Original Article

Expression of BCL-2 in Renal Cell Carcinoma and Its Association with Histopathological Grade

https://doi.org/10.70357/jdamc.2024.v0802.03

Anjum M[1]*, Nasrin M[2], Hossain S[3], Hasan MZ[4], Snigdha SS[5], Jeba R[6], Khan RR[7], Hossain MS[8], Hossain MF[9]

Abstract

Background: Renal cell carcinoma (RCC) is a quite common cancer worldwide and it is difficult to determine prognosis only with staging and grading as spontaneous metastasis or late metastasis can occur after curative surgery. BCL-2, an apoptosis inhibitor gene expression is randomly found in specimen of RCC. Over expression of BCL-2 can help in determining the progression of RCC. **Aim:** The aim of the study was to determine the expression of BCL-2 in histopathologically diagnosed renal cell carcinoma and its association with histopathological grade. **Materials and methods:** This cross-sectional study was conducted in the Department of Pathology, Dhaka Medical College, Dhaka, from March 2021 to February 2023 among purposively included 60 histopathologically diagnosed RCC patients. After taking informed written consent data were collected by face-to-face interview-medical records reviewing by using a pretested semi-structured questionnaire and check list. Immuno-staining with BCL-2 antibody was also done and findings were recorded. Statistical analysis was carried out using the SPSS software version 25. **Results:** The mean±SD age of the patients was 57.2 (±11.7) years, majority 20 (33.3%) were within 55-64 years of age and majority 42 (70%) patients were male. Regarding histopathological type, majority 44 (73.4%) had clear cell type followed by papillary 14 (23.3%) and chromophobe 2 (3.3%) type of RCC. According to ISUP grade, majority 31 (51.6%) belonged to grade 2 followed by 15 (25%), 10 (16.7%) and 4 (6.7%) patients belonged to grade 1, grade 3 and grade 4 RCC respectively. Positive expression of BCL-2 was detected in 37 (61.7%) of cases while negative BCL-2 expression was detected in 23 (38.3%) of patients. Significant statistical association was present between BCL-2 expression with grading of RCC ($p<0.05$). **Conclusion:** BCL-2 expression was positive in about two-third of renal cell carcinoma patients and expression was more frequent among patients with low grade RCC.

Key words: BCL-2 expression, renal cell carcinoma, histopathological grading

Introduction

Renal cell carcinoma (RCC) arises from the modification of epithelial cells of proximal convoluted tubule.[1] It is a quite common cancer all over the world and occurs in both men and women. Among the most frequently diagnosed cancer it was found 6th position in male and 10th position in female.[2] According to WHO, worldwide among the cancer deaths RCC ranks as the 13th most common cause.[3] For both male and female, the cumulative incidence risks of RCC are 0.6% and 0.3% respectively for both eastern & western Asia.[4] In Bangladesh the incidence rate of kidney cancer was found 0.8 per 100000 population and mortality rate due to kidney cancer was found 0.7 per 100000 population.[5]

Several risk factors along with some genetic causes and mutations involved in protein over-expression and up regulation; which was detected as a factor to develop renal cell carcinoma.[1] To determine the prognosis of RCC several factors are examined such as pathologic

Author's Affiliation:

1. *Munira Anjum, Assistant Professor, Department of Pathology, Marks Medical College, Mirpur -14, Dhaka.
2. Mehnaj Nasrin, Medical Officer, Department of Pathology, Mugda Medical College, Dhaka.
3. Sonia Hossain, Pathologist, Dhaka Medical College, Dhaka.
4. Md. Zahirul Hasan, Lecturer, Department of Pathology, Shaheed Tajuddin Ahmed Medical College, Gazipur
5. Shyla Sharmin Snigdha, Assistant Professor Department of Pathology Zainul Haque Sikder Women's Medical College & Hospital, Dhaka.
6. Professor Dr Ruksana Jeba, Professor and Head Department of Pathology Dhaka Medical College, Dhaka.
7. Rizwana Rahman Khan, Assistant Professor Department of Pathology Dhaka Medical College, Dhaka.
8. Md. Shahadat Hossain, Department of Pathology Dhaka Medical College, Dhaka.
9. Mohammad Farid Hossain, Assistant professor Department of Glaucoma National Institute of Opthalmology & Hospital Sher- E- Bangla Nagar, Dhaka

Address of Correspondence : *Dr. Munira Anjum; MD (Pathology), Assistant Professor, Department of Pathology, Marks Medical College, Mirpur -14, Dhaka, Contact: 01742191226, munirarmc87@gmail.com

grade and stage, tumor size, nuclear morphometry and deoxyribonucleic acid (DNA) content.[6] As tumour growth depends on cell proliferation and apoptosis, any unusual behaviour of apoptosis may cause abnormal cellular proliferation and development of carcinoma. It was known that RCC has unique clinical presentations like spontaneous metastasis after removal of the original tumour, late metastasis after curative surgery. Moreover, it is highly resistant to conventional chemotherapy & radiotherapy. For this reason, difficulties arise during determination of prognosis solely by grading.[7] So regulators of apoptosis can help in diagnosis of progression of RCC.[8]

The B cell leukaemia/lymphoma-2 gene (BCL-2) is an inner mitochondrial membrane protein that inhibits programmed cell death or apoptosis.[9] Neoplastic cell development is influenced by BCL-2 through prolongation of cell survival, as inhibition of apoptosis occurs. This proto-oncogene does not cause cell proliferation.[10]

In the presence of cell positivity along with the suppression of BCL-2, the inhibition of programmed cell death occurs, potentially leading to the initiation of malignant transformation.[11] BCL-2 expression was found commonly in different cancers. In case of RCC patient's specimen, expression of BCL-2 proto-oncogene is randomly found. Over expression of BCL-2 without any mutation of p53 which is a tumour suppressor gene may induce renal cancers.[12]

Although conventional clinical and pathological parameters such as tumor grade, vascular and lymphatic involvement provide important information about prognosis of renal cancer, expression of BCL-2 also may enlighten the physicians to determine the patients who are in risk for progression of RCC.

Materials and methods

This cross-sectional study was a part of a large study. This part was conveyed in the Department of Pathology, Dhaka Medical College (DMC), Dhaka with the aim to evaluate the expression of BCL-2 in histopathologically diagnosed renal cell carcinoma and its association with histopathological grade during the period from March 2021 to February 2023. Total 60 histopathologically diagnosed renal cell carcinoma cases irrespective of age and sex were enrolled in this study by purposive sampling technique. Patients who had history of receiving any type of chemotherapy or radiotherapy, autolyzed tissue and necrotic tumour were excluded. After describing detail purpose and procedure of the study informed written consent was taken from each patient, data was collected through face-to-face interview and medical records review by a pre-tested semi-structured questionnaire and check list. Data regarding socio-demographic and clinical profile including radiology & imaging findings were recorded.

One section from representative paraffin block for each case was selected for immunohistochemical stain with BCL-2 at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University. Brownish cytoplasmic staining of tumor cells was considered as BCL-2 scoring. BCL-2 was evaluated based on the percentage of positively stained cells (proportion) and staining intensity compared with positive control. Tonsil was used as positive control. The proportion score was determined as 1 (<30% of tumor cells) and 2 (≥30% of tumor cells). The intensity score was determined as 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). The intensity score and proportion score were multiplied to obtain the total score. Total scores were as follows: 0 to 2 (negative) and 3 to 6 (positive).

Histopathological diagnosis was made and grading was done according to WHO/ISUP grading system. ISUP grade was subdivided into two subgroups-1) Low grade: ISUP grade 1 and 2. 2) High grade: ISUP grade 3 and 4.

All the data was checked thoroughly for any inconsistency followed by coding, categorizing and tabulation using the SPSS software version 25. For descriptive statistics mean, median, standard deviation were analyzed for numerical data and frequencies & proportions for categorical data. Inferential statistics were performed by chi-square test and Fisher's exact test. Statistical significance was set as 95% confidence level. A *p*-value of less than 0.05 was considered statistically significant. Ethics was maintained strictly at every point of the study. Written permission has also been taken from concerned department where study was undertaken. Ethical clearance was obtained from Ethical Review Committee (ERC) of DMC.

Results

In this cross-sectional study 60 cases of histopathologically diagnosed renal cell carcinoma were included with the aim to evaluate the expression of BCL-2 in renal cell carcinoma and their association with histopathological grade. Details of the study results are described below.

Table-1: Distribution of participants according to age (n=60)

Age group (in years)	Frequency (n)	Percentage (%)
24-34	03	5
35-44	03	5
45-54	15	25
55-64	20	33.3
65-75	19	31.7
Mean (±SD) =57.2 (±11.7)		

Majority 33.3% of participants (n=20) were in the age group 55-64 years. Mean (±SD) age was 57.2 (±11.7) years presented in (Table-1).

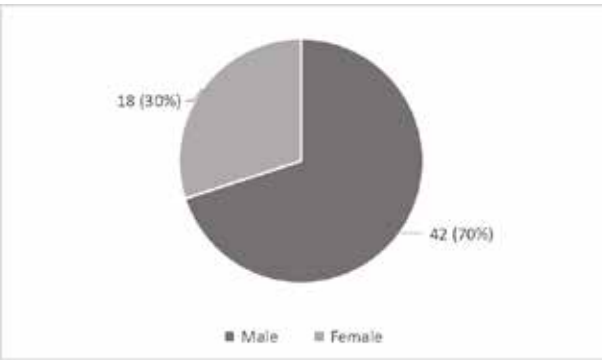

Fig-1: Distribution of participants according to sex (n=60)

Among the participants, majority 70% patients (n=42) were male showed in Figure-1.

Table-2: Distribution of participants according to clinical presentation (n=60)

Presenting complains	Frequency (n)	Percentage (%)
Hematuria	60	100
Flank pain	35	58.3
Abdominal mass	28	46.7

*Multiple response

Regarding clinical presentation it was found that all 100% patients (n=60) had hematuria, 58.3% patients (n=35) and 46.7% patients (n=28) presented with flank pain and abdominal mass respectively stated in Table-2.

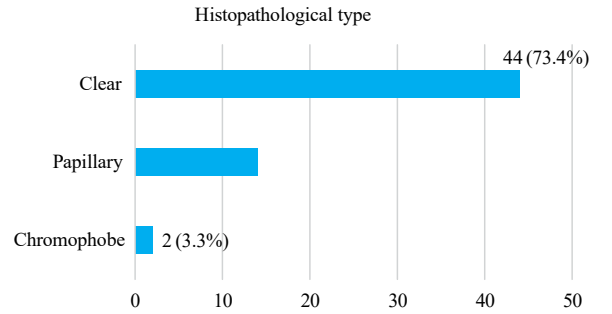

Fig-2: Distribution of participants according to histopathological type of renal cell carcinoma (n=60)

Figure-2 illustrated that clear cell renal cell carcinoma was found in majority 73.4% of participants (n=44); followed by papillary renal cell carcinoma in 23.3% of participants (n=14) and chromophobe renal cell carcinoma comprised rest of the cases 3.3% (n=2).

Table-3: Distribution of participants according to histopathological grading of renal cell carcinoma (n=60)

Grade	Frequency (n)	Percentage (%)
1	15	25.0
2	31	51.6
3	10	16.7
4	4	6.7

All tumors were graded according to the scoring system of the International Society of Urological Pathology (ISUP). Majority 51.6% patients (n=31) were reported as grade 2, followed by 25% (n=15), 16.7% (n=10) and 6.7% (n=4) of patients reported as grade 1, grade 3 and grade 4 renal cell carcinoma respectively presented in Table-3.

Table-4: Distribution of participants by BCL-2 expression (n=60)

BCL-2 expression	Frequency (n)	Percentage (%)
Positive	37	61.7
Negative	23	38.3

Regarding BCL-2 expression, among majority 61.7% of patients (n=37) it was detected positive expression and the rest 38.3% of patients (n=23) it was detected negative expression showed in Table-4.

Table-5: Association between histopathological type and grade with BCL-2 expression (n=60)

Histopathological type	BCL-2 expression		p-value
	Positive	Negative	
Clear	26 (59.1%)	18 (40.9%)	
Papillary	9 (64.3%)	5 (35.7%)	0.775[b]
Chromophobe	2 (100%)	0 (0%)	
Grading			
Low grade (1 and 2)	32 (69.6%)	14 (30.4%)	0.031*
High grade (3 and 4)	5 (35.7%)	9 (64.3%)	

[b]Fisher Exact test performed to measure the level of significance

*Chi-square test was performed to measure the level of significance

Patients who had clear type of renal cell carcinoma, majority 59.1% (n=26) showed positive BCL-2 expression and 40.9% (n=18) showed negative expression. On the other hand, in case of papillary renal cell carcinoma, majority 64.3% patients (n=9) were detected as positive expression while 35.7% patients (n=5) showed negativity. All 100% patients (n=2) of chromophobe type renal cell carcinoma showed positive BCL-2 expression. No significant statistical association was found between BCL-2 expression with histopathological type.

Among the patients who had low grade (1 & 2) renal cell carcinoma, majority 69.6% patients (n=32) showed positive BCL-2 expression. While, patients who had high grade (3 & 4) renal cell carcinoma, majority 64.3% patients (n=9) showed negative BCL-2 expression. This differences of histopathological grade between BCL-2 expression were statistically significant ($p<0.05$) presented in Table-5.

Discussion

Renal cell carcinoma (RCC) is the Commonest diagnosed cancer among all urogenital malignancy and causes 30-40% mortality recorded in 2022.[13,14] Diagnosis of RCC in early stage is very crucial for the treatment of patients and for decreasing mortality.[13] Though BCL-2 is found invariably in specimen of RCC, but depending on grading prognosis of RCC is made mostly. Aim of this cross-sectional study was to evaluate the expression of BCL-2 proto-oncogene in renal cell carcinoma (RCC) and its association with histopathological grade among 60 patients.

In this study mean age of the participants was found 57.2 (±11.7) years. Age of majority 33.3% participants (n=20) were within 55-64 years and male 70% (n=42) predominance was observed. Mean age of RCC patients was 58 years and 68.31% were male patients revealed in another study which presented similarity with current study.[7]

Patients presented with haematuria in 100% cases (n=60), followed by flank pain in 58.3% (n=35) and abdominal mass in 46.7% (n=28) of cases in present study. Hofbauer et al conducted a study in Vienna, Austria stated that among 633 patients of RCC 73% (n=317) were completely asymptomatic and 11% patients (n=68) had flank pain, 10% (n=62) and <1% (n=1) had painless haematuria and palpable tumour respectively. Both flank pain and haematuria were present in 3% of patients (n=16).[15]As the later study was conducted over a long period among large number of participants so, dissimilarity found with present study results.

According to WHO, the most common histopathological subtypes of RCC refer to clear cell type (75%), followed by papillary (10%) and chromophobe type (5%).[16] The histopathological findings of the present study revealed clear cell type in 73.4% (n=4), papillary type in 23.3% (n=14) and chromophobe type in 3.3% (n=2) of cases. In Bangladesh, Hossain et al also found the similar result in another study where maximum 74% patients had clear cell type of RCC followed by 24% and 2% had papillary and chromophobe type respectively.[17]

By histopathological grading among 60 patients in this study, majority 51.6% (n=31) reported as grade-2 followed by 25% (n=15) reported as grade-1, 16.7% (n=10) and 6.7% (n=4) reported as grade-3 and grade-4 respectively. Grading created according to the scoring system of the International Society of Urological Pathology (ISUP). Another study findings matched with the current study presented that 50% patients had grade-2, 32% had grade-1 and 18% had grade-3 RCC.[17] Pehlivan et al conveyed in Turkey revealed dissimilarity that 34.2% patients had grade 1, 28.5% had grade 2, 34.2% patients had grade 3 and 3.1% patients had grade 4 RCC.[18] This might be due to difference of study population.

BCL-2 is an anti-apoptotic gene and centrally regulates cell death or apoptosis by a preserved pathway.[19] Its expression can be detected in many organs and changes in their expression may leads to development of neoplasm. Various members of the BCL-2 gene family impose crucial role in development of renal diseases other than neoplasm.[20,21] Expression of BCL-2 was detected positive in majority 61.7% of cases (n=37) in the current study.

Pehlivan et al found BCL-2 expression was positive among majority 70% RCC patients.[18] BCL-2 expression was also detected positive in majority 54.8% and 71.3% of RCC patients in the studies of Girgin et al and Itoi et al respectively.[22,7]

In the present study, majority of the clear (59.1%) and papillary (64.3%) type of RCC were BCL-2 expression positive while all (100%) Chromophobe type patients were BCL-2 positive. Statistically significance was not observed between BCL-2 expression with histopathological type. Similarity was found in another study carried out in Japan, where differences between immunohistochemical staining of BCL-2 with histopathological types of RCC were not statistically significant. Another study conveyed by Demirović et al also revealed that all chromophobe type of RCC were BCL-2 positive with no statistically significant difference between immunohistochemical staining index (ISI) of BCL-2 renal oncocytomas and chromophobe RCCs.[23]

It was detected in current study that in case of low grade (1 and 2) RCC, 32 (69.6%) cases showed positive expression and in case of high grade (3 and 4) RCC, 5 (35.7%) cases were positive with BCL-2 expression. Significant statistical association was observed between BCL-2 expression and grading of RCC ($p<0.05$). Itoi et al. showed similarity in their study where they stated that BCL-2 expression was more common in grade-1 and grade-2 RCC than grade-3 and these differences of grading of RCC with BCL-2 expression was significantly associated.[7] Vasavada et al. revealed significant correlation between high BCL-2 expression with higher tumour grade which was opposite to current study. The dissimilarity may be due to small number of study subjects that is only 28 clear and seven papillary renal cell carcinoma cases.[24]

BCL-2 proto-oncogene expression was detected among

majority of RCC cases previously in several studies.[24] In this present study BCL-2 expression was found frequently in majority of patients who belonged to low grade (grade-1 & grade-2) compared to high grade (grade-3 & grade-4) RCC. Other study also showed that BCL-2 was more frequently detected in tumors with lower nuclear grade.[7] As an apoptosis inhibitor gene BCL-2 induce survival of cell and may facilitate cell differentiation also. But infrequent BCL-2 expression is related to improper cell differentiation and neoplastic progression.[25,26] This study result will create a new comprehension to evaluate BCL-2 expression and to provide therapeutic benefit for the treatment of RCC patients

Limitations of the study:

The study was a single centered study. Specimens from multiple centers all over the country would give more appropriate information. Follow up of the patients was not done to comment on patient's outcome. Staging might add more information regarding the RCC patients.

Conclusion

BCL-2 expression was detected positive in about two-third of renal cell carcinoma patients and expression was more frequent among patients with low grade RCC. The use of BCL-2 immunomarkers in conjunction with histopathological grade may provide significant prognostic information for categorizing high risk patients in renal cell carcinoma.

Declarations

Ethics approval and consent to participate

Before data collection, both verbal and written informed consent was taken from patients.

Consent for publication

All authors have approved this manuscript for publication.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research received no funding from Dhaka Medical College for conduction of the study.

Authors' contributions

MA, MN, and SH participated in the design of the study, data interpretation and drafted the manuscript. MA, MZH, and SSS contributed to the data design, data interpretation and data analysis and RJ, RRK, MSH, MFH did the critical review of the manuscript.

All authors read and approved the final manuscript.

Acknowledgements

These researchers acknowledged for the support of the authority and respondents.

References

1. Cairns Paul. "Renal cell carcinoma." Cancer biomarkers: section A of Disease markers. 2010; vol. 9,1-6: 461-73. doi:10.3233/CBM-2011-0176

2. Sung H, Ferlay J, Siegel R L, Laversanne M, Soerjomataram I, Jemal A, and Bray F. "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries." CA: a cancer journal for clinicians. 2021; vol. 71,3: 209-249. doi:10.3322/caac.21660

3. Ferlay Jacques. "GLOBOCAN 2008 v2. 0, Cancer incidence and mortality worldwide: IARC CancerBase No. 10." http://globocan. iarc. Fr. 2010.

4. Capitanio U, Bensalah K, Bex A, Boorjian S A, Bray F, Coleman J, Gore J L, Sun M, Wood C, Russo P. "Epidemiology of Renal Cell Carcinoma." European urology vol. 2019; 75,1: 74-84. doi:10.1016/j.eururo.2018.08.036

5. Arabsalmani M, Mohammadian-Hafshejani A, Ghoncheh M, Hadadian F, Towhidi F, Vafaee K, Salehiniya H. Incidence and mortality of kidney cancers, and human development index in Asia; a matter of concern. Journal of nephropathology.2017; 6(1),0–42. https://doi.org/10.15171/jnp.2017.06

6. Dinney C P N, Awad S A, Gajewski J B, Belitsky P, Lannon S G, Mack F G and Millard O H. Analysis of imaging modalities, staging systems, and prognostic indicators for renal cell carcinoma. Urology. 1992; 39(2), pp.122-129.

7. Itoi T, Yamana K, Bilim V, Takahashi K, Tomita F. "Impact of frequent Bcl-2 expression on better prognosis in renal cell carcinoma patients." British journal of cancer. 2004; vol. 90,1: 200-5. https://doi.org/10.1038/sj.bjc.6601454

8. Lowe S W, Ruley H E, Jacks T and Housman D E. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell. 1993; 74(6): pp.957-967.

9. Singh R, Letai A and Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nature reviews Molecular cell biology. 2019; 20(3): pp.175-193.

10. Saikrishana P, Sivapathasundharam B, Rafiuddeen I Syed, Krishnan Baba. Expression Of Bcl-2 Oncoprotien In Oral Squamous Cell Carcinoma - An Immunohistochemical Study. Indian Journal of Pathology and Microbiology. July 2002; 45(3):p 283-287,.

11. Bakir W A. The Relationship Between Bcl-2, and P53 Protiens in transitional Cell Carcinoma of the Bladder. Iraqi Journal of Cancer and Medical Genetics. 2011; 4(1).

12. Tomita Y, Bilim V, Kawasaki T, Takahashi K, Okan I, Magnusson K P & Wiman K G. Frequent expression of Bcl-2 in renal-cell carcinomas carrying wild-type p53. International journal of cancer. 1996; 66(3), 322–325. https://doi.org/10.1002/(SICI)1097-0215(19960503)66:3<322::AID-IJC9>3.0.CO;2-0

13. Bahadoram S, Davoodi M, Hassanzadeh S, Bahadoram M, Barahman M, Mafakher L. "Renal cell carcinoma: an overview of the epidemiology, diagnosis, and treatment." Giornale italiano di nefrologia : organo ufficiale della Societa italiana di nefrologia. 2022; vol. 39,3. 20 Jun. 2022. PMID: 35819037

14. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6):394- 424. https://doi.org/10.3322/caac.21492

15. Hofbauer S L, Martino M de, Sreemann C, Zamani N. "Associations between presenting symptoms, clinicopathological parameters, and prognosis in a contemporary series of patients with renal cell carcinoma." Korean journal of urology. 2014; vol. 55,8: 505-10. doi:10.4111/kju.2014.55.8.505

16. Muglia, Valdair F, and Adilson Prando. "Renal cell carcinoma: histological classification and correlation with imaging findings." Radiologia brasileira. 2015; vol. 48,3:166-74. doi:10.1590/0100-3984.2013.1927

17. Hossain M S, Quruni M O, Hossain M S, Saha M K, Afrin S S, Khan Z B, Siddiqua D R, Jinnah M S A, Karim S S, Jeba R and Dewan M R K. Expression of p53 in Renal Cell Carcinoma and its Association with Histological Types, Grades and Pathological Staging. Journal of National Institute of Neurosciences Bangladesh. 2022; 8(1), pp.57-61.

18. Pehlivan F S, Sarı A, Görgel S N, Morgül Y, Balc U, Ermete M and Şefik E. Relationship of COX-2, Bax, Bcl-2, Ki 67, p 53 expression to clinicopathologic parameters and their impact on prognosis in renal cell carcinoma. Journal of Turgut Ozal Medical Center. 2014; 21(3).

19. Korsmeyer S J. Bcl-2 Gene Family and the Regulation of Programmed Cell Death. Cancer Res. 1999, 59, 1693s–1700s.

20. Gobe G, Zhang X J, Cuttle L, Pat B, Willgoss D, Hancock J, Barnard R, Endre R B. Bcl-2 Genes and Growth Factors in the Pathology of Ischaemic Acute Renal Failure. Immunol. Cell Biol. 1999, 77, 279–286.

21. Gobe G, Zhang X J, Willgoss D, Schoch E, Hogg N A , Endre Z H. Relationship Between Bcl-2 Genes, Growth Factors and Apoptosis in Acute Ischemic Renal Injury in the Rat. J. Am. Soc. Nephrol. 2000, 11, 454–467.

22. Girgin R B, Ozkanli S, Engin-Zerk P, Soylemez T, Olgun Z C, Yildirim A, Kir G. "Relation of beclin-1 and bcl-2 expressions with pathological parameters and prognosis in clear cell renal cell carcinomas." Archivos espanoles de urologia. 2022; vol. 75,4:

368-374. doi:10.37554/en-j.arch.esp.urol-20210619-3504-24.

23. DEMIROVIĆ A, Ulamec M, Bezjak M, Belicza M and KRUŠLIN B. Apoptotic markers (P53, BCL-2 and Bax) expression in renal oncocytoma and chromophobe renal cell carcinoma. Periodicum biologorum. 2014; 116(2), pp.173-176.

24. Vasavada SP, Novick AC, Williams BR. P53, bcl-2, and Bax expression in renal cell carcinoma. Urology. 1998; 51: 1057–1061

25. Pierce RH, Vail ME, Ralph L, Campbell JS, Fausto N. Bcl-2 expression inhibits liver carcinogenesis and delays the development of proliferating foci. American Journal of Pathology. 2002;160(5):1555 - 1560.

26. Gupta A, Jain S, Khurana N, Kakar A K . "Expression of p63 and Bcl-2 in Malignant Thyroid Tumors and their Correlation with other Diagnostic Immunocytochemical Markers." Journal of clinical and diagnosticresearch: JCDR. 2016; vol. 10,7: EC04-8. doi:10.7860/JCDR/2016/13899.8157