Tumor-associated macrophages (TAMs) as Biomarker for urinary bladder tumor

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Abstract

Tumor associated macrophage (TAMs) are alternatively activated macrophages that enhance tumor progression by prompting tumor cell invasion ,migration and angiogenesis .The study aimed to detect of the distribution TAM bladder tumor tissues , and to clarify the relationship between the TAMs and clinicopathological grade of bladder cancer. Immunohisochemistry was used to detect CD68 as general marker for TAMs macrophage in tissue . Fifty patients with urinary bladder carcinoma and twenty benign bladder biopsies from patients with urinary bladder diseases (UBD) were included in this study . Our data showed a high positive immunohistochemical CD68 expression of UBC tumor tissues than in UBD tumor tissues (84% versus 45% ; $p \leq 0.01$) , also a significant difference was observed in grades among patients with UBC in CD68+ expression, high grade tumor cases showed positive immunohistochemical CD68 expression in 27(96.4%), while only 15 cases (68.2%) of low grade tumor showed positive CD68 expression . The results showed that the infiltration of CD68-positive may contribute to poor prognosis in advanced urinary bladder carcinoma

Keywords: Tumor associated macrophages, Bladder cancer , CD68

Introduction

Tumor-associated macrophages (TAMs) represent a substantial fraction of the growing tumor mass and are associated with poor prognosis in several human cancers [1]. TAMs exist in two different polarizations state classified as M1 and M2 . M1 macrophages show a protective role in tumor-genesis activating tumor-killing mechanisms and antagonizing the activities of M2. Tumor-associated macrophages (TAMs) generally have M2 phenotype macrophages that are clearly involved in suppression of adaptive tumor-specific immune responses and in promotion of tumor growth, invasion, stroma remodeling and angiogenesis [2-6]. In recent

years, increasing attention has focused on TAMs, unique macrophage populations that play pivotal roles in tumor immunosuppression, and provide a suitable microenvironment for cancer development and progression[7]. TAM infiltration has been found to be correlated with a worse outcome in several malignant tumors [8-13]. The possible mechanism by which TAMs support tumor progression and help the tumor evade immunosurveillance is through the release a spectrum of tumor promoting and immunosuppressive products. Bladder cancer is one of the most widespread cancers afflicting men and women, and its incidence grows exponentially each year. Early studies reported that the macrophages increase in bladder cancer is associated with high survival and invasive capacity [12]. Activated macrophages promote tumor-genesis through the expression of growth factors and matrix proteases, promotion of angiogenesis and suppression of anti-tumoral immune response (Figure 1)

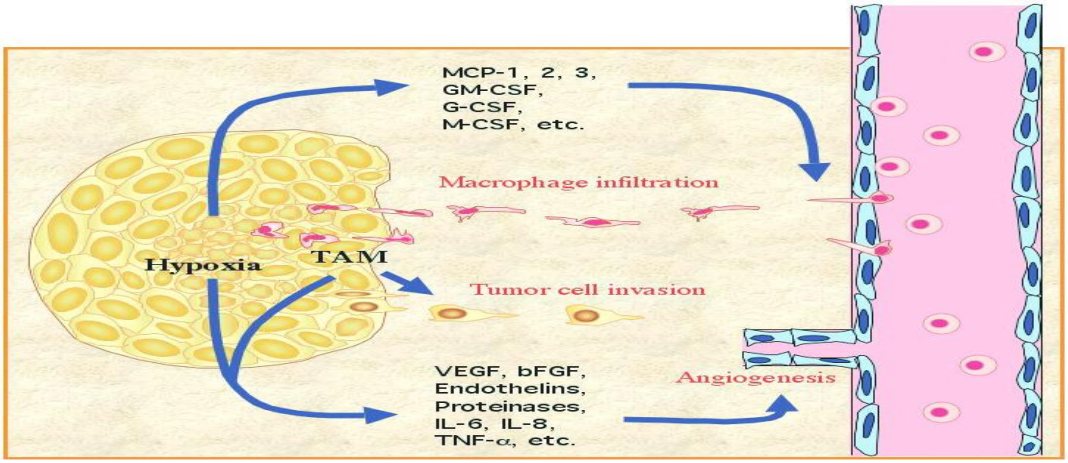


Figure 1: Macrophage infiltration facilitates tumor spreading via induction of tumor angiogenesis and tumor cell invasion [23] .

In Iraq, urinary bladder cancer is the third most common malignancy tumor in both men and women, it's the second most common in men and ninth in women [14]. In Thi-Qar province urinary bladder was the third of all cancer cases according to Weheed *et al*.(2011) [15] ,also in Thi Qar, cancer of the urinary bladder was the top cancer during (2001 –2004) [16] . Few studies to date have described the infiltration CD68+ TAMs macrophages in bladder cancer .

So the aim of this study was to evaluate the infiltration CD68+ tumor-associated macrophages (CD68+TAMs) in urinary bladder tumor as prognostic biomarker .

Materials and Methods

Fifty patients with urinary bladder carcinoma (UBC), 43 (male) and 7 (female) with an age ranged from 31 to 83 years, were included in this study, the patient samples were collected from the histopathology laboratories of Al-Hussain Teaching Hospital and private laboratories in Thi-Qar during the period from January 2014 to September 2014. The diagnosis of these tissue blocks were primarily based on the obtained histopathological records of bladder biopsy samples in hospital laboratory. Confirmatory histopathological re evaluation of each obtained tissue blocks was done by specialist pathologist . In addition twenty benign bladder biopsies from patients with urinary bladder diseases(UBD),they were 16 males and 4 females with an age range of (37-72) years . For each case, one representative section was stained with Hematoxylin and Eosinand the histopathological diagnosis was revised, while other sections were put on positive charged slides and stained immunohistochemically for CD68. Immunohistochemical staining was carried out using the Novocastra TM Polymer Detection Systems (Envision technique) by using commercial kit from Novocastra, Newcastle, UK, RE7150-K , the slides were deparaffinized, rehydrated then blocked. All of the slides were treated with anti CD68 monoclonal antibody, dilution1:100 (Dako, Denemark), then incubated with a post primary block solution for 30 minutes. In the next step the slides were rinsed gently in PBS 2× 5 and tissue sections incubeted with a secondary antibody Novolink TMpolymer mouse and rabbit immunoglobulins) for 30 minutes, washed in PBS 2× 5 with gentle rocking. After washing, the samples were stained with diluted liquid DAB, and then counter stained with hematoxylin . Slides washed , dehydrated then mounting, and examining under light microscope at 10X,20X,40X magnification ...

Results :

We evaluated the expression of CD68 in UBC and UBD tissues , and observed a higher positive immunohistochemical expression of in UBC tumor tissues than in UBD tumor tissues (84% versus 45 % ; $p \leq 0.01$) (Table 1) .

CD68 were detected was detected mostly in the peritumoral tissue and in stromal cell . (**Figure 2**).

In terms of scores, UBC patients with the score +++ represented the highest frequency (38%). While, the score scatter ≤ 10 represented the highest frequency in UBD patients (55.6%) . However, (Table 2) showed the frequency of distribution of CD68 scores in patients groups . The results showed a high statistical difference ($p \leq 0.01$) between urinary

bladder carcinoma and other urinary bladder diseases for CD68 IHC scores in tissue sample taken from each case .

Regarding the tumor grade of UBC, There was a high significant association between the grade of UBC and the immunohistochemical expression of CD68($p \leq 0.01$) , the majority of high grade tumor cases showed positive immunohistochemical CD68 expression 27(96.4%), while only 15 cases (68.2%) of low grade tumor showed positive immunohistochemical CD68 expression (Table 3) .Dense infiltration of CD68+ macrophages in UBC correlated with high clinical grade and the distribution of CD68 scores among tumor grades for UBC showed a high significant correlation between each score and tumor grade ($P \leq 0.05$) (Figure 1,2).

Table (1): CD68 expression in bladder patients groups

Study groups		CD68 EXPRESSION		Total	Chi-square	P value
		positive	negative			
UBC	**No**	42	8	50	10.988	** 0.00092
	Percentage%	84	16	100		
UBD	**No**	9	11	20		
	Percentage%	45	55	100		
Total	**No**	51	19	70		
	Percentage%	72.9	27.1	100		

** ($P \leq 0.01$) high significant

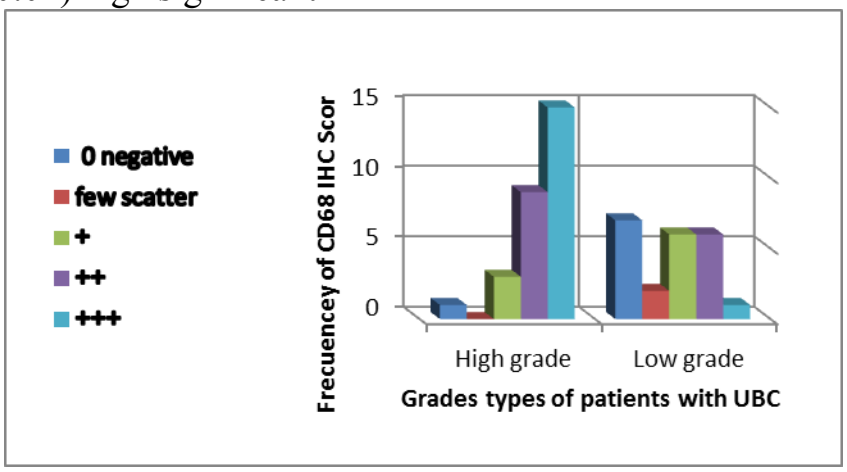
Table (2): Frequency of CD68 IHC scores in patients groups

CD68 Score	UBC		UBD	
	No.	%	No.	%
+++	16	38	0	0
++	15	35.8	2	22.2
+	9	21.4	2	22.2
Scatter ≤ 10	2	4.8	5	55.6
Total	42	100	9	100
P value X^2 value	0.0005** 17.767			

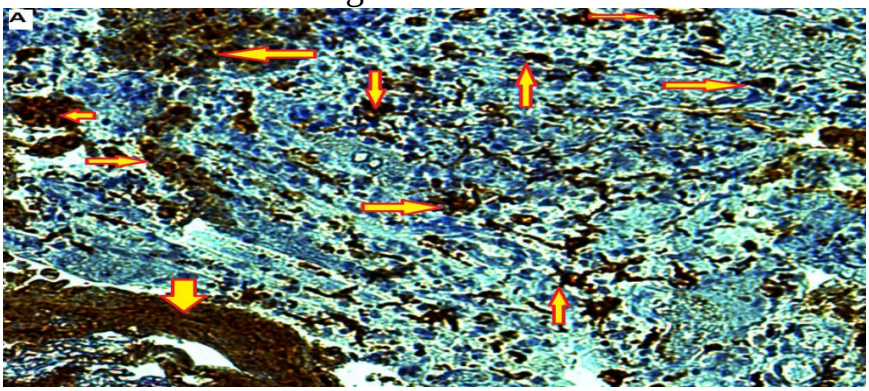
Table (3) Association between CD68 IHC expression and tumor grade

parameter		CD68 EXPRESSION		Total	P-value $\chi 2$ –value
		positive	negative		
Grade of UBC	Low grade (G≤ 1)	15 (68.2%)	7 (31.8%)	22	7.314 P=0.0068**
	High grade (G≥ 2)	27 (96.4%)	1 (3.6%)	28	
	Total	42(84%)	8 (16 %)	50	

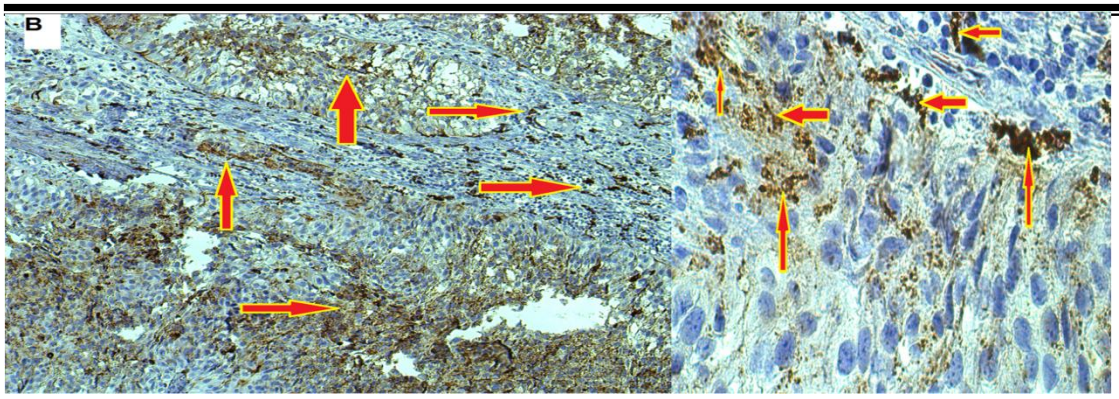
** (P≤ 0.01) high significant



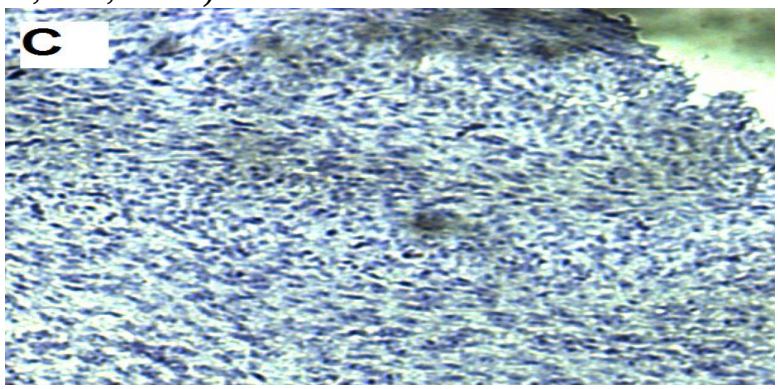
Figure(1) : The IHC Score of CD68 expression in bladder carcinoma patients in relation to the tumor grade**.**



Figure(2): A:Invasive transitional cell carcinoma , poorly differential(Grade II) showing strong positive CD68 staining (Score +++ ,brown)(20X,arrow)



Figure(2): B:Invasive transitional cell carcinoma , showing moderate macrophage density, determined by positive CD68 staining (Score ++,brown)(10,40X,arrow)



Figure(2): C: Immunohistochemical tissue expression of CD68 in bladder tissues showing no detectible CD68 - staining (score 0 negative) (10 X) .

Discussion

CD68 is a pan-macrophage marker frequently used as a marker for TAMs . In our study infiltration of (CD68+)TAMs were the most abundant in patients with UBC compared to patient with UBD ,also the presence of infiltrating CD68+ TAMs was significantly associated with high clinical grade . These results agree with Hanada *et al*. (2000) who found that CD68+ TAMs expression in invasive bladder cancers (154.22+/-11.98) was significantly higher than in superficial bladder cancers (49.05+/-7.76; P<0.0001) [12] . There is persuasive clinical and experimental evidence that macrophages promote cancer initiation and malignant progression. During tumor initiation, they create an inflammatory environment that is mutagenic and promotes growth. As tumors progress to malignancy, macrophages stimulate angiogenesis, enhance tumor cell migration and invasion, and suppress antitumor immunity. At metastatic sites, macrophages prepare the target tissue for arrival of tumor cells, and then a different subpopulation of macrophages promotes tumor cell extravasation,

survival, and subsequent growth [17] . In many human neoplasms, including , prostate, melanoma, and pancreas cancers, the presence of extensive TAM infiltrate correlates with poor prognosis [11,18,20] . CD68 scores among tumor grades for UBC showed a high significant correlation between each score and tumor grade these results agreed with et al. (2014) who found the prognostic effect of TAM counts in tissue sections from muscle-invasive (MI) tumor in urothelial carcinoma (UC) [21] .

One of the most important roles for macrophages in tumor growth seems to be in promoting angiogenesis. Tumor development begins with an "avascular phase"characterized by a limited number of cells and acquisition of nutrients by simple diffusion. As the tumor becomes larger, the metabolic demands increase and a more developed vascular infrastructure is required. This transition to this "vascular phase", termed the "angiogenic switch", is stimulated by macrophages in the tumor [17 ,22] .

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البلاعم الكبيرة المرتبطة بالورم كمعلم حيوي لأورام المثانة

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الخلاصة

تعمل البلاعم الكبيرة المرتبطة سرطانيا كبديل عن البلاعم الكبيره الفعالة التي تعزز تعاقب السرطان من خلال تعزيز نمو الاوعية الدموية المغذية ,هجرة و انتشار الخلايا السرطانية. هدفت الدراسة الى تحديد انتشار البلاعم الكبيرة المرتبطة بالورم في انسجة الاورام السرطانية وتوضيح العلاقة بينها وبين المراتب المرضية السريرية لسرطان المثانة . أستخدمت تقنية التصبيغ المناعي الكيمونسيجي واستخدم CD68 كعلامة لتحديد البلاعم الكبيرة في الأنسجة الورمية بصورة عامة . تضمنت الدراسة 50 مريض يعانون من سرطان المثانة البولية ضمن مراحل ورتب مختلفة , 20 مريض يعانون من أمراض المثانة البولية غير السرطان (أورام حميدة). وقد وجدت الدراسة أن مستوى التعبير المناعي الكيمونسجي الموجب للبلعلم الكبير المرتبط بالورم الـ +CD68 كان مرتفع نسبيا مع فرق معنوي احصائي في مرضى سرطان المثانة البولية مقارنة مع مرضى أورام المثانة البولية الحميدة ($p \leq 0.01$; 84% versus 45%).

وبينت النتائج أن هناك علاقة معنوية بين مراتب مرضى سرطان المثانة البولية والتعبير المناعي الكيمونسيجي للـ +CD68 اذ أظهرت المراتب المتقدمة تركيز عالي للـ +CD68 (96.4%)27مقارنة بالمراتب الابتدائية (68.2%)15. بينت النتائج إن نتشار التعبير المناعي الكيمونسيجي الموجب للبلعم الكبير المرتبط بالورم +CD68 في المراحل المتقدمة لمرضى سرطان المثانة ربما يساهم في التطور السلبي للسرطان .