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RESEARCH ARTICLE

ROLE OF HAEMATOLOGY BIOMARKERS IN URINARY BLADDER AND RENAL CELL CANCER PATIENTS TREATED WITH IMMUNE-ONCOLOGICAL DRUGS

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Abstract

Background: Urinary bladder (UB) and Renal Cell Cancer (RCC) are common in men than women with poor outcome. The novel immunotherapy drugs like Immune Check Point Inhibitors (ICIs) are effective but expensive. However, a cost-effective and reliable biomarker to predict response and clinical outcomes is lacking.

Objective: To study the association of pre-treatment haematological parameters with clinical outcome in urinary bladder and renal cell cancer patients treated with ICIs.

Method: In a retrospective study, we included 52 patients with urinary bladder and RCC treated with ICIs from Jan 2008 to Dec 2019. Clinical evaluation and laboratory investigations were performed as a part of standard protocol. CBC parameters such as WBC, TLC, DLC, Hb, Platelet, Neutrophil:Lymplocyte Ratio(NLR), Platelet:Lymphocyte Ratio (PLR), Lymphocyte:Monocyte Ratio(LMR) were studied at the time of TMC enrolment(first) and before the start (pre-treatment) of the ICIs therapy(io).

Results: Amongst the CBC parameters, WBC count, when categorized based on cut off from ROC at the time of TMC enrolment(first) and the pre-treatment of the ICIs therapy(io) were found to be significantly associated with progression of disease with p- value 0.012 with Hazard Ratio (HR) - 2.52 (95% C.I. - 1.2-5.31) & p- value 0.060 with Hazard Ratio (HR) – 1.99 (95% C.I. – 0.96-4.12) respectively. The ROC - based cut off for WBC at the time of TMC enrolment(first) and the pre-treatment of the ICIs therapy(io) were found to be 7.16 X10e3/µL (AUC of 72%) with 71% sensitivity and 72% specificity and 6.48 X10e3/µL (AUC of 74%) with 67% sensitivity and 72% specificity respectively to predict progressed cases with respect to non-progressed cases. Along with WBC, the pre-treatment Absolute Neutrophil Count (ANC) (>3.67)& Absolute Monocyte count (AMC)(>0.42) pre-treatment of the ICIs therapy(io)) showed a significance with Hazard Ratio (HR) – 3.09(95% C.I.– 1.26-7.6) with p-value 0.010, Hazard Ratio (HR) – 3.48(95% C.I. - 1.6-7.57)and p-value <0.001 respectively. The ROC-based cut off for ANC at the pre-treatment of the ICIs therapy(io) were found to be 3.67 X10e3/µL (AUC of 68.4%) with 82.4% sensitivity and

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50% specificity. Similarly The ROC- based cut off for AEC at the time of TMC enrolment(first) and AMC at the pre- treatment of the ICIs therapy(io) were found to be 0.22 X10e3/µL (AUC of 69.6%) with 55.9% sensitivity and 82.3% specificity and 0.42 X10e3/µL (AUC of 74.7%) with 72.7% sensitivity and 72.2% specificity respectively to predict progressed cases compared to non-progressed cases. Of 52 patients, 34 (65.4%) had progression. The median (m) PFS was 8.67 months (95% CI 2.26 - 15.09).The one year PFS rate was 46.5 (95% CI 31.2 -60.5). The mPFS in WBC <=7.16 was 26.48 months (95% CI 0 - 53.08) higher than the mPFS of 5.75 (95% CI 0.21 - 11.29) in WBC >7.16 with P value 0.012 at the time of TMC enrolment. Similarly, the mPFS in WBC pre- treatment of the ICIs therapy(io))<=6.48 was13.11 months (95% CI 0 – 28.95) higher than the mPFS of 6.18 (95% CI 2.67-9.68) in WBC >6.48 with P value 0.060.Along with WBC, the mPFS in ANC at the pre- treatment of the ICIs therapy(io)<=3.67 was 27.4(95% CI 0-55.21) higher than the mPFS of 6.18(95% CI 1.47-10.88)in ANC >3.67 with p value 0.010. The mPFS in AMC at the pre-treatment of the ICIs therapy(io)< =0.415 was 27.4 (95% CI 9.41-45.39) higher than the mPFS of 3.91 (95% CI 0.06-7.76) in AMC >0.415with p- value <0.001.We observed mPFS in immunotherapy cycles >=6 to be 15.9 (95% CI 6.98-24.82) was higher than the mPFS of 2.23 (95% CI 0.99-3.481) in immunotherapy cycles<6 with p value 0.025. The patients were followed for a period of 40.35 months (95% CI 26.98 - 53.71). Of 52 patients, 32(61.5%) patients deceased with median OS of 18.46 months (95% CI 10.12 - 26.80) and the one year OS rate was 57.6 (95% CI 41.6 - 70.6).

Conclusion: Simple, routinely available and cost effective WBC (TLC), Absolute Neutrophil Count (ANC) ,Absolute Monocyte Count (AMC) and Absolute Eosinophil Count (AEC) from CBC test has a great potential to be used as a biomarker to predict response and clinical outcomes in patients with urinary bladder cancer and RCC patients treated with ICIs.

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Introduction:-

Genitourinary cancer refers to cancers of the urinary system of men and women and the reproductive organs in men. Urinary cancers are diseases resulting from abnormal neoplastic growth of cells in the prostate, bladder, kidney, adrenal gland, urethra, or other parts of the urinary tract system. Bladder cancer is four times more common in men than women, with a respective incidence of 9.6/100,000 among men and 2.4/100,000 among women worldwide. Among men, bladder cancer is the sixth most incident and ninth most deadly neoplasm [1]. Bladder cancer begins when healthy cells in the bladder lining, most commonly urothelial cells change and grow out of control, forming a mass called tumor. Urothelial cells also line the renal pelvis and ureters. Malignant growth arising in the renal pelvis and ureters is also included in urothelial cancers and often called upper tract urothelial cancer and usually treated similar to bladder cancer.

Worldwide, there are over 400,000 new cases of Renal Cell Cancer (RCC). Over 170,000 deaths occur annually due to kidney cancer [2]. RCC is approximately twofold more common in males compared to females [3]. Clear cell Renal Cell Carcinoma (ccRCC) is the most common kidney cancer diagnosis. It can be aggressive and grow faster than other kidney cancers. Taking account of morbidity and mortality increase, it is evident that searching for independent, low-cost, standardized, reliable and reproducible prognostic biomarkers is needed.

The recent discovery of immune checkpoint inhibitors (ICIs) such as PD-1 inhibitors, CTLA4 inhibitors, etc., has revolutionized the therapeutic dynamics in many solid cancers with poor clinical outcomes including genitourinary cancer. However, these novel immunotherapy drugs are very expensive and hence less affordable in low and middle-income countries like India. Moreover, data shows that these novel immunotherapy drugs are not effective in a subset of patients. Hence, it is more relevant to use these expensive therapies in selective patients who will benefit

from such therapies. Unfortunately, no routinely available biomarker can be used to evaluate the effectiveness of these ICIs in patients with Urinary bladder (UB) and Renal Cell Cancer (RCC).

Therefore, there is an urgent requirement to identify routinely used cost-effective, reliable, reproducible biomarkers to predict therapeutic response and clinical outcomes in patients with genitourinary cancers treated with ICIs. Furthermore, a universal prognostic factor that can predict survival regardless of the type of cancer will help to simplify the management of cancer patients.

Thus, in this study, we aimed to investigate the clinical utility of haematological and other laboratory biomarkers in the therapeutic response assessment of immune checkpoint protein inhibitors in patients with Urinary bladder (UB) and Renal Cell Cancer (RCC).

Objective:-

To study the association of pre-treatment haematological parameters with clinical outcome in urinary bladder and renal cell cancer patients treated with ICIs.

Material and Methods:-

This is a retrospective study. The patients of urinary bladder and RCC, who are enrolled at TMC from Jan 2008 to Dec 2019 were included. Amongest these 52 patients of urinary bladder and RCC, the first treatment with ICIs started from November 2016 through the first patient registered at TMC in Jan 2008. Clinical evaluation and laboratory investigations were performed as a part of standard protocol. All patients had Complete blood count (CBC) collected on the date of TMC enrollementand within 7 days before start of immunotherapy. CBC parameters such as White blood cell (WBC),Total leucocyte count(TLC), Differential leucocyte count(DLC), Hb, Platelet, Neutrophil:Lymplocyte Ratio(NLR), Platelet:Lymphocyte Ratio(PLR), Lymphocyte:Monocyte Ratio(LMR),Neutrophil :Eosinophil Ratio(NER),Monocyte: Lymphocyte Ratio (MLR) were studied at the time of TMH enrolment(first) and before the start (pre-treatment) of the ICIs therapy(io). Patient's demographic data, treatment history, and laboratory data were obtained from Electronic Medical Record (EMR) of Tata Memorial Centre (TMC).

The combination of two immune checkpoint inhibitors—ipilimumab (Yervoy) and nivolumab (Opdivo) — has been approved for the treatment of advanced kidney cancer in first line therapy. Two combinations of targeted therapy plus an ICI have been approved for people with advanced kidney cancer in first line therapy.

1. Pembrolizumab (Keytruda), plus the targeted drug axitinib (Inlyta)
2. Pembrolizumab plus lenvatinib
3. Nivolumab plus cabozantinib

In second line therapy single agent immunotherapy (Nivolumab) has been used.

For patients with urinary bladder cancer, Pembrolizumab or Nivolumab or Atezolizumab were used either in first line or second line therapy.

Inclusion Criteria:

• Patients diagnosed with urinary bladder and kidney cancer in TMC from Jan 2008 to Dec 2019 and treated in adult medical oncology.
• Patients treated with ICIs in TMC between 2008 to Dec 2019.
• Patients treated during palliative intent ICIs either in first/ second line therapy of their course.

Exclusion criteria:

• Patients with inadequate clinical/demographical data available in TMC EMR.
• Patients receiving ICIs in adjuvant setting.

The TMC Haematopathology laboratory has received the peripheral blood samples for CBC at the time of TMC enrolment(first) and before the start (pre-treatment) of the ICIs therapy(io). CBC parameters are analyzed using two automated haematology analysers i.e. ADVIA 2120i and DxH 800.

Statistical analysis:
Demographic data was summarized using descriptive statistics. The normality of absolute, percentages and ratios of CBCs at the time of TMC enrolment(first) and before the start (pre-treatment) of the ICIs therapy(io), was assessed using Kolmogorov Smirnov's test for normality. Median and IQR are reported for each CBC.

ROC is used to obtain the AUC and optimal cut off for classifying progression cases. CBCs were categorized based on cut off, as less than equal to or more than the cut off.

The Mann-Whitney test is used to compare the distribution of CBC between progressed and non-progressed cases.

The categorized CBC over cut off from the ROC method are compared for association with the occurrence of progression using the univariate Cox Proportional HazardsRegression and hazard ratio with 95% CI is reported. Kaplan Meier method is used to obtain the survival estimates. Log-rank test is used to compare the survival curves between two or more groups. regression and an odds ratio with 95% CI is reported. Multivariate Cox regression using the backward elimination method is performed using the parameters found significant in univariate analysis.

A p-value < 0.05 is considered as statistically significant.

The distribution of CBC in progressed and non-progressed cases was demonstrated using boxplots. The ROC curve was used to demonstrate the AUC of significant CBC.

All statistical analysis was performed using IBM SPSS version 29.

Sample size:
Retrospectively patients diagnosed with urinary bladder and kidney cancer in TMC from Jan 2008 to Dec 2019 and treated with ICIs from November 2016 were included in the analysis.

Results:-

Within the cohort, tumour histology includes 26 (50%) urinary bladder cancers and 26 (50%) renal cell carcinomaTable (2).The sub classification of both the cancer cases was shown in Table (1)according to histopathology report. Amongst urinary bladder cancer patients, papillary urothelial carcinoma and high grade urothelial carcinoma cases were highest. Clear cell renal cell carcinoma patients were around 65% in renal cell cancer patients.

Patients had median age of 64.5 (IQR 59.3-72) years, 42(81%) were men and 10(19%)were women.Amongst 52 patients, the median immunotherapy cycles was 12(IQR 6-16). Response was evaluated for 52 patients with computed tomography or magnetic resonance imaging, 34 (65%) patients were found to be with disease progression and 18(35%) patients had no disease progression Table (2).

GENDER	**MALE**	**FEMALE**
	42	**10**
MALIGNANCY	**UB**	**RCC**
	26	**26**
RESPONSE	**PROGRESSED**	**NO PROGRESSION**
	34 (65.4%)	**18 (34.6%)**
AGE	**Median - 64.5 (IQR 59.3 – 72) Years**	
IMMUNOTHERAPY CYCLES	**Median - 12 (IQR 6 -16)**	

Table (2):- Demographic of urinary bladder cancer (UB) and renal cell cancer (RCC) patients.

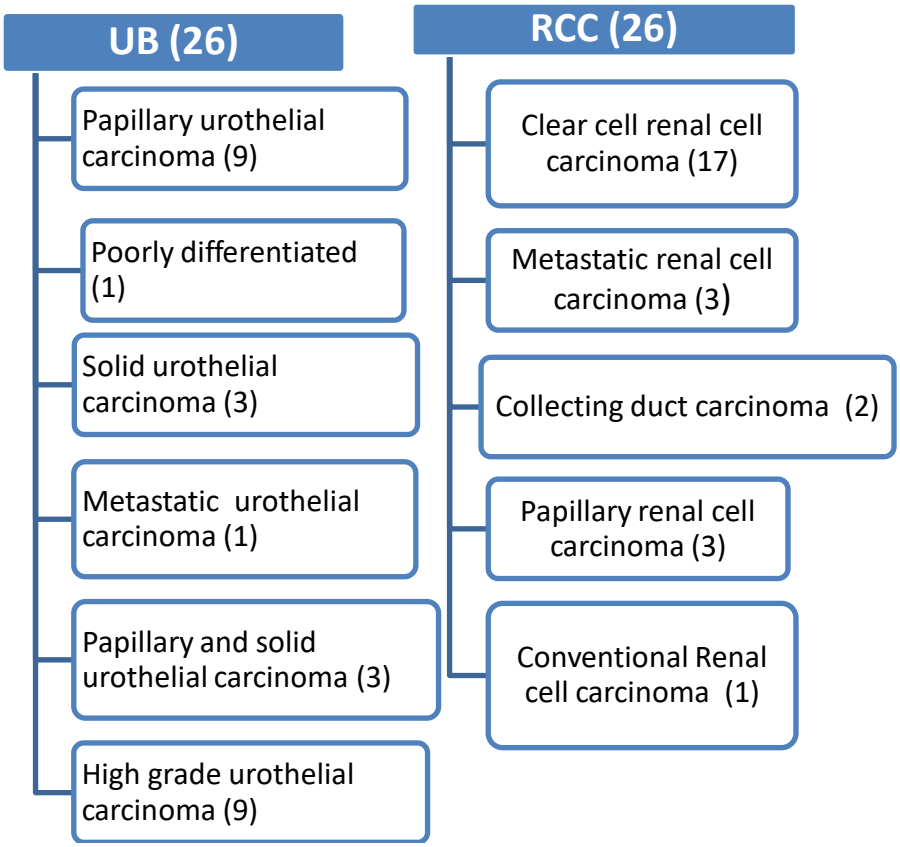

Table (1):- Sub classification of urinary bladder cancer (UB) and renal cell cancer (RCC) patients.

The disease progression of urinary bladder and RCC patients was based on radiology reports (PET-CT, CECT, and MRI). When we compared two diseases groups i.e. urinary bladder (UB) and RCC with diseases progression, the number of progressed cases in RCC was 14 (41.2%) and in UB 20 (58.2%) with p value = 0.080. The hazards of developing progression in UB as compared to RCC patients using Cox Regression with HR 1.295 (95% CI 0.648 – 2.587) P-value 0.464 shows no significant difference.The median (m)PFS in urinary bladder patients was 8.44 months (95% CI 0.0-17.10)whereas in RCC patients, it was 9.56months (95% CI 0.0-31.35) was found. When wecompared these cohorts using log-rank test, p-value was 0.462 (statistically not significant)indicating that there is no significant difference in mPFS in both groups (Table3). Therefore we combined these two cohorts to evaluate the role of hematology biomarkers, when treated with immune oncological drugs.

Diagnosis		Disease non-progressed	Disease progressed	Total	p-value	HR (95% CI)	p - value	Median PFS(in months) (95% CI)	p - value
UB	count	6	20	26	0.08	1	0.464	8.444(0.0-17.10)	0.462
	% within Disease Progression	33.3%	58.8%	50.0%					
RCC	count	12	14	26		1.295(0.65-2.59)		9.561(0.0-31.35)	
	% within Disease Progression	66.7%	41.2%	50.0%					
Total - Overall	count	18	34	52				8.674(2.26-15.09)	
	% within	100.0%	100.0%	100.0					

	Disease Progressio n			%					

Table (3):- Comparison of diseases group i.e. urinary bladder (UB) and RCC with diseases progression and PFS.

When CBC parameters were compared with progressed and non-progressed group, WBC count at the time of TMC enrolment(first) and the pre- treatment of the ICIs therapy(io) were found to be significant with Median (IQR) 8.08 (6.91 - 9.65) and 7.86 (5.71 - 10.6)respectively. Along with WBC, the pre-treatment Absolute Neutrophil Count (ANC) with Median (IQR)5.28 (4.04 - 7.94), Absolute Monocyte count (AMC) with Median (IQR)0.46 (0.36 - 0.66) at pre- treatment of the ICIs therapy(io) &Absolute Eosinophil Count (AEC) with Median (IQR)0.23 (0.13 - 0.34) at the time of TMC enrolment(first) showed significant association (Table 4).

Parameters	Non-Progressed (N=18)		Pogressed (N=34)		P value
	Median (IQR)	Min - Max	Median (IQR)	Min - Max	
WBC Counts_first	**6.75 (6.01 - 7.9)**	**3.61 - 9.7**	**8.08 (6.91 - 9.65)**	**3.66 - 28.12**	**0.009**
WBC Counts_io	**5.71 (4.13 - 7.25)**	**2.25 -11.47**	**7.86 (5.71 - 10.6)**	**4.08 - 18.83**	**0.006**
HB_abs_first	11.1 (9.85 - 13.05)	9.1 - 15.3	12.05 (11.28 - 13.63)	7.4 - 15.3	0.136
HB_abs_io	9.85 (8.68 - 12.53)	5.8 - 14.7	10.9 (9.25 - 12.05)	6.3 - 16	0.574
N_per_first	65.2 (57.9 - 69.08)	42.8 - 87.3	64.8 (58.05 - 71.2)	34.3 - 92.1	0.878
N_per_io	65.05 (53.28 - 78.55)	40.9 - 89.9	71.7 (62.7 - 75.95)	51.1 - 85.8	0.380
LY_per_first	24.25 (16.9 - 29.48)	3.8 - 47.7	21 (17.53 - 29.5)	3 - 51.8	0.577
LY_per_io	19.7 (9.78 - 31.83)	3.8 - 47.7	17.4 (12.75 - 22.4)	6.8 - 37.7	0.581
Mono_per_first	6.81 (5.11 - 7.81)	3.56 -11.02	6.44 (4.66 - 8.05)	2.27 -19.76	0.847
Mono_per_io	6.23 (5.18 - 8.02)	2.65 -12.79	6.36 (4.59 - 9.11)	2.76 -18.78	0.767
EO_per_first	2.3 (0.88 - 3.1)	0.4 - 8.2	2.55 (1.55 - 4.63)	0.5 - 13.95	0.160
EO_per_io	1.7 (0.95 - 2.68)	0.1 - 5.4	1.8 (1 - 3)	0.1 - 8.5	0.601
Neu_abs_first	4.37 (3.61 - 5.33)	1.55 - 6.9	5.23 (3.74 - 6.67)	1.89 - 25.9	0.184
Neu_abs_io	**3.84 (2.05 - 5.5)**	**1.12 - 8.03**	**5.28 (4.04 - 7.94)**	**1.89- 14.86**	**0.022**
Lymp_abs_first	1.48 (0.97 - 1.94)	0.22 - 2.85	1.54 (1.08 - 2.08)	0.16 - 4.91	0.795
Lymp_abs_io	1.04 (0.57 - 1.61)	0.22 - 2.91	1.26 (0.94 - 1.53)	0.18 - 4.81	0.139
Mono_abs_first	0.44 (0.34 - 0.59)	0.17 - 0.78	0.52 (0.35 - 0.76)	0.17 - 1.07	0.181
Mono_abs_io	**0.33 (0.27 - 0.44)**	**0.17 - 0.8**	**0.46 (0.36 - 0.66)**	**0.18 - 2.51**	**0.004**
EO_abs_first	**0.15 (0.05 - 0.21)**	**0.02 - 0.8**	**0.23 (0.13 - 0.34)**	**0.04 - 1.33**	**0.024**
EO_io	0.08 (0.05 - 0.17)	0.01 - 0.38	0.15 (0.07 - 0.23)	0.01 - 0.46	0.162
Platelet_first	271 (186.5 - 318.5)	112 - 408	297 (213 - 362)	126 - 551	0.273
Platelet_io	234.5 (159.5 - 298.5)	112 - 412	302 (193 - 364)	49 - 589	0.129
NLR_first	2.73 (1.98 - 3.94)	0.9 - 22.97	2.92 (2.1 - 5.92)	0.66 -75.25	0.740
NLR_io	3.29 (2.33 - 6.26)	0.9 - 22.97	4.2 (2.91 - 7.88)	1.39- 40.68	0.305
PLR_first	196.03 (104.06 - 248.4)	66.78 - 1235.5	201.81 (116.14 - 271.25)	36.02 - 1784.4	0.617
PLR_io	252.37 (146.29 - 356.42)	66.78 - 1235.5	244.48 (154.24 - 311.18)	25.86 - 664.84	0.723
LMR_first	3.55 (2.26 - 4.91)	0.49 -10.15	3.12 (1.9 - 5.08)	0.22 - 8.56	0.595
LMR_io	3.16 (1.85 - 4.23)	0.49- 10.15	2.82 (1.66 - 3.54)	0.49 - 9.21	0.430

NER_first	26.57 (18.53 - 72.01)	6.68-218.22	22.7 (13.8 - 43.33)	4.27 -211.71	0.204
NER_IO	36.99 (20.08 - 81.73)	8.74 - 810.34	46.73 (25.05 - 78.42)	7.8 - 775.35	0.890
MLR_first	0.28 (0.2 - 0.45)	0.1 - 2.09	0.32 (0.19 - 0.52)	0.07 - 4.69	0.758
MLR_IO	0.32 (0.24 - 0.55)	0.1 - 2.09	0.36 (0.28 - 0.6)	0.11 - 2.01	0.442

Table (4):- Comparison between the progressed and non-progressed cases with CBC parameters, Median (IQR) reported for both groups.(Per- percent, abs- absolute- Neutrophil, Lymp-Lymphocyte, Mono-Monocyte, Eo-Eosinoplhils)

The distribution of CBC parameters with significant differences among the progressed and non-progressed cases is demonstrated using boxplots also.(Fig.1)

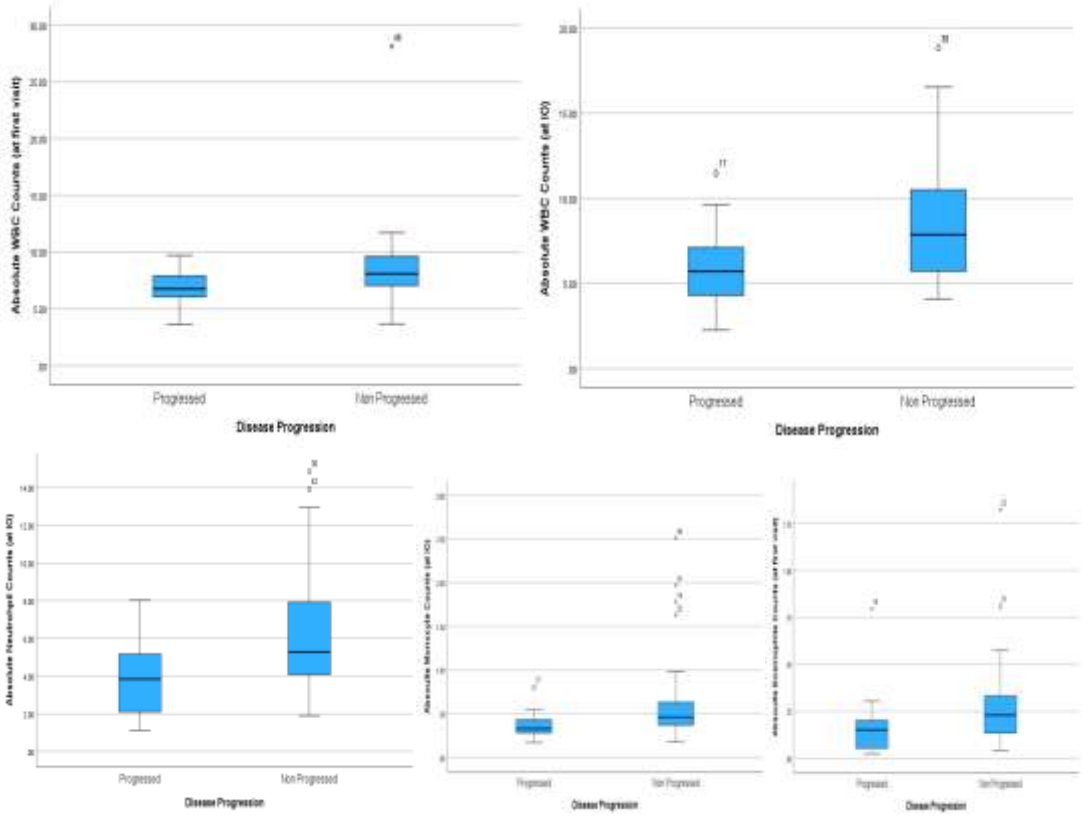

Figure 1:- Boxplots showing the distribution of CBC parameters with significant difference between the progressed and non-progressed groups.

Amongst the CBC parameters, WBC count, when categorised based on cut off of ROC at the time of TMC enrolment(first) and the pre-treatment of the ICIs therapy(io) were found to be significantly associated with progression of disease with p- value 0.012 with Hazard Ratio (HR) - 2.52 (95% C.I. - 1.2-5.31) & p- value 0.060 with Hazard Ratio (HR) – 1.99 (95% C.I. – 0.96-4.12) respectively. The ROC- based cut off for WBC at the time of TMC enrolment(first) and the pre- treatment of the ICIs therapy(io) were found to be 7.16 X10e3/µL (AUC of 72%) with 71% sensitivity and 72% specificity and 6.48 X10e3/µL (AUC of 74%) with 67% sensitivity and 72% specificity respectively to predict progressed cases with respect to non-progressed cases.

Along with WBC, the pre-treatment Absolute Neutrophil Count (ANC) (>3.67)& Absolute Monocyte count (AMC)(>0.42)showed a significance with Hazard Ratio (HR) – 3.09(95% C.I.– 1.26-7.6) with p-value 0.010, Hazard Ratio (HR) – 3.48(95% C.I.- 1.6-7.57)and p-value <0.001 respectively. The ROC- based cut off for ANC at the pre-treatment of the ICIs therapy(io) were found to be 3.67 X10e3/µL (AUC of 68.4%) with 82.4% sensitivity and 50% specificity. Similarly The ROC- based cut off for AEC at the time of TMC enrolment(first) and AMC at the pre-treatment of the ICIs therapy(io) were found to be 0.22 X10e3/µL (AUC of 69.6%) with 55.9% sensitivity and 82.3%

specificity and 0.42 X10e3/µL (AUC of 74.7%) with 72.7% sensitivity and 72.2% specificity respectively to predict progressed cases compared to non-progressed cases(Table 6)(Figure 2).

Of 52 patients 34 (65.4%)) had progression. The mPFS was 8.67 months (95% CI 2.26 - 15.09). The mPFS in WBC <=7.16 was 26.48 months (95% CI 0 - 53.08) higher than the mPFS of 5.75 (95% CI 0.21 - 11.29) in WBC >7.16 with P value 0.012 at the time of TMC enrolment. Similarly the mPFS in WBC pre-treatment of the ICIs therapy(io))<=6.48 was13.11 months (95% CI 0 – 28.95) higher than the mPFS of 6.18 (95% CI 2.67-9.68) in WBC >6.48 with P value 0.060.Along with WBC, the mPFS in ANC at the pre- treatment of the ICIs therapy(io)<=3.67 was 27.4(95% CI 0-55.21)) higher than the mPFS of 6.18(95% CI 1.47-10.88)in ANC >3.67 with p value 0.010. The mPFS in AMC at the pre-treatment of the ICIs therapy(io)< =0.415 was 27.4 (95% CI 9.41-45.39) higher than the mPFS of 3.91 (95% CI 0.06-7.76) in AMC >0.415 with p- value <0.001.

Amongst the 34 progressed patients, 20 patients (58.8%) received immunotherapy for <6 months and 14 patients (41.2%) received immunotherapy >=6 months.The mPFS in immunotherapy cycles >=6 was at 15.9 (95% CI 6.98-24.82) which was higher than the mPFS of 2.23 (95% CI 0.99-3.481) in immunotherapy cycles<6 with p value 0.025.

Out of 34 progressed patients, 24(70.6%) patients received the immunotherapy for <1 year and 10(29.4%) patients received the immunotherapy for >1 year. The one year mPFS rate was 46.5 (95% CI 31.2 -60.5).The mPFS rate in WBC <=7.16 was 59.1 (95% CI 32.2 -78.3) which was higher than the mPFS rate of 37.2 (95% CI 19.5 -55.1) in WBC >7.16 at the time of TMC enrolment. Similarly the mPFS rate in WBC pre- treatment of the ICIs therapy(io)<=6.48 was 56.2 (95% CI 30.2 -75.8) which was higher than the PFS rate of 37.3 (95% CI 19.1 -55.5) in WBC >6.48. Along with WBC, the mPFS rate in ANC at the pre-treatment of the ICIs therapy(io)<=3.67 was 82.1 (95% CI 44.4 -95.3) which was higher than the mPFS rate of 33.9 (95% CI 18.3 -50.2) in ANC >3.67. The mPFS rate in AMC at the pre-treatment of the ICIs therapy(io)< =0.415 was 73.9 (95% CI 47.6 -88.4) whch was higher than the mPFS rate of 24.9 (95% CI 10.2 -42.8) in AMC >0.415.For patients who received immunotherapy >=6 months,the PFS rate was found to be 58.5 (95% CI 38.2 -74.2) which was higher than the mPFS rate of 22.5 (95% CI 5.9 -45.6) in immunotherapy cycles<6(Table 5).

In multivariate model, we adjusted the effects for four parameters found significant on univariate analysisnamely WBC pre-treatment of the ICIs therapy(io),AMC at the pre-treatment of the ICIs therapy(io), number of immunotherapy cycles received by the patients and the type of cancer(urinary bladder cancer and renal cell cancer).Using the backward likelihood ratio elimination method,the model was reduced to AMC(>0.415) at the pre-treatment of the ICIs therapy(io)with and number of immunotherapy cycles(>6 cycles) received by the patientsp-value 0.004 with Hazard Ratio (HR) – 3.211 (95% C.I. - 1.46-7.07) & p-value 0.024 with Hazard Ratio (HR) – 0.43 (95% C.I– 0.21-0.90) respectively. The other variables were insignificant and therefore eliminated from the model(Table 5).

The patients were followed up for a period of 40.35 months (95% CI 26.98 - 53.71). Of 52 patients 32 (61.5%) patients deceased with median OS of 18.46 months (95% CI 10.12-26.80) and the one year OS rate was 57.6 (95% CI 41.6 - 70.6).

Variables	Categories	Total N	No of Progressions	Median PFS(in months) (95% CI)	1 Year PFS Rate (95%CI)	P (Log Rank Test)	HR (95% CI)	P Univariate Cox Regression	HR (95% CI)	P Multivariate Cox Regression	HR (95% CI)
Overall PFS		52	34	8.67 (2.26 - 15.09)	46.5 (31.2 - 60.5)						
WBC_first	<=7.16	23	10	26.48 (0 - 53.08)	59.1 (32.2 - 78.3)	0.012	1.000	0.015	1		
	>7.16	29	24	5.75 (0.21 - 11.29)	37.2 (19.5 - 55.1)		2.52 (1.2 - 5.31)		2.45(1.06-5.66)		
WBC_io	<=6.48	24	11	13.11 (0 - 28.95)	56.2 (30.2 -	0.060	1.000	0.065			

					75.8)						
	>6.48	27	22	6.18 (2.67 - 9.68)	37.3 (19.1 - 55.5)		1.99 (0.96 - 4.12)				
Neu_abs_io	<=3.67	15	6	27.4 (0 - 55.21)	82.1 (44.4 - 95.3)	0.010	1.000	0.014			
	>3.67	37	28	6.18 (1.47 - 10.88)	33.9 (18.3 - 50.2)		3.09 (1.26 - 7.6)		1		
Mono_abs_io	<=0.415	22	9	27.4 (9.41 - 45.39)	73.9 (47.6 - 88.4)	<0.001	1.000	0.002	2.47 (1.09 - 5.54)	0.004	3.211 (1.46-7.07)
	>0.415	29	24	3.91 (0.06 - 7.76)	24.9 (10.2 - 42.8)		3.48 (1.6 - 7.57)				
EO_first	<=0.22	30	16	9.56 (0.21 - 18.91)	49.5 (28.7 - 67.4)	0.247	1.000	0.250			
	>0.22	21	18	7.62 (3.48 - 11.76)	42.3 (20.5 - 62.7)		1.49 (0.76 - 2.92)				
No_of_immunotherapy_cycles	>6	35	20	15.9 (6.98 - 24.82)	22.5 (5.9 - 45.6)	0.025	1.000	0.029	1	0.024	0.43 (0.21-0.90)
	<=6	17	14	2.23 (0.99 - 3.48)	58.5 (38.2 - 74.2)		2.19 (1.09 - 4.42)		3.08 (1.39 - 6.80)		

Table 5:- Progression free survival estimates with univariate and multivariate hazards.

Parameters	Cut off	AUC (%)	Sensitivity (%)	Specificity (%)	Pvalue
WBC (first)	7.16	72.3	70.5	72.2	0.005
WBC (io)	6.48	73.6	66.7	72.2	0.010
N abs (io)	3.67	69.4	82.4	50	0.018
Mono abs (io)	0.42	74.7	72.7	72.2	0.003
Eo abs (first)	0.22	69.6	55.9	82.3	0.022

Table (6):-The ROC-based significant cut offs to differentiate between progressed and non-progressed cases.

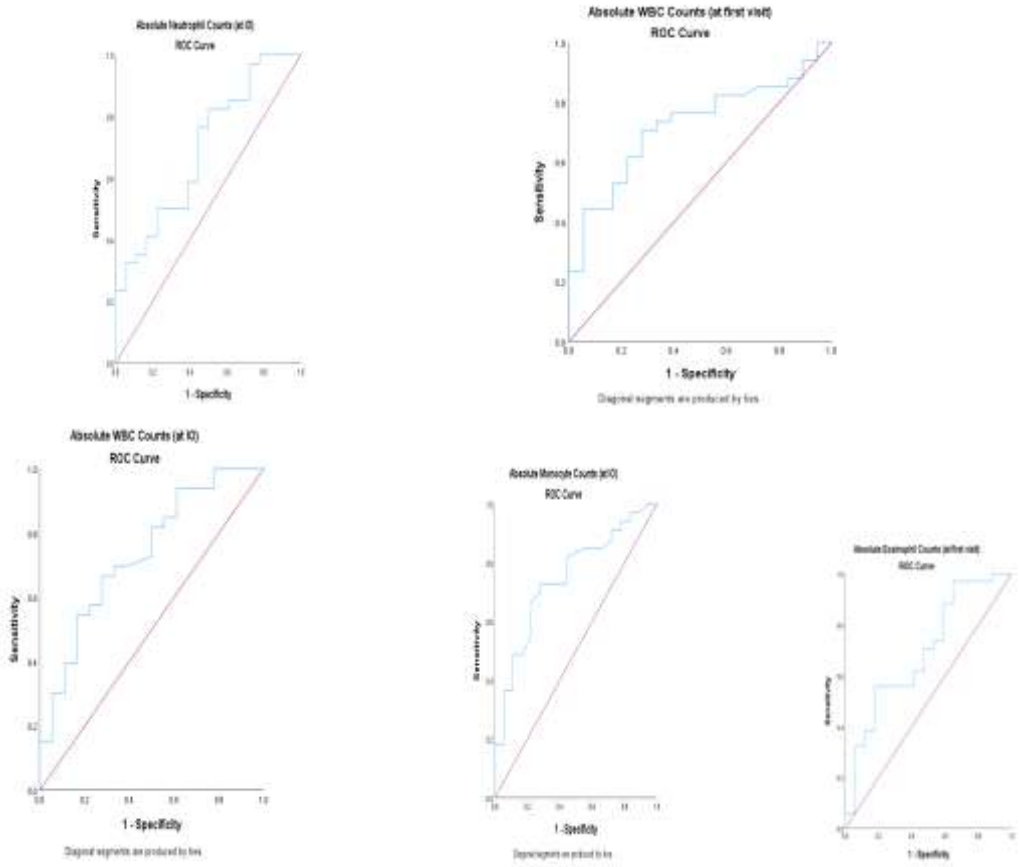

Figure 2:- ROC used to obtain the AUC and optimal cutoff for classifying progression cases.

Discussion:-

The recent discovery of immune checkpoint inhibitors (ICIs) such as PD-1 inhibitors, CTLA-4 inhibitors, etc., has revolutionized the therapeutic dynamics in many solid cancers with poor clinical outcomes including genitourinary cancer. It has become a major focus of kidney cancer treatment research. In resourceful countries, most patients with advanced kidney cancer receive ICIs at some point during their treatment. A small minority of people with clear-cell kidney cancer and other rarer types of the disease have their tumors disappear entirely during treatment with these drugs.

Patients with urinary bladder cancer are also treated with immunotherapy that includes Bacillus Calmette-Guerin (BCG) vaccine as well as ICIs. BCG causes an immune system reaction that directs germ-fighting cells to the bladder and helps in cancer cell killing. More recently, ICIs are also approved to treat bladder cancer. Pembrolizumab or Nivolumab or Atezolizumab is being used either in first line or second line therapy. These drugs help the immune system to identify and fight cancer cells.

However, these novel immunotherapy drugs are very expensive and hence less affordable in low and middle-income countries like India. Moreover, data shows that these novel immunotherapy drugs are not effective in a subset of patients. Hence, it is more relevant to use these expensive therapies in selective patients who will benefit from such therapies. Unfortunately, no routinely available biomarker can be used to evaluate the effectiveness of these ICIs in patients with genitourinary cancers. Therefore, there is an urgent requirement to identify routinely used cost-effective, reliable, reproducible biomarkers to predict therapeutic response and clinical outcomes in patients with genitourinary cancers treated with ICIs. Furthermore, a universal prognostic factor that can predict survival regardless of the type of cancer will help to simplify the management of cancer patients.

In our study, when we compared two diseases group i.e. Urinary Bladder (UB) and RCC with diseases progression, the p value was 0.080 (near to significance). With increased sample size, the results may be significant. We

demonstrated using multivariate analysis that the absolute monocyte count and number of immunotherapy cycles received by the patients were significantly associated with prediction of response and clinical outcome. WBC at the baseline and at the pre- treatment, Absolute neutrophil count (ANC), Absolute monocyte count (AMC) when categories based on cut off of ROC at the pre-treatment of the ICIs therapy(io) were found to be significant.

Many studies have shown that inflammatory markers such as CRP, albumin and NLR have prognostic value in UB and RCC (14, 15). In a recent study, the authors monitored the pre-treatment hematological biomarkers (neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio, and modified Glasgow prognostic score) were predicative biomarkers of prognosis in bladder cancer patients (17). Another study showed Pre-treatment NLR < 5.5 is associated with superior PFS and OS. NLR is a biomarker that can inform prognosis for patients with mRCC (18). While limited in scope due to small cohorts, such studies show that biomarkers have potential for clinical application.

The main limitations to our study are the small cohort size and retrospective design, which are susceptible to selection bias in data analysis.

Conclusion:-

Simple, routinely available and cost effective WBC (TLC), Absolute Neutrophil Count (ANC) and Absolute Monocyte Count (AMC) from CBC test have a great potential to be used as a biomarker to predict response and clinical outcomes in patients with urinary bladder cancer and RCC patients treated with ICIs.

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Competing interests statement:
The authors declare no competing interests.

Abbreviations:
ACTREC- Advance Centre for Treatment, Research and Education in Cancer
AEC- Absolute Eosinophil Count
AMC- Absolute Monocyte count
AUC- Area under the Curve
BCG - Bacillus Calmette-Guerin
CBC- Complete Blood Count
ccRCC- cell Renal Cell Carcinoma
CTLA4- Cytotoxic T- Lymphocyte associated protein 4
EMR- Electronic Medical Record
Hb- Hemoglobin
ICI- Immune Checkpoint Inhibitors
IQR- Interquartile Range
LMR- Lymphocyte Monocyte Ratio
MLR- Monocyte: Lymphocyte Ratio
mPFS- median Progression Free Survival
mRCC- metastatic Renal Cell Carcinoma
NER- Neutrophil :Eosinophil Ratio
NLR- Neutrophil Lymphocyte Ratio
OS- Overall Survival
PD-1- Programmed cell Death protein 1
PD-L1- Programmed Death-Ligand 1
PFS- Progression Free Survival
PLR- Platelet Lymphocyte Ratio
RCC- Renal Cell Cancer
ROC- Receiver operating characteristic Curve
TLC- Total Leucocyte Count
TMC- Tata Memorial Centre

TMH- Tata Memorial Hospital
UB- Urinary bladder
UC- Urothelial Carcinoma
WBC- White Blood Cell

Author contributions:
Shilpa Kushte collected and analyzed the data and wrote the paper. Dr. Prashant Tembhareparticipated in the writing. Dr. Amit Joshi, Dr. Mahendra Kumar Verma, Dr. Kumar Prabhash, Dr. Vanita Narona , Dr. Nandini Menon, Dr. P. G. Subramanian, Dr. Sumeet Gujral, Dr. Prashant Tembhareassisted in the design of this study. Ms. Priti Nikam contributed in data collection. Mr. Akash Pawar ensured the integrity of the data and the accuracy of the data analysis. All authors critically revised the manuscript.

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