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University of Lucknow
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Diagnostic and prognostic significance of MMP-2 and MMP-9 in NMIBC and MIBC patients

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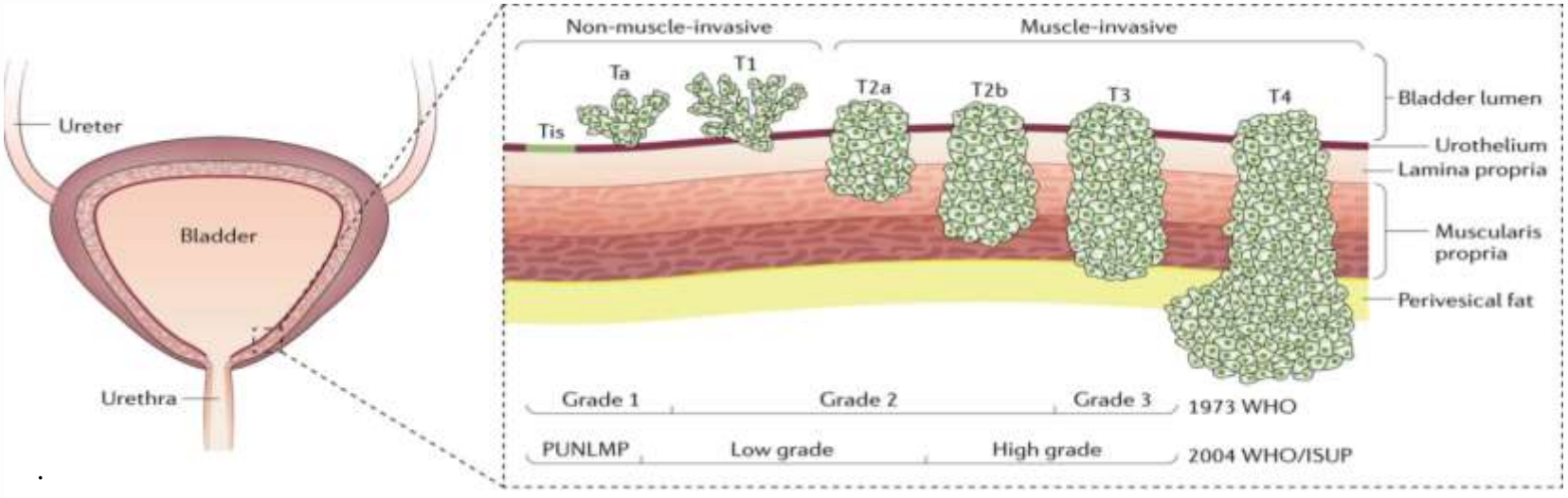
Introduction

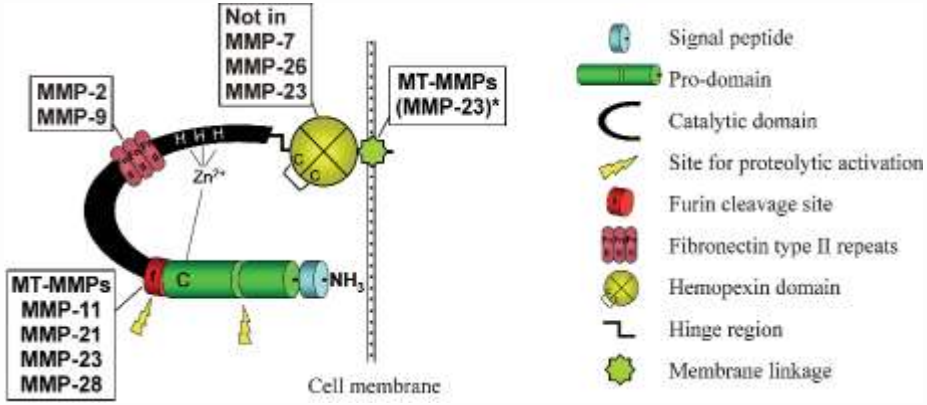
- Urothelial Bladder cancer is the 10th most commonly diagnosed cancer worldwide.
- Approximately 573,000 new cases and 213,000 deaths worldwide (Globocan 2020. It is the second most common genitourinary disease.
- India alone had 18,921 new cases of urothelial carcinoma of bladder in 2020, with an incidence rate of 2.4 in males and 0.7 in females per lakh population.
- Hematuria is most common symptom reported in UCB patients.

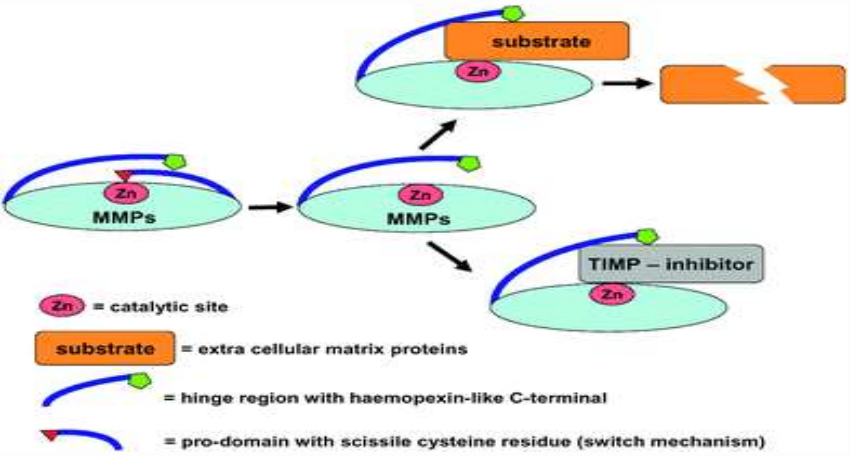


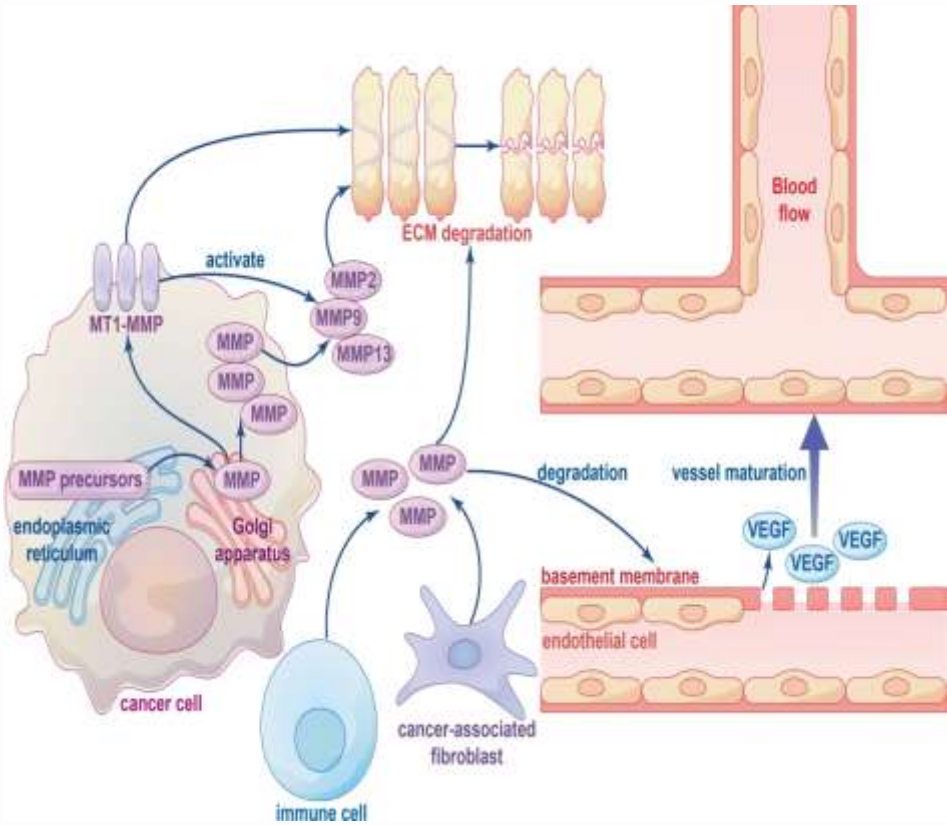
Subtypes of Urothelial Bladder Cancer

- Tumor metastasis involves strong interactions between the invading cancer cells and tumor microenvironment, which produce specialized proteolytic enzymes promoting degradation of the extracellular matrix (ECM).

- Matrix metalloproteinases (MMPs), a family of zinc and calcium-dependent proteolytic enzymes, degrade different components of ECM including collagen, laminin, fibronectin, vitronectin, elastin, and proteoglycans.







Purpose of the study

- 70–80% of the UCB patients are diagnosed as NMIBC.
- Transurethral resection of bladder tumor is the treatment of choice, nevertheless 50–90% of the patients suffer recurrence.
- There is still lack of unanimity among the clinicians regarding the mode of treatment based on radical cystectomy or conservative approach for high grade NMIBC.
- 20–30% UCB patients are diagnosed with MIBC. The standard treatment for MIBC patients is neo adjuvant chemotherapy followed by radical cystectomy, however 50% of such patients experience disease relapse.
- Due to high risk of recurrence and progression life long surveillance is required which makes UCB clinically expensive to manage.
- Owing to the given cost/ expertise required of standard diagnostic procedures, it is deemed necessary to investigate for molecular markers like MMP-2 and MMP-9 of clinical significance which may improve understanding of tumor behavior and disease diagnosis, prognosis.

Methods

Tumor biopsy samples obtained from patients undergone TURBT at Department of Urology at KGMU and classified as NMIBC (70) and MIBC (40)

RNA extraction from tumor and normal tissues followed by cDNA preparation

Real Time quantitative PCR (RT-qPCR)

To evaluate the average fold change expression of MMP-2,MMP-9 in NMIBC and MIBC at transcript level by Livak Method

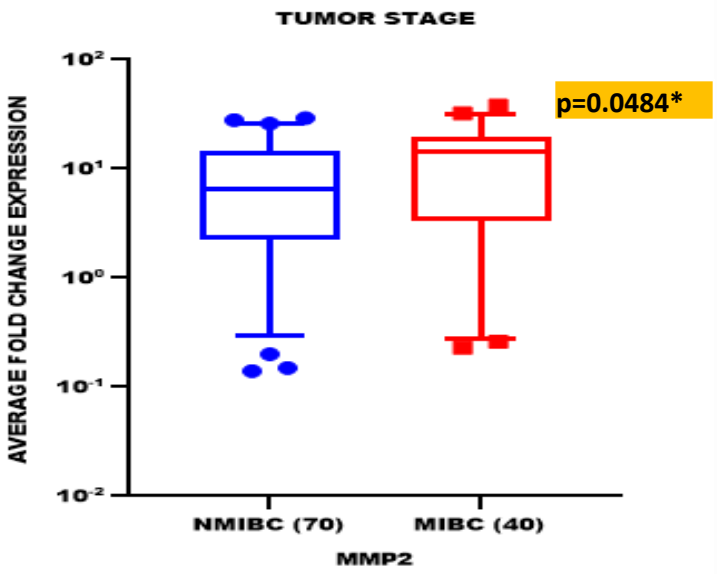
Immunohistochemical staining (IHC)

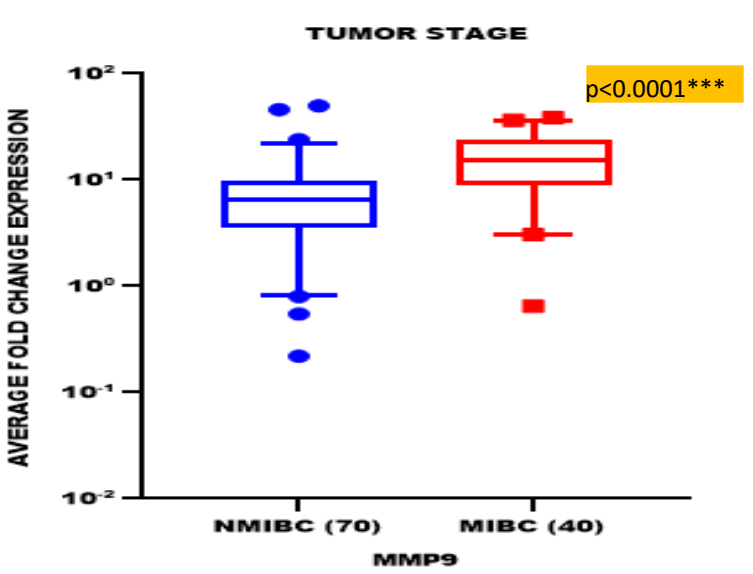
Sections were analyzed microscopically (400X) to calculate Total immunostain score (TIS) Staining intensity was graded as:1 (weakly stained), 2 (moderately stained) or 3 (strongly stained).

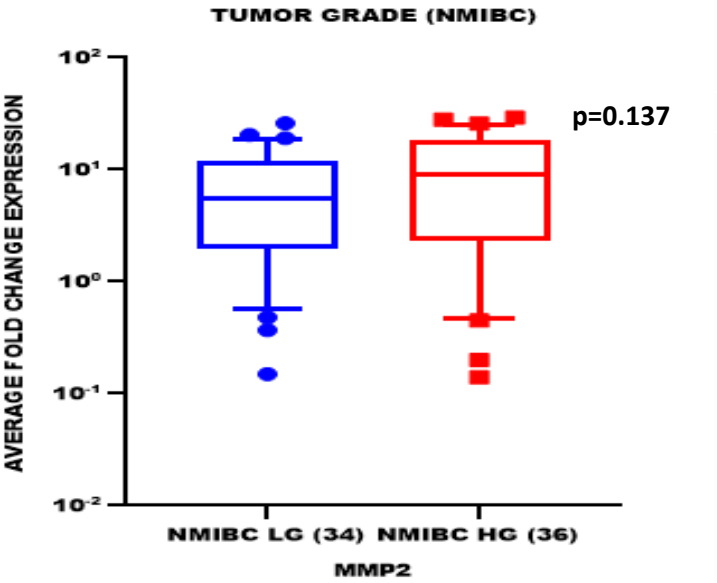
TIS=Intensity of cell staining multiply percentage of stained neoplastic cells .
TIS range from 0 to 12 as (0-3)Weak, (4-6) Moderate and (8-12) Strong expressions

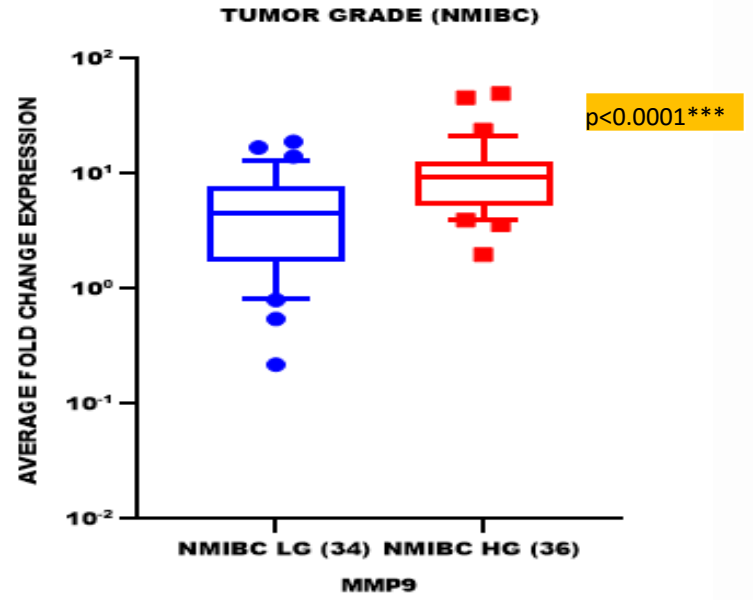
Statistical Analysis
Mann Whiteny test, Fisher's exact Test, Kaplan Meier along with log rank test

Real Time qPCR expression of MMP-2 and MMP-9



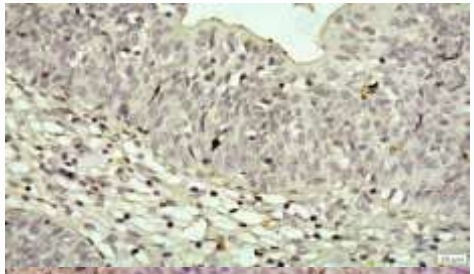




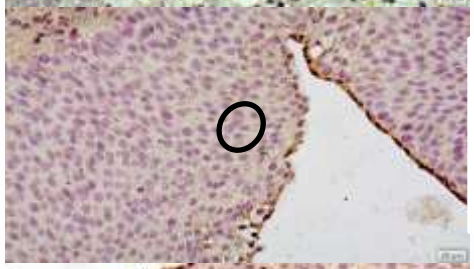


Immunohistochemical Analysis of MMP-2 and MMP-9

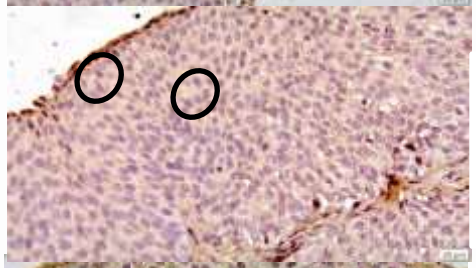
MMP-2



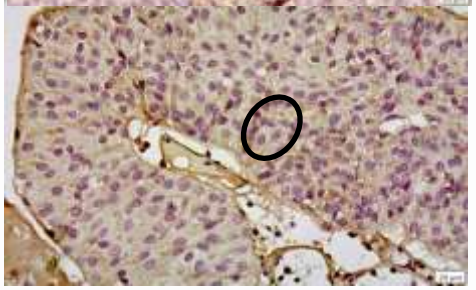
NORMAL
(TIS:1)



NMIBC LOW GRADE
(TIS:3)

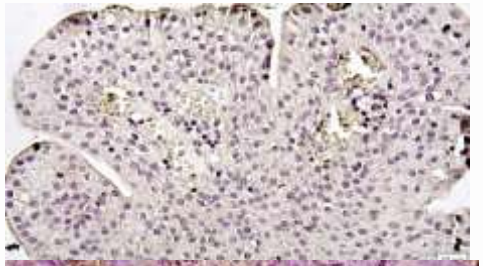


NMIBC HIGH GRADE
(TIS:4)

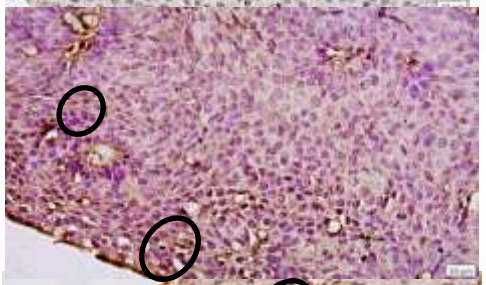


MIBC
(TIS:8)

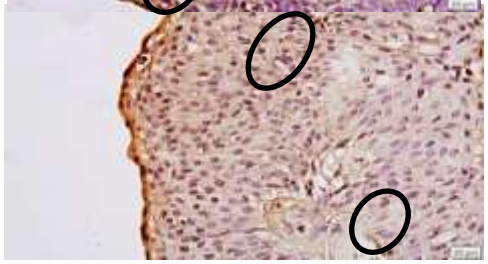
MMP-9



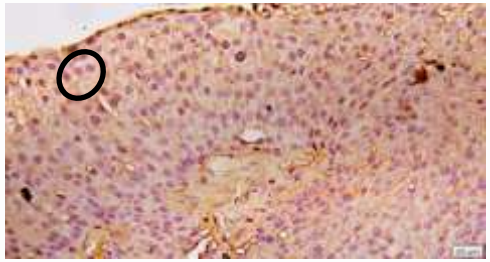
NORMAL
(TIS:1)



NMIBC LOW GRADE
(TIS:2)

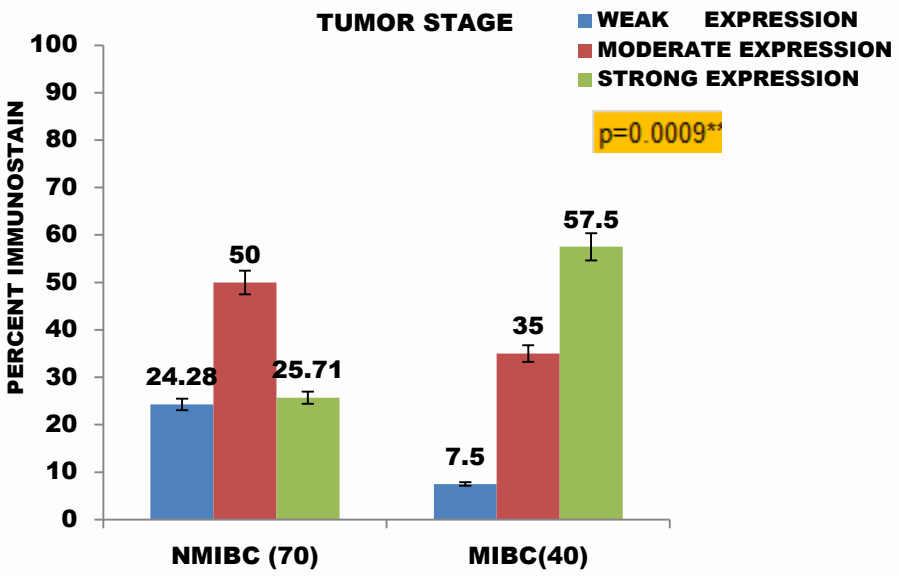


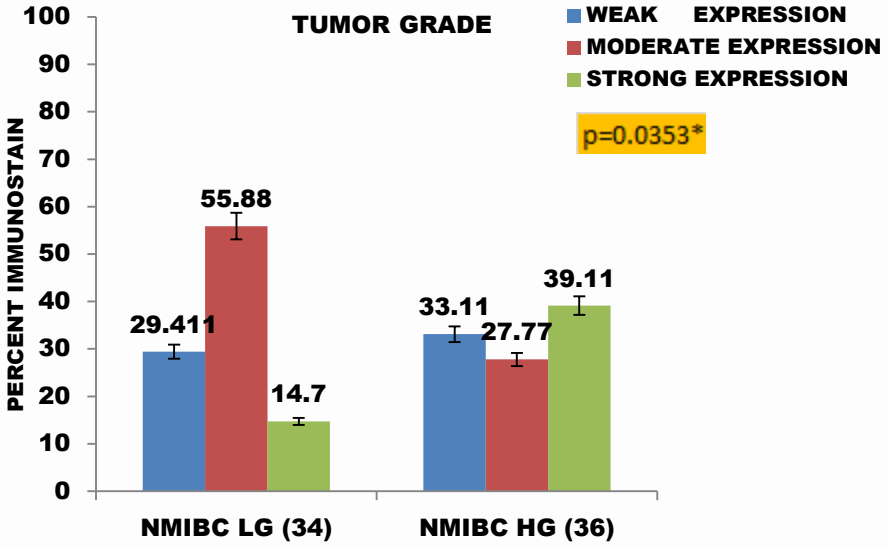
NMIBC HIGH GRADE
(TIS:6)



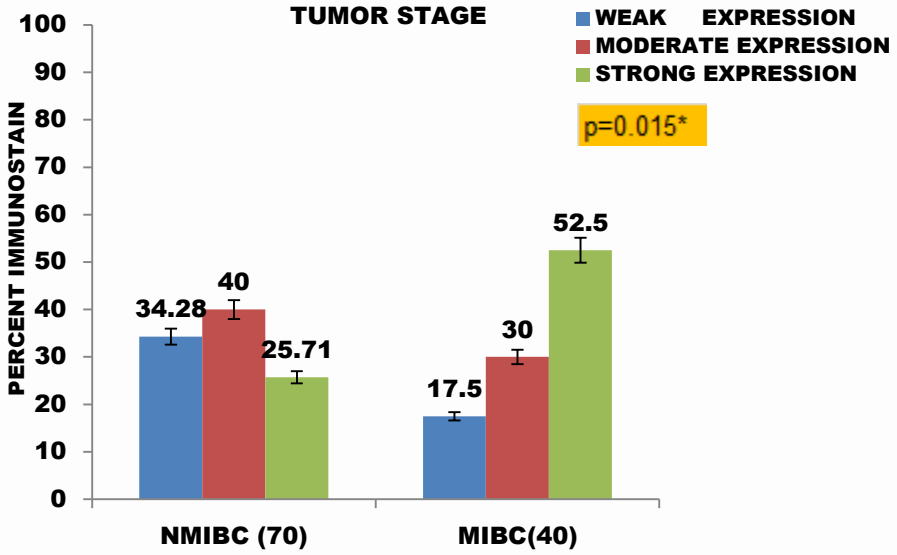
MIBC
(TIS:12)

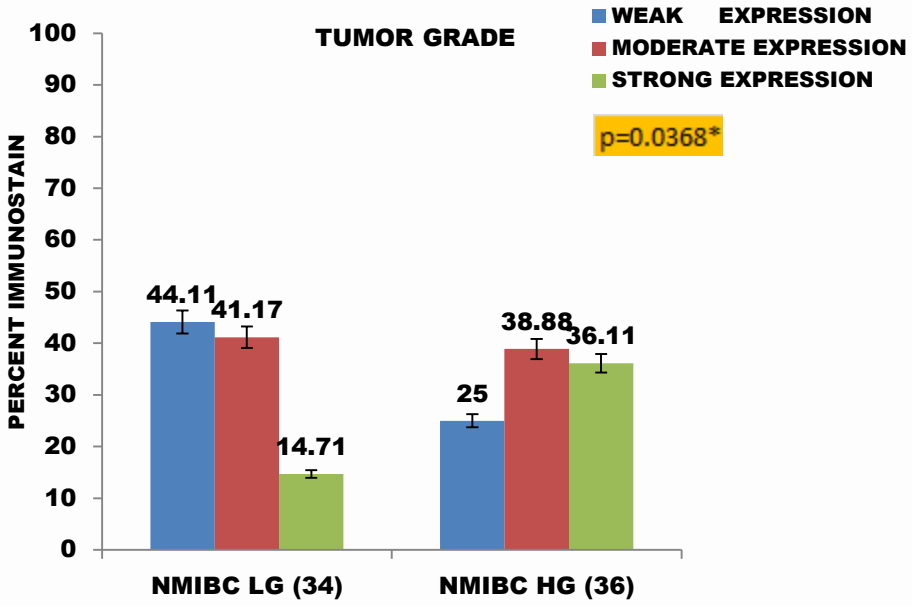
MMP -2

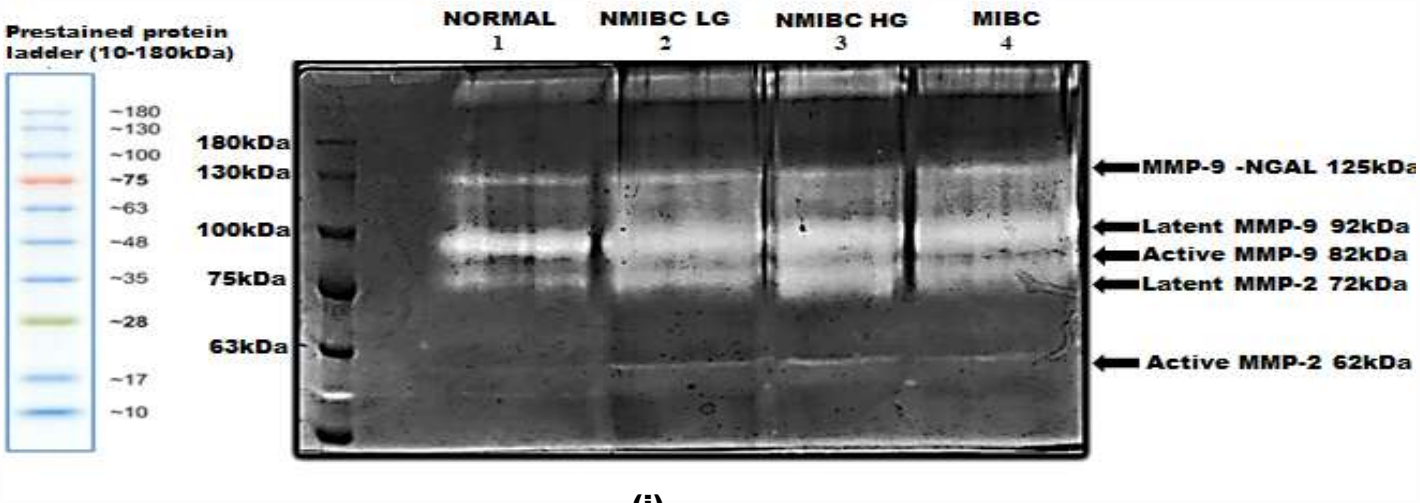

TUMOR STAGE
WEAK EXPRESSION
MODERATE EXPRESSION
STRONG EXPRESSION
p=0.0009**
PERCENT IMMUNOSTAIN
100
90
80
70
60
50
40
30
20
10
0
24.28
50
25.71
7.5
35
57.5
NMIBC (70)
MIBC(40)


TUMOR GRADE
WEAK EXPRESSION
MODERATE EXPRESSION
STRONG EXPRESSION
p=0.0353*
PERCENT IMMUNOSTAIN
100
90
80
70
60
50
40
30
20
10
0
29.411
55.88
14.7
33.11
27.77
39.11
NMIBC LG (34)
NMIBC HG (36)

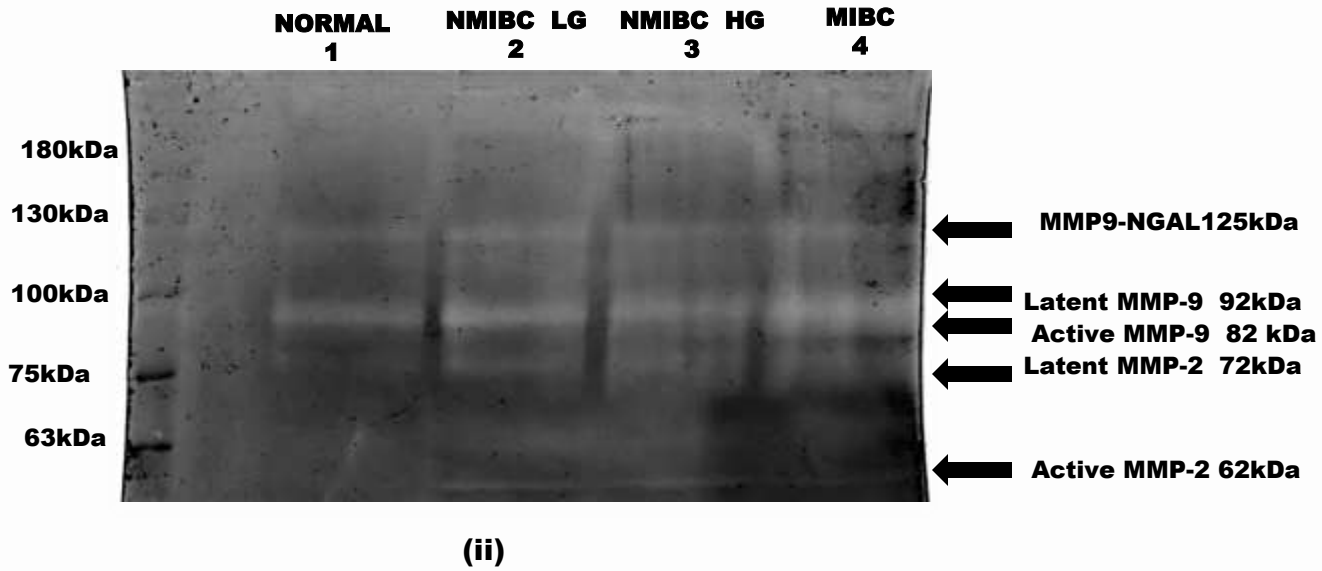
MMP - 9


TUMOR STAGE
WEAK EXPRESSION
MODERATE EXPRESSION
STRONG EXPRESSION
p=0.015*
PERCENT IMMUNOSTAIN
100
90
80
70
60
50
40
30
20
10
0
34.28
40
25.71
17.5
30
52.5
NMIBC (70)
MIBC(40)

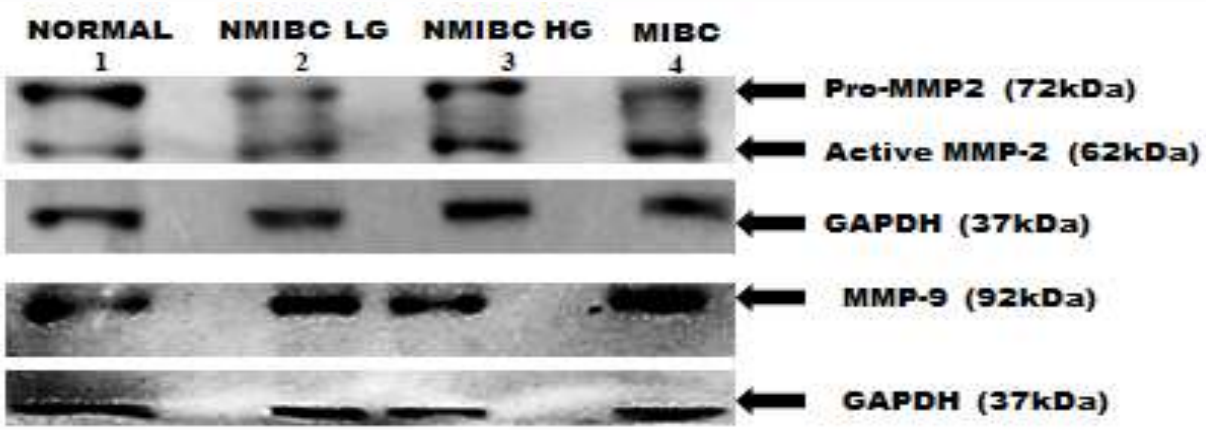

TUMOR GRADE
WEAK EXPRESSION
MODERATE EXPRESSION
STRONG EXPRESSION
p=0.0368*
PERCENT IMMUNOSTAIN
100
90
80
70
60
50
40
30
20
10
0
44.11
41.17
14.71
25
38.88
36.11
NMIBC LG (34)
NMIBC HG (36)


Prestained protein ladder (10-180kDa)
~180
~130
~100
~75
~63
~48
~35
~28
~17
~10
NORMAL 1
NMIBC LG 2
NMIBC HG 3
MIBC 4
180kDa
130kDa
100kDa
75kDa
63kDa
MMP-9 -NGAL 125kDa
Latent MMP-9 92kDa
Active MMP-9 82kDa
Latent MMP-2 72kDa
Active MMP-2 62kDa

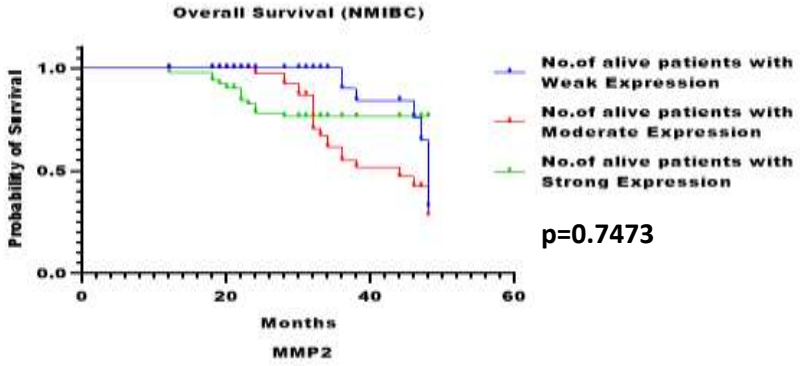
(i)

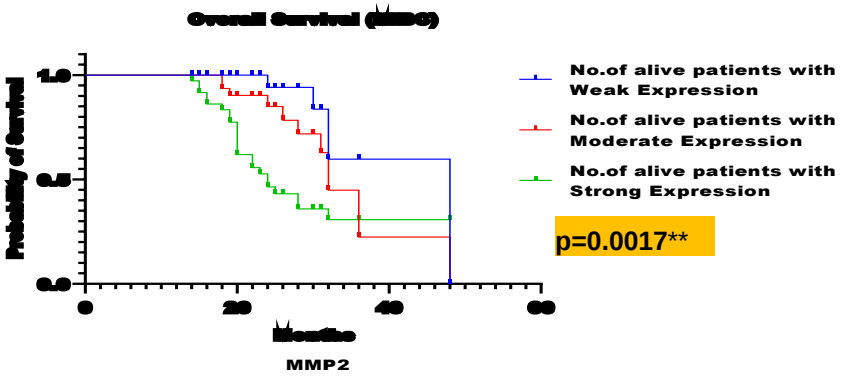

NORMAL 1
NMIBC LG 2
NMIBC HG 3
MIBC 4
180kDa
130kDa
100kDa
75kDa
63kDa
MMP9-NGAL125kDa
Latent MMP-9 92kDa
Active MMP-9 82 kDa
Latent MMP-2 72kDa
Active MMP-2 62kDa

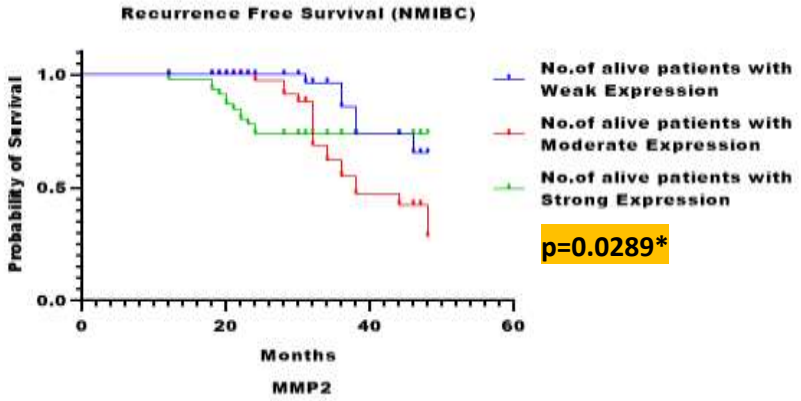
(ii)

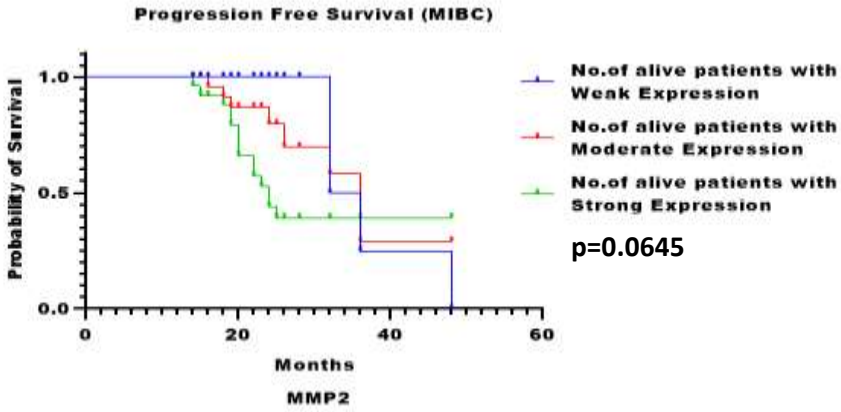

NORMAL
1
NMIBC LG
2
NMIBC HG
3
MIBC
4
Pro-MMP2 (72kDa)
Active MMP-2 (62kDa)
GAPDH (37kDa)
MMP-9 (92kDa)
GAPDH (37kDa)

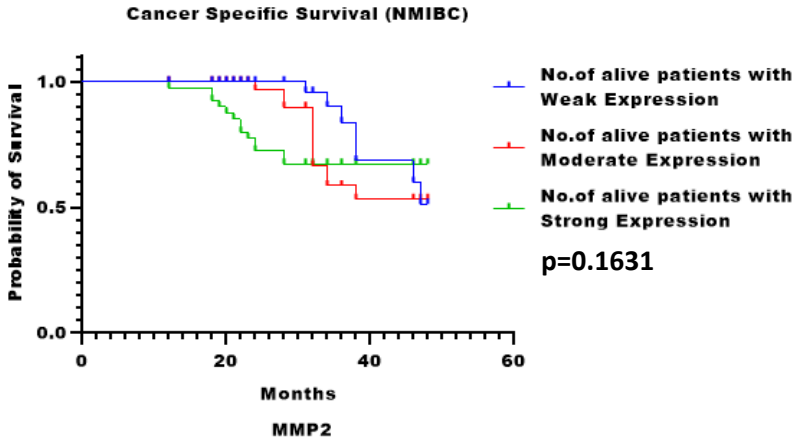
Survival Analysis of MMP-2

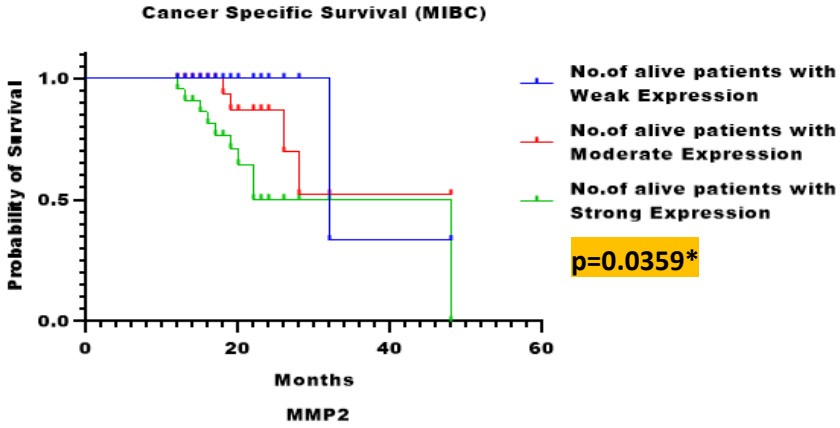




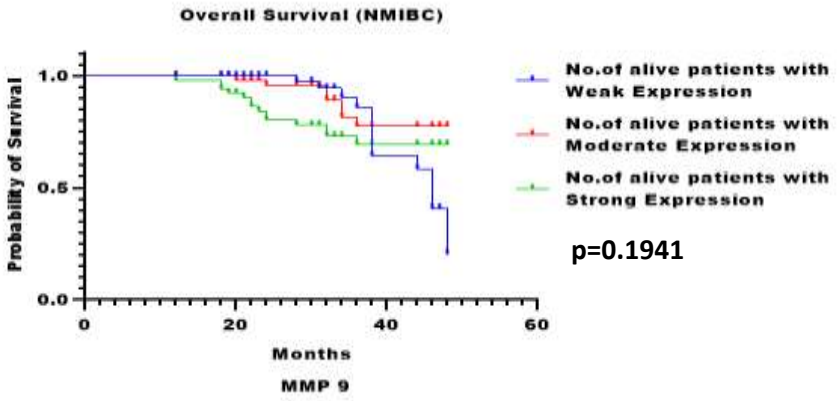


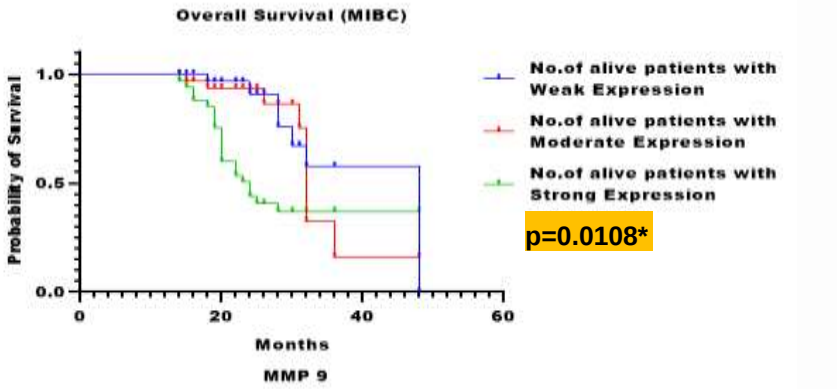


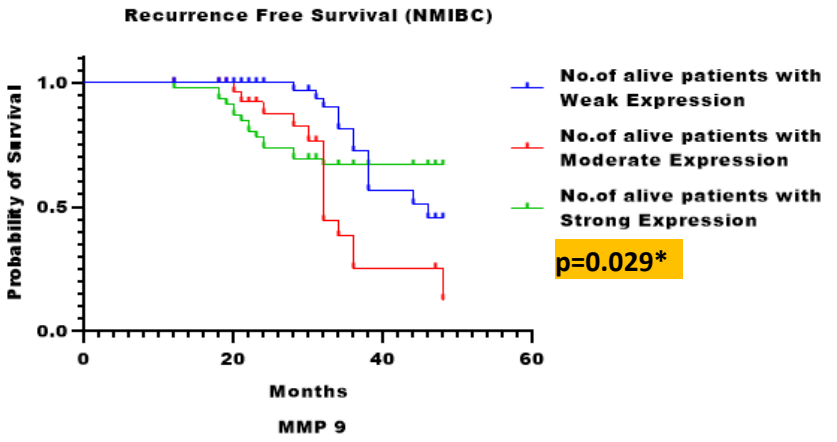


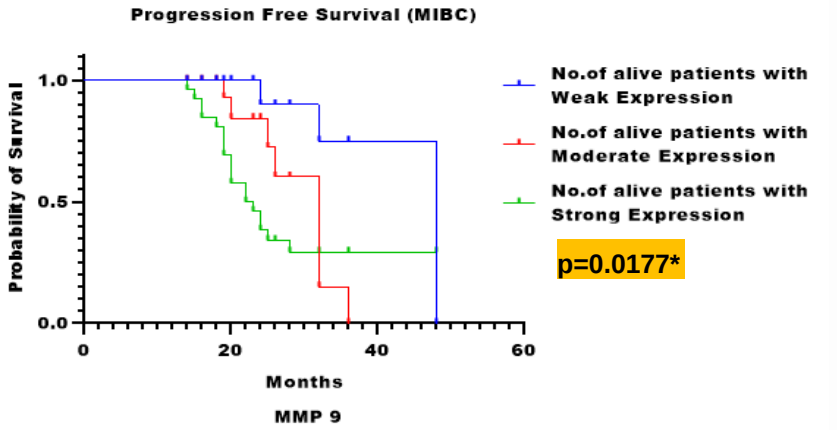


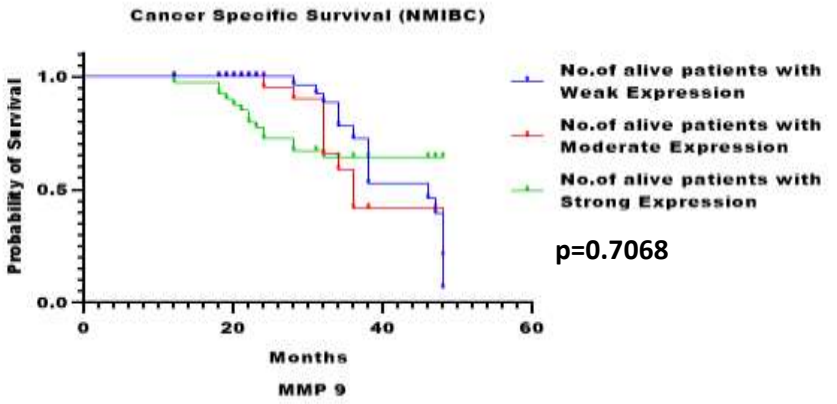
Survival Analysis of MMP-9

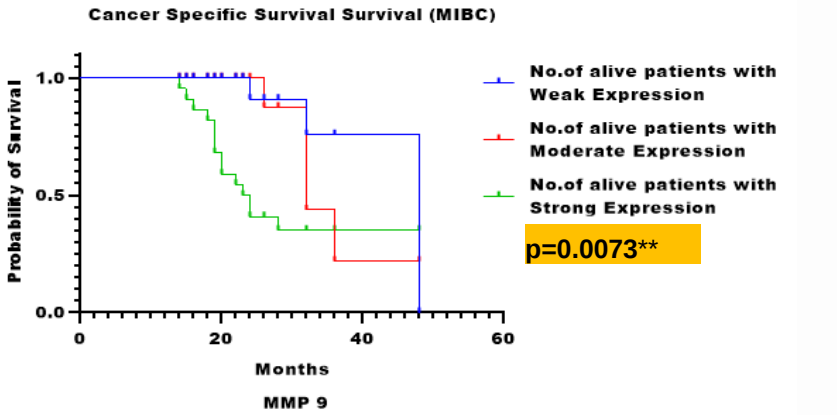




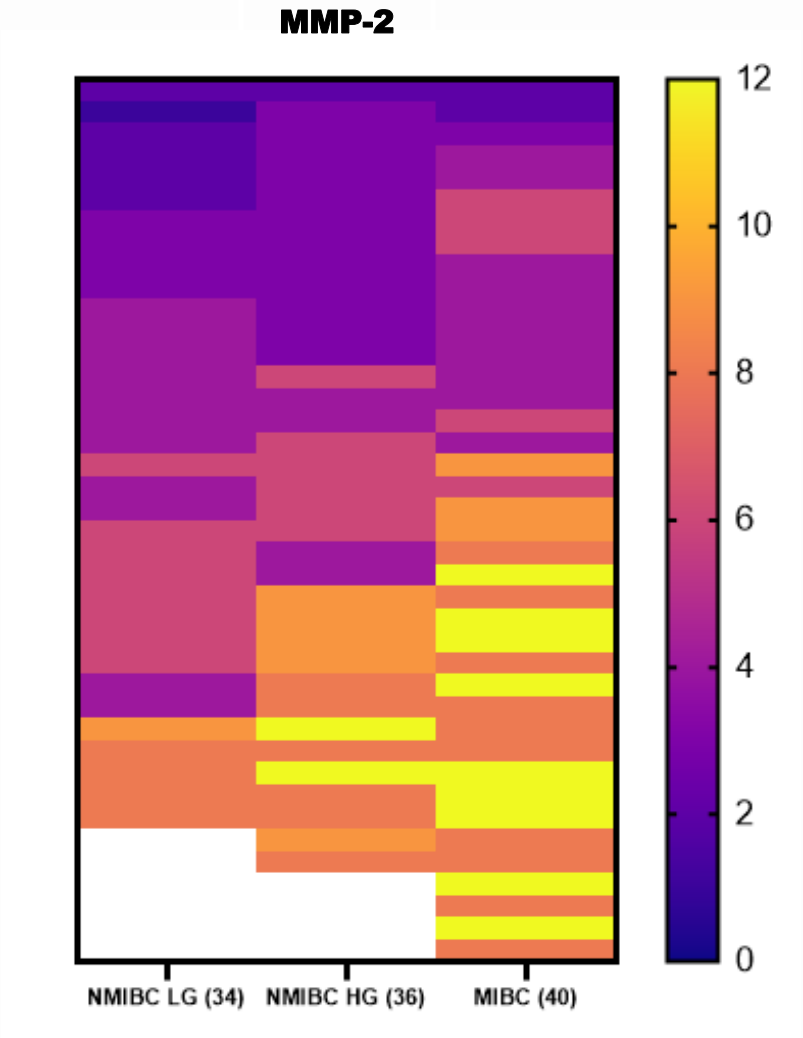


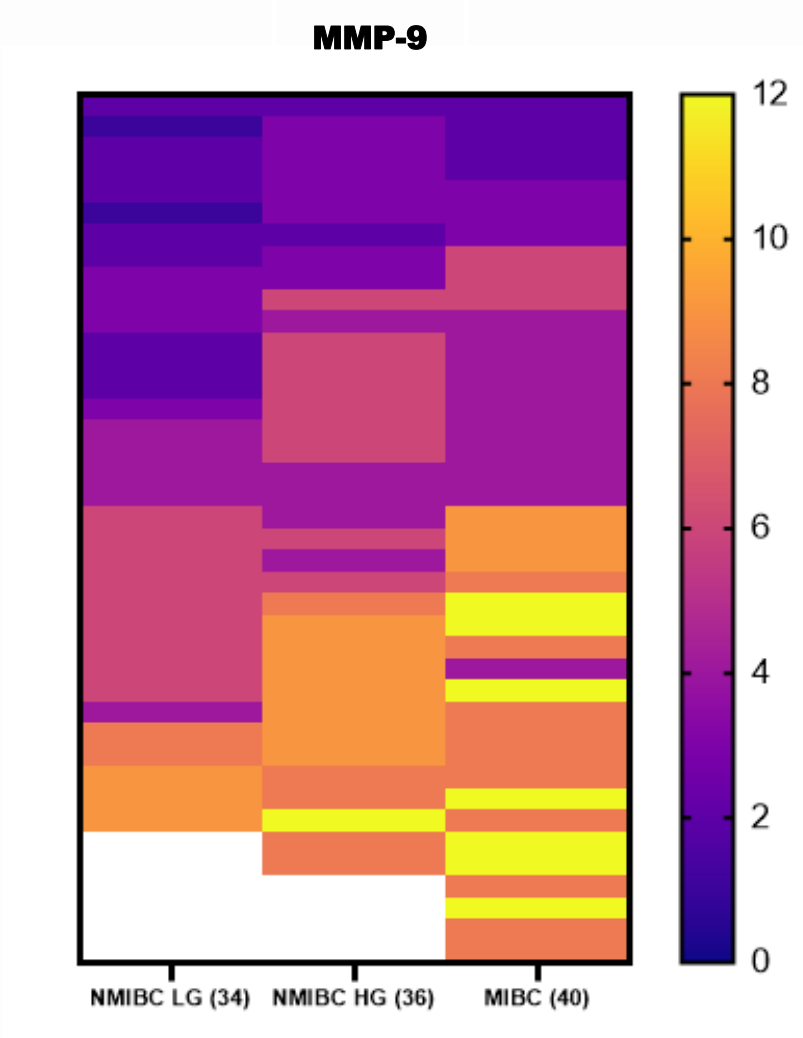




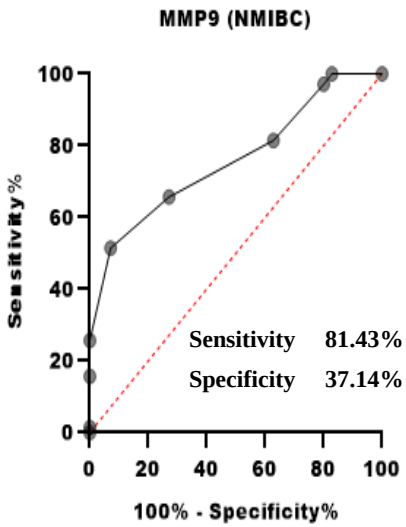


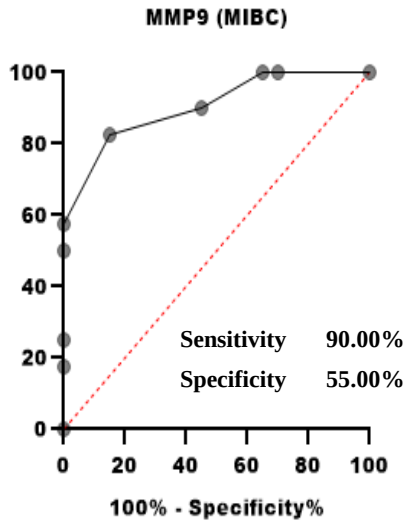
Heat Map representing proteomic expression of MMP-2 and MMP-9 in UCB patients

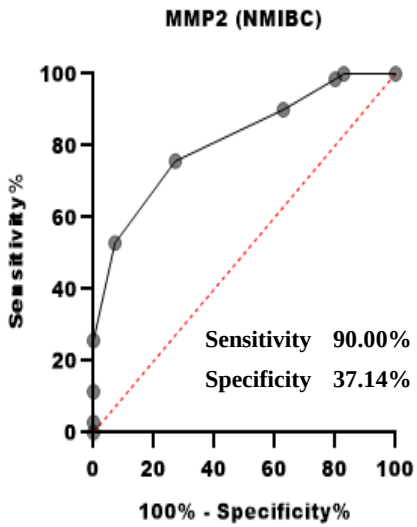


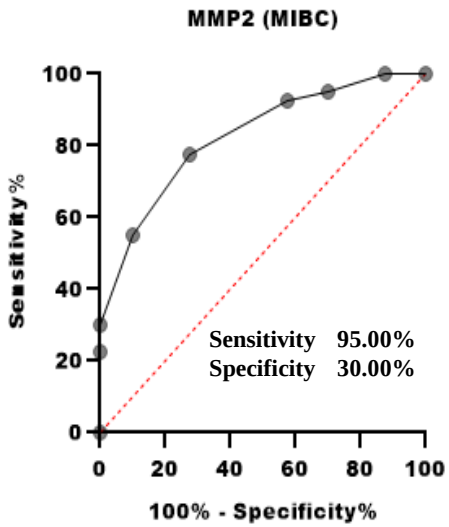


ROC curve Analysis of MMP-2 and MMP-9 expression in UCB patients

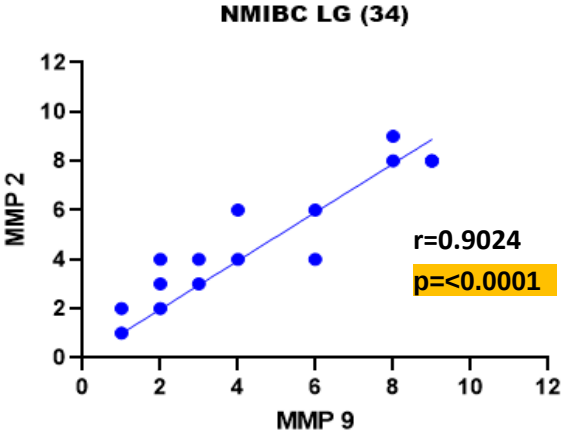


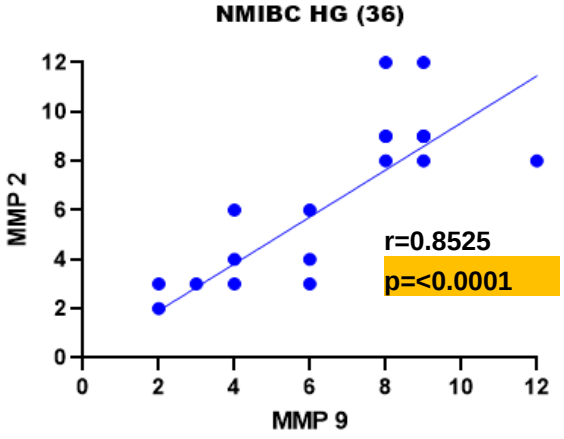


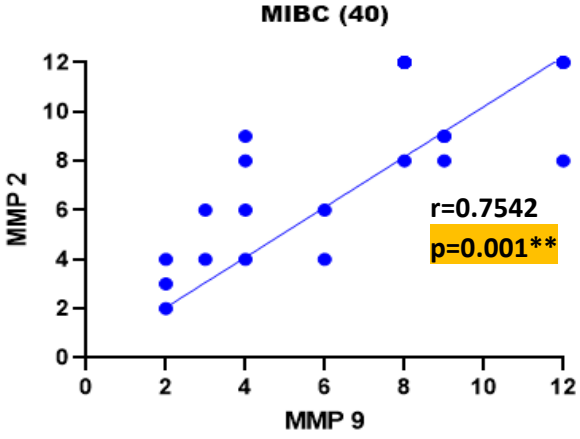




Correlation Analysis of MMP-2 and MMP-9 expression in UCB patients







Discussion and Conclusion

- Tumor metastasis is a crucial event that severely affects the survival of patients, and may influence the determination of appropriate therapeutic strategies.

- Overexpression of MMP-2 and MMP-9 induces the degradation of the major components of ECM, allowing the escape of tumor cells, promoting subsequent metastasis and tumor neo angiogenesis in tumor microenvironment.

- Higher expression of MMP-2 and MMP-9 were found in tumor tissues compared with adjacent normal tissues in several cancer such as Breast cancer, Prostrate cancer ,Oral cancer, Colorectal cancer, Renal cancer ,Head and Neck cancer and Urothelial Bladder cancer.

- Our study reports statistical significance (Transcriptomic and proteomic expressions of MMP-2 and MMP-9) with tumor stage, grade, size, type, and tobacco chewing/ smoking status in UCB patients and are in concordance with results reported in Breast cancer, recurrent glioma, Colon cancer, Pancreatic cancer, Non small Lung carcinoma and Urothelial bladder cancer studies.

- Log rank test along with Kaplan Meier analysis exhibited MMP-2 as the strong predictor of short recurrence free survival in NMIBC cohorts while short overall and poor cancer specific survival in MIBC.

- Levels of MMP-9 were observed to be the significant predictor of poor recurrence free survival in NMIBC cohort, short overall survival, poor progression free survival and short cancer specific survival in MIBC cohorts.

- Association of MMPs with the features of biologically and clinically aggressive UCB as well as poor survival outcomes potentially identify them as preferred marker of clinical significance in given cohort of UCB patients disease diagnosis and prognosis.

- Multicenter studies are required to further confirm its significance in Urothelial bladder cancer

Acknowledgements

- Prof. Dr. Apul Goel ,Department of Urology, King George's Medical University, Lucknow.
- Prof. Dr. Atin Singhai, Department of Pathology, King George's Medical University, Lucknow.
- Dr. Niraj Kumar, Translational Health Science and Technology Institute ,Faridabad, Haryana.
- Prof. Dr. Sudhir Mehrotra, Head of Department, Department of Biochemistry, University of Lucknow
- Mentor: Dr. Minal Garg, Molecular Oncology Laboratory, Department of Biochemistry, University of Lucknow
- Indian Council of Medical Research (ICMR), Govt. of India, New Delhi.

THANK YOU FOR YOUR KIND ATTENTION