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# Role of MMP-2, MMP-9 and VEGF as serum biomarker in early prognosis of renal cell carcinoma

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**KEYWORDS**
RCC;
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MMP-9;
VEGF;
Biomarker

**Abstract**
*Introduction:* Renal cell carcinoma epitomizes a diversified group of tumors which contributes more than 15,000 deaths annually worldwide. In spite of tremendous efforts to identify prognostic factors apart from grade, histology and tumor size, they are not so obvious yet to fulfill the requirement. In this study, the prognostic role of serum matrix metalloproteinase (MMP)-2, 9, and vascular endothelial growth factor (VEGF) levels in patients with pre and postoperative renal cell carcinoma are evaluated to use as biomarker.
*Patients and methods:* A total of 100 patients with a diagnosis of renal cell carcinoma included in the study. Additionally, hundred healthy kidney donors enrolled as control, serum MMP-2, MMP-9, and VEGF levels were analyzed in the serum of post and preoperative patients and parallel in control serum samples by ELISA method.
*Result:* Most of the patients with RCC were found to have high concentrations of serum MMP-2, MMP-9, and VEGF. The levels of MMP-2 in the serum of preoperative patients ranged from 627 to 1117 ng/ml (833.90 ± 111.91), postoperative MMP-2 range 302–913 (553.02 ± 150.08), control range 122–384 (228.33 ± 72.52). In MMP-9 pre-operative range 619–1233 (862.32 ± 119.77), post-operative range 124–909 (552.88 ± 151.91) and control range 42–467 (245.44 ± 116.52 and in VEGF preoperative range was 0.792–2.214 (1.35 ± 0.36), postoperative range was 0.315–1.917 (0.81 ± 0.46) and in control it was 0.01–0.39 (0.10 ± 0.09). We observed that preoperative levels of all three markers, were significantly increased if compared with postoperative and control levels (P = 0.001) however, no any significant correlation found when the levels correlated with grade, stage, size, and type for MMP-2 and MMP-9, but VEGF shows some significance in comparison.

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*Conclusion:* The present data shows relevance and strong significant decrease in the level of MMP-2, MMP-9, and VEGF after surgery, so they could use as biomarkers in early disease diagnosis and also in monitoring disease recurrence.



## Introduction

Renal cell carcinoma (RCC) is also known as hypernephroma, which is the cancer of the kidney and generally begins from very upper layer of the proximal convoluted tubule, which filters the blood to remove waste products from the body. Nearly three to four percent of all solid tumors represent RCC and, it is supposed to be the sixth escorted reason of carcinoma linked fatality, owing to the lack of therapeutic remedies for locally advanced or in case of metastatic disease [1]. Tumors limited within renal parenchyma mostly favor five years survival rate in up to sixty to seventy percent cases, but this is low considerably where metastases occurred outside renal parenchyma, so restricting the tumor at its initial stage is the prime target these days as the disease is mostly resistant to chemotherapy and also to radiation therapy, only a few cases are seen to respond with immunotherapy. It is very piteous that no corroborated RCC marker is known for detection of first-degree disease or the disease without any symptoms in selected populations for the prognostic or treatment effectiveness monitoring point of view [2]. High-quality detection techniques are required for its preliminary identification in an initial stage and for closely watching the recurrent tumors to cop them in the preliminary phase after surgery either by total or partial nephrectomy [3]. Several markers like CD44, VEGF and plasma amyloid-alpha have been experimented as a prospective tool for prognostic or indicative factors for RCC [4–8], though they still need to validate in rigorous trials. It is still an urgent need of RCC tumor biomarker for detecting renal cell carcinoma in its early stage. Biomarkers especially humoral tumor markers using blood serum could be ideal for the routine checkup of RCC in scheduled follow-ups intended to perceive any progression. In this context, we reviewed and observed some literature and local studies to presume that some markers like MMP-2, MMP-9 and, VEGF have potential features which originate as well as facilitate the progression of RCC. Individually if we see, the (vascular endothelial growth factor) has a pivotal role in couple of physiological process, in the development of new blood, in embryo formation, skeletal growth, wound repair mainly, VEGF is a leading cause of pathological angiogenesis and responsible for malignancies, inflammation, diabetic retinopathy, rheumatoid arthritis and many conditions [9]. VEGF is also responsible in propagation and voyage of endothelial cells physiologically as well as pathologically through binding with two congruent VEGF receptors of vascular endothelial cells (VEGF receptor-1 and 2) and is a glycoprotein of approximately 45 kDa. VEGF's are mostly seen expressing in neutrophils, fibroblasts, epithelial cells, activated macrophages, vascular smooth muscle cells which all are combined and involved in renal cell activities and lead to renal cell carcinoma when influenced with overexpression of VEGF and other factors [10,11]. On the other hand, MMP (matrix metalloproteinase) is a family of structural-related zinc-dependent endopeptidase which generally does its actions by degrading macromolecules of the extracellular matrix and has around 28 members in the family, all comprise different types of actions.

All together they are responsible for remodeling of tissues and in many physiological as well as pathological developments like arthritis, cirrhosis, angiogenesis, tissue repair, morphogenesis etc. MMP-2 and MMP-9 are mostly considered and thought to be responsible in case of metastatic disease [12]. MMP and VEGF are massively studied to enumerate their versatile role in angiogenesis as well as metastasis in cases of malignant tumors and also in RCC. Furthermore, it is now also known that MMP and VEGF are most active in the progression of the disease through sequential steps like extravasations and intravasations [13]. MMPs generally arbitrate basement membrane and extracellular matrix humiliation during the very early stage of tumor genesis, which usually contributes to the development of a microenvironment that encourages the tumor growth. MMPs are also active in the later stages of cancer progression in which they result in metastasis of the disease [14]. Latest indications recommend that MMPs and VEGF are moreover significant in the initial stage of tumor maturity until metastasis takes place. Serum level of MMP-2, MMP-9, and VEGF were reported to be significantly elevated in tumor tissue and in the urine sample from patients with RCC [15,16]. But no such studies were reported in our Indian population. Due to the aggressiveness of RCC, most cases are presenting with metastatic disease, due to which patients could not find better survival and die in a very short time. Surgical resection is the only management available at the time of diagnosis but the devious nature of the disease is reflected by the rate of the majority of recurrences after surgery. For this reason, the study was designed to identify disease or recurrence potential patients at an early stage by using biomarkers, which may help to prescribe additional therapy of anti-MMP-2, anti-MMP-9, and anti-VEGF at exact time required for treatment, which is looking effective in the improvement of overall and progression-free survival of the patient. For this purpose, the preoperative and postoperative serum level of MMP-2, MMP-9, and VGEF in RCC patients are measure, and simultaneously marker level has been seen in healthy controls also and compared. With the correlation of levels in pre vs. post vs. control, the levels checked against the stage, grade, size of the tumor and different types of RCC tumor to see if any correlation seen between the levels and co-factors for more exploratory information.

In our knowledge, this is the first study from India about the level of MMP-2, MMP-9, and VEGF in pre-operative and post-operative renal cell carcinoma, for analyzing the pattern of increasing activities in conditions and to verify whether they may have potential as biomarker in providing useful clinical information in recurrence or having maximum probability of RCC. In the present study, we

aimed to determine MMP-2, MMP-9 and VEGF activity levels in serum from patients with the Clear cell, Papillary, Chromophobe and some other type of renal cell carcinoma using pre-coated MMP-2, MMP-9, and VEGF kits.

## Patients and methods

In this case-control study, 100 outpatients with histological and related scan confirmation for renal cell carcinoma recruited between January 2011 to May 2013 from the Outpatient Department of Urology, Sanjay Gandhi Post Graduate Institute of Medical Sciences and King George Medical College, Lucknow, India. Both these medical institutes are renowned tertiary care multispecialty hospitals with medical college facility in the northern part of India. The enrolled patients included 71 male and 29 female with the mean age of 53 years (range 21–79). The patient recruited for the study had a progressive, inoperable histological confirmed renal cell carcinoma. Before patient surgery, a full medical examination including chest X-ray, abdominal–pelvic ultrasonography, computerized tomography, bone scan and blood biochemistry was carried out. The patient enrolled in the study after taking their consent on the proper guidelines of the ethics committee of King George's Medical College, Lucknow-India, ethics committee approved informed consent document. All the patients enrolled in the study were having no any other malignancy other than renal malignancy. They were in average ECOG status $\leq 2$ and in better hepatic and bone marrow function. Patient characteristics are summarized in Table 1.

**Table 1** Clinical characteristics of 100 patients with renal cell carcinoma.

| Variable | n | % |
|---|---|---|
| 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 to 7 | 60 | 60 |
| Metastatic site | | |
| None | 60 | 60 |
| Brain | 04 | 04 |
| Liver | 11 | 11 |
| Lungs | 13 | 13 |
| Bone | 03 | 03 |
| Recurrence | 09 | 09 |
| Addiction | | |
| None | 57 | 57 |
| Smoker | 06 | 06 |
| Alcoholic | 06 | 06 |
| Tobacco chewer | 15 | 15 |
| Multiple | 16 | 16 |

The control group enrolled to compare the results between disease and normal. For the purpose 100 healthy kidney donors selected after tested against any malignancy or disease and they recruited from the same outpatient departments The control group comprises healthy kidney donors without any comorbidities. They also enrolled after taking proper informed consent form. The mean age of recruited control was 46, ranges between 24 to 71 years.

5 ml of venous blood collected from the patient immediately (30 min $\pm$ 10 min) before their planned surgery of kidney, radical nephrectomy or partial nephrectomy. Blood collected in EDTA vacutainer and serum-separating tube II vacutainer and, stored at $-80\,^{\circ}$C within 15 min of collection. Again after surgery 5 ml of venous blood collected from the same patient within 48 h after the surgery and, stored accordingly under the labeling of patient study code as preoperative samples and postoperative samples. The control blood samples collected from healthy kidney donors and, stored in the same manner as in RCC patients after labeling them as control sample with their code identifier.

### *Determination of serum matrixmetalloproteinase-2, 9 and VEGF levels by ELSA*

Quantitative analysis of serum MMP-2, MMP-9, and VEGF has been done by using commercial Human Puregene ELISA kits as per the manufacturer's instructions (Genetix Biotech Asia Pvt., Ltd.). Briefly, Enzyme-Linked Immunosorbent Assay (ELISA) is a diagnostic tool which is now a day widely used in the medical sector. It is working on the principle of antigen-antibody interaction in which antigen from any specific sample is attached to the surface of the kit, and an additional specific antibody is applied over the surface which binds to the antigen. Finally, a substrate added, which contains a specific enzyme, altogether they produce reactions and detectable signals as a change in the colour of the substrate. Working on the same principle our ELISA kits also based on a two-site ELISA sandwich format. Standards and serum samples incubated in a microwell plate precoated with anti-MMP-2, anti-MMP-9 and, anti-VEGF antibody.

At the start of laboratory work standardization of positive controls (known value samples) done as per the given protocol, after that in first 8 well which concludes a single row of 12 rows of ELISA kit (96 wells in total), controls transferred in increasing concentration. From the second row onward patient serum samples transferred in duplicate (each patient sample kept in two wells), finally high purified IgG known as conjugate added in all wells and after that kit kept for incubation as per protocol required time. MMP-2, MMP-9, and VEGF present in samples bound to their specific antibodies used in MMP-2, MMP-9, and VEGF kits. Extensive washing through kit specific washing detergent used to remove non targeted molecules which remain unattached on wells after that kits coated with peroxidise-labelled antibody and again incubated. Finally, the reaction stopped using stop solution and the absorbance was read at 450 nm in a microtiter plate of ELISA Reader. Serum concentrations of MMP-2, MMP-9, and VEGF obtained from the corresponding standard curves and values received in each case of pre-operative, post-operative cases and controls.

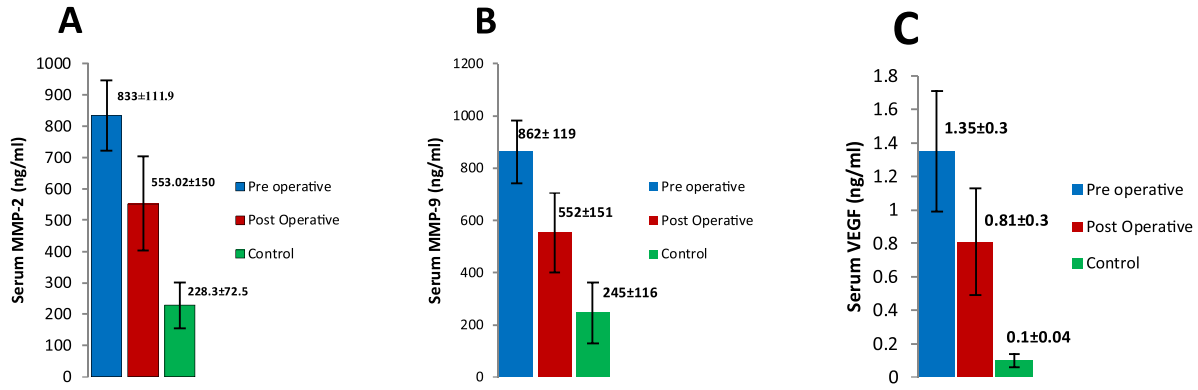


**Figure 1** A. Serum MMP-2 level in pre operative, post operative RCC patients and controls. B. Level difference of MMP-9 in pre, post operative RCC patients and in controls. C. Comparision of levels of VEGF in pre operative-post operative and in controls.

**Table 2** Serum level MMP-2, MMP-9, and VEGF in patients and control group.

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

*Statistical analysis*

The study data presented as the mean and the standard deviation and analyzed with the statistical analysis software SPSS v20.0, SPSS corp. Elevated serum levels of MMP-2, MMP-9, and VEGF defined in pre-operative levels above the 95th percentile of the distribution against post-operative and control subjects. Differences were evaluated using the Wilcoxon rank sum test, two-sample t-test, ANOVA and independent sample test for analyzing the level difference between pre and postoperative MMP-2, MMP-9, and VEGF with control. The Mann–Whitney U test (Kruskal–Wallis test) was used in evaluating the association case. The statistical significance of paired differences between pre-treatment and follow-up was measured using the unpaired t-test. All p-values are two-tailed, no any adjustment did to compare other factors.

## Results

*Patient characteristics*

The clinical data of 100 enrolled subjects abridged in Table 1, out of 100 patients 71 (71%) were male and 29 (29%) were female. The majority (84%) of patients were below age 65 and 39% of the T2 stage, 74% had clear cell histology, 60 out of 100 (60%) were non-metastatic, other were metastatic most commonly involving the lungs (13%). The majority number of the patients detected with big tumor of more than or equal to 7 cm size (60%), 57% of them were non addicted apart from that 15% were addicted to tobacco chewing. Follow-up blood sample has been collected for all patients.

*Pre-post-control levels of MMP-2, MMP-9 and VEGF*

In case of MMP-2,the result of levels achieved in preoperative renal cell carcinoma patients was 833.90 ± 111.91 ng/ml (Mean ± SD), which fall down in postoperative cases upto 553.02 ± 150.08 ng/ml (Mean ± SD), while in control values obtained 28.33 ± 72.52 ng/ml (Mean ± SD). The p-value of pre vs post was highly significant (0.001)-graphical comparison of levels can be seen in Fig. 1A. Similarly, in pre-operative cases of MMP-9, the level was 862.32 ± 119.77 ng/ml (Mean ± SD), while, in post-operative this level drops down to 552.88 ± 151.91 ng/ml (Mean ± SD), in control, the level was 245.44 ± 116.52 ng/ml (Mean ± SD) The p-value found highly significant (P = 0.001 – see Fig. 1B).

In preoperative cases of VEGF, the level was 1.35 ± 0.36 ng/ml (Mean ± SD) while, in postoperative cases the level was 0.81 ± 0.46 ng/ml and in control group, the level found was 0.10 ± 0.09, this result was also highly significant (P = 0.001 – see Fig. 1C).

Summary of result is in Table 2.

*Correlation between tumor grade and levels of MMP-2, MMP-9 and VEGF*

There is no correlation observed when MMP-2 ($P = 0.268$), MMP-9 ($P = 0.011$), and VEGF ($P = 0.008$) levels correlated with grade of tumor individually, i.e. low grade, intermediate and high grade with

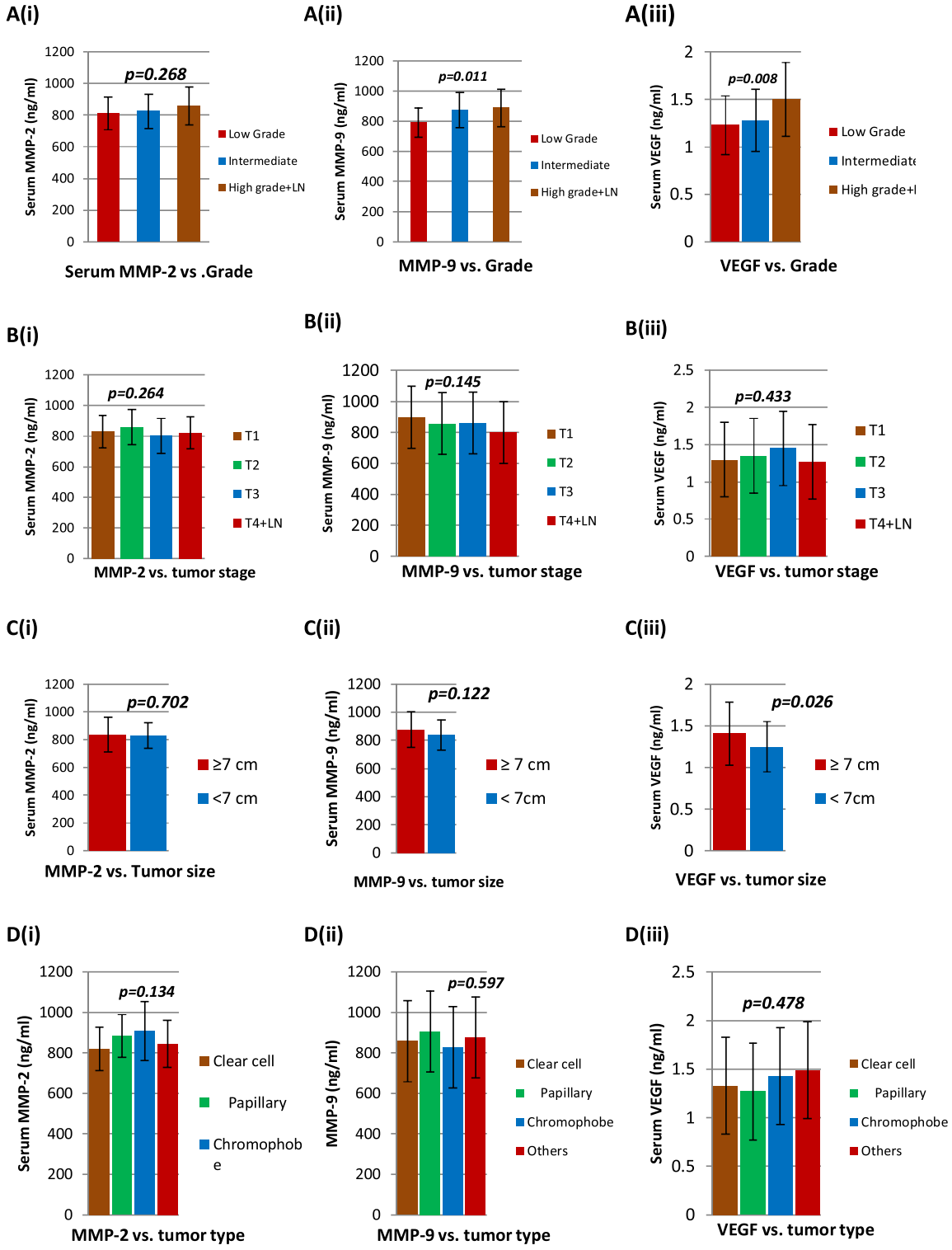


**Figure 2** A(i) Correlation estimation between serum MMP-2 and grade of the tumor, in Fig. 2A(ii) correlation has been seen in case of MMP-9 concentration and grade of the tumor, similarly in Fig. 2A(iii) correlation is seen between VEGF concentration and grade of the tumor. B(i) Correlation estimation between serum MMP-2 and stage of the tumor. Fig. 2B(ii) correlation has been seen in case of MMP-9 concentration and stage of the tumor. Fig. 2B (iii) correlation is seen between VEGF concentration and stage of the tumor. C(i) Correlation estimation between serum MMP-2 and size of the tumor. Fig. 2C(ii) correlation has been seen in case of MMP-9 concentration and size of the tumor. Fig. 2C (iii) correlation is seen between VEGF concentration and size of the tumor. D(i) Correlation estimation between serum MMP-2 and size of the tumor. Fig. 2D(ii) correlation has been seen in case of MMP-9 concentration and size of the tumor. Fig. 2D (iii) correlation is seen between VEGF concentration and size of the tumor.

**Table 3** MMP-2, MMP-9 and VEGF correlation with tumor grade, stage, size and type.

| Variable | n | MMP-2 Mean ± SD | (Sig) | MMP-9 Mean ± SD | (Sig) | VEGF Mean ± SD | (Sig) |
|---|---|---|---|---|---|---|---|
| Grade | | | | | | | |
| Low grade | 20 | 812.02 ± 102.60 | ***(0.268)*** | 791.97± 97.70 | ***(0.011)*** | 1.23 ± 0.31 | ***(0.008)*** |
| Intermediate | 46 | 825.35 ± 108.32 | | 874.70 ± 115.83 | | 1.28 ± 0.33 | |
| High grade + LN | 34 | 858.34 ± 120.43 | | 886.95 ± 124.12 | | 1.50 ± 0.39 | |
| Stage | | | | | | | |
| T1 | 28 | 828.97 ± 105.91 | ***(0.264)*** | 896.73 ± 115.93 | ***(0.145)*** | 1.30± 0.31 | ***(0.433)*** |
| T2 | 39 | 859.06 ± 114.86 | | 856.49 ± 102.02 | | 1.35 ± 0.37 | |
| T3 | 22 | 801.40 ± 114.57 | | 859.94 ± 152.11 | | 1.45 ± 0.40 | |
| T4 + LN | 11 | 822.27 ± 104.83 | | 800.14 ± 99.30 | | 1.27 ± 0.36 | |
| Size | | | | | | | |
| ≥7 cm | 60 | 837.42 ± 124.79 | ***(0.702)*** | 877.45 ± 126.10 | ***(0.122)*** | 1.41 ± 0.38 | ***(0.026)*** |
| <7 cm | 40 | 828.63 ± 90.46 | | 839.63 ± 107.13 | | 1.25 ± 0.30 | |
| Cell type | | | | | | | |
| Clear cell | 74 | 820.36 ± 107.26 | ***(0.134)*** | 857.78 ± 125.69 | ***(0.597)*** | 1.33 ± 0.35 | ***(0.478)*** |
| Papillary | 09 | 883.26 ± 104.94 | | 905.1 ± 123.44 | | 1.27 ± 0.34 | |
| Chromophobe | 06 | 907.95 ± 145.0 | | 827.8 ± 77.22 | | 1.43 ± 0.51 | |
| Others | 11 | 844.21 ± 117.10 | | 876.5 ± 93.67 | | 1.49 ± 0.33 | |

lymph nodes (Fig. 2A.i–iii). Mann–Whitney U test (Kruskal–Wallis test) used in evaluating the association case.

*Correlation between tumor stage and levels of MMP-2, MMP-9 and VEGF*

There is also no correlation was seen in the case of MMP-2 ($P = 0.264$), MMP-9 ($P = 0.145$), and VEGF ($P = 0.433$) levels when compared with stages of tumor like T1, T2, T3 and T4 + lymph nodes (Fig. 2B.i–iii), the output result suggests that higher or lower the stage of tumor does not reflect a high density of MMP-2, MMP-9, and VEGF. Mann–Whitney U test (Kruskal–Wallis test) was used in assessing the association.

*Correlation between tumor size and levels of MMP-2, MMP-9 and VEGF*

The correlation also checked through the Mann–Whitney U test (Kruskal–Wallis test) and here also found that there is no any correlation in case of MMP-2 ($P = 0.702$), MMP-9 ($P = 0.122$), and VEGF ($P = 0.026$) levels with size of i.e. tumor size less than 7 cm and size more than or equal to 7 cm (Fig. 2C.i–iii). Findings suggested that more or less the size of the tumor could not summarize the concentration of MMP-2, MMP-9 or VEGF within.

*Correlation between tumor size and levels of MMP-2, MMP-9 and VEGF*

To ensure any correlation the level of markers in patient serum and tumour cell type i.e clear cell, papillary, chromophobe and other types of the tumor Mann–Whitney *U* test (Kruskal–Wallis test) applied, we got no correlation result (MMP-2: P = 0.134, MMP-9: P = 0.597, VEGF: P = 0.478), see Fig. 2D.i–iii.

The correlation observation was the secondary objective of the study and it suggests that no any association is embroiled with overexpression of MMP-2, MMP-9, and VEGF when they correlate with various types of tumor characteristics like tumor grade, stage, size and type. See Table 3.

## Discussion

In recent years, MMP's and VEGF are the most talkable topic when we are looking over various stages of cancer progression. In many of the recent approach, MMP's and VEGF action was inhibited by using synthetic inhibitors which seems to open a new era of cancer treatment [17,18]. Annually, worldwide around 134,000 deaths are being recorded due to this disease [19].

At present, no any diagnostic method is available for initial diagnosis of RCC, rather than detected incidentally sometimes during some routine tests or tests suggested for other means and similarly, no modality is available to observe the recurrence or effectiveness of treatment given. Biomarkers are easily quantifiable substances which can be used to surveil habitual as well as abnormal biological function. Lamentably, no existing biomarker is available till date for detecting RCC. Throughout the course in current years, the salient role of MMPs and VEGF in diversified stages of cancer progression worked over tremendously and found that obstructing the activity of MMPs and VEGF using synthetic inhibitors could be a recent approach in the treatment of cancer [20].

Small molecule MMP and VEGF inhibitors like MMPI, marimastat, neovastat,prinomastat, and VGA 1155 have been studied in advanced phase clinical trials for the treatment of various types of cancers [21–24].

Recently, some encouraging results received from clinical trials, in which synthetic anti MMP called synthetic MMPIs like marimastat used for treating advanced gastric cancer, temozolomide [25,26] for treating recurrent and progressive glioblastoma multiforme has been used similarly anti VEGF were used in clinical trials and supports FDA to give approval for anti VEGF drugs like Clark et al. [27], Ellis et al. [28] Yang et al. [29] etc.

Albeit about 300 differential proteins have been recognized from various renal tumor tests, cell lines or patient's serum and from their particular controls by proteomic considers, but specified RCC biomarkers have not been accessible for the detection and anticipation for early reactions to treatment up until this point [30].

In some studies, 3-azido withaferin-A induced MMP-2 inhibition was found very much effective in the treatment of prostate cancer and cervical cancer [31].

Sorafenib and Sunitinib which are orally administered tyrosine-kinase inhibitor (TKI) which inhibit VEGFR for disease management, they are widely using medicine for metastatic RCC nowadays, these drugs are well tolerated and have significant disease stabilizing activity and contribute in increasing progression-free survival (PFS) [32,33]. Some aggravating preliminary clinical retort data with VEGF inhibition in RCC has provided a prospect for treatment press forward for this historically resistant malignancy [34].

Despite improving almost 5-year survival rates, clinicians and researchers are looking for ways to better guide therapy by improving outcomes and limiting toxicities. New techniques in surgical procedure and advancement in radiologic imaging are helping a lot but, establishing and associating biomarker in treatment line, could result in most accurate treatment on time when exactly required and may give healthier treatment outcomes. Looking through all prospective and the need of biomarkers in RCC we have gone through many works of literature and observed that MMP-2, MMP-9, and VEGF activity is found most active in RCC and, hence in this paper we are trying to establish the role of MMP-2, MMP-9 and VEGF as biomarker for prognosticating renal cell carcinoma in its early stage.

Statistically, significantly higher level of MMP-2, MMP-9, and VEGF was present in pre-operated patients when compared with post-operated patients and controls.

Renal cell carcinoma introduces a diversified group of cancers that come in existence from the proximal convoluted tubules of the kidney. In comparison to other solid tumors, limited studies are available for prognosticating the presence of renal cell carcinoma in its early stage, for this reason, diagnosis is delayed and leads to a huge number of disease-related deaths worldwide, the reason why biomarkers are very much required to identify renal cell carcinoma at an early stage.

MMP-2, MMP-9 and VEGF's generally associated with pathways creation for tumor generation, invasion and facilitate the tumor to spread, in present work, we considered the levels of MMP-2, MMP-9, and VEGF for diagnosing renal cell carcinoma in an early stage. For the purpose serum sample of hundred patients of renal cell carcinoma were collected before and after the surgery, conversely, hundred control samples are collected from healthy kidney donors before their surgery.

Pre and post-operative levels of patient MMP-2, MMP-9, and VEGF compared individually and against control samples, furthermore, according to result it is found that these biomarkers can help in screening disease and detecting recurrence of RCC, and could be helpful for starting the treatment in the early stage of diagnosis, to increase progression-free and overall survival of the patient.

In addition to this, we have also studied that whether the levels have any impact on the stage, grade, size, and type of the tumor.

Elevated serum MMP-2, MMP-9, and VEGF levels noted in RCC patients before surgery and in comparison with healthy control, also found associated with low progression-free and overall survival of the patient. Taken as a whole, the results of our study positively propounding that MMP-2, MMP-9, and VEGF have important roles from initiation to the rapid progression of renal cell carcinoma and therefore establishing a healthy reference range could be useful in identifying elevated levels and start treatment accordingly to stop the development and progression of RCC. Though, a limited number of patient and control in this study, some larger studies are needed to confirm our findings. Some more factors can be looked which we have not touched, like the level of MMP-2, MMP-9 and VEGF can be seen all together in patient serum, tissue and urine. In conclusion, our findings provide substantiation that MMP-2, MMP-9, and VEGF together have important roles at different phases of metastatic spread. Measurement of these markers in diagnostic as well as in follow up stages of the patient may be a milestone in detecting RCC as well as for patients which are at high risk of progression.

## Conclusion

In summary, the present study has validated preliminary data showing that circulating MMP-2, MMP-9, and VEGF are elevated in patients with RCC and furthermore associated with poor prognosis. We suppose it reflects an association between tumor progression and elevation of tumor-derived proteases which aggravates the disease. Further characterization of MMP-2, MMP-9 and VEGF in humans, as well as the results of enduring clinical trials, will help explicate the role of MMP-2, MMP-9, and VEGF in patients with renal cell carcinoma and its diagnosis in early stage and can be established as biomarkers for better disease management and for achieving better patient survival.

## Conflict of interest

The authors declare no competing financial interests.

## Authors' Contributions

*S. Ahmad*: Contributed in envisage of presented idea of study and performed the scientific calculations, carried out sample collection, storage and performed experiments, data collection, statistical analysis, drawing figures and took the lead in writing manuscript with inputs of all authors.

*S. Ahmad is a PhD student at King George Medical University and this work is submitted in partial fulfilment of the requirement for PhD.*

*V. Singh*, *R.J. Sinha*, *A. Srivastava*, *A. Mandhani*: Envisage the presented idea build-up the theoretical framework, identification of patient and control, taking their consent and providing blood samples.

*V. Singh*, *R.J. Sinha*, *A. Mandhani*: Analyzed the data, supervised the study and findings of this work.

All authors discussed and endowed with their critical feedback to assist in outlining the research, its analysis, and manuscript.

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Pradesh, India and from Maya Clinicals, Hyderabad-India, which is a contract research organisation. The terms of this arrangement have been reviewed and approved by King George Medical University at Lucknow, Uttar Pradesh-India.

### Ethical Committee Approval

The study ethically approved from the ethical committee of King George Medical University (Chhatrapati Sahuji Maharaj Medical University), Lucknow-Uttar Pradesh-India-226003 with approval number ***1405/R.Cell-10 dated 31-Aug-2010/Chairman (Faculty In-charge) = Prof. Shally Awasthi***.

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