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# ASSOCIATION OF MITOCHONDRIAL CYTOCHROME B GENE MUTATIONS WITH SCHISTOSOMIASIS AND AGING IN BLADDER CANCER PATIENTS IN SUDAN

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## ABSTRACT

**Background:** Mutation in Mitochondrial DNA (mtDNA) are increasingly recognized as key events in carcinogenesis. The Cytochrome b (Cyt B) gene, encoding a subunit of respiratory complex III, is a hotspot for mutations in various cancers due to the active role in reactive oxygen species (ROS) generation. **Objective:** This study aimed to detect Cytochrome b gene mutations in bladder cancer tissues using immunohistochemistry (IHC) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and to correlate these alterations with clinicopathological features, particularly Schistosoma infection and aging. **Materials and methods:** A retrospective cohort study was conducted on 90 formalin-fixed paraffin embedded (FFPE) Bladder cancer specimen s from Khartoum state hospitals (2020-2022). IHC was performed using an anti-Cytochrome b antibody. PCR-RFLP with AluI digestion was used to detect mutations. Statistical analysis employed Chi-square tests, with significance at $p < 0.05$. The cohort was predominantly male (65.6%), with a mean age of 62.2 years. IHC Revealed Cytochrome b overexpression in 27.8% (25/90) of samples, while PCR detected mutations infection ($p = 0.043$) and older age ($p = 0.03$). no significant associations were found with sex or tumor grade. Agreement between IHC and PCR was moderate and statistically significant ($p < 0.001$). **Conclusion:** Mitochondrial Cytochrome b gene mutations are highly prevalent in Sudanese bladder cancer patients and are strongly associated with schistosomiasis and advancing age. These findings suggest that chronic inflammation and oxidative stress drive Cytochrome b mutagenesis, positioning it as promising biomarker for early detection and risk stratification, particularly in endemic regions.

**KEYWORDS:** Bladder cancer, Cytochrome b, Immunohistochemistry, Mitochondrial DNA, PCR-RFLP, Schistosomiasis, Sudan.

## INTRODUCTION

Bladder cancer is a major cause of cancer-related morbidity and mortality worldwide, with a particularly high burden in regions where Schistosoma haematobium infection is endemic.[1,2] In Sudan, bladder cancer is significant public health issue health issue, and the interplay between parasitic infection, chronic inflammation, and genetic alterations is critical area of research.[3]

Mitochondria are central to cellular energy metabolism and apoptosis. Mitochondrial DNA (mt DNA) is highly susceptible to oxidative damage due to proximity to electron transport chain (ETC) and lack of protective histones.[4,5] Mutations in mtDNA accumulate with age

and are exacerbated by inflammatory conditions, contributing to the multistep process of carcinogenesis.[6]

The Cytochrome b (CYTB) gene is a component of respiratory complex III (ubiquinol-cytochrome c reductase). It is a frequent target of somatic mutations in cancer, partly because complex III is a major site of reactive oxygen species (ROS) production.[7,8] A well-studied 21-bp deletion in the CYTB gene has been shown to increase ROS levels, promote cell cycle progression, and enhance tumor growth in bladder cancer models.[9] Epidemiological studies have linked chronic inflammation, such as that caused by Schistosomiasis, to increased mtDNA damage, particularly in CYTB gene.[10]

This study aimed to investigate the prevalence of Cytochrome b gene mutations in Sudanese bladder cancer patients and to asses their association with Schistosoma infection, aging, and other clinicopathological parameters using IHC and PCR-RFLP.

## MATERIALS AND METHODS

### Study Design and Area

A descriptive retrospective cohort study was conducted at Radiation and 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 diagnosed with bladder cancer were included. Specimens with other cancer types were excluded.

### Immunohistochemistry (IHC)

Sections (5 mictometer) were deparaffinized, rehydrated, and subjected to antigen retrieval in sodium citrate buffer (Ph 6.0). Endogenous peroxidase was blocked with 0.3% $H_2H_2$. Primary antibody against Cytochrome b (dilution 1:2000) (Abcam, USA) was applied for 1hour. Detection used the LSAB method with DAB as chromogen. Positive staining (brown) in >5 cells per high-power field was considered overexpression.

### DNA Extraction and PCR-RFLP

Genomic DNA was extracted from microdissected tumor areas (20 µm transverse section) using proteinase K digestion.

PCR amplification for cytochrome b (440 bp fragment) used forward primer TCATCGACCTCCCCACCCCATC, Reverse, CGTCTCGAGTGATGTGGGCGATT. PCR products were digested with AluI: wild-type mtDNA showed asingle band at 440bp, while mutant mtDNA yield two fragments at 273bp and 167 bp. Fragments were resolved on 12% polyacrylamide gel.

### Statistical Analysis

SPSS version 20.0 was used. Chi-square test were applied; $p < 0.05$ was considered significant.

### Ethical Approval

Ethical approval was obtained from relevant institutional review bords.

## RESULTS

The study included 90 bladder cancer patients (65.6% male, mean age 62.2 years). Transitional cell carcinoma was predominant type (84.4%). Schistosoma infection was present in 26.7%.

### Immunohistochemistry and PCR Result

IHC showed Cytochrome b overexpression in 27.8% (25/90) of samples. PCR detected Cytochrome b mutations in 33.3% (30/90) of samples (Table1).

| Method | Positive n (%) | Negative n (%) | Total |
|---|---|---|---|
| IHC | 25 (27.8%) | 65 (72.2%) | 90 |
| PCR | 30(33.3%) | 60 (66.7%) | 90 |

### Association with clinicopathological factors

Cytochrome b mutations (PCR) showed a statistically significant association with Schistosoma infection: 55% (11/20) of infected patients had mutations versus 31% (19/60) of non-infected (p= 0.043). mutations were also significantly associated with older age (> 60 years) (p = 0.03). No significant associations were found with gender or tumor grade (Table 2). Agreement between IHC and PCR was moderate and statistically significant ($p < 0.001$).

**Table 2: Association between Cytochrome b mutation (PCR) with clinicopathological parameters.**

| Parameter | Positive (n =30) | Negative (n= 60) | P-value |
|---|---|---|---|
| Male | 19 (32.2%) | 40 (67.8%) | 0.0875 |
| Age > 60 | 18 (48.6%) | 19 (51.4%) | 0.03 |
| High-grade tumor | 12 (34.3%) | 23 (65.7%) | 0.824 |
| Schistosoma positive | 11 (55.0%) | 9 (45.0%) | 0.043 |

Significant at p< 0.05

## DISCUSSION

This study demonstrates a high prevalence of mitochondrial Cytochrome b gene mutations in Sudanese bladder cancer patients, detected in 33.3% of cases by PCR. This frequency is consisted with previous international studies, including those by Guney et al. (Turkey) and Dasgupta et al. (USA), which reported frequent CYTB alteration in bladder cancer.[11,12] The higher mutation rate of CYTB compared to ATPase 6 (11.1) in our cohorts highlights the particular vulnerability of complex III to mutagenesis, likely due its role as a primary source of ROS within the ETC.[7]

This most compelling finding of this study is the strong and significant association between Cytochrome b mutations and Schistosoma infection ($p = 0.043$). This provide molecular evidence linking chronic parasitic inflammation to mitochondrial genomic instability. S.haematobium eggs deposited in the bladder wall elicit a robust granulomatous inflammatory response, generating high levels of ROS and reactive nitrogen species. These species can directly damage mtDNA, because mt DNA repair mechanisms are limited, mutations accumulate over time.[3,10] This chronic oxidative milieu likely selects for cells with CYTB mutations that further increase ROS production, creating vicious cycle that drives carcinogenesis, as previously demonstrated by Dasgupta et al.[9,12]

The significant association between CYTB mutations and older age ($p = 0.03$) aligns with the known accumulation of somatic mtDNA mutations over a lifespan due to cumulative oxidative damage and declining mitochondrial repair efficiency.[13] This age-related accumulation may compound the effects of chronic Schistosomiasis, increasing the risk of bladder cancer in older Sudanese populations.

The moderate but significant agreement between IHC and PCR for CYTB ($p < 0.001$) suggests that protein overexpression, detected by IHC may also capture cases where proteins stability is altered without underlying mutations in the screened restriction site, support the utility of IHC as cost-effective screening tool for mitochondrial dysfunction in routine pathology laboratories.

The lack of association with tumor grade suggests that CYTB mutations may occur early in carcinogenesis, potentially serving as initiator rather than driver of progression. This supports the potential of CYTB mutations as early detection biomarker, especially in high-risk populations.[15]

## CONCLUTION

Mitochondrial Cytochrome b gene mutations are highly prevalent in Sudanese bladder cancer patients and are significantly associated with Schistosoma infection and aging. This study highlights the pathogenic link between chronic inflammation, oxidative stress, and mitochondrial mutagenesis. CYTB mutations emerge as promising candidate biomarker for early detection and risk stratification, particularly in Schistosomiasis-endemic regions. Larger prospective studies employing whole-genome sequencing are warranted to validate these findings and explore the functional consequences of specific mutation.

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