Evaluation of Prostatic Semeno Microprotein and CD68 Immunohistochemical Expression in Renal Allograft Rejection

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Abstract:

Rejection is considered the major obstacle to long-term allograft survival. Many studies suggest that macrophage recruitment plays an important role in the development of graft rejection and could express CD68 immunohistochemical marker. The persistent inflammation mediated by macrophages may lead to fibrosis and chronic rejection in renal allograft. PSMP is a newly identified protein which can bind to c-c motif chemokine receptor 2 (CCR2) leading to macrophage recruitment. In this study, 50 cases of renal allograft rejection were collected from urology and nephrology center, Mansoura university to evaluate both PSMP and CD68 immunohistochemical expression. The present study showed statistically significant association between CD68 score and borderline TCMR cases **(P<0.001)** while PSMP score showed significant association with CABMR cases **(P=0.004).** Also, a high statistically significant positive correlation was found between PSMP and CD68 scores among studied patients **(P =0.001). (r =0.51).** PSMP was found to have an important role in renal allograft rejection so target therapy directed against PSMP could help to delay the process of rejection.

Keywords: Transplantation – Allograft-Rejection- PSMP- Chemokines- Macrophages.

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Introduction

Renal transplantation is an effective treatment option for patients with end-stage renal disease**.**In Egypt, the first kidney transplantation was performed in Mansoura in 1983, and the first pediatric transplant was performed in 1989. Following that more than 3000 transplantations were carried out in Egypt during the most recent 15 years **(Bakr *et al.,* 2020)**. Renal allograft biopsy is the gold standard tool for the diagnosis of rejection that can be used to assess both the type and degree of rejection**(Jeong 2020).**Rejection could be classified according to severity of rejection into either acute/ active or chronic and according to mechanism of rejection into cell mediated or humoral (antibody mediated)**(Van Loon *et al.,* 2017).**

The diagnosis of acute and chronic T cell mediated rejection (TCMR) largely depends on morphologic changes in the tubulointerstitial compartment. Diagnostic criteria for acute TCMR

have not changed significantly since Banff 1991 and are graded according to active inflammation of non-atrophic tubules and interstitium and vessels. However, the criteria for chronic active TCMR were updated in Banff 2017 **(Roufosse *et al.,* 2018).** To diagnose chronic active TCMR, tubulointerstitial inflammation with a score > 2 should be present in areas of atrophic tubules and interstitial fibrosis, as well as in non-scarred tubules and interstitium **(Nankivell *et al.,* 2018)**

The diagnosis of antibody-mediated rejection (ABMR) is crucial and requires specific treatment, and if improperly managed leads to graft loss**(Jimenez *et al.,* 2012).** Chronic active antibody-mediated rejection (CAAMR) is an intermediate process that occurs during the development of chronic antibody-mediated rejection (CAMR), which has been recognized recently. CAAMR is considered an obstacle to the long-term survival of renal allografts **(Sablik *et al.,* 2019).**Although the diagnostic criteria for CAAMR were defined in the revised Banff 2017 criteria **(Roufosse *et al.,* 2018),** many morphological lesions associated with CAAMR and CAMR appeared similar and it's difficult to distinguish these two phases in clinical practice**(Haas 2018).**

Increasing attention has been paid to macrophage graft infiltration in the immunopathological characteristics of chronic allotransplantation rejection **(Li *et al.,* 2019).** Macrophages are a type of innate immune cell that participate in adaptive immunity through antigen presentation, co-stimulation, tissue repair, and the production of pro-inflammatory cytokines**(Guillén-Gómez *et al.,* 2017).**

Macrophages may be recruited to the rejection site by a variety of chemokines with augmenting the immune response and promoting renal glomerular and tubular injury.The persistent inflammation mediated by macrophages may lead to fibrosis and chronic rejection in renal allograft **(Guillen-Gomez *et al.*, 2017)**.

CD68 is a marker of mature macrophages. it can be expressed on M1 and M2 macrophages and other myeloid cells **(Zhang *et al.,* 2016)**. The chemokine PC3-secreted microprotein (PSMP) is a newly identified chemokine found in the PC3 cell line and malignant prostate tumors **(Pei *et al.,* 2014)**. PSMP has a similar affinity for C-C motif chemokine receptor 2 (CCR2) and its ligand 2 (CCL2). Whatever the type of macrophages, PSMP is a damage-induced chemotactic cytokine that can recruit them in tissues **(Zhan *et al.,* 2021),** so,playing an important role in the pathogenesis of the inflammation-related diseases **(She *et al.,* 2020).** Although it has been implicated in the pathogenesis and progression of many inflammatory, fibrotic and neoplastic diseases**(Kalnins *et al.,* 2015; Pei *et al.*, 2019; Wei *et al.*, 2022) ,** the significance of PSMP protein in renal allograft rejection remains unclear.

.**Materials and methods**:

Patient selection:

Retrospective study was done for 50 patients , who underwent kidney biopsy between 2021 and 2023 at Urology & Nephrology center, Mansoura university. The original diagnosis of rejection was made and then reevaluated according to the 2020 revised Banff criteria **(Jeong 2020).**The

clinical data regarding the age, sex, clinical and laboratory data, history of previous lesions, data of the donor, timing of rejection were retrieved from the patient's medical files. All patients underwent ABO-compatible renal transplantations from first relative living donor, and biopsies were obtained from all patients for clinical surveillance due to elevated creatinine or proteinuria.

Inclusion criteria:rejection cases of both types (TCMR & ABMR).

Exclusion criteria: Cases with incomplete formalin-fixed paraffin-embedded (FFPE) slides, unsatisfactory sample and incomplete clinical data were excluded from the study.

II- Histopathological study:

Paraffin blocks of all cases were sectioned at 3–4-micron thickness and stained with hematoxylin and eosin stain, Periodic acid-Schiff, and Masson Trichrome for histopathological assessment for detection of Banff score of rejection.

II-Immunohistochemical study

Representative blocks from all the studied cases were stained with C4d immunostains for diagnosis of ABMR, PSMP and CD68 and the results were analyzed, tabulated, and recorded.

I- Primary antibody: The primary antibody for CD68 (DAKO, ready to use) and PSMP (Bio SB, Netherland, clone EP203,igG rabbit monoclonal antibody with 1:25- 1:100 dilution).

II- Universal kit: Super-sensitive (Bio SB) IHC detection system. An improved Biotin-Streptavidin amplified (B-SA) detection system (Mouse/ Rabbit Poly-Detector DAB HRP Brown, REF: BSB 0205). Designated to detect specific antigens in formalin-fixed, paraffin-embedded tissue.

III) Interpretation of PSMP and CD68 expression(Paoletti *et al.,* 2019):

Cytoplasmic CD68 and PSMP expression are considered positive in interstitial macrophages. The number of PSMP & CD68 positive interstitial cells detected in non-scarred areas of the cortex was analyzed in 10 high power fields (HPF) (X40), excluding sub-capsular, glomerular, and vascular areas. Staining scores were attributed as follows**:**0: no positive cells**,** 1:1-10 cells/HPF**,** 2:11-20 cells/HPF**,** 3:>20 cells/HPF.

Statistical analysis: Data analysis was performed using Statistical Package for the Social Sciences version (SPSS) 25 software (IBM Corporation, Armonk, NY, USA). Descriptive statistics: mean ± SD were reported for continuous variables, while frequency and percentage for categorical variables. Interferential statistics: At level of significance value (P value): $P > 0.05$ (Non-significant), $P \leq 0.05$ (Significant). For qualitative data: Chi-square test and Fisher's exact test were used, while quantitative data that was normally distributed: ANOVA test was used and not normally distributed data: Kruskal-Wallis's test was used after testing of normality using Shapiro-Wilk test. Spearman rank correlation was used to assess correlations between variables.

RESULTS:

Table 1: statistical correlation of CD68 and PSMP with demographic, clinical data and type of rejection

Variable		Patients (n=50)		CD68 score			P-value	PSMP score				P value
		No	%	Score 0 (n=1)	Score 1 (n=36)	Score 2 (n=13)		Score 0 (n=1)	Score 1 (n=23)	Score 2 (n=19)	Score 3 (n=7)	
Age (years)	*mean± SD (range)*	32.6±11.1 (11 – 57)		52 -	33.1± 10.9 (17 – 57)	29.9± 10.9 (11 – 54)	0.2	24 -	34.7± 11.75 (17-57)	33.6± 10.6 (17-55)	24.6± 7.46 (11-33)	0.1
	≤ *31years*	27	54 %	0 (0%)	19 (70.4 %)	8 (29.6 %)	0.5	1 (3.7 %)	12 (44.4 %)	9 (33.3 %)	5 (18.5 %)	0.6
	>31 years	23	46 %	1 (4.3 %)	17 (73.9 %)	5 (217 %)		0 (0 %)	11 (47.8 %)	10 (43.5 %)	2 (8.7%)	
Sex	*Male*	35	70 %	1 (2.8 %)	26 (72.2 %)	9 (25%)	1.0	1 (2.8 %)	20 (55.6 %)	11 (30.6 %)	4 (11.1 %)	0.1
	Female	15	30 %	0 (0%)	10 (71.4 %)	4 (28.6 %)		0 (0 %)	3 (21.4 %)	8 (57.1 %)	3 (21.4 %)	
Time to transplantation (years)	*mean± SD (range)*	10.6±7.1 (3-31)		27 -	9.3±6.3 (3-31)	12.8± 7.5 (4-27)	0.09	4 -	11.8± 8.8 (3-31)	9.5±4.9 (4-22)	10.4± 6.1 (3-21)	0.7
DM	- *Present*	1	2%	0 (0%)	1 (100 %)	0 (0%)	0.8	1 (2 %)	23 (46.9 %)	18 (36.7 %)	7 (14.3 %)	0.6
	- *Absent*	49	98 %	1 (2%)	35 (71.4	13 (26.5 %)		0 (0	0 (0%)	1 (100	0 (0%)	

					%)				%)		%)		
HTN	- *Present* - *Absent*	33 17	66 % 34 %	1 (3%) 0 (0%)	26 (78.8 %) 10 (58.8 %)	6 (18.2 %) 7 (41.2 %)	0.2	0 (0 %) 1 (3 %)	5 (29.4 %) 18 (54.5 %)	7 (41.2 %) 12 (36.4 %)	5 (29.4 %) 2 (6.1%)	0.09
Last creatinine	*mean± SD (range)*	3.3±2.2 (0.8-9.8)		27 -	3.4±2.1 (1.1-9.6)	3.1±2.6 (0.8-9.8)	0.2	2.1 -	3.6±1.9 (1.1-7)	3.2±2.4 (1.1-9.6)	2.9±3.1 (0.8-9.8)	0.7
Last proteinuria	*mean± SD (range)*	2.7±3.6 (0.3-22.14)		0.97 -	3.1±4.2 (0.4-22.1)	1.8±1.2 (0.3-3.8)	0.5	0.9 -	3.6±0.5 (0.4-22.1)	2.1±1.4 (0.3-5)	1.4±1.2 (0.4-3.6)	0.4
Improvement	*Yes* *No*	7 43	(14 %) (86 %)	0 (0%) 1(14.3%)	32 (74.4 %) 4 (57.1 %)	11 (25.6 %) 2 (28.6 %)	**0.04**	1 (2.3 %) 0 (0 %)	20 (46.5 %) 3 (42.9 %)	17 (39.5 %) 2 (28.6 %)	5 (11.6 %) 2 (28.6 %)	0.7
ABMR	Chronic Chronic active	62	12 % 4%	0 (0%) 0 (0%)	2 (33.3 %) 0 (0%)	4 (66.7 %) 2 (100 %)	0.07 0.1	0 (0 %) 0 (0 %)	1 (16.7 %) 1 (50%)	1 (16.7 %) 1 (50%)	4 (66.6 %) 0 (0%)	**0.004** 0.9
TCMR	Acute Chronic Borderline	6 17 19	12 % 34 % 38 %	0 (0%) 1 (5.8 %) 0 (0%)	3 (50%) 12 (70.6 %) 19 (100	3 (50%) 4 (23.5 %) 0 (0%)	0.40 0.45 **<0.001**	0 (0 %) 0 (0 %) 1 (5.3	3 (50%) 7 (41.2 %) 11(57.9%)	2 (33.3 %) 9 (52.9 %) 6 (31.6	1(16.7%) 1 (5.9 %) 1 (5.3 %)	0.99 0.35 0.18

				%)				%)			%)	

Table 2: statistical correlation of CD68 and PSMP with parameters of rejection

Variable		Patients (n=50)		CD68 score			P-value	PSMP score				P-value
		No	%	Score 0 (n=1)	Score 1 (n=36)	Score 2 (n=13)		Score 0 (n=1)	Score 1 (n=23)	Score 2 (n=19)	Score 3 (n=7)	
Total Glomeruli no.	*median (range)*	10.5 (2 – 23)		15 (15-15)	10(2-23)	11 (6-19)	0.7	11 (11-11)	9 (2-19)	11 (6-19)	12(10-23)	0.2
Normal glomeruli	*median (range)*	6 (0 – 19)		12 (12-12)	5.5 (0-19)	6 (1-11)	0.3	10 (10-10)	5 (1-19)	6 (0-17)	6 (1-18)	0.7
(GS)	*median (range)*	2 (0 – 18)		1 (1-1)	2 (0-18)	3 (0-13)	0.4	1 (1-1)	1 (0-6)	3 (0-18)	4 (0-13)	0.05
(SS)	*median (range)*	1 (0 – 8)		2 (2-2)	1 (0-8)	2 (0-6)	0.5	0 (0-0)	1 (0-8)	1 (0-4)	2 (0-6)	0.4
(cg)	*median (range)*	0 (0 - 3)		0 (0-0)	0 (0-3)	0 (0-2)	0.9	0 (0-0)	0 (0-3)	0 (0-2)	0 (0-2)	0.7
Tubulitis	*t 1*	18	36 %	0 (0%)	15 (83.3 %)	3(16.7 %)	0.4	0 (0%)	8(44.4 %)	9 (50%)	1 (5.6 %)	0.4
	t 2	4	8%	0 (0%	3 (75%	1 (25%)	1.0	0 (0%	2 (50%)	1 (25%	1 (25%	0.8

				)	)			)		)	)	
Tubular atrophy	*ct 1* *ct 2*	22 6	22 6	0 (0%) 0 (0%)	16 (72.7%) 3 (50%)	6(27.3%) 3 (50%)	1.0 0.4	0 (0%) 0 (0%)	10 (45.5%) 1 (16.7%)	7 (31.8%) 5 (83.3%)	5 (22.7%) 0 (0%)	0.3 0.2
Interstitial inflammation	*i 1* *i 2*	27 17	54% 34%	0 (0%) 0 (0%)	21 (77.8%) 12 (70.8%)	6 (22.2%) 5 (29.4%)	0.4 0.8	0 (0%) 0 (0%)	11 (40.7%) 9 (52.9%)	11 (40.7%) 6 (35.3%)	5 (18.5%) 2 (11.8%)	0.5 0.9
Interstitial fibrosis	*ci 1* *ci 2*	22 4	44% 8%	0 (0%) 0 (0%)	13 (59.1%) 4 (100%)	9 (40.9%) 0 (0%)	0.051 0.6	0 (0%) 0 (0%)	10 (45.5%) 1 (25%)	7 (31.8%) 3 (75%)	5 (22.7%) 0 (0%)	0.3 0.4
Acute vasculitis	*v 1* *v 2*	1 1	2% 2%	0 (0%) 0 (0%)	1 (100%) 0 (0%)	0 (0%) 1 (100%)	1.0 0.3	0 (0%) 0 (0%)	1 (100%) 0 (0%)	0 (0%) 0 (0%)	0 (0%) 1 (100%)	1.0 0.2
Chronic vasculitis	*cv 1* *cv 2* *cv 3*	27 3 1	54% 6% 2%	1 (3.7%) 0 (0%) 0 (0%)	19 (70.4%) 2 (66.7%) 1 (100%)	7 (25.9%) 1 (33.3%) 0 (0%)	1.0 1.0 1.0	1 (3.7%) 0 (0%) 0 (0%)	10 (37%) 1 (33.3%) 0 (0%)	12 (44.4%) 1 (33.3%) 1 (100%)	4 (14.8%) 1 (33.3%) 0 (0%)	0.5 0.6 0.5
Hyalinos	*h1*	11	22	0 (0%	9 (81.8	2 (18.2	0.8	0 (0%	6 (54.5	2 (18.2	3 (27.3	0.2

is	*h 2*	11	%	)	%)	%)	0.3	)	%)	%)	%)	0.5
	h 3	3	22 % 6%	0 (0%) 0 (0%)	6 (54.5 %) 3 (100 %)	5 (45.5 %) 0 (0%)	0.6	0 (0%) 0 (0%)	6 (54.5 %) 2 (66.7 %)	5 (45.5 %) 1 (33.3 %)	0 (0%) 0 (0%)	1.0
C4d	*Positive*	4	8%	0 (0%)	2 (50%)	2 (50%)	0.3	0 (0%)	3 (75%)	1 (25%)	0 (0%)	0.8
	Negative	46	92 %	1 (2.2 %)	34 (73.9 %)	11 (23.9 %)		1 (2.2 %)	20 (43.5 %)	18 (39.1 %)	7 (15.2 %)	

Table 3: CD68 score in relation to PSMP findings among studied patients

Variable		**CD68 score**			**P value**	**R**
		Score 0 (n=1)	**Score 1 (n=36)**	**Score 2 (n=13)**		
PSMP	**Score 0 (n=1)**	**0 (0%)**	**1 (100%)**	**0 (0%)**	**0.001**	**0.51**
	Score1 (n=23)	**1 (4.3%)**	**20 (87%)**	**2 (8.7%)**		
	Score2 (n=19)	**0 (0%)**	**14 (73.7%)**	**5 (26.3%)**		
	Score 3 (n=7)	**0 (0%)**	**1 (14.3%)**	**6 (85.7%)**		

**Fisher-exact test, P ≤0.05: significant, P >0.05:non-significant*

Table (3) shows a statistically significant correlation between CD86 and PSMP score among studied patients *(P =0.001) (r=0.51).*

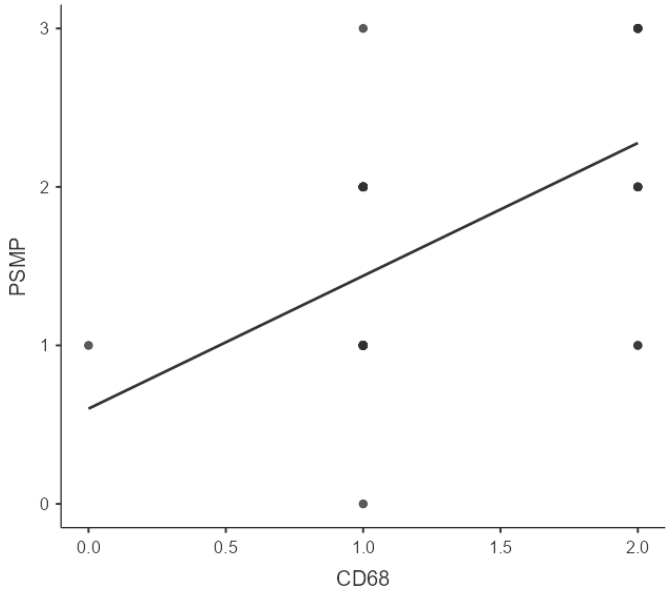

Figure 1: Correlation between CD68 score and PSMP score

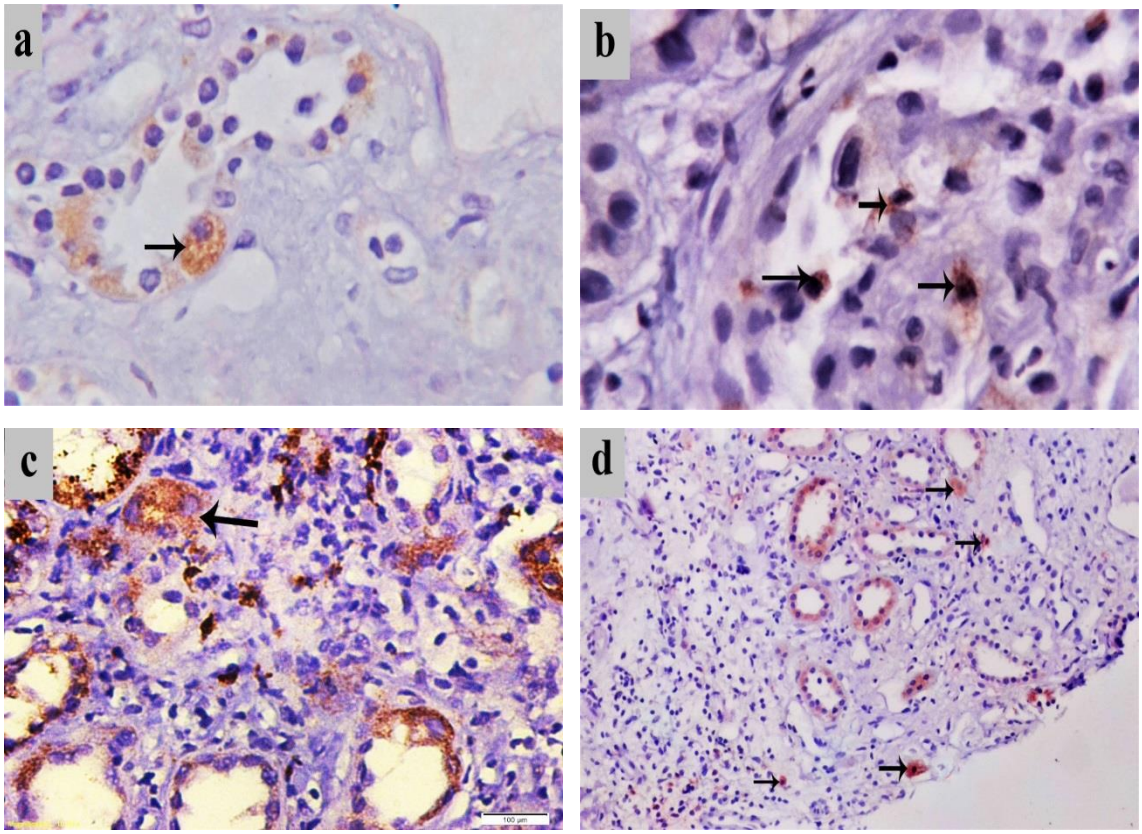

Figure 2: a) Kidney core biopsy from a case of borderline TCMR (CD68 score1) showing positive cytoplasmic CD68 immunostaining in interstitial macrophage infiltrate encroaching into

a tubule (arrow) (CD68 IHC x400). b) Kidney core biopsy from a case of chronic TCMR (CD68 score 1) showing interstitial macrophage infiltration with positive cytoplasmic CD68 immunostaining (arrow). (CD68 IHC x400). c) Kidney core biopsy from a case of acute TCMR (CD68 score 2) showing interstitial macrophage infiltrate with positive cytoplasmic CD68 immunostaining (arrow). The surrounding tubular cell positivity is considered a non-specific reaction. (CD68 IHC x400). d) Kidney core biopsy from a case of chronic active ABMR (CD68 score 2) showing positive cytoplasmic CD68 immunostaining in interstitial macrophage (arrow). The surrounding tubular cell positivity is considered a non-specific reaction. (CD68 IHC x200).

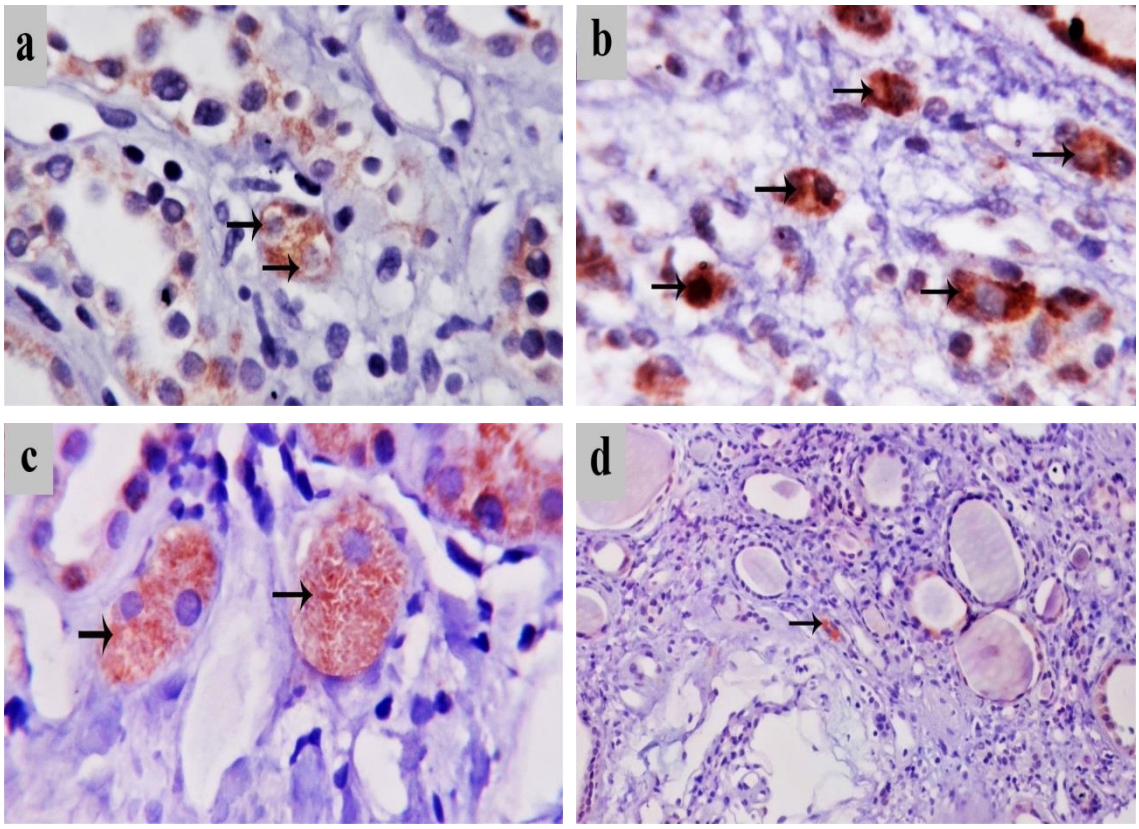

Figure 3: a) Kidney core biopsy from a case of borderline TCMR (PSMP score 1) showing positive cytoplasmic PSMP immunostaining in interstitial macrophage (arrow). (PSMP IHC x400). b) Kidney core biopsy from a case of chronic TCMR (PSMP score 2) showing interstitial macrophage infiltration with positive cytoplasmic PSMP immunostaining (arrow). (PSMP IHC x400). c)Kidney core biopsy from a case of chronic ABMR (PSMP score 3) showing positive cytoplasmic PSMP immunostaining in interstitial macrophages (arrow). (PSMP IHC x400). d) Kidney core biopsy from a case of chronic active ABMR (PSMP score 1) showing positive cytoplasmic PSMP immunostaining in interstitial macrophage (arrow). (PSMP IHC x200).

DISCUSSION:

Renal allograft rejection, clinical or subclinical is the major causes of kidney allograft loss.. Increasing evidence focused on the profound meaning of innate immune cells, such as NK cells, macrophages, and neutrophils **(Zhang *et al.,* 2021).**The number of macrophages and T cells generally correlates with the loss of tissue function in various forms of diseases due to uncontrolled immune response initiated by these cells **(Segerer and Nelson 2005).**Macrophages have a critical role in innate immunity in acute and chronic allograft rejection **(Chen *et al.,* 2023).** The macrophages' destiny relies on various environmental conditions that fuel polarization to any of the classically triggered pro-inflammatory M1 immune response or anti-inflammatory M2 response. M1 or M2 polarization is a precisely regulated process comprising several key signaling pathways, transcriptional epigenetic and post-transcriptional regulatory networks **(Zhang *et al.,* 2021; chen *et al.,* 2023).**

In the present study, statistical correlation between PSMP expression and CD68 as a marker for macrophages detection, was carried out in 50 cases of renal allograft rejection. PSMP and CD68 expression analysis were carried out with different clinicopathological data. Cytoplasmic PSMP and CD68 expression were considered positive in interstitial macrophages.

In this study, statistical analysis for PSMP and CD68 expression in relation to demographic or clinical data showed no statistically significant association (P>0.05).This comes in agreement with a previous study done by Zhan and his colleagues, who reported no significant difference among baseline characteristics including age, gender, infections, diabetic nephropathy, time post-transplant to biopsy and PSMP expression ($p > 0.05$) **(Zhan *et al.,* 2021).**

The present study showed statistically significant association between CD68 score and worsening in laboratory data (graft function/ serum creatinine and proteinuria) among studied patients **(P=0.04),** whileno significant association was found between PSMP score and laboratory data among studied patients (P >0.05). This result is consistent with the previous study done by **Toki et al. (2014)**whonoticed that patients performed post transplantation biopsy at one year and three years with lower CD68 score had a significantly higher estimated glomerular filtration rate compared with those with high CD68 score (Group I (CD68<400/mm) with 87±29 mL/min vs. Group II (CD68>400/mm); 64± 19 mL/min, p= 0.014)**(Toki 2014).**Another finding in keeping with the results of this study was reported by **Bergler et al. (2016)**, who concluded that patients with poor clinical outcome (reflected by significantly elevated creatinine values and a significantly shortened graft survival) up to month 36 after transplantation had a significantly increased CD68 infiltration ($p < 0.0001$)**(Bergler 2016).** Also,**Bräsen (2017)**, found out that CD68 macrophage marker density significantly contributes to the prediction of allograft function and associated with a significantly lower GFR**(Bräsen JH 2017).** In addition,Azad and his colleagues found that the macrophage score in protocol biopsies 6 months following transplant predicted histological damage at 24 months after transplant with sensitivity of 91.6% and specificity of 83%. They suggested that the macrophage score could be applicable as a "rule-out test" for long-term graft loss. That is, patients with low macrophage scores may be candidates for less aggressive immunosuppression, limiting the treatment-related side effects. **(Azad *et al.,* 2018).** Moreover,Guillén-Gómezand his colleaguesfound that the elevated numbers of CD68-positive cells at baseline in deceased donor kidneys correlated inversely with

medium- and long-term renal function. This is indicative of the impact of kidney inflammatory status before transplantation on graft outcome **(Guillén-Gómez*et al.*, 2017).**

Current study showed that there was a statistically significant association between CD68 score and borderline TCMR **(P<0.001)** as all cases of borderline TCMR had score 1 CD68 expression. There was no statistical association between CD68 score and other subtypes of TCMR or ABMR (p>0.05).

In contrast to this result, a study carried out by **Bergler et al. (2016)** founda significant increase in glomerular CD68 infiltration in patients diagnosed with marked TCMR with arteritis as well as ABMR in comparison with normal histology. This controversycould be explained by different sample size, with increased number of TCMR in our study (84%), especially border line TCMR cases, compared with that of ABMR cases (16%). Also, the fact that **Bergler et al. (2016)** did compartmental CD68 score while this study focused on interstitial score could be contributed to this controversy**(Bergler 2016)**. In addition, **Shao (2024)** found a significant macrophage infiltration in post-renal transplant ABMR and suggested that macrophages may work as reference indicator for early predicting post-renal transplant AMR **(Shao, 2024).**

It has been suggested that macrophage infiltration augments the inflammatory response and promotes tubular injury **(Qi *et al.* 2008),** that might participate in the formation of interstitial fibrosis and tubular atrophy, the main lesion seen in chronic kidney allograft injury. **(Mantovani *et al.* 2013).**

Analysis of the various inflammatory cells present in the renal allograft during acute TCMR demonstrated an abundance of macrophage-associated transcripts **(Mueller et al., 2019)**, and a predominance of macrophages, comprising 32–60% of inflammatory cells **(Yu and Lu, 2022).** Also, biopsies of patients with acute TCMR demonstrated increased transcript levels of granulocyte monocyte colony stimulating factor (GM-CSF)**,** which increase in monocyte surface expression of major histocompatibility complex (MHC) class II (HLA-DR) and the costimulatory molecules CD40 and CD80 **(Singh *et al.,* 2009).** In acute TCMR, Intrarenal macrophages display a proinflammatory phenotype and secrete various cytokines, including IFNγ, IL-1β, IL-12, IL-18, and TNFα, which can activate endothelial cells and promote cytotoxic T-cell generation **(Wilson *et al.,* 2020).** Additionally, activated inflammatory macrophages can produce reactive oxygen species (ROS) that can aggravate allograft injury. Taken together, renal tubular epithelial cell injury promotes monocyte chemotaxis and upregulates costimulatory molecules on infiltrating monocytes during acute TCMR, which can facilitate T-cell activation and amplify TCMR **(Yu and Lu 2022).**

In active AMR, a clinical study of protocol biopsies performed one year after transplant, intrarenal macrophages correlated with interstitial fibrosis and renal dysfunction at one year and three years. The vast majority (92%) of tissue macrophages were identified as having an M2 phenotype in this study **(Ikezumi *et al.,* 2015).** Also, a clinical study with protocol biopsies performed at one, five, and ten years, demonstrated that increased intrarenal M2 macrophages corresponded to increased interstitial fibrosis and worsened renal function. **(Kamal *et al.,* 2015).** In addition, in CAABMR**,** molecular analysis demonstrated that macrophage-associated

transcripts are among the most abundant transcripts**(Hayde *et al.,* 2013),** and the presence of glomerular M2 polarized macrophages corresponded to elevated production of IL-1β, IL-6, and TNFα, higher TG levels and worse renal function at the time of biopsy and at three months following biopsy **(Kim, *et al.,* 2018).**

As regards PSMP score and type of rejection, this study found that 66.6% of CABMR cases had PSMP score 3 in comparison to 33.4%with score two and one collectively. This difference was statistically significant **(P=0.004).**There was no statistically significant association between PSMP score and CAABMR or TCMR cases (P >0.05).

This result is inconsistent with that reported by**Zhang et al. (2021),** who found that the immunohistochemical analysis of PSMP protein was strongly expressed in CAAMR group compared with the control and CAMR groups ($p < 0.01$). They also noticed that PSMP mRNA was detected at significantly higher levels in the biopsy tissues of CAAMR patients compared with those from the Control group ($p < 0.05$)**(Zhang *et al.,* 2021).**

This difference may be attributed to the difference in sample size, type of antibody used and other factors and molecules that may affect PSMP production and metabolism in the studied cases**(Zhang *et al.,* 2021).**

This study detected no significant association between CD68 or PSMP score and glomerular, tubulo-interstitial and vascular findings and C4d immunohistochemical staining among studied patients (P >0.05). In contrast to these results, **Zhang et al. (2021)** found a significant correlation between the expression level of PSMP and v-lesion detection in CAAMR. This controversy could be explained by the small size of the (v>0) cases including only three cases with v>0 with no chronic vasculitis or hyalinosis included in his study. So, more samples remain necessary to verify this relationship between v-lesion and PSMP **(Zhang *et al.,* 2021).** Also, In contrast to our results, **Shao (2024)** noticed significant macrophage infiltration in post-renal transplant ABMR, and closely related to the severity of IF/TA and the degree of tubulointerstitial inflammatory cell infiltration**(Shao, 2024).**

In this study, a high statistically significant positive correlation was found between PSMP and CD68 score among studied patients **(P =0.001). (r =0.51).** This comes in agreement with **Zhang et al. (2021)** who found a significant correlation between PSMP expression and the number of infiltrating CD68+ cells ($p < 0.05$), in CAAMR patients**(Zhang *et al.,* 2021).**

The kidney contains a complex array of phagocytes with macrophage and antigen-presenting cell characteristics **(Woollard and Pusey, 2014)**.Their recruitment is largely directed by chemokines and their cognate receptors **(Mannon 2012).**PSMP is a recently discovered chemokine that has an affinity for CCR2. CCR2 has been reported to be an essential chemokine receptor that aids the entry of bone-marrow residing monocytes into the bloodstream and migration to the affected tissue to mediate inflammation process **(Pei *et al.,* 2017).** CCL2, a well-known ligand of CCR2 had been reported to be expressed on monocytes and macrophages **(Takada *et al.,* 2010).** The CCL2-CCR2 axis is currently the most well-studied chemokine pathway in many diseases **(Tamargo and Kant, 2023).** A recent study by **Song et al. (2024),** reported

thatoverexpressed human PSMP in the mouse kidney could reverse the improvement of kidney injury in a CCR2-dependent manner, and this effect could be achieved without CCL2 involvement (**Song *et al.,* 2024)**.

The chemokine PSMP was also expressed in the colonic mucosa of patients with colitis and significantly up-regulated in the initial stage prior to the expression of IL-6, TNF-α and CCL2 in a dextran sulfate sodium induced colitis in mice **(Zhang *et al.,* 2017).** In the liver, IL-1β, the damage-associated molecular pattern (DAMP) molecules IL-33 and HMGB-1 could induce mouse primary hepatocytes to produce PSMP. Also, PSMP was upregulated in fibrotic/cirrhotic liver tissues in a CCR2-dependent manner. Promising reduction in liver fibrosis development (in vivo) was established by using PSMP antibodies, indicating that PSMP antibodies may be a potential therapeutic agent for liver fibrosis **(She *et al.,* 2020).**

Bone marrow derived macrophages can promote renal fibrosis by both direct and indirect mechanisms. In a direct way, a part of macrophages transition into myofibroblasts, which express CD68 and α-SMA through macrophage- myofibroblast transition and produce collagen I, that is responsible for renal fibrosis. TGF-β1-Smad3 signal pathway is the main signaling pathway of macrophage-myofibroblast transition. Signaling-associated inhibitors had been proven to provide protection in animal models **(Wei *et al.*, 2022).**In prostate cancer tissues, results demonstrated that PSMP secreted by the tumor cells promoted the growth and survival of prostate cancer cells and epithelial mesenchymal transition by directly stimulating tumor cells. **(Pei *et al.,* 2019).**

In ovarian cancer,PSMP expression is induced with prolonged anti-VEGF therapy, specifically under hypoxia, and has an important proangiogenic role in treatment-resistant ovarian tumors **(Mitamura *et al.*, 2018)**. Also **Ruiz et al. (2022),** revealed that CD5L, EGFL6, and PSMP contribute to the progression of ovarian cancer, and the inhibition of these proteins by their antibodies, RNAi, and/or aptamers can reduce tumor growth and reverse adaptive resistance in ovarian cancer **(Ruiz *et al.*, 2022).**

In conclusion, the data in the present study indicated that macrophage infiltration correlated with development of graft rejection and poor graft function. PSMP is highly correlated with macrophage infiltration and plays an important role in chronic antibody mediated rejection. Further studies are recommended to understand the pathway of PSMP production and metabolism. Antibodies targeting PSMP could be useful in limiting graft fibrosis and improve graft outcome.

Conflicts of interest: Non to declare.

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