Histopathological and Immunohistochemical Studies on the Prognostic Significance of Angiogenesis in Renal Cell Carcinoma

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Abstract: In the present study, evaluation of tumour angiogenesis has been carried out in a group of patients with renal cell carcinoma. The retrospective study included 97 patients for whom radical nephrectoly was carried out between 1997 and 1999 in the Urology and Nephrology Center belonging to Mansoura University, Egypt. Patients were stratified according to grade, stage, age, lymph node status, tumour size and angiogenesis. Angiogenesis was evaluated by measuring the mean microvessel density (MVD). Microvessels were immunohistochemically stained using monoclonal antibody that reacted with endothelial cells linning the wall of blood vessels and was called CD34. Microvessels were counted in active areas of angiogenesis (hot spots) under magnification of X250 and the mean of 3 counts (MVD) was used for univariate and multivariate statistical analysis. The results of the present study indicated that, evaluation of microvessel density is of no appreciable value in the assessment of prognosis for renal cell carcinoma. Tumour stage could provide more objective tool for better judgment on the patient survival and might help in choice of the more convenient therapy for the individual patient.
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1. Introduction

Renal cell carcinoma (RCC) is a relatively rare tumour affecting six of every 100.000 people and accounting for approximately 3% of adult malignancies and 1.4 of cancer related deaths. It is more common among urban population and among males with a male-to-female ratio of approximately 2:1. The tumour arises from the proximal convoluted tubules of the kidney and is characterized by abundant neovascularization and arteriovenous-venous fistula formation. Growth of solid tumours requires angiogenesis. The new proliferating vessels supply oxygen and nutrition for the tumour cells and promote their growth. Experimental evidence shows that, the dynamics of hematogenous metastasis are dependant on the access of the tumour cells to the microvasculature. RCC is a tumour with unpredictable behavior **(Dekel *et al*., 2002)**. Radical nephrectomy is the curative treatment option for renal cell carcinoma. If the tumour is small and nephron sparing is required, partial nephrectomy is the procedure of choice **(Dekel *et al*., 2002)**. Clearly there is a need for more precise prognostic markers for predicting the risk of developing metastatic RCC.

Because RCC is one of the most highly vascularized solid malignancies, it seems logical that factors that regulate the process of angiogenesis and invasion would be correlated to its pathogenesis **(Slaton *et al*., 2001).** The prognostic of RCC remains poor. One third of the patients already have metastasis when first consulting the hospital. Another 30-40% of patients develop metastases after surgical excision of the primary tumour. RCC are radioresistant and more than 8% are chemoresistant **(Stassar *et al*., 2001)**. Angiogenesis is the growth of new vessels from the existing vasculature, a process central to tumour growth and metastasis **(Jones and Fujiama, 1999).** The measurement of intratumoural microvessel density (MVD) has been proved to be an important prognostic indicator for many malignant neoplasms. The value of MVD as a predictor of patient prognosis in RCC is controversial. Certain reports revealed a direct correlation between MVD and survival, others revealed an inverse correlation and one report showed no correlation **(Sabo *et al*., 2001).**

There is considerable experimental evidence that angiogenesis plays a crucial role in the growth of solid tumours over 1 to 2 mm. in diameter (about 10^6 cells), and in gaining access to the existing vasculature. A multitude of new angiogenesis inducers and inhibitors have been isolated and characterized, and attempts to control tumour growth by inhibiting angiogenesis have generated much interest. The results of early clinical trials are just now becoming available **(Fujioka *et al*., 1998).**

The aim of the present study is:

1. To determine whether the degree of vascular complexity has a predicative value in the prognosis of RCC or not.

2. To evaluate the relationship between angiogenesis and the different clinical and pathologic parameters such as age, sex grade and lymph node status.
3. To perform multivariate analysis to detect the independent prognostic factors that can affect the patients survival.

2. PATIENTS AND METHODS

1. Patients:

Between January 1997 and December 1999, 97 patients were diagnosed as renal cell carcinoma and were admitted to Mansoura Urology and Nephrology Center. The study group consisted of these patients for whom radical nephrectomy was performed for the treatment of renal tumour. The patients of this reterospective study were chosen on the basis of availability of complete archival material. At the time of nephrectomy, all specimens were examined according to the same pathological protocol. Tissue sections were obtained from the tumour.

1. a. Age:

Patients' age ranged from 5 to 76 years for the whole study population. For men (mean ± SD = 49±12.2 years) but for women (mean ±SD= 50.7±13.3 years).

1. b. Sex:

There were 69 (71.1%) men and 28 (28.9%) women and the male to female ratio 2.46: 1.

2. Histopathology:

The hematoxylin and eosin stained slides were reviewed to confirm the diagnosis. Conventional type of renal cell carcinoma was diagnosed in 42 patients (43.3%), chromophobe type in 19 patients (19.6%), papillary type in 33 patients (34.0%) and unclassified type in 3 patients (3.1%).

2. a. Grade:

Tumours were graded using Fuhraman's grading system **(Fuhrman *et al.*, 1982)** into four grades 1, 2, 3 and 4. The percents of grades of our study were found to be (41.2%), (40.2%), (15.5%) and (3.1%) respectively.

2.b. Stage:

The International Union Against Cancer and the American Joint Committee on Cancer proposed a revision of TNM system that is now the recommended staging system for RCC **(Guinan *et al.*, 1997).** This system was used in the present study for pathological staging of the tumours where:

Stage I: Tumour within capsule.

Stage II: Tumour invasion of perinephric fat (confined to gerota's fascia).

Stage III: Tumour involvement of regional lymph nodes and/or renal vein and cava.

Stage IV: Adjacent organs or distant metastases.

The percents of the stages of the patients were found to be (67.0%), (16.5%), (13.4%) and (3.1%) respectively.

3. Tumour size:

By reviewing the pathological reports, the tumour size was assessed by determination of the largest diameter. The tumours were categorized according to size into 2 groups, equal and less than 7 cm and more than 7 cm.

4. Histopathological examination:

After radical nephrectomy, the tumour was immediately fixed in 10% neutral buffer formalin for 24 hours. Several pieces of 3-5 mm thickness were taken from the tumour and fixed tissues were chemically processed through the tissue processor (VIP Tissue-Tek apparatus, Japan). These processed tissues were embedded in molten paraffin wax and the paraffin blocks were sectioned at 3 µm for hematoxylin and eosin staining and at 4 µm for immunohistochemical staining with Reichert Jung 2030 microtome (West Germany) using disposable blades.

5. Tumour angiogenesis:

Angiogenesis of the tumours was assessed after highlighting the microvessels using the immunohistochemical staining technique.

5. a. Immunohistochemistry:

The avidin-biotin immunoperoxidase technique was applied (Guesdon, 1979). Four micron thick sections from retrieved tumour blocks were dewaxed and rehydrated through graded alcohols. These sections were mounted on coated slides by Histo-Grip (Zymed, USA) and were washed in water. Antigen sites were unmasked using microwave antigen retrieval technique (Kok and Boon, 1995). After their cooling, the slides were rinsed with phosphate buffered saline (PBS). Endogenous peroxidase activity was removed by submersing slides in blocking solution which was prepared by adding one part of 30% H_2O_2 to nine parts of methanol. Non-specific binding was blocked with blocking serum (reagent A of Histostain®- plus bulk kit, Zymed, USA). It was left for 10 minutes in contact with the slides. After removing the excess blocking serum, the monoclonal mouse anti-CD34 which is an antibody concentrate that will provide qualitative demonstration of the CD34 protein in formalin-fixed paraffin embedded human tissue sections was applied.

CD34 was applied at a dilution of 1: 100 in phosphate buffer saline (PBS, 10 mM sodium phosphate, 140 mM sodium chloride, PH 7.2) and was incubated for 60 min. The slides were rinsed well

with PBS for 3 minutes. Biotinylated secondary antibody solution (reagent B of Histostain®- plus bulk kit, Zymed, USA) was incubated with the slides for 10 minutes. This was followed by a further wash in PBS. Enzyme conjugate solution (ready to use streptavidin-peroxidase HRP which is reagent C of Histostain®plus bulk kit, Zymed, USA) was applied to the slides for 10 minutes. The slides were washed with buffer solution, then the final steps of this procedure i.e. addition of chromogen substrate di-amino-benzidine (DAB) [Sigma chemical company, UK] and light counter-stain with haematoxylin were performed. The result is brown stain to the microvessels:

5. b. Assessment of microvessel density:

The microvessel density, defined as capillaries and small venules, was assessed in areas with a solid tumour morphology away from any artifact or necrosis and without prior knowledge of patient outcome. The vessel counting was performed in three areas of maximal neovascularization where the highest number of discrete microvessels was stained (hot spots) (Dickinson *et al*., 1994). Low power light microscopy at X40 magnification was used to scan the often heterogeneous tumour sections for these areas. At X250 magnifications, counts were made of all distinct brown staining endothelial cells or cell clumps. Sclerotic areas within the tumour which showed less neovascularization, and adjacent benign tissue were not considered in vessel counts. Microvessels found in unaffected areas adjacent to tumour infiltrated tissue were not counted but were used as an internal control in assessing quality (Jaeger *et al*., 1995). To be defined as an individual vessel, cell nests showing immunostaining had to be clearly separate from adjacent microvessels, tumour cells or connective tissue elements. Vessel lumina, or intratumoural red blood cells were not necessary to define microvessels. Larger muscular walled vessels were excluded from the assessment. The microvessel density (MVD) was defined as the mean of the highest 3 counts.

6. Follow-up:

Patients were kept under regular clinical review. Follow-up, including mortality data, were obtained for all patients except one patient. They were followed regularly and examined for treatment failure depending on clinical findings and radiological evidence. The mean follow-up was 62.34±35.87 months (range =106) for the whole study groups. It was 61±37 (range-105), 63.87±35.04 (range =105), 69.94±32.53 (range= 93) and 12±10.81 (range= 21) for conventional, papillary, chromophobe and unclassified renal cell carcinoma (RCC) types respectively.

7. Prognostic variables:

The following clinical, morphologic, and biologic variables were studied with respect to prognosis: age, sex, histopathologic grade, tumour stage and lymph node status, beside tumour angiogenesis.

8. Statistical analysis:

The statistical analysis was performed using SPSS (Statistical Package of Social Science) program version 11(SPSS inc., Chicago, IL, USA). The quantitative data were presented in the form of mean, standard deviation and range. Student-t-test and one way-ANOVA (Analysis of variance) were used in the analysis. Kaplan-Miere survival curves were plotted and the log-rank test was used to determine statistical differences between life table curves (Kaplan and Meier, 1958). The period of disease-free survival was defined as the time between the date of surgery and death (from cancer) or the development of local recurrence or distant metastasis. Death from unknown cause was considered death from cancer. Censored survival values represent patients who were alive without clinical evidence of disease at the time of last follow-up. To simplify the statistical analysis, the patients were divided into groups according to age, tumour size and MVD.

Cox's proportional hazard analysis was used to study the simultaneous effects of the different factors in survival (Cox, 1972). The included variables were only those which were significant with log rank test. This multivariate analysis was performed to study the effect of one factor on survival, while the influence of others was controlled.

To simplify the statistical analysis in Kaplan-Meire survival curves and because of the little number of cases in both stage 4, grade 4 and unclassified type, we concluded them with stage 3, grade 3 and papillary type respectively.

3. Results

In the present study, the clinico-pathologic features of the study population are enlisted in table (1). Total cases were 97; conventional type accounted 43.3% of cases, papillary type 34%, chromophobe type 19.6% and unclassified type 3.1%.

Types:

1.a. Conventional renal cell carcinoma:

There were 42 patients with conventional type renal cell carcinoma included in this study. Most of them were men (69%). Age ranged from 5 to 76 years (mean ± SD=50.76 ± 13.27). The median follow up period was 82 months (range=105). The

regional lymph nodes were positive in 12 cases and negative in 30 cases.

1.b. Papillary renal cell carcinoma:

There were 33 patients with papillary type renal cell carcinoma included in this study, most of them were men (78.78%). Age ranged from 24 to 75 years (mean ± SD=48.81 ± 11.32 years). The median follow up period was 69 months (range=105). The regional lymph nodes were positive in 8 cases and negative in 25 cases.

1.c. Chromophobe renal cell carcinoma:

There were 19 cases with chromophobe renal cell carcinoma included in this study. Most of them were men (68.42%). Age ranged from 33 to 62 years (mean ± SD= 50.36 ± 9.05). The median follow up period was 82 months (range=29). The regional lymph nodes were positive in 5 cases and negative in 14 cases.

1.d. Unclassified renal cell carcinoma:

There were 3 cases only with unclassified renal cell carcinoma. Two females and one male. The first female was 24 years old with follow up period 21 months and died of disease. The second female was 68 years old with follow up period 15 months and died of disease. The male case was 14 years old and unfollowed.

2- Lymph node status:

For all cases, there were 25 cases with positive lymph nodes, and 72 cases with negative lymph nodes as shown in table (2).

3- Angiogenesis in renal cell carcinoma:

The slides of 97 patients were suitable for pathological interpretation after immunohistochemical staining for tumour angiogenesis (figs. 1-12) Descriptive analysis of microvessel density (MVD) for the four types of renal cell carcinoma which were included in our study are shown in table (3).

4- Angiogenesis and Clinicopathologic characteristics:

There was no significant relationship between MVD and age, sex, grade, stage, and tumour size. Type had a significant relationship with MVD (Table 4). Conventional type had significantly higher MVD than that of papillary type and chromophobe type. Unclassified type had a median vascularity. A significant relationship between MVD and lymph node status was obtained too. Negative cases had higher MVD mean than positive cases.

Correlation between angiogenesis (MVD) and age was slightly positive (increasing in age increased MVD slightly) as shown in fig. (13). Correlation coefficient (R)= -0.07 ($-1 \leq R \leq 1$).

Correlation between angiogenesis (MVD) and tumour size was slightly negative (increasing in tumour size decreased MVD slightly) as shown in fig (14). Correlation coefficient (R) = 0.117 ($-1 \leq R \leq 1$).

5- Other significant relations:

5-a. Relations between age and stage was significant (P value=0.004). High ages had significantly higher stages.

5-b. Relation between tumour size and stage was significant (P value=0.03). Increasing in tumour size increased tumour stage.

5-c. Relation between tumour size and grade was significant (P value=0.01). Increasing in tumour size increased tumour grade.

5-d. Relation between stage and grade was significant (P value=0.0001).

Most stages had low grades and vice verse.

5-e. Relation between tumour size and survival time was significant (P value=0.009) (Fig. 15). As the tumour size increased the survival time decreased. Correlation coefficient (R)= -0.265 ($-1 \leq R \leq 1$).

6. Survival of patients with renal cell carcinoma (Table 5):

6. a. Overall survival:

The 5-year survival rate was 72.16%. The risk of treatment failure (tumour recurrence or death from tumour) was high in the first 2 years of follow up (Fig. 16).

6.b. Survival and sex:

Males had better survival than females. This difference reached statistical significance (p=0.02). The 5-year survival for males 79.71% while it was 53.57 for females (Fig. 17).

6. c. Survival and age:

Age was categorized in four groups to simplify the statistical analysis: <40 years, 40-50 years, 50-60 years and >60 years. The number of patients in each group was 16, 36, 26 and 19 patients receptively. There was no significant relationship between age and the survival of the patients (P=0.28) (Fig. 18).

6. d. Survival and stage:

Tumour stage had a significant impact on patients survival ($P < 0.0001$). There were only 3 cases in stage four, therefore we categorized stages 3 and 4 in one group. The 5-year survival rate was 83.08%, 68.75% and 31.25% for stage 1, 2 and 3 and 4 respectively (Fig. 19).

6.e. Survival and grade:

A significant relationship was observed between tumours grade and survival (P=0.001). As in stage, the number of grade four patients were 3 cases, thus we categorized grade 3 and 4 in one group. The 5-year survival rates for patients with grade 1, 2 and (3 and 4) were 80.00%, 76.92% and 44.44% respectively (Fig. 20).

6. f. Survival and type:

There was no significant relationship between the type and survival of the patients (P=0.65). Unclassified type was 3 cases only, thus we added them to papillary type to simplify statistical analysis. The 5-year survival rates were 69.05%, 78.95% and 72.22% for conventional, chromophobe and (papillary and unclassified) types respectively (Fig. 21).

6. g. Survival and lymph node status:

There was a significant relationship between the lymph node status and the survival of patients (P=0.04). The 5-year survival rates for patients with negative and positive lymph nodes were 77.78% and 56.00% respectively (Fig 22). The presence of regional positive lymph nodes which led to metastasis to the patients decreased the survival rates to these patients.

6. h. Survival and tumour size:

Tumour size of the patients was divided into 2 groups: >7 cm and ≤ 7 cm. The first group (> 7cm) number was 65 patients and had a 5-year survival rate 63.08% and the second group (≤ 7 cm) number was 32 patients and had a 5-year survival rate 90.63%. There was a significant relationship between lymph node status and survival of the patients (P=0.004) (Fig. 23).

6. i. Survival and microvessel density (MVD):

Microvessel density (MVD) was divided into 3 groups: < 20, 20-30 and >30. The number of patients in the 3 groups were 49, 37 and 11 patients respectively. The 5-year survival rates for the 3 groups were 77.55%, 62.16% and 81.82% respectively. There was no significant relationship between MVD and survival of the patients (P=0.157) (Fig. 24).

6.j. Multivariate analysis:

Multivariate analysis wase performed, using Cox's proportional hazard analysis, for the significant variables in unvariate analysis (sex, stage, grade, lumph node status and tumour size). Sex and lymph node status could not sustain its significance, while stage proved to be independent variable for prognosis and sustained its significance (Table 6). Grade and tumour size were considered as dependent variables, although their (P value) slightly increased above the critical value 0.05.

Table (1): Characteristics of the study population

	Total cases	Conventional type	Papillary type	Chromophobe type	Unclassified type
Number	97	42	33	19	3
Male to female ratio	69:28	29:13	26:7	13:6	1:2
Mean age±SD range	49.54±12.56 71 (5-76)	50.76±13.27 71 (5-76)	48.81±11.32 51 (24-75)	50.36±9.05 29 (33-62)	35.33±28.72 54 (14-68)
Grade:					
1	40	24	6	10	-
2	39	11	20	8	-
3	15	7	7	1	-
4	3	-	-	-	3
Stage:					
1	65	29	19	17	-
2	16	4	9	1	2
3	13	8	4	1	-
4	3	1	1	-	1
Lymph node status					
Negative	72	30	25	14	3
Positive	25	12	8	5	-
Tumour size					
≤ 7 cm	32	18	8	6	-
> 7 cm	65	24	25	13	3

Table (2): Positive and negative lymph node percents.

	Frequency	Percent	Valid Percent	Cumulative percent
Positive	25	25.8	25.8	25.8
Negative	72	74.2	74.2	100.0
Total	97	100.0	100.0	

Table (3): Descriptive analysis of MVD for types of RCC

Type	Minimum MVD	Maximum MVD	Range	SUM	Mean	SD
Conventional	17	42	25	1101	26.21	6.919
Papillary	14	29	15	621	18.81	3.58
Chromophobe	15	32	17	372	19.57	3.93
Unclassified	18	29	11	71	23.66	5.50

Table (4):Clinico-pathological characteristics of RCC and their relation to the tumour angiogenesis.

Variable	No. of patients	Microvessel density mean (MVD)	P value
1) Type			
Conventional	42	26.21	
Papillary	33	18.81	0.001
Chromophobe	19	19.57	
Unclassified	3	23.66	
2) Age	97	22.3	0.25
3) Sex			
- Male	69	22.13	0.9
- Female	28	22.78	
4) Grade			
- G1	40	23.9	
- G2	39	21.25	0.19
- G3	15	20.6	
- G4	3	23.66	
5) Stage			
- S1	65	22.75	
- S2	16	20.87	0.76
- S3	13	21.92	
- S4	3	22.33	
6) Lymph node			
- positive	25	20.36	0.03
- negative	72	23.18	
7) Tumour size	97	22.3	0.48

Table (5):Kaplan-Meier estimate of 5-year disease-free survival in relation to patient and tumour characteristics.

Characteristic	No. of patients	Mean survival	5 year survival rate%	95% confidence interval (95% CI)	P value
1- Total	97	6.70	72.16	6.00, 7.91	
Sex					
Male	69	7.16	79.71%	6.42, 7.91	0.02
Female	28	5.48	53.57%	4.10, 6.86	
Stage					
- 1	65	7.46	83.08%	6.76, 8.15	
2	16	5.94	68.75%	3.94, 7.93	<0.0001
3, 4	16	3.87	31.25%	2.04, 5.69	
Grade					
1	40	7.27	80.00%	6.31, 8.24	
2	39	7.19	76.92%	6.24, 8.15	0.0001
3, 4	18	3.60	44.44%	1.83, 5.36	
Age					
< 40	16	7.23	81.25%	5.78, 8.69	
40-50	36	7.18	77.78%	6.13, 8.22	0.28

50-60 > 60	26 19	6.54 5.02	69.23% 57.89%	5.23, 7.86 3.43, 6.60	
Type Conventional Papillary and unclassified Chromophobe	 42 36 19	 6.41 6.60 7.23	 69.05% 72.22% 78.95%	 5.34, 7.48 5.45, 7.74 5.83, 8.63	 0.65
Lymph node status Positive Negative	 25 72	 5.80 7.00	 56.00% 77.78%	 4.31, 7.28 6.24, 7.75	 0.04
Tumour size > 7 cm ≤ 7 cm	 65 32	 5.95 8.03	 63.08% 90.63%	 5.04, 6.87 7.25, 8.81	 0.004
MVD < 20 20-30 > 30	 49 37 11	 7.12 5.91 7.40	 77.55% 62.16% 81.82%	 6.21, 8.03 4.69, 7.12 5.71, 9.09	 0.157

Table (6):Results of the proportuional hazard analysis (Cox's regression) of disease free survival in RCC

Characteristics	Regression	Standard error	Relative risk (95% CI) Exp (B) (lower, upper)	P value
Grade G1 G2 G3,4	 -0.4088 0.7612	 0.5092 0.5272	 0.6645 (0.2449, 1.8024) 2.1409 (0.7618, 6.0170)	0.0515 0.4220 0.1488
Stage S1 S2 S3,4	 0.6935 1.2752	 0.5498 0.4654	 2.0007 (0.6811, 5.8767) 3.5794 (1.4377, 8.9115)	0.0232 0.2071 0.0061
Tumour size	1.2628	0.6530	3.5353 (0.9831, 12.7130)	0.0531

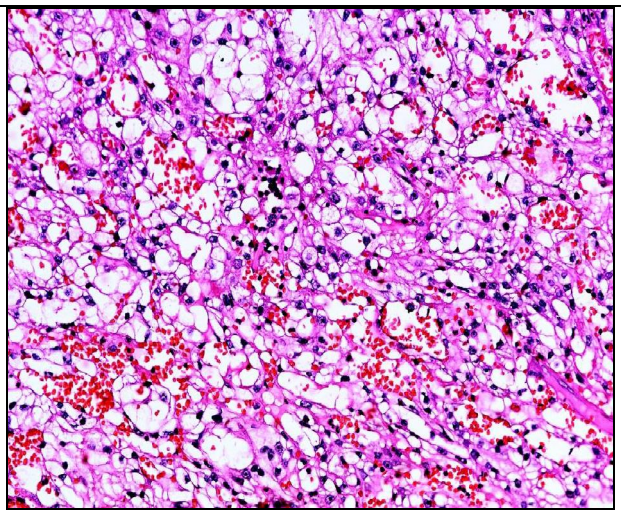

Fig. (1): Conventional type of renal cell carcinoma (RCC) (H&E X250).

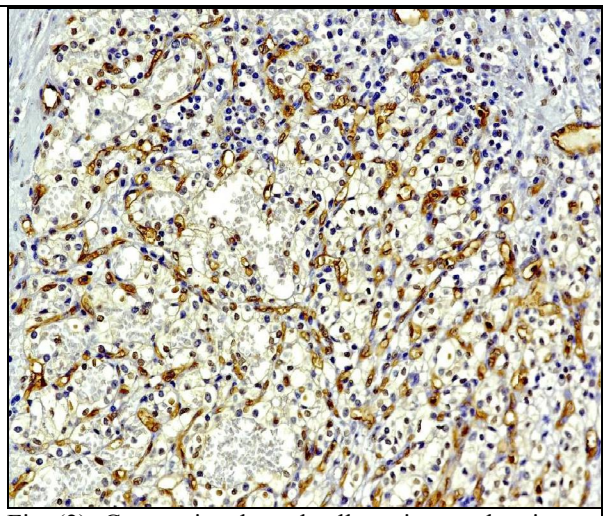

Fig. (2): Conventional renal cell carcinoma showing high vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

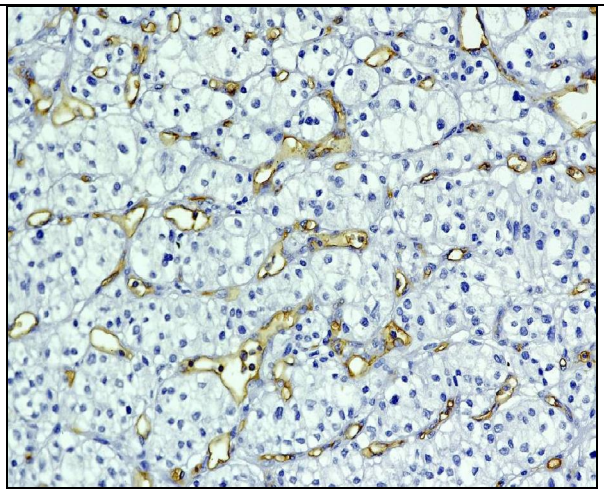

Fig. (3): Conventional renal cell carcinoma showing median vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

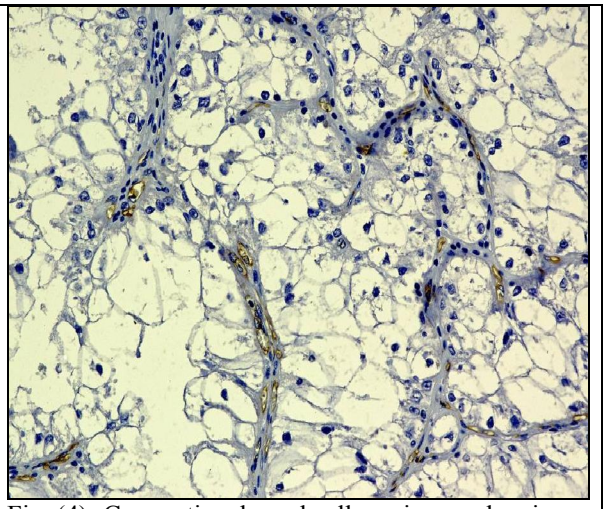

Fig. (4): Conventional renal cell carcinoma showing low vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

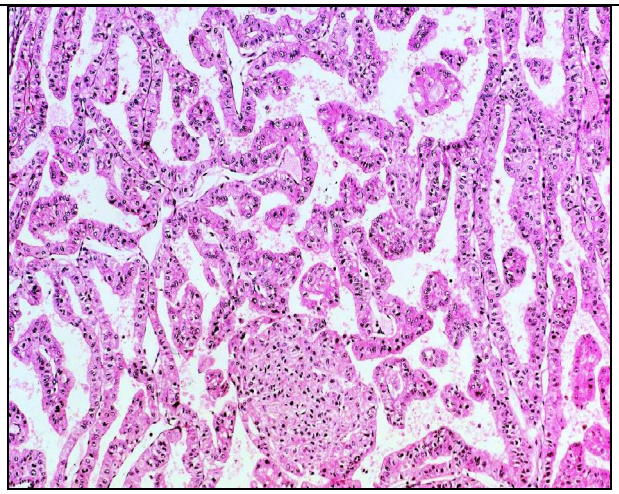

Fig. (5): Papillary type of RCC (H&E X100).

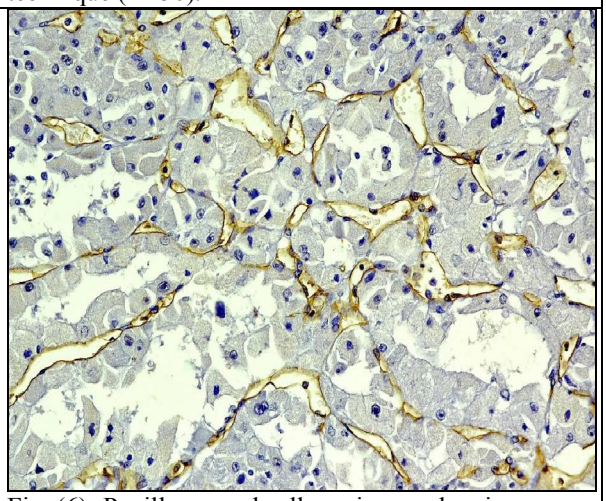

Fig. (6): Papillary renal cell carcinoma showing median vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

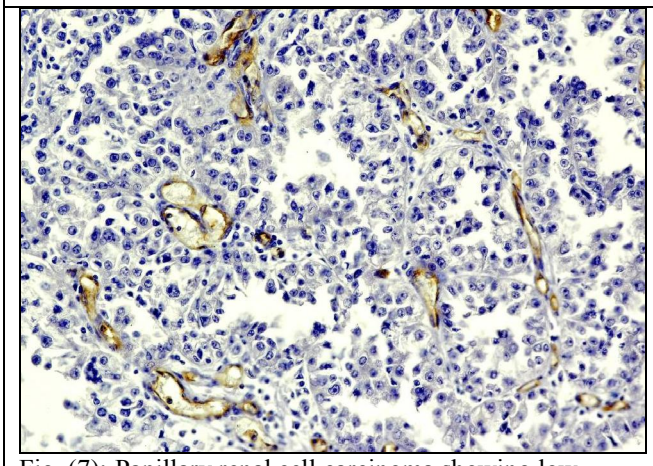

Fig. (7): Papillary renal cell carcinoma showing low

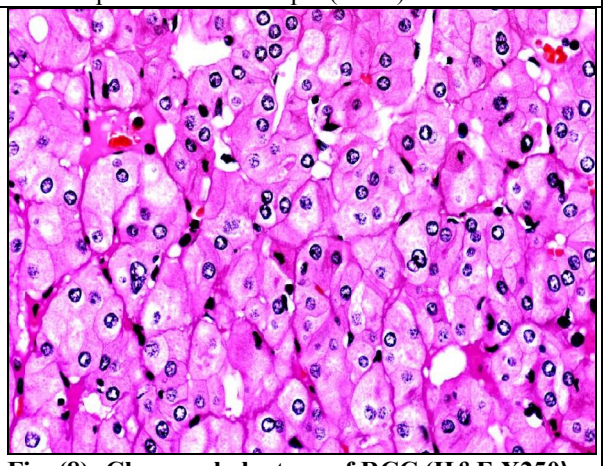

Fig. (8): Chromophobe type of RCC (H&E X250).

vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

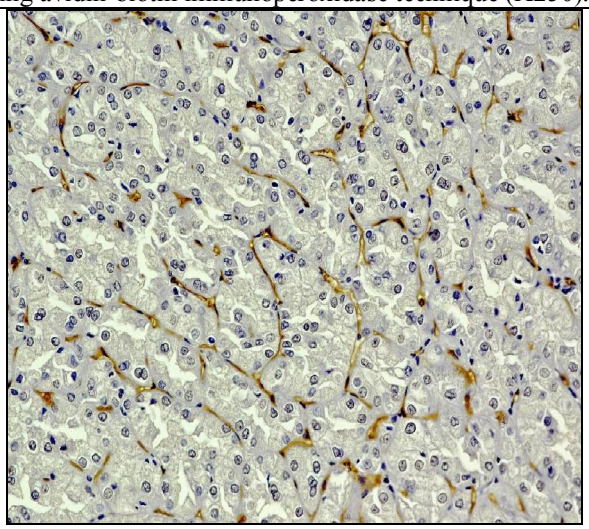

Fig. (9): Chromophobe renal cell carcinoma showing high vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

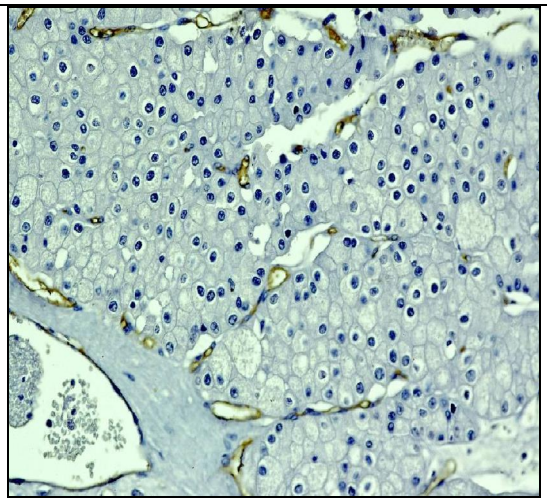

Fig. (10): Chromophobe renal cell carcinoma showing low vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

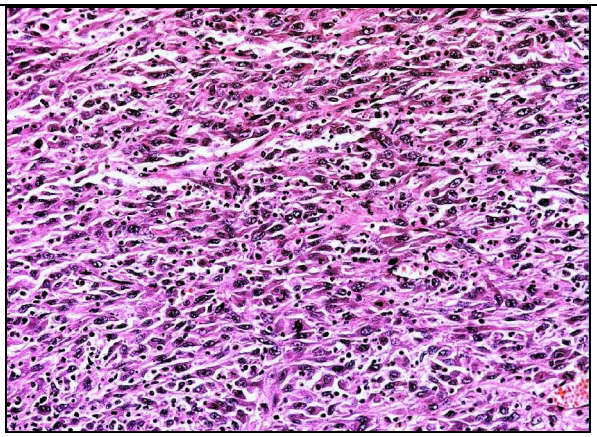

Fig. (11): Unclassified type of RCC (H&E X250).

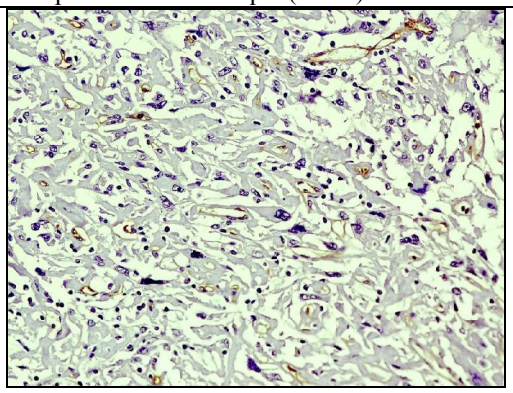

Fig. (12): Unclassified renal cell carcinoma showing median vascularity stained with anti-CD34 monoclonal antibody using avidin-biotin immunoperoxidase technique (X250).

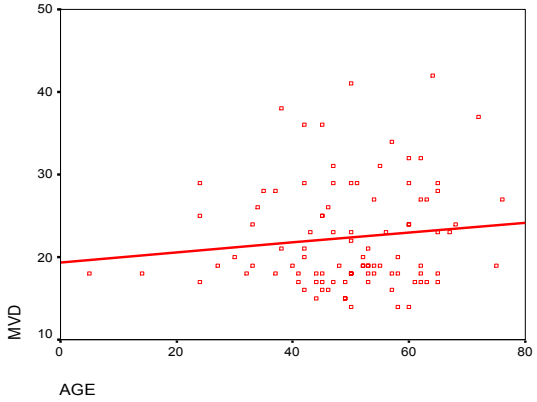

Fig. (13): Correlation between MVD and age. MVD (vessels/X 250 field)

Fig. (14): Correlation between MVD and tumour size. MVD (vessels/ X 250 field)

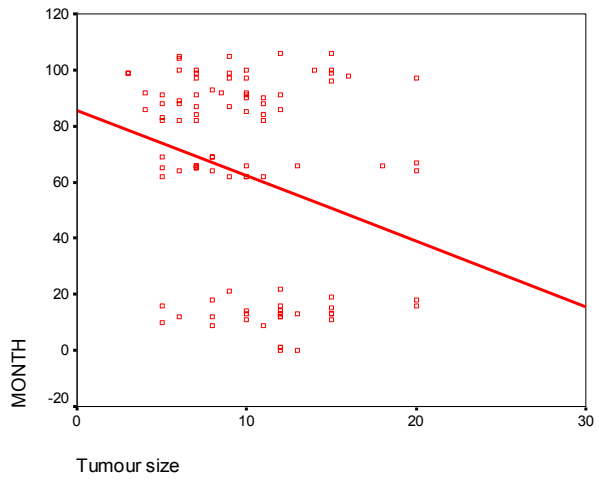

Fig. (15): Relation between tumour size and survival time

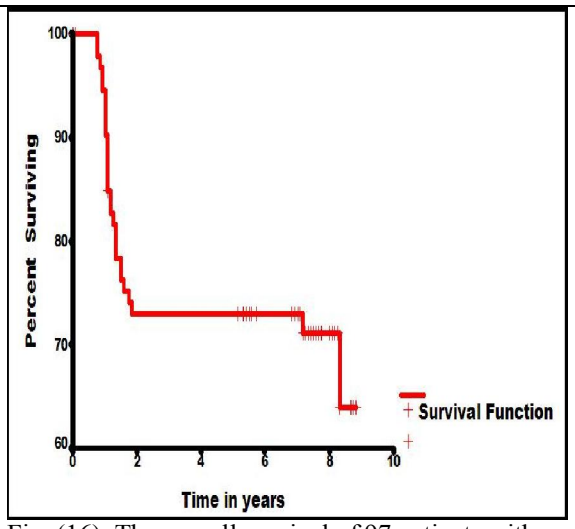

Fig. (16): The overall survival of 97 patients with RCC.

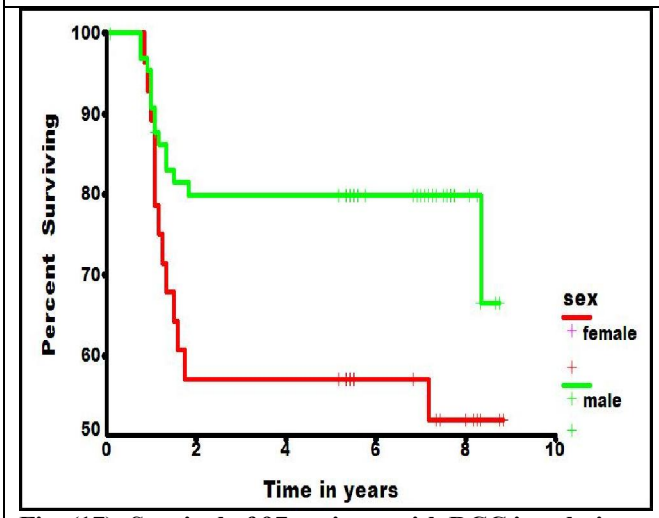

Fig. (17): Survival of 97 patients with RCC in relation to sex (P value= 0.02).

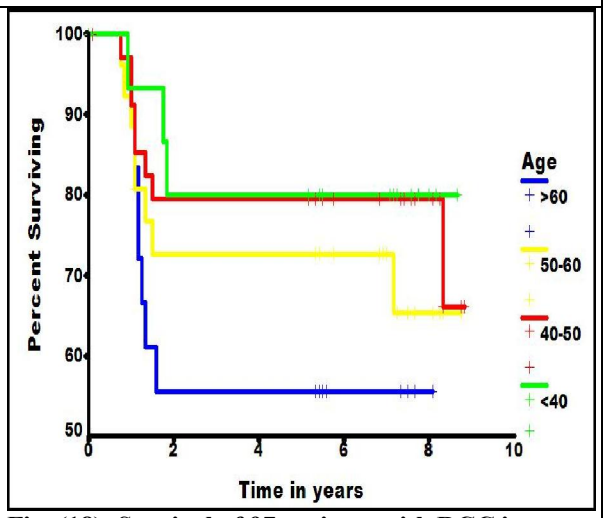

Fig. (18): Survival of 97 patients with RCC in relation to age (P value= 0.28)

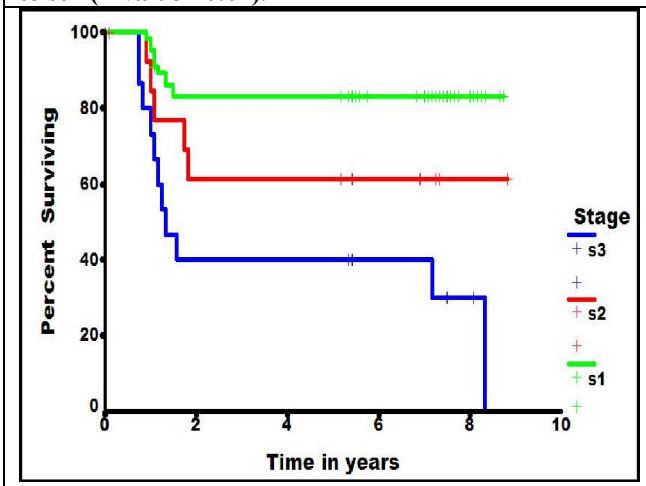

Fig. (19): Survival of 97 patients with RCC in relation to stage (P value= 0.28)

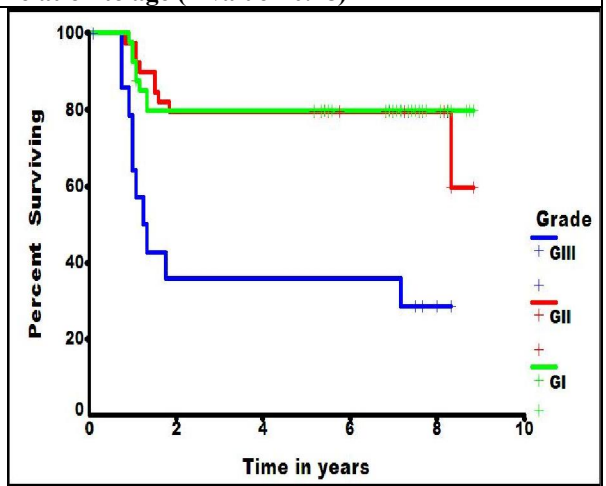

Fig. (20): Survival of 97 patients with RCC in relation to grade (P value= 0.0001).

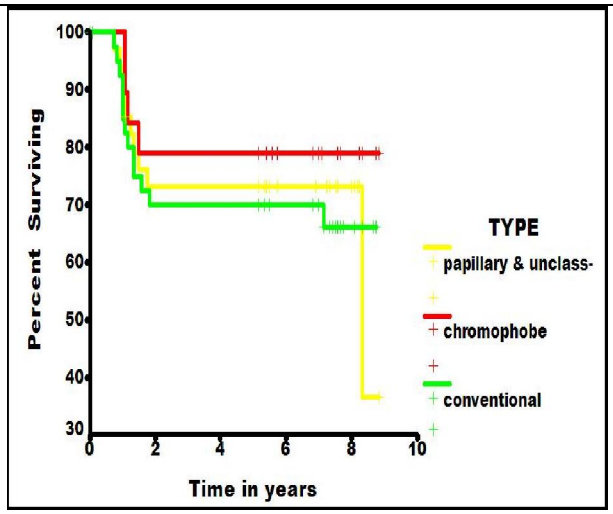

Fig. (21): Survival of 97 patients with RCC in relation to type (P value= 0.65).

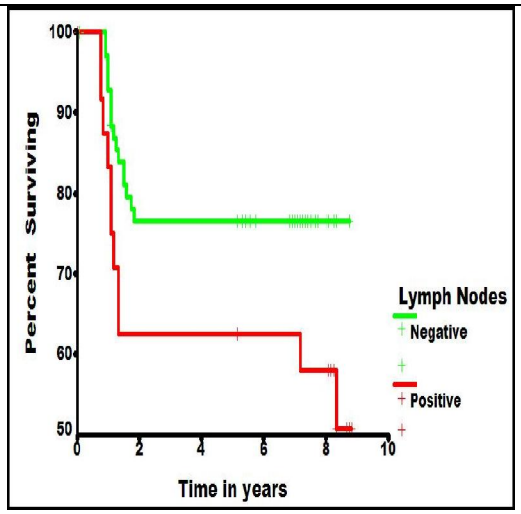

Fig. (22): Survival of 97 patients with RCC in relation to lymph node status (P value= 0.04).

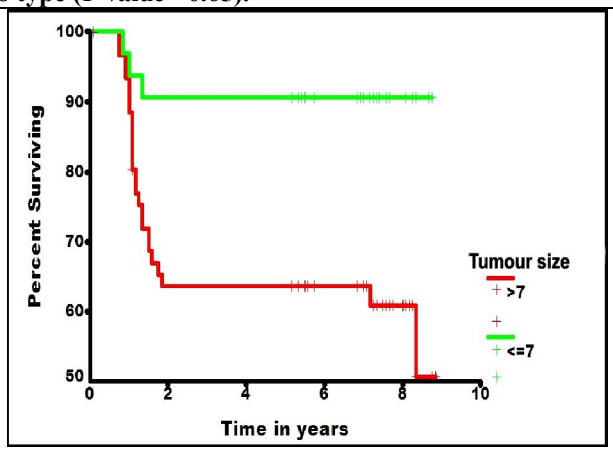

Fig. (23): Survival of 97 patients with RCC in relation to tumour size (P value= 0.004).

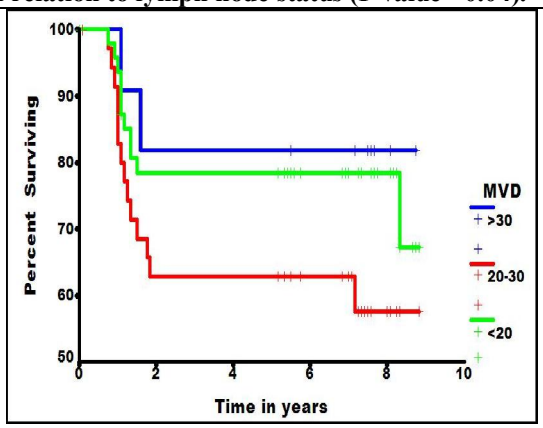

Fig. (24): Survival of 97 patients with RCC in relation to microvessel density (MVD) (P value= 0.157)

4. Discussion

In the present study, the male to female ratio is 2.46:1 which is nearly similar to most studie. The average age of the study group was 49.5 ± 12.57 years. In the west, mean age is higher, for example 65.3 years **(Roosen *et al.*, 1994)** and 65.1 years **(Dekel *et al.*, 2002)**. The overall 5-year survival in 97 patients in this report is 72.16%, with a mean survival 6.70 years, while the overall 5 and 10-year survival rates for patients with RCC were rather similar among the different series and ranged from 50 to 70% and 30 to 50% respectively **(Giuliani *et al.*, 1990; Sene *et al.*, 1992 and Giberti *et al.*, 1997)**. In the present reterospective study, we analyzed various parameters with the aid of univariate and multivariate analysis with survival rate to determine the most effector prognostic indicators.

The prognostic role of microvessel density as assessed by microscopy for renal cell carcinoma in our study is to determine the relationships between microvessel density and other parameters such as age, stage, grade, type, sex, lymph node status and tumour size, and comparing our results with other results. Male to female ratio in our study was 2.46:1. This ratio differed in most studies but males number in most studies was higher than females number. In some studies, male to female ratio was high such as 4.6:1 **(Suzuki *et al.*, 2001)** and 4:1 **(Kinouchi *et al.*, 2003)**, and in some other studies this ratio decreased such as 1.9:1 **(Sabo *et al.*, 2001)** and 1.5:1 **(Dekel *et al.*, 2002)**.

Methods for the quantification of blood vessels in previous studies of angiogenesis have usually involved some element of subjectivity, mainly in the selection of the area of highest vascularity (hot spot area). **Joo *et al.* (2004)** found that, individual microvessels were counted in the area of highest vascularity at (X200) in three selected microscopic fields. Any brown staining endothelial cell or cluster that was separated from the other nearby

microvessels was counted. Large anastomosing sinusoidal vessels were counted as a single vessel. Large vessels with thick muscular walls were excluded from the count. The microvessel count was expressed as the mean number of vessels in the selected area. **Sabo *et al.* (2001)** found that the five most vascularized microsopic fields (hot spots) of the RCC tumours were selected for analysis using medium-sized magnifying lens (X100). **(Nativ *et al.*, 1998)** found that, in all tumour sections, individual microvessels were counted in the area of highest vascularity at (X400) magnification of five randomaly selected microscopic fields. In our study we counted microvessels using magnifying lens (X250) we compared our results with other results according to the relationships between MVD and other prognostic factors in RCC.

In the present study we found that there was no significance in the correlation between stage and MVD (P value=0.794). Similar results were previously reported **(Mac Lennan *et al.*, 1995; Slaton *et al.*, 2001 and Joo *et al.*, 2004)**. Other results found that there was a significant relationship between stage and MVD **(Sabo *et al.*, 2001 and Kinouchi *et al.*, 2003)**. The 5-year survival rates of stages I, II, III and IV were 83.08%, 68.75% and 31.25% respectively in our study. The 5-year survival following nephrectomy in some studies was 60% to 80% in stage I, 40% to 70 % in stage II, 10% to 40% in stage III and 5 % or less in stage IV **(Sene *et al.*, 1992 and Thrasher and Paulson, 1993)**. These results are in agreement with our results.

Univariate analysis in our study identified tumour stage as a significant prognostic factor for cancer specific survival (P< 0.00001); Multivariate analyses indicated that T. stage was an independent prognostic factor. Similar results were reported in some other studies **(Miyata *et al.*, 2003)**. Some studies reported a significant relationship in univariate analyses but not in multivariate analyses **(Joo *et al.*, 2004)**.

In our study we found that, there was no significance(P value= 0.061) between grade and microvessel density. Similar results were previously reported **(Mac Lennan *et al.*, 1995; Slaton *et al.*, 2001 and Joo *et al.*, 2004)**. Some other studies found that, there was a significant relationship between grade and MVD **(Nativ *et al.*, 1998 and Kinouchi *et al.*, 2003)**. **Sabo *et al.* (2001)** found that although MVD was higher in low-grade as opposed to high grade tumour, the difference was not statistically significant (P value = 0.12).

The 5-year survival rates for grades I, II, III and IV were 80.00%, 76.92% and 44.44% respectively in our results. **Medieros *et al.* (1997)** found that, the 5-year survival rates for grade 1 through 4 were 76%, 72%, 51% and 35% respectively, with significant differences observed between tumours of grade 1 and 2 and those of grades 3 and 4 in survival rates.

In our results we found that, there was significant differences between tumours of grades 1 and 2 and those of grades 3 and 4 in survival rates (P value= 0.0001) in univariate analyses. Multivariate analyses identified tumour grade as a dependent prognostic factor. Similar results were reported in some other studies **(Miyata *et al.*, 2003)**. **Joo *et al.* (2004)** found that, there was no significance between grade and survival rates in both univariate and multivariate analysis.

Our results revealed that there was no significance in the correlation between MVD and tumour size (P value= 0.481). Similar results were previously reported **(Nativ *et al.*, 1998 and Joo *et al.*, 2004)**. **Kinouchi *et al.* (2003)** found that there was a significant relationship between MVD and tumour size.

The 5-year survival rates for patients with tumour size ≤ 7 and > 7 were 90.63% and 63.08% respectively. Giuliani *et al*. (1990) reported 5-year survival rates of 84% for patients with tumour size less than 5 cm, 50% for tumours between 5 and 10 cm, and 0% for tumours more than 10 cm in diameter. Such tumours have been associated with more than 90% 5-year survival rates, whether they are managed with nephron-sparing surgery or radical nephrectomy **(Butler *et al.*, 1994 and Lerner *et al.*, 1996)**.

In our study we found that, there was significant differences between tumours ≤ 7 and > 7 in 5-year survival rates (P value= 0.004) in the univariate analysis. Multivariate analysis indicated that tumour size was a dependent factor (P value = 0.0531). Similar results were reported in some other studies, **Joo *et al.* (2004)** found that, there was significantly differences between tumour size groups in survival rates by univariate analyses (P value= 0.0105), but multivariate analyses indicated that tumour size was a dependent factor (P value = 0.415). **Roosen *et al.* (1994)** found that, there was insignificant differences in tumour size groups in univariate analyses (P value = 0.65), and identifies tumour size as a dependent factor in multivariate analyses (P value=0.97).

In our study we found that, there was insignificant relationship between sex and microvessel density (MVD) (P value=0.997). Similar results were previously reported by **Nativ *et al.* (1998)** who found that, there was no significant relationship between MVD and sex.

The 5-year survival rates for males and females were 79.71% and 53.57% respectively in our study. There was significantly differences between males and females using univariate analyses (P value=

0.0243) when multivariate analyses was performed, the survival difference between males and females disappeared.

In Western, studies have contested and supported the adverse impact of male gender in patients with RCC. **Ljungbery *et al.* (1988) and Green *et al.* (1989)** found no significant difference in survival between 81 men and 55 women with RCC. **Lieber and associates (1981)** reported that men had poorer survival rate than women. Many investigations found that, there was an insignificant relationship between sex and survival using univariate analyses and sex was identified as dependent factor using multivariate analyses **(Roosen *et al.*, 1994 and Suzuki *et al.*, 2001)**. **Lieber and associates (1981)** found that, there was significant differences between males and females using univariate analyses, but when multivariate analyses were performed, the survival difference disappeared.

Many investigations have evaluated the prognostic value of age of the patients with RCC. Our results indicated that, there was no significance relationship between age and MVD (P value =0.254). Although increasing in age increased MVD as shown in the results, this relationship was statistically insignificant. Similar results were noted between angiogenesis and age by **Nativ *et al.* (1998)**.

The 5-year survival rates for age < 40, between 40 and 50, between 50 and 60 and > 60 are 81.25%, 77.78%, 69.23% and 57.89% respectively. Univariate analyses indicated that there were no significant differences between age groups (P value=0.2828), Similar results were obtained using univariate and multivariate analysis **(Roosen *et al.*, 1994)**. Opposite results reported that, differences between age groups were significant using univariate analysis (P value =0.019), but when multivariate analyses was performed significantlty differences between age groups disappeared and age became a dependent factor **(Suzuki *et al.*, 2001)**. **Dehner *et al.* (1970)** reported 64.3% year actuarial survival rate for 15 children treated with nephrectomy. **Lieber *et al.* (1981)** found no significant differences in survival according to age at diagnosis. The overall 3-year survival, 5-year survival and 10-year survival were 60%, 55% and 47% respectively.

The present study indicated that, there was a significant relationship between lymph node status and MVD (P value= 0.03). Similar results were reported by **Dekel *et al.* (2002) and Joo *et al.* (2004)**. Opposite results were obtained by **Slaton *et al.* (2001)** who found that, there was no significant differences between the two groups of lymph node status in microvessel density. The 5-year survival rates for positive and negative lymph nodes were 56% and 77.78% respectively, this difference was significant using univariate analyses (P value= 0.04), but multivariate analyses indicated that lymph node status was a dependent factor. Lymph node involvement had long been recognized as a dire prognostic sign as it is associated with 5-year and 10-year survival rates of 5% to 30% and 0% to 5% respectively **(Bassil *et al.*, 1985)**. **Gusliani and associates (1990)** reported 52% 5-year survival for 25 patients with nodal positive disease managed with radical nephrectomy and extensive lymph node dissection which was better than historical controls. Other studies suggested improved survival in patients with pathologic stage NO disease managed with lymph dissection **(Golimbu *et al.*, 1986 and Herrlinger *et al.*, 1991)**.

The present study indicated that, there was a significant correlation between the histological type and microvessel density (P value= 0.001). There was significant differences between conventional type and both of papillary and chromophobe types. The mean microvessel count in conventional type was higher than that in both papillary and chromophobe types. **Mac Lennan and Bostwick (1995)** found that, there was a positive correlation of clear cell pattern and chromophobe pattern with increased MVD (P value= 0.047). **Kinouchi and his associates (2003)** found that, there was a significant correlation between type and MVD (P value= 0.009). The clear type was significantly higher than non clear type in the mean microvessel density. **Nativ *et al.* (1998)** found that, there was no significance between angiogenesis and cell type or histologic architecture. The 5-year survival rates for conventional type, chromophobe type and papillary and unclassified types were 69.05%, 78.95% and 72.2% respectively. There were no significant differences between all types in survival rates using univariate analyses (P value = 0.6539). **Dekel *et al.* (2002)** found that, the vessel count in papillary type was lower than in other histologic types. Giuliani *et al*. (1990) found that, there was not any statistical differences in survival between different types. **Suzuki *et al.* (2001)** found that, the correlation between survival and histologic examination was unclear.

The value of MVD as a predictor for patient prognosis in RCC is controversial because certain reports revealed a direct correlation between MVD and survival. Others revealed an inverse correlation and one report showed no correlation **(Sabo *et al.*, 2001)**. The 5-year survival rates for patients with low MVD < 20, median (20-30) and high > 30 were 77.55%, 62.16 and 81.82% respectively in our study. The differences between groups were insignificant using univariate analyses (P value= 0.1571).

Regarding prognosis and angiogenesis in RCC, reports showed that MVD is an independent

prognostic factor, MVD is a risk factor for distant metastasis **(Suzuki *et al*., 2001)**. They found that there was no correlation between angiogenesis and survival (P value= 0.112). Similar studies classified MVD into few, moderate and large amounts like our study and found no correlation between MVD and survival using univariate and multivariate analysis **(Roosen *et al*., 1994)**. **Nativ *et al*. (1998)** found that, there was a significant relationship between MVD and survival in univariate analysis (P value=0.041) and in multivariate analysis. For patient survival they found that the only significant and independent predictors were MVD (P value= 0.00014). The 10-year survival rate for patients with low and high microvessel count was 91% and 46% respectively.

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