of 50 consecutive resected cases of CRC carried out at Department of Pathology in a tertiary health care centre from January 2010 to Sep 2012. Only those cases with adenocarcinoma or its variants were selected. Cases of lymphoma, gastro intestinal stromal tumor (GIST), and signet ring cell carcinoma were not included in the study. An excel sheet was made after reviewing the grossing notes and the relevant gross details of the tumor like site, size, type of growth whether ulcerative / proliferative, and involved nodes were noted. The sections submitted for H &E were reviewed. Those sections with maximum depth of tumor invasion at normal and tumor tissue interface were selected and their blocks were retrieved for performing IHC. The tumors were staged according to TNM classification. Relevant data from oncology files regarding the patient was noted. Immunohistochemistry was done using 4µm sections from selected blocks using poly lysine coated slides. The antibodies (DAKO) CD34: Monoclonal Mouse anti-human CD34, (clone QBEnd10, ready-to-use) and CD105: mouse monoclonal IgG2a antibody were used.

After immunohistochemistry the sections were screened for 'hot spots', (i.e the area which appear to have maximum vessel density) in both intratumoral and extratumoral area. Images from five high power fields (400×) in the hot spot area were recorded for each sample. Microvessels in these hot spot areas were marked using Microsoft paint software. A single microvessel was defined as any brown immunostained endothelial cell separated from adjacent microvessels, tumor cells and other connective tissue elements. Branching structures were counted as a single vessel, unless there was a break in the continuity of the structure. In this way, the number of vessels was noted in five fields in the "hot spots" at 400x i.e. an area of 0.289 mm².

Morphometric analysis was performed on CD-34 and CD105 immunostained sections using a computerized digital photomicrograph system (Dewinter Optical Inc. with Digi Eye 330 digital photomicrography camera and Biowizard 4.2 Image analysis software). The measuring scale of the image analysis software was properly calibrated with the standard scale, as per instruction given in the software manual. The images were processed using the software to get sharp microvessel boundary before performing the measurement.

MVD was obtained by dividing the total number of microvessels by the total area of the five hotspots. Final MVD was calculated in terms of number of vessels per $1mm^2$. The microvessel caliber (VC) was measured using the software. It was considered that the cross section of a microvessel is circular. In the histological section a microvessel can be cut in any possible direction, hence it may appear as circular, elliptical, parallel lines (when the VC was large and cut longitudinally near the central axis), as an solid bar (when the VC was small and cut longitudinally near the surface) and as an irregular structure (when the microvessel had kinks in the section) [6,7]

Therefore, we measured VC in the following five ways:
(a) diameter of a circle
(b) minor axis in an ellipse
(c) average distance between parallel lines
(d) average thickness of a solid bar, and
(e) minimum feret diameter of an irregular structure

To measure the VC, the cursor was traced on the microvessel and then the software automatically measured its length. After measurement, the data was transferred to MS Excel sheet for further analysis. VCSA and TVA were calculated from the formula given below.

% Total vessel area (TVA) = {sum of all microvessels area appeared on five 400x sections ÷ Total area of the five sections} x 100.

VCSA (micro vessel cross-sectional area) were calculated from its microvessel caliber (VC) by using the formula, VCSA=$\pi VC^2 /4$

Statistics:

Statistical analysis was done using IBM SPSS (Statistical Package for Social Sciences) statistics software version 20

IV. Results

The most common age group affected by CRC in this study was patient in seventh and eighth decade with mean age of 60 years. 52% of cases were belonging to this age group. Out of total 50 cases 41 (82%) cases were males and 9(18%) cases were females. In our study most common site involved by colorectal carcinoma was rectum and anal canal. 15 cases presented in the rectum and anal canal. Another 14 cases presented with growth in ascending colon and hepatic flexure. In general left sided (30/50) tumors were common than right sided tumors. Margins were involved in 2 of the 50 cases. Grossly 37 tumors were ulceroproliferative growth, 12 tumors were circumferential growth and one tumor was variegated growth. Maximum dimensions of the tumor were ranging from 2 cm to 21.5 cm with an average of 6.1cm. The tumors were graded according to the AJCC/UICC TNM protocol [8]. Most of the cases were classified as moderately differentiated adenocarcinoma.

Out of total 50 cases, 27 were moderately differentiated tumor. 16 and 7 cases were well differentiated and poorly differentiated adenocarcinoma respectively. The predominant group of tumors in our study belongs to stage II (20 cases), 6 cases had metastasis presented with stage IV, 18 cases were stage III and 12% cases were stage I. 18 cases show presence of nodal metastasis. 5 cases showed perineural invasion, 17 cases showed lymphangioinvasion. 4 cases showed both perineural and lymphangioinvasion. 2 cases showed metastatic deposits in liver. 16 cases showed serosal, soft tissue and peritoneal deposits. (Table 1)

Morphometry results:

In the intratumoral area by CD34, the mean MVD was 246/mm^2, having minimum MVD=156/mm^2 and maximum MVD = 451/mm^2. The mean VC obtained by CD34 in our study, in intratumoral areas was 13.00µm with a minimum of 8.15µm and a maximum of 19.83µm. The mean MVD in intratumoral area by CD105 was 204/mm^2 with a minimum of 83 and maximum of 378 /mm^2. The mean VC by CD 105 in intratumoral area was 13.51 µm with a minimum of 8.70 µm and a maximum of 20.39 µm. In our study the mean intratumoral MVD, VC, %TVA and VCSA was significantly higher as compared to extratumoral MVD by both markers, CD 34 as well as CD 105 with a significant p value(Table 2). In our study the number and size of blood vessels in intratumoral area were more as compared to extratumoral area.

The mean intratumoral MVD was almost similar in three grades by both markers (Table 3). There was no significant positive or negative correlation of MVD with increasing grade of the tumor by both tumor. In our study, cases without perineural invasion (Table 4) had significantly higher MVD (253/mm^2) than cases with perineural invasion (186/mm^2) by CD34. However there is not much significant difference between MVD by CD105 in between cases with perineural invasion and cases without perineural invasion. Our study did not show any statistically significant difference of mean MVD, VC, %TVA in between cases with regional lymph node metastasis, lymphangioinvasion, metastatic liver deposits and cases without regional lymphnode metastasis, lymphangioinvasion and metastatic liver deposits by both markers. (Table 4 and 5) There was no statistically significant difference between stage of the tumor and MVD observed in our study by using one way ANOVA test (p value 0.983) by both CD 34 and CD 105. Fig 1a&1b represents the distribution of MVD according to stage of tumor in study subjects by CD34 and CD105

DIFFERENCE BETWEEN CD34 AND CD 105:

In our study, on comparing morphometrical parameters obtained between CD34 and CD 105 IHC, the mean intratumoral MVD (204 ± 60/mm^2**)** obtained by CD105 was significantly lower than the mean intratumoral MVD (246±73/mm^2) obtained by CD34(Figure 2 & 3ab). The mean VC by CD 105 (13.51µm) was slightly more as compared to mean VC by CD34 (13µm). Hence the number of blood vessels marked by CD 105 perunit area (i.e MVD) was less as compared to CD 34, however the caliber of microvessels (i.e VC) stained by CD105 was more(Figure 3ab). The mean %TVA by CD105 was also low as compared to the mean %TVA (8.95) by CD34.

V. Discussion

Angiogenesis is central to the development of tumor and its progression. Angiogenesis of both tumors and pathological processes have been studied extensively all over the world. MVD is the gold standard method for studying angiogenesis. In our study we used CD 34 a pan endothelial marker and CD 105 a relatively new marker for endothelial cells which highlights endothelial cells in neo angiogenic vessels only. In our study most of our cases (52%) were in seventh and eighth decade with a mean age of 60 years. This result is similar to an American study where the maximum number of new CRC cases occurred in the age group of 65-79 years in both males and females[9].

In our study, 82% of cases were males with a male female ratio of 4:1. This is in agreement with the study result by Murphy G et al where incidence of CRC was higher in men than in women[10]. In our study most of the cases (60%) presented with left sided lesions. The commonest site involved in our study was rectum and anal canal (30%).

In our study the mean intratumoral MVD was significantly higher as compared to extratumoral MVD by both markers, CD 34 as well as CD 105 with a significant p value. This result is in agreement with a study by Hammodi SR, where the MVD in intratumoral areas was more than the MVD in normal areas[11]. The mean VC obtained by CD34 in our study, in intratumoral areas was 13µm with a minimum of 8.12µm and a maximum of 19.83µm, whereas the maximum VC in extratumoral area was 10.16µm which was less than the mean VC of intratumoral area. Similarly all other parameters like VCSA and %TVA, studied also showed significant difference between intratumoral and extratumoral areas by both markers. Thus in our study the number and size of blood vessels in intratumoral area were more as compared to extratumoral area. This finding suggests that angiogenesis plays a central role in the development of CRCs. As angiogenesis in tumors is less organised than angiogenesis in embryo or wound healing, the newly formed vessels tend to be more distorted, more leaky and more dilated than mature normal blood vessels[12]. Hence, intratumoral blood vessels are reported to have more vessel caliber (VC) than normal extratumoral blood vessels. This was also seen in our study.

The findings from our study suggest that grade and stage does not have linear positive correlation with MVD, VC, %TVA or VCSA. The mean MVD was almost similar in three grades. Our study was contradictory to most of the studies in this field suggesting that grade and stage have significant positive correlation of tumor grade with MVD by both CD34 and CD 105. This discordance may be because we studied only 50 cases.

In a study in Thailand Yodavudh S et al demonstrated mean MVD by CD31 and mast cell density as a prognostic marker as well as positive linear correlation with grade and stage in CRC[13]. They also found significantly higher MVD in cases with lymphangioinvasion and perineural invasion than cases without lymphangioinvasion and perineural invasion. In our study, cases without perineural invasion had higher MVD ($253/mm^2$) than cases with perineural invasion ($186/mm^2$). It might suggest that cases with perineural invasion might have additional factors influencing the invasive properties of the tumor in addition to the angiogenesis. In an Iraqi study Hammodi SR et al have obtained higher mean MVD in cases with lymphnode metastasis than cases without lymph node metastasis[11]. However, our study did not show any statistically significant difference of mean MVD, VC, %TVA in between cases with regional lymph node metastasis, lymphangioinvasion, metastatic liver deposits and cases without regional lymphnode metastasis, lymphangioinvasion and metastatic liver deposits by both CD34 and CD105. The above mentioned results are in contrast to a study by Saad RS et al, who showed that CD105 MVD had positive correlation with the presence of angiolymphatic invasion, lymph node metastases, tumor grade, tumor stage and hepatic metastases[4].

On comparing morphometrical parameters obtained between CD34 and CD 105 IHC, the mean intratumoral MVD obtained by CD105 was significantly lower than the mean intratumoral MVD obtained by CD34. In a study in Japan Tanaka F et al showed CD 105 to be more specific and a superior marker than CD34 for angiogenesis in non small cell carcinoma lung[14]. As CD 34 is a pan endothelial marker it highlights all blood vessels both already existing and newer ones. Whereas CD105 highlights the neoangiogenic blood vessels and hence it has less MVD. In our study, the mean vessel caliber VC and mean %TVA obtained by both IHC markers did not show any significant difference. The mean VC by CD 105 (13.51µm) was slightly more as compared to mean VC by CD34 (13µm).

VI. Conclusion

As angiogenesis has a central role in development and progression of tumor. Intratumoral MVD was significantly more as compared to extratumoral MVD in our study. However, intra tumoral MVD, VC, and %TVA does not differ among different grades and stages of tumor. Intratumoral MVD and %TVA were increased uniformly irrespective of TNM grading, staging, lymphnode metastasis, perineural and lymphangioinvasion. As neo angiogenic vessels are marked by CD105, CD 105 was a better marker than CD 34 for studying neo angiogenesis. In cases with perineural invasion, in addition to angiogenesis additional factors might influence the invasive properties of the tumor which needs to be explored that will help in prognostication of the tumor. Further interventional studies are needed to determine definite role of CD 105 as a target molecule for anti angiogenic therapy similar to bevacizumab.

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References

[1]. Weitz J, Koch M, Debus J, et al. *Colorectal cancer, Lancet 2005*; 365:153–65.
[2]. Hermanek P, Gospodarowicz MK, Henson DE, et al. Prognostic Factors in Cancer. *Berlin: Springer* 1995:64–79.
[3]. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971; 285: 1182–86
[4]. Saad RS, Liu YL, Nathan G, et al. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in colorectal cancer. *Modern Pathology* 2004; 17:197–03
[5]. Weidner N, Semple JP, Welch WR, et al. Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med* 1991; 324:1–8
[6]. "Morphometric study of microvessels in primary CNS tumors and its correlation with tumor types and grade" Prabal Deb, Dibyajyoti Boruah, Vibha Dutta, Microvascular Research 84(1), 34-43(2012)
[7]. "Morphometric study of microvessels, epidermal characteristics and inflammation in psoriasis vulgaris with their correlations" Dibyajyoti Boruah, Nikhil Moorchung, Biju Vasudevan, Ajay Malik, Manas Chattarjee Indian Journal of Dermatology, Venereology and Leprosy 79(2), 216-223(2013)
[8]. Edge SB, Byrd DR, Compton CC, et al. AJCC Cancer Staging Handbook, 7th edition. New York: Springer, 2010; 173-06
[9]. Siegel R, DeSantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014; 64:104-117
[10]. Murphy G, Devesa SS, Cross AJ et al. Sex Disparities in Colorectal Cancer Incidence by Anatomic Subsite, Race and Age. *Int J Cancer*. 2011; 128(7):1668– 75
[11]. Hammodi SR, Mohammed DR. The Significance of Angiogenesis in the Biological Behaviour of Colorectal Carcinoma and its Metastatic Lymph Nodes Using CD34 Antibody. *Kufa Med Journal* 2009; 12:2:267-72
[12]. Conway EM, Collen D, Carmeliet P. Molecular mechanisms of blood vessel growth. *Cardiovascular Research* 2001; 49:507–21
[13]. Yodavudh S, Tangjitgamol S, Puangsa S. Prognostic Significance of Microvessel Density and Mast Cell Density for the Survival of Thai Patients with Primary Colorectal Cancer. *J Med Assoc* Thai 2008; 91(5):723-32
[14]. Tanaka F, Otake Y, Yanagihara K, et al. Evaluation of Angiogenesis in Non-small Cell Lung Cancer: Comparison between Anti-CD34 Antibody and Anti-CD105 Antibody. *Clin Cancer Res 2001*; 7:3410-15

Table l: Distribution of cases according to node metastasis, angioinvasion and deposits.

Features	Present (No of cases)	Percentage of cases N=50*
Lymph node deposits	18	36%
Perineural invasion	5	10%
Lymphangioinvasion	17	34%
Serosal, liver, peritoneal deposits, or direct extension	22	44%
Margins involvement by tumor	2	4%
Liver deposits	2	4%

Number of studied samples = 50

Table 2: MVD , VC, VCSA and %TVA by CD34 and CD 105 in intra tumoral and extratumoral area

. Parameter (unit)		**CD34 Mean ± Std. Deviation (Minimum-Maximum)**	**CD105 Mean ± Std. Deviation (Minimum-Maximum)**	**P value for results between intra tumoral and extratumoral area**
MVD (No of vessels /mm^2)	Intratumoral	246±73 (156-451)	204 ± 60 (83-378)	< 0.001*
	Extratumoral	135±42 (43-243)	70 ± 31 (14-156)	
VC (µm)	Intra tumoral	13.00 ±2.68 (8.15-19.83)	13.51 ± 2.73 (8.70 - 20.39)	
	Extratumoral	7.09 ±1.47 (4.77-10.16)	7.71 ± 1.58 (5.00 - 11.49)	
VCSA (µm^2)	Intratumoral	173.76±78.51 (54.62.-436.46)	190.51±87.29 (72.24-422.15)	
	Extra tumoral	45.47±18.32 (19.69-88.49)	55.81±24.67 (20.70-142.39)	
%TVA	Intra tumoral	8.95±4.00 (2.30-17.94)	8.54 ± 3.95 (3.21 - 20.60)	
	Extratumoral	1.06± 0.67 (0.24-3.10)	0.77± 0.53 (0.14-2.77)	

Number of studied samples = 50

Table 3: Intra tumoral Micro vessel density by CD 34 and CD105 in relation to grade of the tumor

Grade of tumor	No of case	Mean ± Std Dev (Min-Max) MVD(per mm^2) CD34	P value	Mean ± Std Dev (Min-Max) MVD(per mm^2) CD105	p Value*
1	17	246±83 (163-451)	0.976	195±60 (121-357)	0.353
2	27	248±65 (156-374)		214±58 (107-378)	
3	6	240±88 (163-326)		180±60 (83-253)	
Total	50	247±73 (156-451)		203±60 (83-378)	

Table 4: Correlation of Intra tumoral morphometry parameters by CD34 in relation to lymphnode metastasis, lymphangioinvasion and perineural invasion.

Feature		**N***	**Mean MVD ± Std Dev (MIN-MAX) No of Vessels/µm²**	**Mean VC ± Std Dev(MIN-MAX) µm**	**Mean %TVA± Std Dev (MIN-MAX)**
Lymph node metastasis	Present	18	255±85 (163-451)	13.17±2.96 (9.20-19.83)	9.55±4.59 (2.30-17.94)
	Absent	32	242±67 (156-381)	12.90±2.56 (8.15-17.77)	8.61±3.66 (3.02-17.64)
	p value		0.561	0.741	0.461
Lymphangio invasion	Present	17	233±78 (156-374)	13.51±2.21 (9.44-17.07)	9.57±4.40 (4.00-17.94)
	Absent	33	253±71 (163-451)	12.73±2.90 (8.15-19.83)	8.63±3.81 (2.30-17.64)
	p value		0.380	0.295	0.461
Perineural invasion	Present	5	**186±25 (163-222)**	13.99±1.45 (12.61-16.43)	7.52±3.79 (5.25-14.18)
	Absent	45	**253±74 (156-451)**	12.88±2.78 (8.15-19.83)	9.10±4.03 (2.30-17.94)
	p value		**0.001**	0.19	0.41

Number of studied samples = 50

Table 5: Correlation of Intra tumoral morphometry parameters by CD105 in relation to lymphnode metastasis, lymphangioinvasion and perineural invasion)

Feature		**N***	**Mean MVD ± Std Dev(MIN-MAX) (No of vessels/ µm²)**	**Mean VC ± Std Dev(MIN-MAX) (µm)**	**Mean %TVA± Std Dev (MIN-MAX) (%)**
Lymph node metastasis	Present	18	198±44 (125-274)	12.66±2.17 (10.07-17.07)	7.37±2.31 (3.51-10.93)
	Absent	32	206±68 (83-378)	13.99±2.93 (8.70-20.39)	9.20±4.53 (3.21-20.60)
	p value**		0.61	0.07	0.06
Lymphangio invasion	Present	17	210±45 (146-298)	13.38±2.47 (10.07-19.09)	8.99±3.12 (4.19-16.85)
	Absent	33	200±67 (83-378)	13.58±2.89 (8.70-20.39)	8.31±4.35 (3.21-20.60)
	p value**		0.51	0.80	0.52
Perineural invasion	Present	5	165±54 (107-253)	13.14±1.41 (11.90-15.39)	7.12±1.95 (4.79-9.53)
	Absent	45	208±60 (83-378)	13.55±2.85 (8.70-20.39)	8.70±4.10 (3.21-20.60)
	p value**		0.155	0.6	0.17

* N – number of cases ** p value obtained by unpaired T test

Number of studied samples = 50

Figure 1a : Intra tumoral Microvessel density(in per mm^2) in relation to the stage of tumor by CD 34

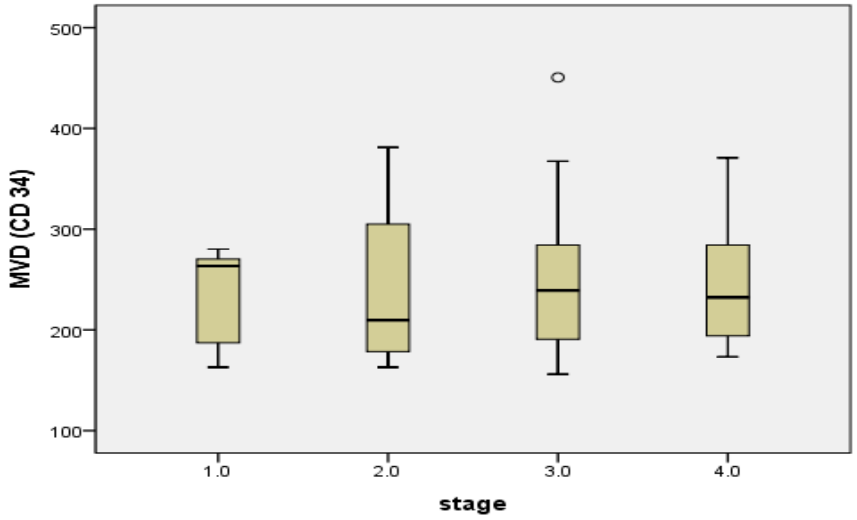


Figure 1b : Intra tumoral Microvessel density(in per mm^2) in relation to the stage of tumor by CD 105

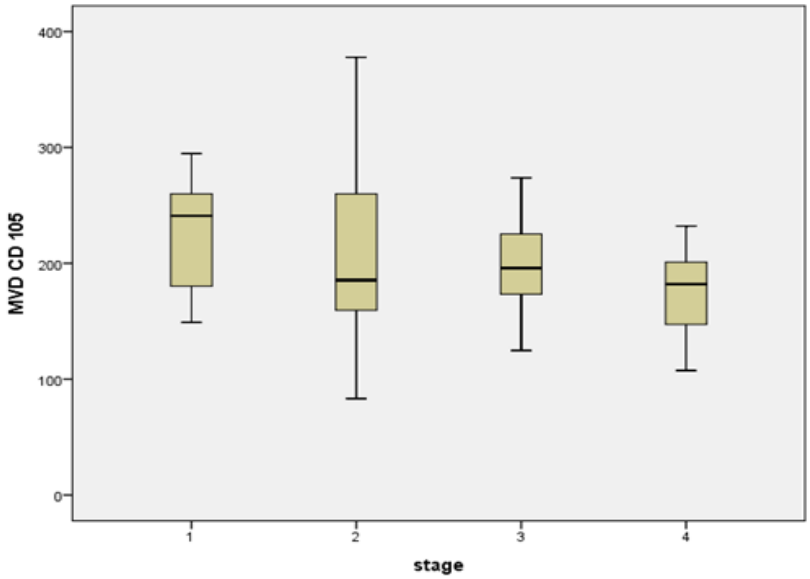


Figure 2: mean intratumoral MVD obtained by CD105(green) was lower than MVD obtained by CD 34

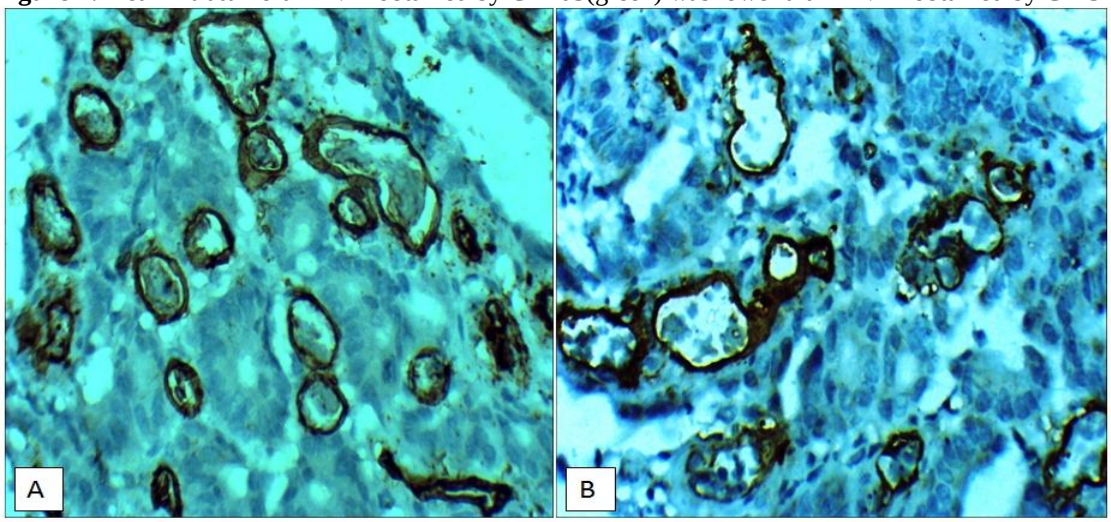
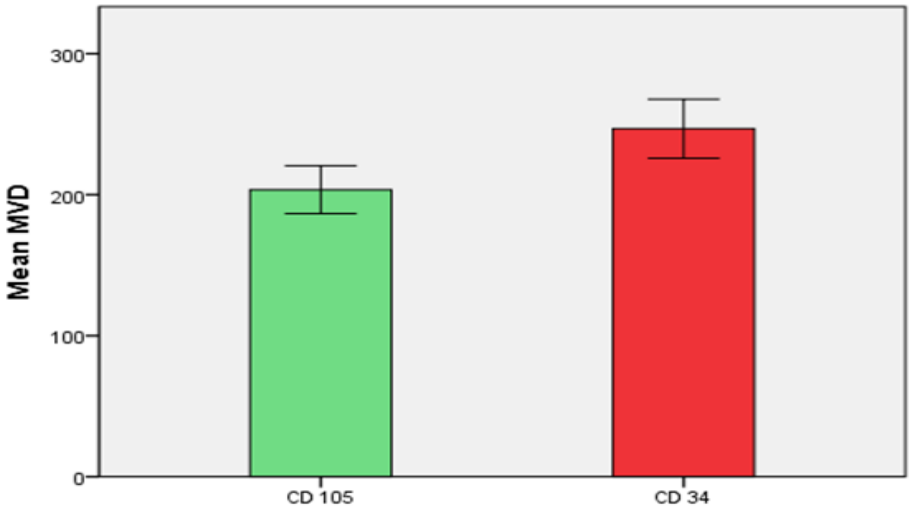


Figure 3 a and b: Intratumoral blood vessels highlighted by CD34 were more in number as compared to Figure B where blood vessels are highlighted by CD105



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