Correlation of Fibrosis with Tumor Size and International Society of Urological Pathology (ISUP) Grading of Clear Cell Renal Cell Carcinoma

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Background: Clear cell renal cell carcinoma (ccRCC) is one of the most aggressive sub-types of renal carcinoma that causing deaths from all urogenital cancers. Handling of ccRCC has been hampered due to the radioresistance and chemoresistance. This is thought to be due to the increasing involvement of the extracellular matrix produced by Cancer Associated Fibroblasts (CAF) and cancer cells. Fibrosis is an extracellular matrix accumulation process that can increase the tumor progression and metastasis.
Materials and methods: This study was a cross sectional study conducted on 21 patients with ccRCC that taken from their formalin-fixed paraffin embedded tissue blocks, each stained with hematoxylin and eosin to assess ISUP grading and Masson trichrome's stain to assess fibrosis. Spearman correlation test ($p < 0.005$) was performed to asses the correlation of fibrosis with tumor size and grading ISUP.
Results: Among 21 specimens of ccRCC patients, the most cases were 8 (38.1%) severe fibrosis found in tumors> 7 cm 5 (62.5%) and at grade IV 6 (75%). It was found that no significant correlation of fibrosis and tumor size ($p = 0.135$) and ISUP grading ($p = 0.285$) according to spearman correlation test
Conclusion: Although no significant correlation was found from the statistical analysis, there appeared to be the increasing impression of fibrosis which were also followed by the increasing of tumor size and ISUP grading.
Keywords: Clear cell renal cell carcinoma, fibrosis, tumor size, ISUP grading.

INTRODUCTION

Kidney cancer is one of the world health issues with various incidents in various countries. The incidence of this cancer increases every year. This tumor is the leading cause of death from cancer in male urogenital tract (1). Worldwide, Kidney cancer is ranked 15th (2.2%) of 36 cancers (2). Indonesia does not have complete kidney cancer data yet, but according to data from Global Burden Cancer (GLOBOCAN) in 2012, the prevalence of kidney cancer in Indonesia ranges from 0.82 to 1.8 per 100,000 population. (2). Data from Cipto Mangunkusumo Central Hospital and Dharmais Cancer Hospital showed that there were 81 cases of renal cell carcinoma (RCC) during January 1995-December 2008 (4). Clear cell Renal Cell Carcinoma (ccRCC) is the highest RCC sub-type (80%), while the other 20% is a non-ccRCC sub-type (5–7). This sub-type of kidney cancer is the most aggressive cancer that often found at an advanced stage and the leading cause of death(3).

The International Consensus of the Urological Pathology (ISUP) and WHO classification in 2016 established several prognostic parameters for RCC, including: histological subtypes, ISUP / Furhman grading, sarcomatoid and rabdoid differentiation, necrosis, microvascular invasion, tumor staging, tumor size and tumor free tissue (4). In addition, several independent prognostic factors have been also discovered, one of them is the relationship between stromal cells in a Tumor Microenvironment (TME) with the proliferation, progression and resistance of cancer therapy (5). TME consists of stromal cells and Extracellular Matrix (ECM) around tumors that have different and overlapping functions (6–8). Cancer Associated Fibroblasts (CAF) are mesenchymal cells that originate from the mesoderm layer and play the most important roles for synthesizing, secreting, collecting and modifying ECM composition and organization (9).

Extracellular matrix is normally a stable structural component, functioning to maintain the mechanical strength of the tissue (10). Increased ECM accumulation in the stroma can disrupt the process of homeostasis. Disturbances of the homeostasis process can include: synthesis, modification, remodeling and degradation. This process is called fibrosis (11). In chronic fibrosis, tissue stiffness driven feedback which increases the chronicity of the disease (12). Whereas in cancer, rigid ECM will induce tumor development and activate myofibroblastic cells or CAF which will maintain desmoplastic reactions and ultimately promote tumorigenesis (13).

Fibrosis is an important factor in predicting the prognosis. Recent research reveals that stromal cells and increased ECM accumulation are causes of treatment resistance and an inseparable part in the management of cancer eradication, so that many studies have begun to develop antifibrosis targeting therapy that focus on their role in preventing the development of cancer (14). Many studies abroad have examined the role of fibrosis in various organs such as breast, lung and liver. One study conducted by Won et al. in 2018 stated that there was a significant correlation between fibrosis and Furhman grading (15). As far as researchers observe in many sources, with a change in a better RCC grading system, studies linking fibrosis and ISUP grading have not been found particularly in Indonesia.

MATERIALS AND METHODS

This research is an analytical study with cross sectional approach. It has received an approval from the ethics committee of the implementation of health research at the Faculty of Medicine, Universitas Sumatera Utara, Medan. The sample in this study was a slide from patients who diagnosed as clear cell renal cell carcinoma in Haji Adam Malik Central Hospital Medan in the period of 2012-2019 and taken a total sampling of 21 cases. The inclusion criteria in this study were tissue results of biopsy and renal surgery that were histopathologically diagnosed as clear cell renal cell carcinoma, complete clinical data including: age, sex, tumor size and ISUP grading. The exclusion criteria is slides that had been excised could not / difficult to assess again the focus of the tumor and fibrosclerotic with HE staining.

Slides from the cutting of formalin-fixed paraffin embedded tissue blocks were carried out by hematoxylin and eosin staining to assess ISUP grading and Masson's Trichromes staining (ScyTek Laboratories, Inc., USA) to assess fibrosis. Then all slides were reviewed with two pathologists using an Olympus CX23 microscope. Grading assessment is based on consensus of the International Society of Urological Pathology (ISUP) which is divided into 4 grades: grade 1 if the nucleoli is not visible or the nucleoli is unclear and basophilic at 400x magnification, grade 2 if the nucleoli is conspicuous and eosinophilic at x400 magnification and can be seen although not prominent at 100x enlargement, grade 3 if the nucleoli is clearly visible and eosinophilic color at 100x enlargement and grade 4 if the severe nucleus pleomorphisms, multinucleated giant cells, sarcomatoid and rhabdoid differentiation (16,17).

Intratumoral and peritumoral fibrosis is assessed semi-quantitatively by looking at a number of collagen deposits between tumor cells and located at the tumor boundary. Fibrosis assessment is based on the amount of intensity and extent of fibrosis. Fibrosis intensity was assessed in the visual field with the area with the highest intensity of fibrosis (15). Categorized as follows: score 1: If there appear to be less dominant collagen fibers and fibrous connective tissue, score 2: If a similar proportion of collagen fibers and fibrous connective tissue is seen, score 3: If thick and sclerotic collagen fibers appear to be almost identical more dominant. The extent of fibrosis was assessed by looking at the percentage of fibrosis in the entire field of view, categorized as follows: Score 0: if found ≤5% fibrosis of the entire field of view. Score 1: If found 6-25% fibrosis of the entire field of view. Score 2: If found 26-50% fibrosis of the entire field of view. Score 3: If ≥50% of fibrosis is found in entire fields of view. Then the assessment of fibrosis was calculated from the sum of the fibrosis intensity scores and area extend of fibrosis, than grouped into mild if the total score is 0-2, moderate if the total score is 3-4 and severe if the total score is 5-6.

RESULT

Based on clinical data from medical records / pathology archives, the highest sample of male sex was obtained in 15 (71.4%) cases, followed by female sex in 6 (28.6%) cases. The mean age of patients was 47.05 with deviation standar 16.32 years, the youngest age was 20 years and the oldest is 81 years. Most cases in the range 51 to 60 years in 6 (28.6%) cases, followed by ages 31-40 years and 41-50 years respectively 4 (19%) cases, 21-30 years in 3 (14.3%) cases, age >70 years in 2 (9.5%) cases and at least ages 10-20 years and 61-70 years respectively in 1 (4.8%) case. No patients found at age ≤ 10 years. All samples were obtained from operative measures, 2 (9.5%) cases came from tumor incision biopsy and 18 (90.5%) cases from total nephrectomy.

The average tumor size was around 8.58 with the largest tumor size being 17 cm and the smallest being 2 cm. Most tumors were> 7 cm in size with 11 cases (52.4%), followed by a size of ≤ 7 cm in 10 (47.6%) cases. The majority of patients were found in grade 4 as 13 (61.9%) cases, followed by grade 3 in 6 (28.6%) cases and grade 2 in 2 (9.5%) cases, while grade 1 was not found in this study. Fibrosis was assessed based on the accumulation between the extent of fibrosis and the intensity of fibrosis. Severe fibrosis were found in 8 (38.1%) cases, followed by moderate fibrosis in 7 (33.3%) cases and mild fibrosis in 6 (28.6%) cases (table 4.1).

Table 1. Characteristics distribution of clear cell renal cell carcinoma.

Characteristics	Amount = n	Percentage (%)
Number of Samples	21	100
Operation procedure		
Nephrectomy	19	90,5
Incision biopsy	2	9,5
Sex		
male	15	71,4
Female	6	28,6
Ages group, mean ± SD years	47,05±16,32	
≤ 10 years	0	0
11-20 years	1	4,8
21-30 years	2	9,5
31-40 years	5	23,8
41-50 years	4	19
51-60 years	6	28,6
61-70 years	1	4,8
>70 years	2	9,5
Tumor sizes, mean± SD cm	8,58±4,91	
≤ 7 cm	10	47,6
>7 cm	11	52,4
ISUP grading		
Grade 1	0	0
Grade 2	2	9,5
Grade 3	6	28,6
Grade 4	13	61,9
Fibrosis		
mild	6	28,6
moderate	7	33,3
severe	8	38,1

SD-Standard Deviation

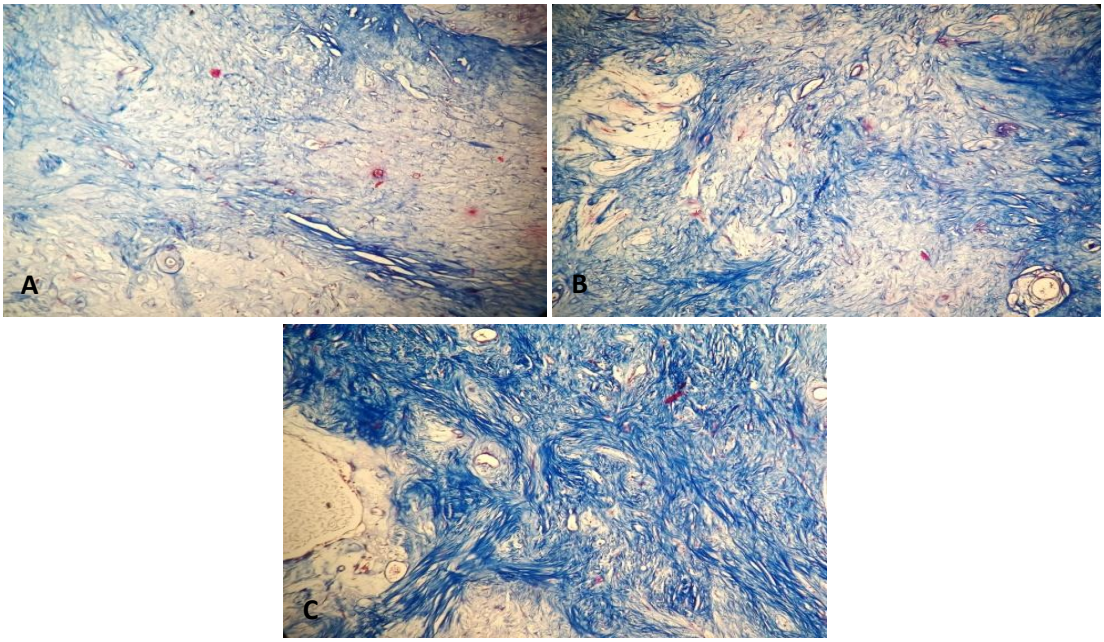

Figure 1. Intra- and peri-tumoral fibrosis. A. score 1, consists mostly of loose fibrous connective tissue. B. score 2, consists of an equal number of loose fibrous connective tissue and collagen fibers, C. Score 3, consists of mostly solid collagen fibers (Masson's Trichrome staining, x200).

A total of the 21 ccRCC samples, the most of mild fibrosis were found in tumor's size of ≤7 cm as 5 (83.3%) cases. The most moderate and severe fibrosis were found in tumor's size of >7 cm as 5 cases with the percentage 71 , 4% and 62.5%, respectively. All level fibrosis which were mostly found in grade 4 in 3 cases with a percentage 50%, 57.1% and 75%, respectively. Based on the results, a Shapiro-Wilk normality test was performed and an abnormal data distribution was obtained (p> 005). Then the Spearman correlation test was performed and found no significant correlation between fibrosis with tumor size and ISUP grading because p-values were found respectively 0.135 and 0.245 (p<0.05). Although no significant association was found, a positive correlation coefficient value indicates an increase in fibrosis followed by an increase in tumor size and grading, or vice versa, but with a weak correlation strength (table 4.2).

Table 2. Correlation of fibrosis with tumor size and grading in clear cell renal cell carcinoma.

Characteristic	Fibrosis						*r*	*p-value*
	Mild		Moderate		Severe			
	n=6	%	n=7	%	n=8	%		
Tumor size							0,316	0,135
≤7 cm	5	83,3	2	28,6	3	37,5		
>7 cm	1	16,7	5	71,4	5	62,5		
ISUP grading							0,245	0,285
Grade I	0	0	0	0	0	0		
Grade II	1	16.7	1	14.3	0	0		
Grade III	2	33.3	2	28.6	2	25		
Grade IV	3	50	4	57.1	6	75		

r-coeeficient correlation; p-value <0,005

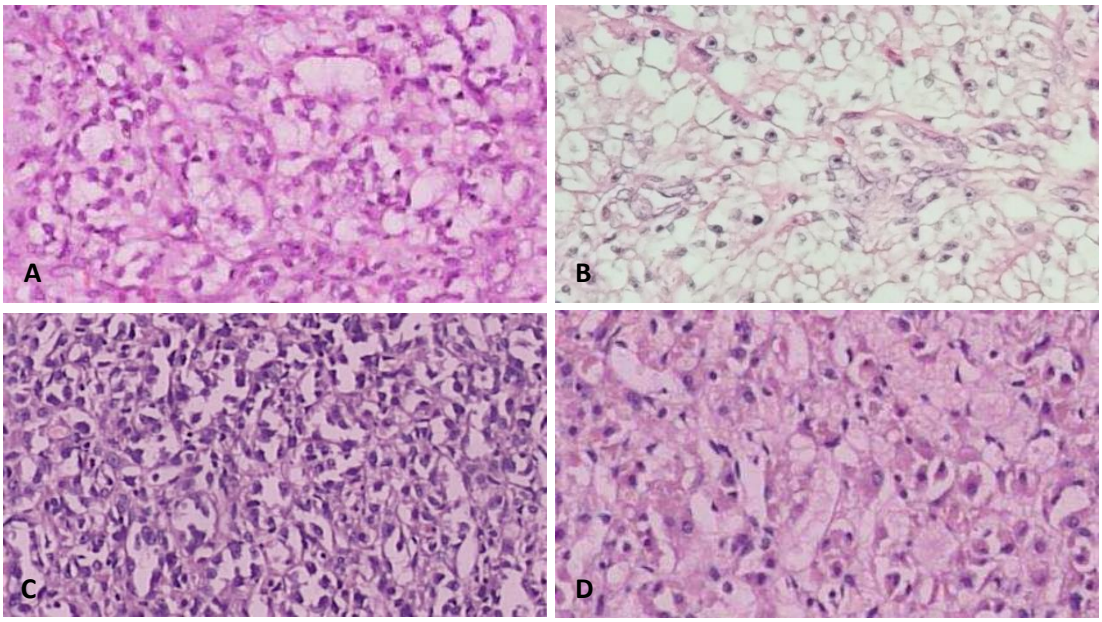

Figure 2. Grading histopathology ISUP, no grade 1 was found in this study. A. Grade 2, consists of tumor cells with nucleoli clearly visible on strong enlargement, but not prominent, B. Grade 3, consists of tumor cells with protruding nucleoli and easily visible on weak enlargement, C & D. Grade 4, consit with rhabdoid differentiation (HE, x400).

DISCUSSION

Clear cell renal cell carcinoma (ccRCC) is a solid organ cancer originating from the proximal tubule of the kidney (18,19), the first most common sub-type with an incidence of about 65-75% of all RCC sub-types which is then followed by papillary renal cell carcinoma (20,21). Rini et al. in 2009 stated that the ccRCC sub-type is the highest contributor to the death rate of all malignancies in the urinary tract (22). This cancer is often much more found in male than female with a ratio of 2.75: 1 (23). López et al. in 2016 found that ccRCC cases were found to be older than this study, with an average age of 66 years (24). There are several other medical conditions that are

associated with ccRCC, including: chronic kidney disease, acquired kidney cystic disease, hemodialysis and kidney transplantation (25).

Clear cell RCC can occur sporadic (> 96%) or familial (<4%) (26). Deletion of the chromosome 3p and inactivation of the VHL suppressor gene are the initial events in the majority of sporadic ccRCC cases that cause dysregulation of HIF-1α. Whereas in familial ccRCC, malignancies often occur due to mutations of the VHL gene suppressor tumor (21,26–28). Young patients of ccRCC were also found in this study, which was 20 years old in 1 case. This was also reported by Won et al., in 2018 who found a ccRCC sufferer at the age of 27 years (15). The ccRCC case found at a young age in this study was probably related to hereditary renal syndrome (familial). This is supported by some literature which states that familial. Clear cell renal cell carcinoma at a young age usually found multifocal or bilateral in both kidneys (21,29).

Generally, kidney tumors can be diagnosed early with radiological imaging. Ultrasonography (USG), Computed Tomography Scanning (CT-Scan) and Magnetic Resonance Imaging (MRI) tests can detect kidney tumors incidentally by about 60-80%. These examination can also distinguish which is solid or cystic tumors, and even these examinations can assess the extent of local invasion, invasion of lymph nodes and distant metastases (17,25). This study found the average size of the tumor about 8.59 cm with a range of 2-17 cm and most found in the highest cut off point (> 7 cm). López et al., in 2016 found that most cases of ccRCC had an average size of 6.22 cm with a range of 1-19 cm and the highest cut off point> 4 cm was 68.8% (24). This is in contrast to the study of Won et al. in 2018, he explained the mean tumor size of 4.48 cm with a range of 0.5 -19 cm, but did not divide a specific cut-off point for tumor size (15). Leibovich, et al. in 2010 also found different results from this study, where they found the most tumors measuring <5 cm (3). The difference in tumor size may be influenced by the degree of proliferation of tumor cells that characterized by grading. In higher grades tumor size will be found larger than patients who have lower grade. Study by Mukhopadhyay et al. in 2015 found a significant correlation between tumor cell proliferation index and Furhman grading (30). Another co-founding factor that affects tumor size is tumor invasion into the walls of the vena cava and renal vein. So far there is no provision to determine the size of a kidney tumor and the possibility of renal vein thrombus which has also been assessed (31). In addition, in developed countries, tumors are often detected incidentally early during health screening (17), so they often find tumors at an early stage when the tumors still in small size.

Grading systems in kidney malignancies, such as Furhman, Lasky and Limas have been used since 1982 (19), and have not been used anymore for cases of ccRCC and papillary renal cell carcinoma since 2016. Grading systems from the International Society of Urological Pathology has become a substitute for the previous grading system in both sub-types. In this study, the ISUP grading system divides ccRCC into 4 grades based on nucleoli and cell differentiation (severe pleomorphism, rabdoid and sarcomatoid images) (17). The results found most patients are in grade 4. This study is almost similar with the research of Dagher et al. those who used ISUP grading found the majority of patients with grade III in male and the average age group of 64 years (4). In contrast to Research Won et al. in 2018 in using Furhman grading to assess grading in ccRCC and found the most cases were in grade 2 in 57.8% followed by grade III as much as 27% cases and found also patients with grade 1 in 5.9% cases (15). This difference is due to the results of the assessment with Furhman grading different from ISUP grading. Research conducted by Dagher et al. that compare the ISUP grading system and the Furhman system showed ISUP grading can be used as one of the significant prognostic factors for predicting survival rates for patients with ccRCC (4).

Fibrosis is a pathological process which is characterized by the accumulation of extracellular matrix (ECM) which is the result of infiltration and proliferation of mesenchymal cells in the interstitial tissue. ECM in stromal fibrosis that play roles in proliferation and invasion of tumor cells, among others are: collagen, fibronectin, lysyl oxidaxe (LOX), tenascin C, hyaluronan, periostin, osteonectin and proteoglycans (32). The mechanism of fibrosis is thought to be related to many overlapping factors, for example: CXC motif Chemokine 12 (CXCL12), CXC receptor type 3 (CXCR3), CXC receptor type 4 (CXCR4), Tumor Growth Factor-β (TGFβ), Cyclooxygenase-2 (COX-2), Peroxisome Proliferator-activated gamma Receptor (PPAR-gamma), Matrix-metallo Proteinase (MMP), Interleukin-6 (IL-6) and Interleukin-17 (IL-17). These factors are reported have an important role in tumor development and fibrosis (33–38). Formation of fibrosis can change cell polarity, causes proliferation and make an environment condition suitable for the development of tumor cells (39). Stress induces hyperproliferation of tumor cells in the early stages and fibrosis in the advanced stages resulting in mechanical changes in the TME. This change is through 2 different pathways, namely: the β-catenin pathway and the Yes Associated Protein (YAP) / TAZ pathway (40). Transforming growth factor-β (TGF-β) is an important cytokine mediator to induce fibrosis response and activate cancer stroma. These cytokines contribute to changing stromal cells in TME into Cancer Associated Fibroblasts (CAF). CAF secretes and activates TGF-β to expand its population. In addition, the effect of TGF-β is known to induce increased production of ECM rich in Collagen 1 (33).

Most cases in this study were found severe fibrosis at tumor size> 7 cm and grade 4 (table 2). This is different from Won et al. in 2018 in his study of 204 ccRCC sufferers where fibrosis was mostly found in male, the age group ≥60 years, tumors size <7 cm and low Furhman grading (grades 1 and 2). The difference results of this study may be due to the collagen assessment methods and grading assessment systems. In this study, an increasing levels of fibrosis was followed by an increasing size of tumor and ISUP grading. However, after the statistical correlation test with the Spearman correlation test found no significant correlation of fibrosis with tumor size and ISUP grading ($p < 0.005$). Although no significant correlation was obtained, the correlation of fibrosis with tumor size and ISUP grading showed a positive correlation (increased fibrosis followed by an increase in tumor size and ISUP grading and vice versa), but with a weak relationship strength. So far, we have not found a literature linking fibrosis with ISUP grading. However, studies using Furhman grading found no significant relationship between intratumoral fibrosis grade and tumor size and grading. However, the study also explained that there was a significant relationship between fibrosis size> 0.5 cm to Furhman grading. This explains that the presence of fibrosis itself is more meaningful than grouping the degree of fibrosis (15). In addition, the weaknesses in this study might be due to the method used. Farris et al. has conducted a study comparing several methods using several observers to reduce errors in assessing fibrosis. The results obtained that the assessment by visual scoring method with Masson's Trichrome histochemical staining is better than morphometric method with Sirrius red immunohistochemical staining. In that study, it was also explained that Masson's trichrome stain combined with Periodic Acid Shift (PAS) stain can reduce collagen assessment errors with a feature of the brush border of the glomerular tubules and basement membrane that expressing the same color rather than using Masson's trichrome stain. The study also mentions that Masson's Trichrome stain is less sensitive for assessing collagen in the early fibrosis formation (41).

CONCLUSION

We examined 21 samples of clear cell renal cell carcinoma in the Anatomic Pathology Unit of Haji Adam Malik Central Hospital Medan. We found the most clear cell renal cell carcinoma cases were in male with age group 51-60 years, had tumor size> 7 cm and grade 4. In this study we did not find a significant correlation of fibrosis with tumor size and ISUP grading, but it showed an impression of increased fibrosis which was also followed by an increase in tumor size and ISUP grading with a weak correlation.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

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