ORIGINAL ARTICLE

EXPRESSION OF BAP1 IN CLEAR CELL RENAL CELL CARCINOMA

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Background: BAP1 (BRCA1 associated protein 1 on chromosome 3) is a commonly mutated gene in clear cell renal cell carcinoma. Aim of the study was to evaluate the prognostic significance of BAP1 by immunohistochemistry in clear cell renal cell carcinoma. **Methods:** It was a descriptive case series in which data was retrospectively collected. Immunohistochemistry was used to evaluate the loss of nuclear expression of BAP1. **Results:** Loss of BAP1 was observed in 60% of cases of clear cell renal cell carcinoma. 27% of grade 1 tumours, 62% of grade 2 tumours, 65% of grade 3 tumours and 66% of grade 4 tumours showed loss of BAP1. Loss of BAP1 was observed in 54% cases of stage 1 tumours, 72% of stage 2 tumours and 66% of stage 3 tumours. Our study showed loss of BAP1 in 67% of cases with tumour necrosis, in 75% of cases with sarcomatoid features and in 60% of patients with distant metastasis. **Conclusions:** We conclude that the loss of BAP1 nuclear expression is associated with poor prognostic features. i.e., higher grade, higher stage, tumour necrosis, sarcomatoid features and distant metastasis leading to death of patients.

Keywords: BAP1; Clear Cell Renal Cell Carcinoma; bad prognosis

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INTRODUCTION

Renal cell carcinoma in humans originates from specialized cells of the nephron. These tumours have a very high mortality rate.[1] Clear cell renal cell carcinoma generally occurs in adults (average age 55–60 years). Male to female ratio is 2:1 and incidence of bilaterality is 1%.[2] Clear cell renal cell carcinoma comprises 70% of all epithelial renal tumours.[3]

These tumours are characterized by loss of genetic material of short arm of chromosome 3 and mutations in VHL gene. Both kidneys are affected equally and most tumours are solitary.[4] BAP1 (BRCA1 associated protein 1 on chromosome 3) is a hydrolase of ubiquitin carboxy terminal and this participates in cell cycle, differentiation of cell, death of cell and DNA damage.[5] BAP1 attaches to ring finger domain of BRCA1. In BRCA1 growth-controlled pathway, BAP1 is a new tumour suppressor gene.[6] It is a tumour suppressor gene whose mutation is seen in multiple tumours, i.e., uveal melanoma[7] mesothelioma[8] cholangiocarcinoma[9] and melanocytic tumours[10]. Inactivation of BAP1 protein is seen in 15% of renal carcinomas[11] BAP1 mutations are frequently reported in clear cell renal cell carcinoma[12], and are associated with worse prognostic factors[13]. The architecture of these tumours is papillary, tubulopapillary or nested.[14] Traditionally the prognosis of clear cell renal cell carcinoma is determined by tumour size, grade,[11,13] stage, presence of necrosis, lymph node and distant metastasis, sarcomatoid and rhabdoid features[14].

We observed BAP1 expression by immunohistochemistry in various renal cell carcinoma types and corelated its relationship with tumour size, grade, stage, presence of necrosis, sarcomatoid and rhabdoid features and lymph node and distant metastasis due to tumour.

Few studies have been done regarding loss of BAP1 in clear cell renal cell carcinoma and there were no previous studies done in Pakistan on this subject. Moreover, these previous studies on BAP1 were mostly by PCR techniques. We employed immunohistochemistry for evaluation. This will aid in those settings where PCR techniques for evaluation are not available. The results will be useful for relevant caregivers.

MATERIAL AND METHODS

In this descriptive case series, 142 cases were retrospectively obtained from Shaukat Khanum Memorial Cancer Hospital and Research Centre Lahore. (2010–2016). The study was approved by International Review board of the Shaukat Khanum Memorial Cancer Hospital and Research Centre and the requirement for informed consent was waived. Confidentiality of data is maintained. Formalin fixed paraffin embedded tissue samples obtained from surgically resected specimens were collected. Slides were reviewed and representative block for each case was selected.

The patient and tumour characteristics including age, histological tumour type, grade of tumour, stage of tumour, presence or absence of necrosis, sarcomatoid or rhabdoid features, metastasis and follow up data were collected. The renal cell carcinoma was subtyped and graded according to World Health Organization/International Society of Urological Pathology grading system. Sections from the blocks were immune-stained using Bond-III automated immunohistochemistry/*in situ* hybridzation stainer (BioSB). Mouse monoclonal antibody was used derived from cell culture supernatant that is concentrated, dialysed, filter sterilized and diluted in buffer pH7.5, containing BSA and sodium azide as a preservative. BAP1 Clone: BSB-109 was used. Manufacturer's protocol was followed. The tissues were 3–5 microns thick. Section was retrieved with BOND ER2 solution for 40 minutes. Antibody incubation time was 15 minutes. BAP1 expression level was evaluated according to the extent of nuclear staining in tumour cells. The staining was scored as negative (no expression or expression in less than 10% tumour cells) or positive (expression in greater than or equal to 10% of tumour cells)[15] Bap1 expression in intra-tumoural lymphocytes and stromal cells was considered as positive internal control[16] Negative staining of BAP1 was associated with loss of function mutation.[17]

All of the statistical analyses were performed using SPSS version 20. Mean, standard deviation, were calculated for quantitative variables. Frequencies and percentages were calculated for categorical variables. Chi square test was used to examine the relationship between categorical variables. One-way ANOVA test was used to compare the mean difference in age and tumour size versus diagnosis. p-value ≤ 0.05 was considered statistically significant.

RESULTS

In our study, there were 142 cases of which 110 cases were of clear cell renal cell carcinoma, 16 cases were papillary renal cell carcinoma and 16 cases were of chromophobe renal cell carcinoma. Data is summarized in tables-1 and 2. In figure-1, microscopic pictures of different tumours are shown. The ages of patients with clear cell renal cell carcinoma ranged from 20–81 years with a mean age of 52 years. The tumour size ranged from 1.8–16 cm with a mean size of 6.7 cm. The ages of patients with chromophobe renal cell carcinoma ranged from 25–67 years with a mean age of 43 years and the size ranged from 4.5–17.5 cm with a mean size of 10.7 cm. The age of patients in papillary renal cell carcinoma ranged from 14–68 years with a mean age of 52 years and size ranged from 2.5–11 cm with a mean size of 5.4 cm. 11 patients had grade 1 tumours, 79 patients had grade 2, 32 patients had grade 3 and 6 patients had grade 4 tumours. 68 patients had stage 1 tumours, 29 patients had stage 2 tumours and 39 patients had stage 3 tumours. Tumour necrosis was present in 16 cases and sarcomatoid features were present in 4 cases. Regional lymph node metastases were seen in 4 cases. The median follow-up period was 116 months. Out of 142 patients with renal cell carcinoma, 92 patients were alive at the time of analysis, 20 patients had died, and we had lost follow-up of the remaining 30 patients. Twenty had metastatic disease at the time of diagnosis: 4 patients had brain metastasis, 16 patients had lung, liver and bone metastasis. Four patients showed lymph node metastasis out of 7 patients who had lymph node dissection done with the nephrectomy specimen. For the rest of patients, the lymph node status was unknown because lymph node dissection was not done.

BAP1 immunohistochemistry was applied and cases were classified as either BAP1 negative or BAP1 positive. Loss of BAP1 expression was seen in 67 out of 110 cases of clear cell renal cell carcinoma (60%), 14 out of 14 cases of chromophobe renal cell carcinoma (100%) and 5 out of 16 cases of papillary renal cell carcinoma (31%)

Loss of BAP 1 was seen in 3 out of 11 cases of Grade 1 tumours (27%), 49 out of 79 cases of grade 2 tumours (62%), 21 out of 32 cases of grade 3 tumours (65%) and 4 out of 6 cases of grade4 tumours (66%). Loss of BAP1 was associated with 37 out of 68 of stage 1 tumours (54%), 21 out of 29 of stage 2 tumours (72%), and 25 out of 38 of stage 3 tumours (66%).

In 16 cases with tumour necrosis, 11 cases had loss of BAP1 (67%). In 4 patients with sarcomatoid features, 3 had loss of Bap1 (75%). In 4 patients with lymph node metastasis, loss of BAP1 was seen in 3 patients (75%). Out of 142 patients with renal cell carcinoma, 92 patients were alive at the time of analysis and 60 patients showed loss of BAP1. 20 patients had died due to renal cell carcinoma at the time of analysis. 20 patients had died as a result of distant metastasis at the time of analysis and 12 of those showed loss of BAP1. We had lost follow-up of the remaining 30 patients so we cannot comment on metastases in these cases. Among 20 patients who died as a result of distant metastases, 12 patients had loss of BAP1 (60%).

Table-1: Summary of patient and tumour characteristics

Characteristics	Value (n)
Tumour type	
Clear cell renal cell carcinoma	110
Chromophobe renal cell carcinoma	16
Papillary renal cell carcinoma	16
Age	
Clear cell renal cell carcinoma	20–81 years (mean:52 years)
Chromophobe renal cell carcinoma	25–67 years (mean:43 years)
Papillary renal cell carcinoma	14–68 years (mean 52 years)
Size	
Clear cell renal cell carcinoma	1.8–16 cm(mean:6.7 cm)
Chromophobe renal cell carcinoma	4.5–17.5 cm(mean:10.7 cm)
Papillary renal cell carcinoma	2.5–11 cm(mean:5.4 cm)
WHO/ISUP Grade	
Grade 1	11
Grade 2	79
Grade 3	32
Grade 4	6
Cannot be assessed	14
Pathological stage	
Stage 1	68
Stage 2	29
Stage 3	39
Cannot be assessed	6
Tumour Necrosis	
Present	16
Absent	126
Sarcomatoid Features	
Present	4
Absent	138
Lymphnode metastasis	
Present	4
Absent	3
Cannot be assessed	135
Distant Metastasis	
Present	20
Absent	122
Patient Status	
Alive	92
Dead	20
Lost to follow-up	30

Table-2. Association of BAP1 expression with patient and tumour characteristics

	Loss of BAP1	BAP1 positive	Total number of cases	Percentage of cases with loss of BAP1	*p*-value
Tumour type					0.002
Clear cell renal cell carcinoma	67	43	110	60%	
Chromophobe renal cell carcinoma	14	0	14	100%	
Papillary renal cell carcinoma	5	11	16	31%	
Grade of tumour					0.24
Grade 1	3	8	11	27%	
Grade 2	49	30	79	62%	
Grade3	21	11	32	65%	
Grade4	4	2	6	66%	
Stage of tumour					0.16
Stage 1	37	31	68	54%	
Stage2	21	8	29	72%	
Stage 3	25	13	38	66%	
Necrosis	11	5	16	67%	0.48
Sarcomatoid features	3	1	4	75%	0.65
Lymphnode metastasis	3	1	4	75%	0.21
Distant metastasis	12	8	20	60%	0.001
Patient Status					
Alive	60	32	92	65%	0.09
Dead	12	8	20	60%	
Lost follow-up			30		

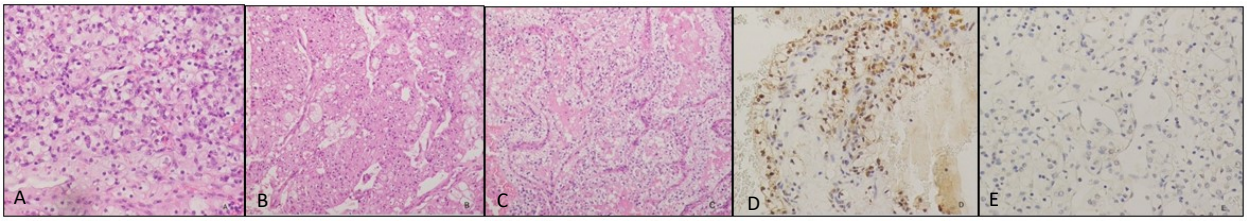

Figure-1: A. Clear cell renal cell carcinoma. B. Chromophobe renal cell carcinoma. C. Papillary renal cell carcinoma. D. Positive expression of BAP1. E. Loss of BAP1 expression

DISCUSSION

BAP1 (BRCA1 associated protein 1 on chromosome 3) is a ubiquitin carboxy terminal hydrolase and is involved in cell cycle regulation, cellular differentiation, cell death and DNA damage response.[5] It is a tumour suppressor gene whose mutation is seen in multiple tumours, i.e., uveal melanoma[7] mesothelioma[8] cholangiocarcinoma[9] and melanocytic tumours[10]. BAP 1 is one of the four common mutations found in clear cell renal cell carcinoma, the other three being VHL, SETD2 and PBRM1.[17] BAP1 mutations are frequently reported in clear cell renal cell carcinoma[12] and are associated with worse prognosis factors[13], i.e., larger tumour size[16], higher tumour grade[15,18], higher TNM stage[16], sarcomatoid features, rhabdoid features[13] coagulative tumour necrosis[16] distant metastasis[19], disease progression[20], tumour recurrence and death[19].

BAP1 mutation evaluation is being done by immunohistochemistry, PCR techniques, western blot and DNA sequencing analysis.[21] We observed BAP1 expression by immunohistochemistry in various renal cell carcinoma types and corelated its relationship with clinicopathological characteristics of patients.

In our study, there were 142 cases, in which loss of Bap1 was observed in 60% of cases of clear cell renal cell carcinoma, 100% cases of chromophobe renal cell carcinoma and 31% cases of papillary renal cell carcinoma. A study by Wi *et al*.[15] showed loss of BAP1 in 18.7% cases of clear cell renal cell carcinoma and 23.1% cases of chromophobe renal cell carcinoma while no Bap1 loss was observed in papillary renal cell carcinoma. A study by Ho *et al*.[21] showed loss of BAP1 in 10% cases of clear cell renal cell carcinoma. In a study by Pena *et al*.[16] there was loss of BAP1 in 14% of cases of clear cell renal cell carcinoma. In a study by Eckel *et al*[22] 20% of cases of clear cell renal cell carcinoma showed loss of BAP1 expression by immunohistochemistry.

In our study, BAP1 was associated with higher grade tumours. 27% of grade 1 tumours, 62% of grade 2 tumours, 65% of grade 3 tumours, 66% of grade 4 tumours showed loss of BAP1. A study by Wi *et al*.[15] showed loss of BAP1 in 0% cases of grade 1 tumour, 16% cases of grade 2 tumour, 35 % of grade 3 tumours and 47% of grade 4 tumours. In a study by Joseph *et al*.[20] there was loss of BAP1 in 0% cases of grade 1 tumours, 1.7% of grade 2 tumours, 20% of grade 3 tumours and 36% of grade 4 tumours.

Loss of BAP1 was observed in 54% cases of stage 1 tumours, 72% of stage 2 tumours and 66% of stage 3 tumours in our study. A study by Wi *et al*.[15] showed loss of Bap1 in 16.9% of stage 1 tumours, 37% of stage 2 tumours and 16.7% of stage 3/4 tumours. In a study by Joseph *et al*.[20] there was loss of BAP1 in 6% cases of stage 1 tumours, 15% of stage 2 tumours, and 21% of stage 3 tumours.

Our study showed loss of BAP1 in 67% of cases with tumour necrosis. In a study by Wi *et al*.[15] there was loss of BAP1 in 22% of cases with tumour necrosis and a study by joseph *et al*.[20] showed tumour necrosis in 26.7% of cases with tumour necrosis. Our study showed loss of BAP1 in 75% of cases with sarcomatoid features while in a study by Wi *et al*.[15] there was loss of BAP1 in 27.7% of cases.

In our study, BAP1 mutations were observed in clear cell renal cell carcinoma and chromophobe renal cell carcinoma, was associated with higher grade of tumour and more frequently associated with stage 2 tumours. Moreover, BAP1 loss was associated with tumour necrosis and sarcomatoid features in our study. These findings are consistent with the previous studies.

In our study, loss of BAP1 was seen in 75% of patients with lymphnode metastasis. However, in the study by Wi *et al*.[15] 16% of patients with lymphnode metastasis showed loss of BAP1. However, in our study the loss of BAP1 was also observed in papillary renal cell carcinoma which is different from the previous studies. Our study showed association of loss of BAP1 in 60% patients with distant metastasis. However previous studies did not establish any significant association of loss of BAP1 with metastatic disease. These differences can be due to difference in the sample size, ethnic and racial differences, difference in the technique of evaluation of BAP1, difference in the clone, fixation time or dilution factor of antibody, difference in the retrieval method utilized or difference in the incubation time of antibody used.

CONCLUSION

We conclude that the loss of BAP1 nuclear expression detected by immunohistochemistry is associated with poor prognostic features. i.e., higher grade, higher stage, tumour necrosis, sarcomatoid features and distant metastasis leading to death of patients. Owing to similarity with contemporary literature, based on molecular testing indicate that BAP1 immunohistochemistry can be applied in routine labs.

Conflict of interest declaration:

The authors declare no conflicts of interest or any financial associations with any organization.

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AUTHORS' CONTRIBUTION

AF: Data collection and writing. SM: Writing and revision. AL: Idea and revision. UH and UNS: Idea and proofreading

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