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MMP-2, MMP-9, AND VEGF LEVELS IN PATIENTS WITH RENAL CELL CARCINOMA: ROLE AS POTENTIAL BIOMARKERS FOR EARLY PROGNOSTICATION OF DISEASE

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ABSTRACT

Aim: The aim of this study was to observe the levels of Matrix Metalloproteinases-2 (MMP-2), Matrix Metalloproteinases-9 (MMP-9), and Vascular endothelial growth factor (VEGF) prospectively, in patients with renal cell carcinoma (RCC), and assess their impact on patient survival, to establish them as biomarkers for prognosticating disease. **Material and Method:** 100 patients with RCC who were planned for surgery included in this study. Their venous blood sample was collected before and after the surgery to analyze the level variation of MMP-2, MMP-9, and VEGF using ELISA (enzyme-linked immunosorbent assay) technique. All patients were followed up at every three months for up to 5 years. Blood samples of healthy kidney donors were taken to serve as the control for the study. The survival analysis was done with Kaplan–Meier survival curves. **Results:** Preoperative MMP-2, MMP-9, and VEGF levels were higher compared to the postoperative levels in patients with RCC (p=0.001).On further analysis, higher marker levels, high-grade tumor, advance stage tumor and tumor size >7 cm were associated with low overall survival and progression-free survival (p=0.001). **Conclusion:** Level variation of MMP-2, MMP-9, and VEGF correlate with survival of patients with RCC in different conditions. They may serve as a useful biomarker for prognosticating patients with RCC in the early stage for achieving better survival.

KEYWORDS: RCC, MMP-2, MMP-9, VEGF, OS, PFS.

INTRODUCTION

Renal cell carcinoma (RCC) constitutes around three percent of all types of cancers in humans.[1] The incidence of this malignancy is increasing by 2% every year.[2,3] The mortality due to RCC is double that of bladder cancer and prostate cancer.[4]

While the localized renal tumors have a favourable prognosis, with survival exceeding 5 years for over 90% of the patients, survival is decreased considerably in case of metastasis.[5] Till now, no biomarker is available which may identify the patients who will progress to metastasis despite presenting with an apparently localized disease.[6,7] Recent work suggests that Matrix Metalloproteinases-2 (MMP-2), Matrix Metalloproteinases-9 (MMP-9), and Vascular endothelial growth factor (VEGF) have a role in the initiation and progression of RCC.[8] Matrix Metalloproteinases (MMP) is a group of 28 member zinc-dependent endopeptidases and among all the members, MMP-2 and MMP-9 are thought to be associated with metastatic RCC.[9] VEGF also participates in physiological processes like initiating new blood vessels from the existing ones and it can also lead to pathological angiogenesis.[10]

So far, there is no study which has focused on establishing a marker that may predict survival in Indian patients with RCC. In the present study, we have explored the role of MMP-2, MMP-9 and VEGF levels in patients with RCC who underwent nephrectomy, for predicting overall survival (OS) as well as progression-free survival (PFS). This has prognostic as well as a potential therapeutic implication as it may usher the development of newer agents seem to be very much effective for increasing overall and progression-free survival of the patient.

MATERIAL AND METHODS

The present study is a case-control study between January 2011 and January 2013, performed on patients diagnosed with RCC (cases) and voluntary kidney donors (controls). It carried out at two tertiary care hospitals in northern India. In each group, 100

individuals included. The study protocol was approved by the Ethics Committee at the outset. Patients with multiple tumors, bilateral tumors and malignancies other than RCC were excluded. Written informed consent taken from each participant. Patient characteristics summarized in **Table -1.**

Table. 1: Characteristics of Patients with RCC.

Variable		Number (n)	Percentage (%)
Patients		100	
Gender			
	Male	71	71
	Female	29	29
Age (Y)			
	Mean (range)	53.29 (21-79)	
	Less than 65	84	84
	More than 65	16	16
Tumor Stage			
	T1	26	26
	T2	39	39
	T3	22	22
	T4+LN	13	13
Tumor Cell Type			
	Clear Cell	74	74
	Papillary	09	09
	Chromophobe	06	06
	Others	11	11
Tumor Size (cm)			
	Less than 7	40	40
	More than or equal to7	60	60

Blood samples (5 mL) were obtained from each patient 30 minutes before surgery and again within 48 hours after surgery in Serum-separating tube II vacutainer and their centrifuged serum were preserved at -80^0 C. Then after levels of MMP-2, MMP-9 and VEGF were estimated by commercial human puregene ELISA (enzyme-linked immunosorbent assay) kits (Genetix Biotech Asia Pvt. Ltd). Patients were followed up for 5 years for analysing survival. The primary outcome of this study was to identify differences in MMP-2, MMP-9 and VEGF levels before and after surgery in patients with RCC. The overall survival of patients studied in the context of change from high to low levels of these markers after surgery, grade of tumor, tumor size, and tumor cell type.

STATISTICAL ANALYSIS

In the present study data were analyzed and expressed as mean values ± standard deviations (SD) to illustrate the baseline patient characteristics and survival patterns. Time to survival was calculated from the date of surgery to the date when the patient progressed as PFS and time to death as OS. Kaplan-Meier method was used to estimate and obtain survival curves. Relationships between outcomes, demographic factors, and survival patterns were assessed using Kaplan-Meier analyses and log-rank comparisons. All statistical analyses were performed using IBM SPSS Statistics Base, version 20.0 (IBM Corp, Armonk, NY).

RESULTS

As shown in **Table 2**, a very significant difference of preoperative and postoperative levels of MMP-2, MMP-9, and VEGF have been seen in comparison (p=0.001), similarly, the levels in control were also very low when compared with preoperative samples (p=0.001).

Table. 2: Serum Level comparisons in preoperative-postoperative patients and control group.

Group		Mean±SD ng/ml	p-value (pre vs. post)	p-value (pre vs. control)
	Patient (Pre OP)	833.90±111.91		
	Patient (Pre OP)	553.02±150.08		
MMP-2	Control	228.33±72.52	<0.001	<0.001
	Patient (Pre OP)	862.32±119.77		
MMP-9	Patient (Pre OP)	245.44±116.52	<0.001	<0.001
	Control	552.88±151.91		
VEGF	Patient (Pre OP)	1.35±0.36	<0.001	<0.001
	Patient (Pre OP)	0.81±0.32		
	Control	0.10±0.04		

Mean±SD= Mean plus minus standard deviation, p=Probability, pre=preoperative, post= postoperative

At a median follow up period of 5.2 years, the overall survival was 49%. One and three-year overall survivals were 83% and 70% respectively. Survival was better with lower levels of MMP-2, MMP-9 and VEGF after surgery suggesting that lower the level of these markers, greater is the survival (p=0.001). **See Figure 1.A, 1.B, 1.C**

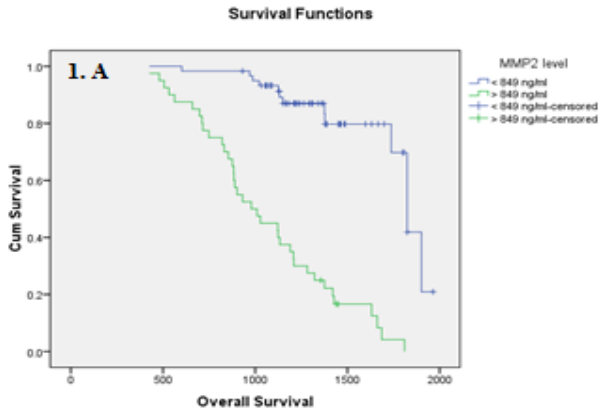

Figure. 1.A: Serum MMP-2 level and overall survival.

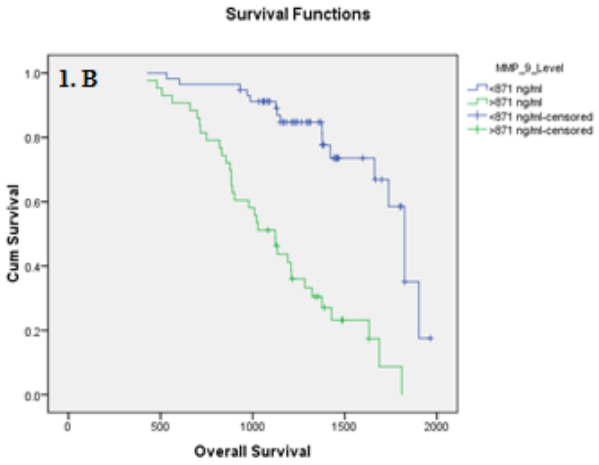

Figure. 1.B: Serum MMP-9 level and overall survival.

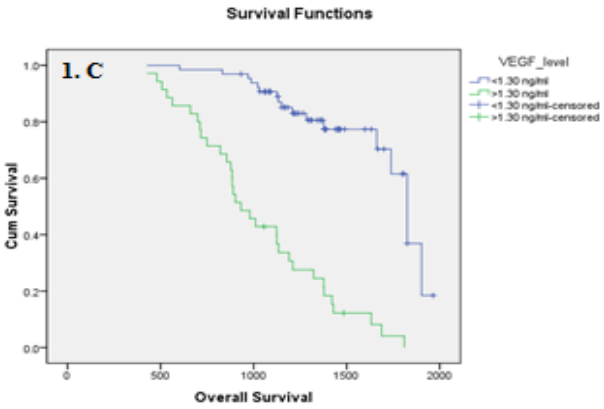

Figure. 1.C: Serum VEGF level and overall survival.

The levels of MMP-2, MMP-9, and VEGF were low in initial nuclear grade, stage and tumor less than 7 cm (<7cm) and higher in high nuclear grade, stage and more than or equal to 7 cm (≥7) cm tumor and lower levels were seen with better survival rather than higher levels (p=0.001). See **Table-3**.

Table. 3: Low and high levels of MMP-2, MMP-9 and, VEGF in different conditions and correlation with patient survival.

Level	Median Survival in days±SD (months)	Median Survival in days±SD (months)	Median Survival in days±SD (months)	p-Value
	Low level	**High level**		
MMP-2 MMP-9 VEGF	1823±52.2 (60) 1825±61.4 (60.8) 1825±73.1 (60.8)	971±50.3 (32.3) 1123±63.8 (37.4) 932±69.6 (31.06)		***0.001***
Stage	**Low**	**Intermediate**	**High+Lymph nodes**	
MMP-2 <849 ng/ml >849 ng/ml **MMP-9** <871 ng/ml >871 ng/ml **VEGF** < 1.30 ng/ml > 1.30 ng/ml	 1662±61.2 (55.4) 1541±72.8 (51.3) 1687±69.3 (56.2) 1216±58.2 (40.5) 1687±69.8 (56.2) 1212±58.2 (40.4)	 1210±59.9 (40.3) 1148±64.5 (38.2) 1210±55.6 (40.3) 1115±61.5 (37.1) 1190±51.8 (39.6) 1021±66.2 (34.0)	 821±52.2 (27.3) 694±71.9 (23.1) 855±56.4 (28.5) 679±61.8 (22.6) 821±63.5 (27.3) 657±70.3 (21.9)	***0.001***
Size	**Tumor size <7 cm**	**Tumor size ≥ 7 cm**		
MMP-2 <849 ng/ml >849 ng/ml **MMP-9** <871 ng/ml >871 ng/ml **VEGF** < 1.30 ng/ml > 1.30 ng/ml	 1421±92.4 (47.3) 1105±77.2 (36.8) 1428±80.7 (47.6) 1136±89.8 (37.8) 1367±84.2 (45.5) 1002±93.7 (33.4)	 877±89.3 (29.2) 695±85.8 (23.1) 902±78.8 (30.0) 687±75.9 (22.9) 877±66.8 (29.2) 703±75.7 (23.4)		***0.001***
Grade	**T1**	**T2**	**T3+T4**	
MMP-2 <849 ng/ml >849 ng/ml **MMP-9** <871 ng/ml >871 ng/ml **VEGF** < 1.30 ng/ml > 1.30 ng/ml	 1665±95.6 (55.5) 1328±111.2 (44.2) 1641±91.8 (54.7) 1306±100.2 (45.5) 1341±63.2 (44.7) 1047±115.4 (34.9)	 1352±83.5 (45.0) 1226±123.7 (40.8) 1318±83.5 (43.9) 1209±111.1(40.3) 1121±74.1 (37.3) 955±123.7 (31.8)	 710±110.0 (23.6) 553±96.3 (18.4) 699±54.8 (23.3) 521±66.5(17.3) 710±71.6 (23.6) 625±78.3 (20.8)	***0.001***
p=probability, SD= Standard Deviation				

PFS was assessed between the duration of patient RCC surgery to the time when disease progressed. The levels of MMP-2, MMP-9 and VEGF were compared in low levels with high levels with their PFS time. See **Table-4**.

Table. 4: Level of MMP-2, MMP-9 and VEGF and progression-free survival of patients.

Progression-free survival	Median Survival in days±SD (months)	p-Value
MMP-2 <530 ng/ml >530 ng/ml	1170±79.5 (39.0) 792±74.4 (26.4)	***0.001***
MMP-9 <550 ng/ml >550 ng/ml	1198±73.0 (39.9) 780±56.9 (26.0)	***0.001***
VEGF < 0.789 ng/ml > 0.789 ng/ml	1106±68.2 (36.8) 802±59.8 (26.7)	***0.001***
****Significant (p<0.05), SD: standard deviation***		

In this case also we received strong statistically significance correlation between the variation of levels and progression-free survival days. See **Figure 2.A, 2.B, 2.C**

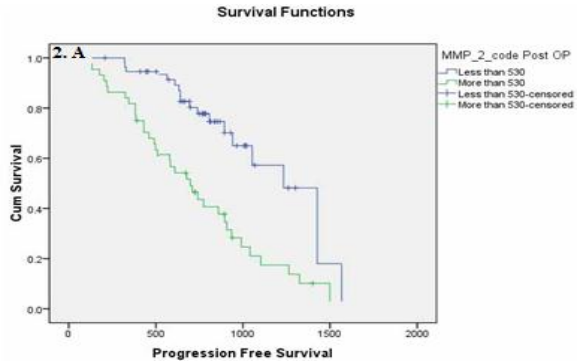

Figure. 2.A: Serum MMP-2 level and progression free survival.

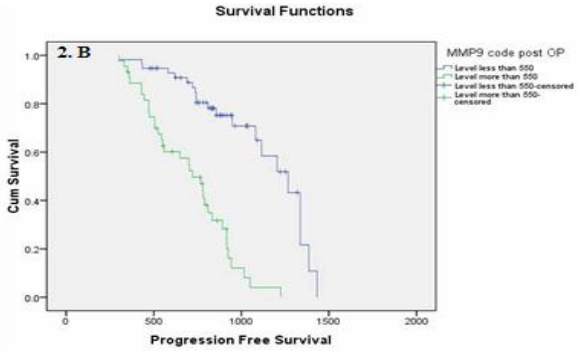

Figure 2.B: Serum MMP-9 level and progression free survival.

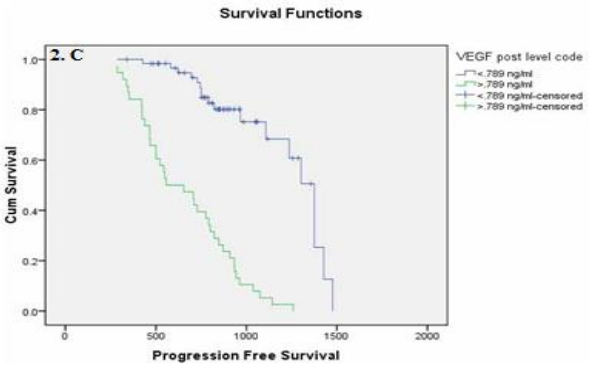

Figure. 2.C: Serum VEGF level and progression free survival.

These results indicate that the levels of MMP-2, MMP-9 and VEGF have an impact on patient progression- free and overall survival.

DISCUSSION

Currently, no diagnostic tool available to detect RCC in its early phase, which makes it very difficult to perceive the recurrence and efficacy of the treatment, in this context biomarkers could be the easily available tool. In recent years, tremendous work has been done on MMPs and VEGF at various stages of cancer progression and it has been observed that restraining the activity of MMPs and VEGF by means of synthetic inhibitors could be a recent approach to cancer treatment.[11] For the treatment of various types of cancers MMP inhibitors like MMPI, marimastat, neovastat, prinomastat, and VEGF inhibitors as VGA 1155, studied in advanced phase clinical trials and received some encouraging results like marimastat used for treating advanced gastric cancer, temozolomide used for treating recurrent and progressive glioblastoma multiforme[12] similarly anti-VEGF clinical trials support FDA (Food and Drug Administration) to give approval for many drugs as orally administered medicine for many carcinomas[13], 3-azido withaferin-A induced MMP-2 inhibition found effective in the treatment of prostate cancer and cervical cancer[14] etc and finally contributing in improvement of overall survival of patients.

MMP-2 has been studied as a biomarker in breast cancer, epilepsy and Glioblastoma apart from RCC[15-18] while MMP-9 has been studied as a biomarker in breast cancer and bladder cancer.[19-21] Similarly, VEGF also studied as a biomarker in ovarian cancer and lung cancer.[22-24]

The pathogenesis of RCC involves the interplay of various oncogenic factors and their inhibitors. Among these are a family of proteins known as metalloproteinases and their inhibitors, the tissue inhibitors of metalloproteinases (TIMP). These proteins involved in the disintegration of the matrix, which is an early step in metastasis of tumor cells. MMP-2 and MMP-9 are supposed to associate with tumor progression in RCC. It is seen that MMP-2 levels are higher in renal tumor compared to normal renal tissue.[25] In another study, it was shown that MMP-2 and MMP-9 levels are higher in advanced RCC compared to localized RCC and that the levels are related to the aggressiveness of RCC. VEGF is another important factor which has a role in the pathophysiology of RCC. Drugs which act against VEGF such as Sunitinib and Sorafenib are already the mainstay of immunotherapy for RCC.[26] MMPs and VEGF have been implicated in several other malignancies. Few studies performed earlier, which have assessed the relationship between the levels of MMPs and aggressiveness of RCC. There is still a paucity of evidence in the literature regarding the role of these markers in predicting survival in patients with RCC. Some studies studied the relation between MMP-9 levels and nuclear grade in patients with incidental localized RCC. They found that strong MMP-9 expression associated with a greater than 7-fold increase in the odds of the high nuclear grade.[27] In one of the study MMP-2 and MMP-9 expression in 153 patients with RCC has been seen where 104 patients belonged to stage 1 and 2 while 49 patients belonged to stage 3 and 4. The mean follow-up was 40 months. It is found that MMP-2 and MMP-9 expression correlated with shortened survival as well as high tumor grade.[28] We too found a significant impact of MMP-2, MMP-9 and VEGF expression on PFS and OS in the present study. Similarly, the association of cancer-specific survival studied in 249

patients with RCC. On immunostaining, it observed that strong MMP-9 staining was associated with poor cancer-specific survival.[29]

The significance of circulating MMPs as predictors of disease progression in patients with metastatic RCC who were on Sunitinib therapy. It is found that the MMP-9/ TIMP-2 ratio predicted disease progression in these patients. Although they found that the baseline MMP-9 levels did not correlate with PFS, the baseline levels of MMP-9 were significantly higher in patients who did not respond to Sunitinib compared to the responders. In our study, the patients had localized RCC unlike the study and hence the baseline biological characteristics of the two studies are not comparable. However, for the association of MMP-9 levels with response to Sunitinib, we have shown the association with survival and a common underlying message from both studies is that MMP-9 levels to determine the aggressiveness of RCC.[29-30]

The strength of the present study is that the levels of MMPs and VEGF have compared with not only the tumor size, grade, and stage but also with the histological type which has not been done in most studies done previously. Further, the follow-up period is over 5 years and we studied both PFS and OS which helps us to study the cancer behavior better than simply using OS. This study is limited by small sample size. However, taken as a whole, the result of the study suggest that establishing a healthy reference range of MMP-2, MMP-9, and VEGF could be useful as biomarkers in patients with RCC who could benefit from immunotherapy in future for increasing progression-free and overall survival in RCC.

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