The Correlation between Clinicopathological Characteristics in Clear Cell Renal Cell Carcinoma (ccRCC) with Immunohistochemical Expression of Light Chain 3 B (LC3-B)

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ABSTRACT

Background
Clear cell renal cell carcinoma (ccRCC) is the most common histological subtype in RCTs. This tumor is very aggressive and is often diagnosed at an advanced stage. LC3-B is a specific autophagy marker that plays a role in ccRCC. Research regarding the relationship between clinicopathological characteristics of ccRCC and LC3-B expression is still very limited and controversial.

Objective
To assess the relationship between clinicopathological characteristics of ccRCC and LC3-B immunohistochemical expression.

Methods
This study used 22 slide samples/paraffin blocks of ccRCC cases that had been diagnosed histopathologically at the Anatomic Pathology Unit of H. Adam Malik General Hospital, Medan from January 2017 to April 2023. All clinicopathological data was taken from medical records/pathology archives. LC3-B expression was assessed in the cytoplasm of tumor cells with assessment semiquantitatively based on the multiplication of intensity and percentage of expressed tumor cells. Statistical analysis was carried out using Fischer's Exact and Kruskal Wallis tests.

Results
Among the 22 ccRCC samples, weak expression of LC3-B was found in the age group ≤60 years, male gender, tumor size >10 cm, positive LVI, negative PNI, grade IV, severe stromal TILs, mild intratumoral TILs and low budding in intratumoral budding tumors. Fischer's Exact and Kruskal Wallis statistical tests showed that there was no significant relationship between gender, tumor size, LVI, PNI, ISUP grading, TILs and peritumoral tumor budding with LC3-B expression.

Conclusion
There was no significant relationship between clinicopathological characteristics of ccRCC with LC3-B expression (The Null Hypothesis is accepted (H_0 $\rho=0$)).

Key words: clinicopathology, ccRCC, LC3-B

INTRODUCTION

Kidney cancer is included in the top 15 most common cancers based on GLOBOCAN data in 2020. The incidence and mortality due to kidney cancer is reported to be increasing. Deaths due to kidney cancer increased from 68.140 cases in 1990 to 138.530 cases in 2017 with a total number of deaths of 89.620 cases in men and 48.910 cases in women. Based on 2018 GLOBOCAN data, the incidence of kidney cancer in Indonesia was reported to be 2.112 cases, then increased to 2.394 cases in 2020, while the death rate rose from 1.225 cases to 1.358 cases in 2020.[1,2]

Clear cell renal cell carcinoma (ccRCC) is a subtype of RCT originating from proximal renal tubule epithelial cells which have heterogeneous cell morphology with clear cytoplasm, sometimes eosinophilic with numerous blood vessels. This cancer is the most common subtype in RCTs. The overall incidence is estimated to be 3.59 cases per 100.000 people per year. Most ccRCCs develop by age 60 years (mean age 62 years). The incidence of ccRCC was significantly higher in men than in women with a ratio of 1.94:1. This cancer is associated with the germline mutation Von Hippel-Lindau syndrome (VHL) which triggers the growth of cancer cells. Tumor size in ccRCC ranges from 23 to 170 mm (mean 63 mm).[3]

The ISUP grading system has been shown to significantly predict cancer-specific survival. In the ISUP grading system, the microscopic image is assessed in one field of view which shows the highest level of cell pleomorphism.[1,3] Lymphovascular Invasion (LVI) is a significant potential prognostic factor in ccRCC. LVI is thought to be associated with a tendency to localized recurrence or metastasis.[4] PNI is associated with reduced survival and is a poor independent prognostic factor.[5] Tumor infiltrating lymphocytes (TILs) are an important prognostic and predictor of response to therapy in various types of cancer.[6] Tumor budding has been proven as a prognostic factor in various types of carcinoma such as esophageal, pancreatic, breast, head and neck, lung and colorectal carcinoma.[7]

Autophagy was first introduced by Christian de Duve in 1966, autophagy is a process that targets material in the cytoplasm and then undergoes degradation and recycling by lysosomes. Autophagy is a catabolic, meaning "self-eating" pathway that facilitates the recycling of nutrients from damaged, aging organelles and other cellular components through lysosomal degradation. Autophagy dysfunction has a close relationship in various pathological processes of cancer. Autophagy has two roles in cancer, where in some conditions autophagy plays a cytoprotective role to prevent the development of cancer. In contrast, in other conditions, autophagy plays a cytotoxic role that contributes to cancer survival. Autophagy plays a role in the growth, invasion, differentiation and metastasis of cancer cells. In ccRCC, regulation of autophagy is directly related to cancer cell function and pathogenesis.[8,9]

Microtubule-associated protein 1 light chain 3(LC3) is a widely used autophagy marker. The LC3 family has 3 isoforms, namely LC3-A, LC3-B and LC3-C. LC3-B is an autophagy marker that plays an important role in ccRCC. Deng et al in his research stated that LC3-B expression in ccRCC was highest in the age group ≤50 years, male gender, no lymph node (KGB) metastases and most low grade in ISUP grading. Mikhaylova et al stated that an increase in grade correlates with an increase in LC3-B expression in ccRCC.[10,11] Research on the relationship between clinicopathological characteristics in ccRCC and LC3-B expression is still very limited and controversial. The aim of this study was to determine the relationship between clinicopathological characteristics of ccRCC and LC3-B immunohistochemical expression.

METHODS

This research is a cross-sectional analytical study in the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra and the Anatomic Pathology Unit of H. Adam Malik Hospital Medan using slides and paraffin blocks that have been diagnosed histopathologically as ccRCC from January 2017-April 2023, meeting the inclusion criteria and exclusion criteria. The sample size was calculated based on the Lemeshow, 1990 formula and obtained 22 samples. LC3-B immunohistochemical staining was performed at the Anatomic Pathology Unit of Teguh Memorial Hospital. The inclusion criteria in this study were complete clinical data including age, gender and tumor size and slide preparations, paraffin blocks from nephrectomy surgery/kidney biopsy diagnosed histopathologically as ccRCC. Meanwhile, the exclusion criteria are paraffin blocks that are damaged/cannot be re-cut serially because the tissue is inadequate, it is difficult to diagnose ccRCC on minimal tissue preparations from kidney biopsy preparations and histopathological diagnosis as metastatic lesions from other organs.

LC3-B expression was characterized by the presence of brownish granules stained in the cytoplasm of tumor cells using rabbit anti LC3-B antibody (Cat No. GTX127375) with a dilution of 1:100. The assessment was carried out

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semiquantitatively based on the multiplication of the intensity and percentage of expressed tumor cells assessed in 4 fields of view of the microscopic preparation at 400 times magnification.[10-13] The intensity of LC3-B expression seen at 400 times magnification was categorized as follows: 0 (negative, no brown pigment found), +1 (weak, yellowish brown pigment found), +2 (medium, light brown pigment found), and +3 (strong, dark brown pigment found). The percentage of LC3-B expression seen at 40 times magnification was categorized as follows: 0 (no positive stains), 1 (<10% positive stains), 2 (10-50% positive stains) and 3 (>50% positive stains). LC3-B expression is calculated from multiplying the intensity score by the percentage which is categorized as follows: weak expression (score ≤4) and strong expression (score >4).[12] Relationship between gender, tumor size, LVI, PNI, ISUP grading, TILs and peritumoral budding tumors with LC3-B expression were analyzed by Fisher's Exact and Kruskal Wallis tests. The statistical test is significant if the p value <0.05.

RESULTS

In this study, 22 samples of ccRCC sufferers were obtained who were diagnosed histopathologically and met the inclusion and exclusion criteria at H. Adam Malik General Hospital, Medan from January 2017-April 2023.

Table 1. Correlation of clinicopathological characteristics in ccRCC with LC3-B immunohistochemical expression

Characteristics	Amount=n	Percentage (%)	LC3-B Expression				p-value
			Weak Expression		Strong Expression		
			N	%	N	%	
Age group, average ± SD year	48,9±9,6						
≤60 years old	18	81.8	15	83.3	3	16.7	
>60 years old	4	18.2	1	25	3	75	
Gender							
Male	14	63.6	12	85.7	2	14.3	0.137[a]
Female	8	36.4	4	50	4	50	
Tumor size, average ± SD cm	14,3± 4,4						
≤ 4 cm	0	0	0	0	0	0	0.675[b]
> 4 - ≤7 cm	1	4.5	1	100	0	0	
> 7 - ≤ 10 cm	5	22.7	3	60	2	40	
> 10 cm	16	72.7	12	75	4	25	
Lymphovascular Invasion (LVI)							
Negative	8	36.4	7	87.5	1	12.5	0.351[a]
Positive	14	63.6	9	64.3	5	35.7	
Perineural Invasion (PNI)							
Negative	17	77.3	13	76.5	4	23.5	0.585[a]
Positive	5	22.7	3	60	2	40	
Grading ISUP							
Grade I	1	4.5	1	100	0	0	0.163[b]
Grade II	6	27.3	6	100	0	0	
Grade III	5	22.7	4	80	1	20	
Grade IV	10	45.5	5	50	5	50	
TILs Stroma							
Mild	6	27.3	6	100	0	0	0.187[b]
Moderate	4	18.2	3	75	1	25	
Severe	12	54.5	7	58.3	5	41.7	
Intratumoral							
Mild	11	50.5	10	90.9	1	9.1	0.172[b]
Moderate	9	40.9	5	55.6	4	44.4	
Severe	2	9.1	1	50	1	50	
Tumor budding peritumoral							
Low budding	9	40.9	9	100	0	0	
Intermediate budding	7	31.8	3	42.9	4	57.1	0.143[b]
High budding	6	27.3	4	66.7	2	33.3	

SD: Standard Deviation
[a]Fischer's Exact, [b]Kruskal Wallis

Based on clinical data from medical records/pathology archives, the mean age of sufferers in this study was around 48.9 ± 9.6 years, where the youngest was 29 years and the oldest was 64 years. The most cases were found in the age group ≤60 years with 18 cases (81.8%) and the age group >60 years with 4 cases (18.2%). Cases of ccRCC were more common in men with 14 cases (63.6%) compared to women with 8 cases (36.4%). Tumor size is taken from macroscopic tissue measurements and CT scans. In this study, samples were taken from patients who underwent nephrectomy and kidney biopsy. The average tumor size ranged from 14.3 ± 4.4 cm with the smallest tumor size being 7 cm and the largest tumor size being 24 cm. In this study, the majority of tumors had a size of >10 cm in 16

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cases (72.7%), followed by a tumor size of > 7 - ≤ 10 cm in 5 cases (22.7%) and a tumor size of > 4 - ≤7 cm in 1 cases (4.5%). In this study, no tumor size ≤ 4cm was found.

In this study, LVI was found to be positive in 14 cases (63.6%) and negative in 8 cases (36.4%). Most of the PNI was found to be negative in 17 cases (77.3%) and positive in 5 cases (22.7%). In this study, the majority of ccRCC had grade IV with 10 cases (45.5%), followed by grade II with 6 cases (27.3%), grade III with 5 cases (22.7%) and the least cases with grade I was 1 case (4.5%). Stromal TILs were mostly found to be severe in 12 cases (54.5%), followed by mild grade TILs in 6 (27.3) and moderate grade TILs in 4 cases (18.2%). Meanwhile, intratumoral TILs were found to be mostly mild with 11 cases (50.5%), followed by moderate TILs with 9 (40.9) and severe TILs with 2 cases (9.1%). The most common peritumoral budding tumors were low budding with 9 cases (40.9%), followed by intermediate budding with 7 (31.8) and high budding with 6 cases (27.3%).

Based on the table above, weak expression of LC3-B was highest in the age group ≤60 years with 15 cases (83.3%) while in the age group >60 there was 1 case (25%). Strong expression of LC3-B in the age group ≤60 years was 3 cases (16.7%) and in the age group >60 years was 3 cases (75%). In this study, of the 22 ccRCC samples, weak expression of LC3-B was found in 12 cases of men (85.7%) and 4 cases of women (50%). Strong expression of LC3-B was mostly found in women, 4 cases (50%) and men, 2 cases (14.3%). The Fischer's Exact test was carried out, obtained p-value = 0.137 (significant p-value < 0.05). So it was concluded that there was no significant relationship between gender and LC3-B immunohistochemical expression. In this study, the most weak expression of LC3-B was found with tumor size >10 cm in 12 cases (75%), followed by tumor size >7 cm - ≤ 10 cm in 3 cases (60%), and tumor size >4 cm - ≤ 7 cm in 1 case (100%). In this study, there was no weak expression of LC3-B with a tumor size <4 cm. Strong expression of LC3-B was highest with tumor size >10 cm in 4 cases (25%) and tumor size >7 cm - ≤ 10 cm in 2 cases (40%). In this study, there was no strong expression of LC3-B with tumor sizes >4 cm - ≤ 7 cm and <4 cm. The Kruskal Wallis test was carried out, obtained p-value = 0.675 (significant p-value < 0.05). So it was concluded that there was no significant relationship between tumor size and LC3-B immunohistochemical expression.

Based on the table above, the most positive LVI was found in weak expression of LC3-B in 9 cases (64.3%) and negative in 7 cases (87.5%). Strong expression of LC3-B was most commonly found in positive LVI in 5 cases (35.7%) and negative in 1 case (12.5%). The Fischer's Exact test was carried out, the p-value = 0.351 (p-value < 0.05 was significant). So it was concluded that there was no significant relationship between LVI and LC3-B immunohistochemical expression. In this study, the most negative PNI was found in weak expression of LC3-B in 13 cases (76.5%) and positive in 3 cases (60%). Strong expression of LC3-B was mostly found in negative PNI in 4 cases (23.5%) and positive in 2 cases (40%). The Fischer's Exact test was carried out, obtained p-value = 0.585 (significant p-value < 0.05). So it was concluded that there was no significant relationship between PNI and LC3-B immunohistochemical expression.

In this study, of the 22 ccRCC samples, the highest weak expression of LC3-B was found in grade II ISUP, 6 cases (100%), followed by grade IV, 5 cases (50%), grade III, 4 cases (80%) and grade 1. as many as 1 case (100%). Strong expression of LC3-B was mostly found in grade IV in 5 cases (50%) and grade III in 1 case (20%). There was no strong expression of LC3-B in grade I and grade II. The Kruskal Wallis test was carried out, obtained p-value = 0.163 (significant p-value < 0.05). So it was concluded that there was no significant relationship between ISUP grading and LC3-B immunohistochemical expression.

Based on the table above, the highest weak expression of LC3-B was found in severe stromal TILs with 7 cases (58.3), followed by mild grade with 6 cases (100%) and moderate grade with 3 cases (75%). Strong expression of LC3-B was mostly found in severe stromal TILs in 5 cases (41.7%) and moderate grade in 1 case (25%). There was no strong expression of LC3-B with mild stromal TILs. The Kruskal Wallis test was carried out, obtained p-value = 0.187 (significant p-value < 0.05). So it was concluded that there was no significant relationship between stromal TILs and LC3-B immunohistochemical expression. In this study, the highest weak expression of LC3-B was found in mild grade intratumoral TILs in 10 cases (90.9%) followed by moderate grade in 5 cases (55.6%) and severe grade in 1 case (50%). Strong expression of LC3-B was mostly found in moderate grade stromal TILs in 4 cases (44.4%), severe grade in 1 case (50%) and mild grade in 1 case (9.1%). The Kruskal Wallis test was carried out, obtained p-value = 0.172 (significant p-value < 0.05). So it was concluded that there was no significant relationship between intratumoral TILs and LC3-B immunohistochemical expression.

In this study, of the 22 ccRCC samples,

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the most weak LC3-B expression was found with low budding in intratumoral budding tumors in 9 cases (100%), followed by high budding in 4 cases (66.7%) and intermediate budding in 3 cases (42 .9%). Strong expression of LC3-B was mostly found in intermediate budding in 4 cases (57.1%) and high budding in 2 cases (33.3%). There was no strong expression of LC3-B with low budding in intratumoral budding tumors. The Kruskal Wallis test was carried out, obtained p-value = 0.143 (significant p-value < 0.05). So it was concluded that there was no significant relationship between intratumoral tumor budding and LC3-B immunohistochemical expression.

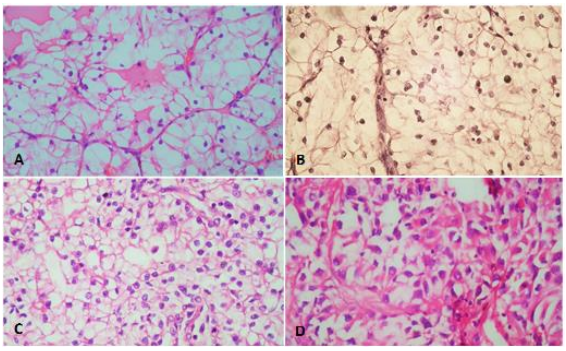

Figure 1. Microscopic appearance of ISUP grading in the study. A. Grade I (H&E, 400 times). B. Grade II (H&E, 400 times). C. Grade III (H&E, 100 times). D.Grade IV, sarcomatoid differentiation (H&E, 400 times).

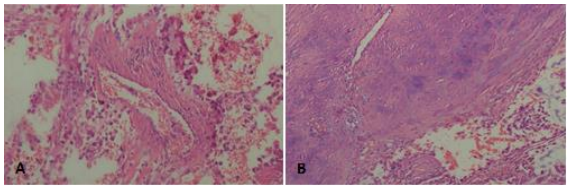

Figure 2. Microscopic appearance of LVI and PNI in the study. A.LVI positive (H&E,100 times). B. PNI positive (H&E, 100 times).

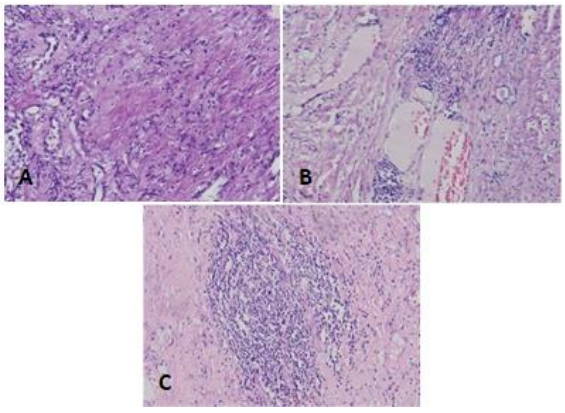

Figure 3. Microscopic appearance of stromal TILs in the study. A.Mild TILs (H&E,200 times). B. Moderate TILs (H&E,200 times). C.Severe TILs (H&E,200 times).

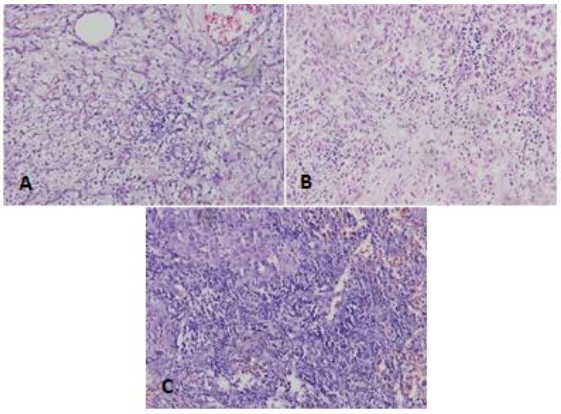

Figure 4. Microscopic appearance of intratumoral TILs in the study. A.Mild TILs (H&E,200 times). B. Moderate TILs (H&E,200 times). C.Severe TILs (H&E,200 times).

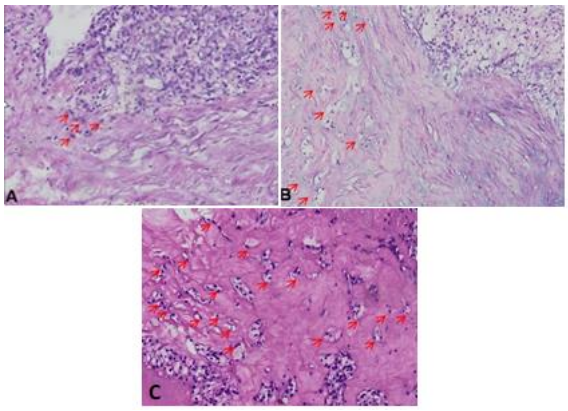

Figure 5. Microscopic appearance of peritumoral budding tumor in the study. A. Low budding (H&E, 200 times). B. Intermediate budding (H&E, 200 times). C. High budding (H&E, 200 times).

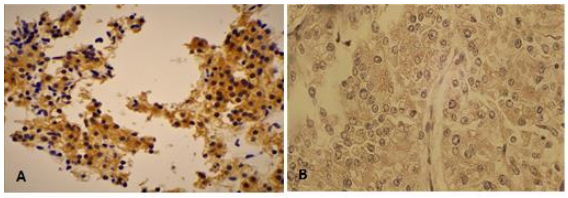

Figure 6. Microscopic appearance of LC3-B expression in the study. A. Strong expression of LC3-B in the cytoplasm (H&E,400 times). B. Weak expression of LC3-B in the cytoplasm (H&E,400 times).

DISCUSSION

In this study, the most cases were found in the age group ≤ 60 years with 18 cases (81.8%) (mean age 48.9 ± 9.64 years) with the youngest age being 29 years and the oldest age being 64 years. The results of this research are in line with the research of Kharismawaty et al which stated that the most cases of ccRCC were in the 51-60 year age group with 7 cases (30.4%).[14] Research by Bi et al stated that the highest number of ccRCC cases was at an

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average age of 58 years.[15] The results of this study are not in line with research by Lopez et al who found that ccRCC cases were more common at older ages, namely an average age of 66 years. [16] In the aging process, the natural function of tissues or organs decreases. The tissue's ability to repair itself, maintain its normal structure and function will slowly disappear, making it very vulnerable to infections and other diseases. In addition, in old age there is an accumulation of free radicals in the body. Free radicals will damage several cell components such as proteins, lipids, carbohydrates and nucleotides as well as other macromolecules. Damage caused by free radicals in cells is associated with various diseases. In old age, histological changes occur in the stroma. The stroma will form a protumorigenic environment caused by the senescence-associated secretory phenotype (SASP) which plays a role in the initiation and progression of age-related cancer.[17]

In this study, ccRCC cases were more common in men with 14 cases (63.6%) and women with 8 cases (36.4%). The results of this research are in line with research by Roldan et al which stated that the incidence of ccRCC was greater in men with 18 cases (69.2%).[18] In line with research by Bi et al which states that ccRCC is more common in men than women with 82 cases (78.1%).[15] Men have a greater risk of developing ccRCC than women because it is related to ccRCC risk factors such as smoking, exposure to environmental pollution (such as asbestos, benzene and tricholoroethylen) which men often inhale while working, diet and poor lifestyle.[3]

In this study, the majority of patients had a tumor size of >10 cm, 16 cases (72.7%) (mean 14.3 ± 4.4 cm) with the smallest size being 7 cm and the largest size being 24 cm. This research is in line with research by Kharismawaty et al who found that the majority of ccRCC sufferers had a tumor size of >10 cm, 30.4% (mean 8.513 cm).[14] This is also in line with research by Thaib et al who found that ccRCC sufferers had a tumor size of >10 cm in 28 cases (73.7%).[19] The results of this study were different from the research of Chang et al which stated that the majority of ccRCC sufferers had a tumor size of <5 cm, 299 cases (67.8%).[20] In the early stages, ccRCC does not produce specific symptoms. Tumors without specific symptoms can be detected through radiological examinations such as ultrasound, CT scan and MRI.[3] Early detection/screening for ccRCC in developing countries is still lacking and often too late so that the tumor when diagnosed is already large and has metastasized to the organs. other. In this study, tumor size was taken from macroscopic tissue measurements and CT scans. Tumor size can only be assessed until T2b staging because tissue samples are also taken from kidney biopsies.

In this study, the majority of positive LVI were found in 14 cases (63.6%). This research is in line with research by Belsante et al where positive LVI was found in 14.3% of all non-metastatic ccRCC patients.[21] The results of this study differ from Dae et al's study, which found negative LVI in 364 cases (93.8%) in non-metastatic ccRCC patients.[22] In the study Chang et al also found negative LVI in 348 cases (73.2%) of ccRCC.[20] LVI is thought to be associated with a tendency for localized recurrence or metastasis because metastasis begins from tumor cells that invade the blood circulation and lymphatic vessels. LVI is a significant independent predictor of cancer specific survival (CSS) and disease-free survival (DFS) in ccRCC.[21,23]

In this study, most of the PNI was negative in 17 cases (77.3%). The results of this study are in line with research by Simsek et al who reported PNI of 2-7% in ccRCC from nephrectomy specimens.[24] The results of this study differ from the study of Capek et al who reported one case of ccRCC with PNI in a spinal lesion located between T12 and L4.[25] In this study, most of the tumors were >10cm in size so that when cutting the tissue there was a possibility that the PNI focus would not be captured. Apart from that, there were 2 samples obtained from kidney biopsies which were small. Perineural invasion (PNI) is an independent prognostic factor in poor overall and disease-free survival. The prevalence of PNI in RCTs is around 2-7% in nephrectomy specimens. The prevalence of PNI can increase from 30% to 82% if actively sought using S100 immuno-histochemistry. Although RCTs tend to metastasize earlier, the time between primary diagnosis of tumor and intraspinal lesion is approximately 16 years.[25]

In this study, the majority of ccRCC had grade IV, 10 cases (45.5%). This research is in line with research by Seker et al where it was found that the majority of ccRCC sufferers had grade IV. [26] The results of this study differ from the research of Evelon et al where the most ccRCC was found in grade II as many as 30 cases (51%).[27] The same results were also obtained in the study of Galtung et al, where the most ccRCC was found in grade II as many as 86 cases (47%).[28] The presence of nucleoli in the ISUP grading assessment role in the process of carcinogenesis. If morphological and functional changes occur in the nucleoli, overproduction and decreased ribosome biogenesis will occur, causing uncontrolled

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proliferation of tumor cells. Ribosomopathy experienced by people with hereditary diseases causes changes in the biogenesis and function of ribosomes which will translate proteins resulting in a particular phenotype.[29,30] In addition, early detection/screening for ccRCC in developing countries is still lacking and is often too late so that the tumor when diagnosed has higher grade.

In this study, the majority of severe stromal TILs were found, 12 cases (54.5%) and mild intratumoral TILs, 11 cases (50.5%). This research is in line with research by Kharismawaty et al which stated that massive nodular lymphocyte infiltration was more dominant in the stroma in ccRCC and negative for intratumoral TILs in ccRCC in 12 cases (52.17%). [31] This study is different from the research of Ghatalia et al in RCC reporting high stromal and intratumoral TILs in ccRCC.[32] Based on the 2014 TILs working group, intratumoral TILs are difficult to assess because they are central to the tumor. Intratumoral TILs usually have a lower prevalence and are detected in fewer cases. Intratumoral TILs are more heterogeneous and difficult to observe on HE-stained slides without using immuno-histochemistry (CD 3 and CD 8) or immuno-fluorescence. Stromal TILs are assessed in the space between tumor nests (stroma) so as not to disrupt tumor density and growth patterns. Neoplastic transformation will change the regular tissue structure and induce an immune response. The ability of tumor cells to escape from immune cells is related to the role of TGF-β, IL-10, ganglioside, adenosine, reactive oxygen stress and thrombospondin. TILs are protumorigenic predictors in ccRCC.[15,33]

In this study, the most low budding cases were found in 9 cases (40.9%). By looking at the characteristics of the tumor and stroma in ccRCC, it is difficult to assess peritumoral tumor budding so immunohistochemical examinations such as pancytokeratin are needed. Researchers have not found research on peritumoral budding tumors in ccRCC so it is difficult for researchers to compare the results of this study on kidney organs. Tumor budding presents pathological features associated with various malignancies. Tumor budding is considered a prognostic biomarker to predict disease progression and unfavorable survival. Tumor budding is considered the histological basis of metastasis and further tumor cell invasion.[34,35]

In this study, the most weak expression of LC3-B was found in the age group ≤60 years as many as 15 cases (83.3%), male gender as many as 12 cases (85.7%) with tumor size >10 cm as many as 12 cases (75%). The relationship between gender and tumor size and LC3-B expression was analyzed using the Fischer's Exact, Kruskal Wallis test and it was concluded that there was no significant relationship between gender and tumor size and LC3-B immunohistochemical expression. Research on the clinicopathology of ccRCC with LC3-B expression is still very limited. In the research of Deng et al stated that the highest expression of LC3-B was found in ccRCC in the age group ≤50 years and male gender.[10]

Autophagy activity decreases with age due to the accumulation of macromolecules and organelles damaged during aging.[36] Autophagy is related to male reproduction, especially spermatogenic and endocrinological processes. Autophagy is known to regulate lipid metabolism through lipophagy to access sources of cholesterol and triglycerides. When autophagy is impaired in Leydig cells, serum testosterone levels decrease. In addition, decreased autophagy leads to accumulation of Na+/H+ exchange regulatory factor 2 (NHERF2), and decreases the expression of scavenger receptor class B, type I (SR-BI). The increase in tumor size is inversely proportional to LC3-B expression due to down-regulation of ATG7, decreased levels of p62, and down-regulation of p53.[10,12]

In this study, the most weak expression of LC3-B was found with positive LVI in 9 cases (64.3%) and negative PNI in 13 cases (76.5%). This data was analyzed statistically and it was concluded that there was no significant relationship between LVI and PNI and LC3-B immunohistochemical expression. The author has not found any research linking LVI, PNI with LC3-B expression in ccRCC. Autophagy can induce certain non-tumor cells to form the tumor microenvironment and promote the growth and invasion of tumor cells through blood vessels and nerves.[37] In this study, weak expression of LC3-B was found mostly in grade II, 6 cases (100%). The highest strong expression of LC3-B was grade IV in 5 cases (50%) and statistically it was concluded that there was no significant relationship between ISUP grading and LC3-B expression. Research by Deng et al stated that increasing grade in ccRCC correlates with low LC3-B expression which is a poor prognosis in ccRCC.[10] Mikhaylova et al states that an increase in grade correlates with an increase in LC3-B expression in ccRCC.[11] Autophagy has two roles in cancer, namely cytoprotective and cytotoxic.[8,9] The dual nature of autophagy is still controversial and difficult to explain in ccRCC.

In this study, weak expression of LC3-B was found mostly in poor grade stromal TILs in 7 cases (58.3%). Intratumoral TILs were found to be mostly mild in 10 cases (90.9%). This data

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was analyzed statistically and it was concluded that there was no significant relationship between stromal and intratumoral TILs and LC3-B immunohistochemical expression. The authors have not found any studies linking TILs to LC3-B expression in ccRCC. Autophagy has an important role in the immune response of tumor cells. Stimulation of autophagy can increase antigen identification and activation of T lymphocytes. In this study, the most weak LC3-B expression with low budding in intratumoral budding tumors was 9 cases (100%) and statistically it was concluded that there was no significant relationship between intratumoral budding tumors with immuno-histochemical expression of LC3-B. The authors have not found any studies linking peritumoral tumor budding with LC3-B expression in ccRCC. Autophagy plays a role in tumor cell migration and invasion by producing pro-migration cytokines, such as IL-, MMP2 and WNT-56A during tumor cell invasion.[37] It can be concluded that in this study, there was no relationship between the clinicopathological characteristics of ccRCC with immunohistochemical expression of LC3- B as described above (Null Hypothesis (H0) is accepted (H0 ρ=0)). Research on the clinicopathology of ccRCC with LC3-B expression is still very limited. Therefore, it is difficult for researchers to compare the results of this study with other studies.

CONCLUSION

There was no relationship between the clinicopathological characteristics of ccRCC and the immunohistochemical expression of LC3-B (Null Hypothesis (H0) was accepted (H0 ρ=0)).

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