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IMMUNOHISTOCHEMICAL AND MOLECULAR DETECTION OF MITOCHONDRIAL ATPASE 6 GENE MUTATIONS IN BLADDER CANCER AMONG SUDANESE PATIENTS

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ABSTRACT

Background: Mitochondrial DNA (mtDNA) dysfunction plays acritical role in carcinogenesis. Mutations in MT-ATPase 6 gene, encoding a subunit of mitochondrial complex V (ATP synthase), have been implicated in various cancers, but their prevalence in bladder cancer, particularly among Sudanese patients, remains unclear. Objective: This study aims to detect ATPase 6 gene mutations in bladder cancer tissue using immunohistochemistry (IHC) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and to correlate these alterations with clinicopathological parameters. Materials and Methods: A retrospective cohort study was conducted on 90 formalin-fixed paraffin-embedded (FFPE). Bladder cancer specimens from three major hospitals in Khartoum state (2020-2022). IHC was performed using an anti-ATPase 6 antibody. PCR-RFLP with HpaII digestion was used to detect mutations. Statistical analysis includes Chi-square test, with significance set (> 0.05). Results: The study comprised male (59 patients, 65.6%) compared to females (31 patients, 34.4%). The patients age ranged from (30 to 95) with mean age of (62.5) years old, the age was categorized as follow (30-51) were 32 patient (35.6%), (52-73) were 43 patient (47.8%), (74- 95) were 15 patient (16.7%). the prevalence of transitional bladder cancer (Seventy-six patients (84.4%) while 14 patients (15.6%) had other bladder cancer (leiomyosarcoma,) diagnoses. Regarding tumour grade, the distributions were as follows: 35 patients (38.9%) had high-grade tumours, 26 patients (28.9%) had low-grade tumours, and 29 patients (32.2%) had invasive tumours. The results also showed that 24 patients (26.7%) were positive for Schistosoma infection, while 66 patients (73.3%) were negative. Immunohistochemical (IHC) analysis showed the presence of expression in 10 out of 90 samples (11.1%), Polymerase Chain Reaction (PCR) results revealed the presence of mutations in the ATPase 6 gene sequence in only 10 out of 90 samples (11.1%), indicating a low mutation rate. No statistically significant association were found between ATPase 6 alterations and gender, age group, tumour grade, tumour type, or Schistosoma infection (p value > 0.05 for all).

Agreement between IHC and PCR was weak and not statistically significant. Conclusion: Mutations in Mitochondrial ATPase6 gene are relatively infrequent in in Sudanese bladder cancer patients and show no significant correlation with standard clinicopathological factors. Despite its low prevalence, the detection of this mutation contribute to the molecular characterization of bladder cancer and further investigation with larger cohorts is recommended.

INTRODUCTION

Carcinoma of the urinary bladder, the fourth most common cancer in men and the ninth most common cancer in women, result in significant morbidity and mortality. It affects more than 400.000 patients annually worldwide.[1,2] Urothelial carcinoma accounts for the majority of cases, while squamous cell carcinoma is linked to chronic inflammation, particularly Schistosoma haematobium infection in endemic regions.[3,4] Early detection remains difficult due to limitation of current diagnostic tools like cystoscopy and urine cytology, necessitating the search for novel biomarkers.[5,6]

Mitochondria are important organelles responsible for energy production, redox homeostasis, oncogenic signaling, cell death, and apoptosis. The mitochondrial genome encodes 37 genes of which 22 are transfer RNA (tRNA), two are ribosomal RNA (12S and 16S ribosomal subunits), and the remaining 13 encode polypeptides involved in OXPHOS.[7] MtDNA mutations tend to be induced by oxidative damage, defects in nuclear genes in mtDNA stability and replication, altered nucleotide biosynthesis or transport, and exogenous sources.[8,9] The MT-ATPase 6 gene encode the a-subunit of ATP synthase (complex V), which is crucial for ATP production. Mutations in this gene have been associated with mitochondrial diseases such as NARP (neuropathy, ataxia, and retinitis pigmentosa) and Leigh syndrome.[10,11] In cancer, mitochondrial dysfunction and ATPase 6 alterations have been reported in various tumors, yet their specific role in bladder cancer remains underexplored, especially in Africa populations.[12]

The study aimed to detect ATPase 6 gene mutations in Sudanese bladder cancer patients using immunohistochemistry (IHC) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and to correlate these findings with demographic and pathological features.

MATERIALS AND METHODS

Study Design

A descriptive retrospective cohort study was conducted at the Radiation Isotopes Center of Khartoum, Ibn-Sina Hospital, and Military Hospital Helipad, covering the period from, 2020 to, 2022.

Study Population and Samples

A total of 90 archived formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients previously diagnosed with bladder cancer were included. Both sexes and age groups were eligible, while specimens with other cancer type were excluded.

Immunohistochemistry

Sections (5 micrometer) were deparaffinized in xylene, then rehydrated via graded dilutions of alcohol followed by running tap water, sections are then pre-treated with boiling sodium citrate, sections treated with 0.3% hydrogen peroxide for 10 min to block endogenous peroxidase, then primary antibody of ATPase 6 with 1: 500. antibodies (Abcam, U.S.A) for 1 h at room temperature, then incubated with a biotin linked secondary antibody for 30 min followed by streptavidin-horseradish peroxidase (30 min) using a reagent kit from Dako (K5001). Immunostaining visualized using DAB for 10 min, followed by light counterstaining with hematoxylin. Positive staining (brown cytoplasmic/nuclear) in more than 5 cells per high-power field was considered overexpression.

DNA extraction and PCR-RFLP

DNA extracted from lesion show malignant feature micro-dissected from, 20 µm transverse sections using fine glass capillaries, transferred to sterile microfuge tubes, each containing 10 µl of cell lysis buffer (50 mMTris–HCl pH 8.5, 1 mM EDTA, 0.5% Tween-20,, 200 µg/ml proteinase K), denature by the proteinase K. The mitochondrial mutation was detected using PCR-RFLP as mtDNA fragment of 402 bp length was amplified with the following primers. For ATPase 6 gene Forward primer 5′-CAACTAACCTCCTCGGAC-3′reverse primer: T8993C Rv 5′-TGAAAACGTAGGCTTGGAT-3′ (9169–9151 bp region) amplified by PCR using 10-20 ng and digested using endonuclease HpaII, gives rise to two bands lengths 224 and 178 bp .mtDNA of normal of unaffected individuals showed only one band of length 402 bp, then separated by 12% polyacrylamide gel electrophoresis. Ethidium bromide-stained gels were then photographed using Geldoc (Bio-Rad Laboratories, Inc., Hercules, CA, USA.

Statistical analysis

Data were analyzed using SPSS version, 20.0. Frequences, percentage, and Chi-square tests were used; $p<0.05$ considered to be statistically significant.

Ethical approval

Ethical clearance was obtained from respective institute and review boards.

RESULTS

A total of 90 bladder cancer patients were included. Male comprised 65.6% (n=59) and female 34.4% (n=31), with a mean age of 62.5 years (30-95). Transitional cell carcinoma accounted for 84.4% (n=76) of cases. Schistosoma infection was present in 26.7% (24) of patients.

Immunohistochemistry and PCR results
IHC showed ATPase 6 overexpression in 11.1% (10/90) of samples. PCR detected ATPase 6 mutations in 11.1% (10/90) of samples.

No statistical significant associations were found between ATPase 6 mutations (by IHC or PCR) and gender, age group, tumor grade tumor type, or Schistosoma infection (Table 2). The agreement between IHC and PCR for ATPase 6 was weak and statistically significant ($p < 0.05$).

Table 1: Frequency of ATPase 6 alterations detected by IHC and PCR.

Method	Positive n (%)	Negative n (%)
IHC	10 (11.1%)	80 (88.9)
PCR	10 (11.1%)	80 (88.9)

Table 2: Association of ATPase 6 mutation (PCR) with clinicopathological parameters.

Parameter	Positive (n=10)	Negative (n=80)	p-value
Male	4(6.8%)	55 (93.2%)	0.07
Age > 60 years	4 (8.3%)	45 (91.8%)	0.486
High grade tumour	5 (14.3%)	30 (85.7%)	0.0376
Schistosoma positive	1 (4.2%)	23 (95.8%)	0.613

DISCUSSION

This study investigated the frequency of mitochondrial ATPase 6 gene mutations in bladder cancer among Sudanese patients using (IHC) and (PCR-RFLP) method. Our result demonstrates a relatively low prevalence of ATPase 6 mutations (11.1 %) of cases by both techniques.

The low mutation rate of ATPase 6 in our cohort is consistent with some earlier studies that suggested ATPase 6 mutations are less frequent than those in other mitochondrial genes like Cytochrome b or D-loop region in certain cancers.[12,13] This may be attributed to the functional constraints of ATPase 6; being acritical component of ATP synthase, sever mutation may be lethal or strongly selected against, whereas milder alterations might not confer.

A significant growth advantage compared to mutations affecting other respiratory complexes.[10] However, a study by Guney et al. reported variable frequences of ATPase 6 mutations in bladder cancer, indicating that population-specific genetic backgrounds and environmental factors may influence mutation spectra.[12]

Our analysis found no statistically significant association between ATPase 6 mutations (by IHC or PCR) and gender, age group, tumor grade tumor type, or Schistosoma infection. This lack of association suggests that while ATPase 6 mutations may contribute to mitochondrial dysfunction, they might not be a primary cause of tumor aggressiveness or progression in this population. The absence of a significant link with schistosomiasis contrast with findings for cytochrome b, possibly because complex V is less exposed to ROS generated by inflammation than complex III, which is a major source of electron leakage.[14]

The weak agreement between IHC and PCR for ATPase 6 highlights the biological differences between these methods. IHC detects protein overexpression, which may result from post-transcriptional regulation or protein stabilization rather than specific restriction site mutations targeted by PCR-RFLP. Furthermore, the use of archival FFPE samples may have compromised DNA quality, potentially leading to false-negative PCR results.[15]

CONCLUSION

Mitochondrial ATPase 6 gene mutations are infrequent (11.1%) among Sudanese bladder cancer patients and show no significant association with age, sex, tumor grade, tumor type, or schistosomiasis infection. While its detection contributes to the molecular profiling of bladder cancer, its role as diagnostic and prognostic biomarker appears limited in the real life. Future studies employing next-generation sequencing are recommended to uncover the mitochondrial mutation in population.

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