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RESEARCH

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Genomic analysis of BAX and Bcl-2 gene mutations in human papilloma virus-associated squamous cell carcinoma of the cervix

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Abstract

Purpose The aim of the current study was to molecularly determine Bcl-2 and BAX gene mutations in HPV-associated squamous cell carcinoma of the cervix.

Methods Formalin-fixed, paraffin-embedded tissue blocks, all consisting of squamous cell carcinoma of the cervix, were used for this study. The nucleic acid amplification technique and various steps for DNA sequencing, including DNA extraction and polymerase chain reaction, were used.

Results Mutations were detected in the Bcl-2 gene of patients with squamous cell carcinoma of the cervix in the 10–860 bp region, while BAX gene mutations were detected in the 10–320 bp region. The nucleotide mutations in the Bcl-2 gene were A > G (50%), C > T (33.33%), and G > A > T (16.67%), while the BAX gene mutations were A > (16.67%), T > (16.67%), G > (16.67%), A > C (16.67%), T > G (16.67%), and T > C (16.67%). The mutations in the BAX gene were Indel (50%), Transversion (33.4%), and Transition (16.6%), while only the Transition mutation (100%) was detected in the Bcl-2 gene. The functional mutations in the BAX gene were only missense mutations (100%), but in the Bcl-2 gene, the functional mutations were missense (50%) and silent (50%) mutations.

Conclusion Our findings revealed genomic mutations of different types and frequencies in the BAX and Bcl-2 genes in squamous cell carcinoma of the cervix, which should encourage further research to better understand these mutations and exploit them for clinical use.

Keywords Cervical cancer, Bcl-2, BAX, HPV, Mutation signatures

Introduction

Cervical cancer is a slow-growing cancer that originates in the cervix of women and is one of the most common malignant cancers globally. It poses a significant threat to the life and health of women, with approximately 604,000 new cases reported annually, 75–80% of which occur in developing countries (Sung et al. 2021). In Nigeria, cervical cancer is the second most common cancer among women aged 15 to 44, with more than 14,000 new diagnoses and more than 20 deaths daily (Ogundipe et al. 2023). The development of cervical cancer typically begins at the junction of the cervical squamous column, progressing from atypical hyperplasia of the cervical epithelium to intraepithelial carcinoma in situ, invasive carcinoma, and ultimately metastatic carcinoma, a process that can last several years or even more than a decade (Ekundina et al. 2021). Research has indicated that factors such as premature or disordered sexual activity, viral infections (e.g., human papillomavirus), dietary habits, and early pregnancy are all associated with an increased risk of cervical cancer (Fowler et al. 2023).

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Human papillomavirus (HPV) infection is a major risk factor for cervical cancer, accounting for more than 90% of cases (Siegel et al. 2017). HPV is the most common sexually transmitted infection and can cause multiple benign or malignant cancers in more than 500,000 individuals annually (Evande et al. 2023). Squamous cell carcinoma and adenocarcinoma represent approximately 70% and 25%, respectively, of all cervical cancers caused by HPV infection (Okolie et al. 2022). Despite the popularity of cervical cytology screening, HPV vaccines, and increased awareness of protection, cervical cancer remains a significant public health concern (Ogundipe et al. 2023). Early prevention and diagnosis are crucial for the effective treatment of cervical cancer. Research has shown that the incidence of cervical cancer is greater among female first-degree relatives, indicating a potential role for genetic factors in the development of this disease (Zhang et al. 2020). This finding has prompted researchers to investigate susceptibility and mutated genes associated with cervical cancer.

B-cell lymphoma 2 (Bcl-2), encoded by the Bcl-2 gene in humans, is a key member of the Bcl-2 family of regulatory proteins that controls cell death (apoptosis) by either inhibiting (antiapoptotic) or inducing (proapoptotic) apoptosis (Ekundina et al. 2021). The Bcl-2 family of proteins shares a similar structure, consisting of a hydrophobic α-helix surrounded by amphipathic α-helices (Hu et al. 2021). Bcl-2 is located on the outer membrane of mitochondria, where it plays a crucial role in promoting cell survival and inhibiting the actions of proapoptotic proteins (Ekundina et al. 2023). Mutations in the Bcl-2 gene have been linked to various cancers, including melanoma, prostate cancer, breast cancer, chronic lymphocytic leukemia, and lung cancer, as well as potential associations with schizophrenia and autoimmunity. Additionally, it can lead to resistance to cancer treatments (Garcia-Aranda et al. 2018). The BAX gene (Bcl-2 associated X-protein) is a pro-apoptotic member of the Bcl-2 gene family, encoding the 21-kDa BAX-alpha protein, which is essential for regulating intrinsic apoptosis in relation to Bcl-2 (Carpenter & Brady 2023). The expression of BAX is upregulated by the tumor suppressor protein p53, and BAX has been shown to be involved in p53-mediated apoptosis. The p53 protein, a transcription factor activated in response to cellular stress, regulates numerous downstream target genes, including BAX. Therefore, p53 likely promotes the apoptotic functions of BAX in vivo as a primary transcription factor (Borrero & El-Deiry 2021).

Persistent infections with high-risk human papillomaviruses (hrHPVs) are the primary cause of cervical cancer. While most Bcl-2 homologs prevent cell death, a subset, such as BAX, is pro-apoptotic. BAX was initially identified as an inhibitory binding partner of Bcl-2 (Rahman et al. 2020). The pro-death function of BAX is activated in response to various harmful events inside or outside the cell, leading to conformational changes, membrane insertion, and oligomerization to form a channel or other structure in the mitochondrial outer membrane (Wolf et al. 2022). The role of Bcl-2-like antiapoptotic proteins is to inhibit their proapoptotic partners, leading to the original rheostat model in which the balance between counteracting anti- and proapoptotic BCL-2 family proteins determines cell fate (Qian et al. 2022). The ratio of Bcl-2 to BAX expression determines survival or death following an apoptotic stimulus (Ekundina et al. 2022). Investigating mutations in the BAX and Bcl-2 proteins in HPV-related squamous cell carcinoma of the cervix is an important step in establishing a new predictor of the outcome of diagnosis and treatment for human cervical carcinoma. Therefore, the purpose of this study was to determine possible mutations in the Bcl-2 and BAX genes in HPV-associated squamous cell carcinoma of the cervix.

Materials and methods

Study design

This research utilized a retrospective survey design to investigate mutations in the Bcl-2 and BAX genes in patients with squamous cell carcinoma of the cervix.

Tissue sample collection

A total of 10 formalin-fixed, paraffin-embedded tissue blocks, all containing squamous cell carcinoma of the cervix, were obtained from University College Hospital (UCH) Ibadan, Oyo State, Nigeria.

Molecular techniques

The molecular techniques for DNA sequencing involve several steps, including DNA extraction, polymerase chain reaction, isolation of the gene of interest, integrity testing, purification, and DNA sequencing.

DNA extraction

DNA extraction from human tissue was performed using the Dellaporta DNA extraction protocol with minor modifications. Briefly, each tissue sample was ground using a sterile mortar and pestle with 500 µl of extraction buffer and then transferred to a sterilized Eppendorf tube. Next, 33 µl of 20% sodium dodecyl sulfate (SDS) was added, and the mixture was vortexed and incubated in a water bath at 65 °C for 10 min. After incubation, 10 µl of 5 M potassium acetate was added at room temperature, followed by vortexing and centrifugation at 1000×g for 10 min. The supernatant was collected in another Eppendorf tube, and 300 µl of cold Iso-propanol

was added, mixed gently, and incubated at 20 °C for 60 min. The DNA was then pelleted by centrifugation at 13,000 × g for 10 min, and the supernatant was carefully decanted without disturbing the pellet. The DNA pellet was washed with 500 µl of 70% ethanol by centrifuging at 1000 × g for 10 min. The ethanol was decanted, and the DNA was air-dried at room temperature until no trace of ethanol was visible in the tube. Finally, the pellet was resuspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA (Ekundina et al. 2023).

Polymerase chain reaction

The PCR sequencing preparation cocktail for all PCRs consisted of 10 µl of 5 × GoTaq colorless reaction buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 pmol of each primer, and 0.3 µl of Taq DNA polymerase (Promega, USA), resulting in 35 µl of sterile distilled water. Additionally, 1.5 µl of DNA template was added. PCR was carried out in a GeneAmp 9700 PCR System Thermocycler (Applied Biosystems Inc., USA) with a specific PCR profile for each primer, as presented in the table below (Evande et al. 2023).

Gene Name	Primer Name	Primer Sequence	PCR profile
Bcl-2	Bcl2F	CCAGACTCACAT CACCAAGT	An initial denaturation at 94 °C for 5 min; followed by a 30 cycle consisting of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 1 min and a final termination at 72°c for 10 min
BAX	BAXF	CACCACTTCCTG CCTCTG	An initial denaturation at 94 °C for 5 min; followed by a 30 cycle consisting of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 1 min and a final termination at 72°c for 10 min
	BAXR	GACACTCGCTCA GCTTCTT	

Integrity

The integrity of the amplified gene fragment was confirmed by electrophoresis on a 1.5% agarose gel. The gel was prepared using 1 × TAE buffer and stained with 3 µl of 0.5 g/ml ethidium bromide. After solidifying, the gel was loaded with 2 µl of 10 × blue gel loading dye and 4 µl of each PCR product. Electrophoresis was carried out at 120 V for 45 min, and the gel was visualized under ultraviolet transillumination. The size of the PCR products was estimated by comparing their mobility with a 100 bp DNA ladder run alongside the samples (Hu et al. 2021).

Purification

Following gel electrophoresis, the amplified fragments were purified using ethanol. The purified fragments were then checked on a 1.5% agarose gel and quantified using a NanoDrop Model 2000 from Thermo Scientific (Ekundina et al. 2023).

Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems with the Big Dye Terminator v3.1 cycle sequencing kit. BioEdit software and MEGA 6 were used for genetic analysis (Ekundina et al. 2023).

Data analysis

The lengths of the Bcl-2 and BAX isoforms were measured using Kapelan Bio-Imaging Solutions software version 2.7.2. The data obtained from the study were analyzed in Microsoft Excel using simple frequencies and percentages. The results are presented in tables, charts, and graphs.

Results

Figure 1 shows an agarose gel with positive amplification of the BAX gene from DNA isolated from 10 tissue blocks using BAX gene-specific primers. The presence of a 550 bp band indicates positive amplification of the extracted DNA. Figure 2 displays an agarose gel with positive amplification of the Bcl-2 gene from DNA isolated from 10 patients using Bcl-2 gene-specific primers. The presence of an 870 bp band indicates positive amplification of the extracted DNA. Figure 3 shows the functional mutations of the BAX and Bcl-2 genes in squamous cell carcinoma. The results revealed two functional mutations in the Bcl-2 gene, missense (50%) and silent (50%) mutations, while the BAX gene had only one missense mutation (100%).

Table 1 showed the demographic characteristics of the subjects studied. Ten female subjects were recruited for this study of which 20% belonged to age group 20 – 30 years, 30% to age group 31 – 40 years and 50% belonged to age group 41 – 50 years. Majority of the subjects were married (70%), Christians (70%), live in urban areas (60%), had secondary education (40%), and were employed (40%).

Table 2 presents the NCBI BLAST results showing the identity of the fragment amplified with BAX-specific primers. The results indicate that squamous cell carcinoma of the cervix has a (100%) Query cover, an E-value of 0%, and a percentage identity of 100%, except for SCC-1, SCC-2, SCC-3, SCC-5, SCC-8, SCC-9, and SCC-10, which show 99.64%, 99.64%, 99.82%,

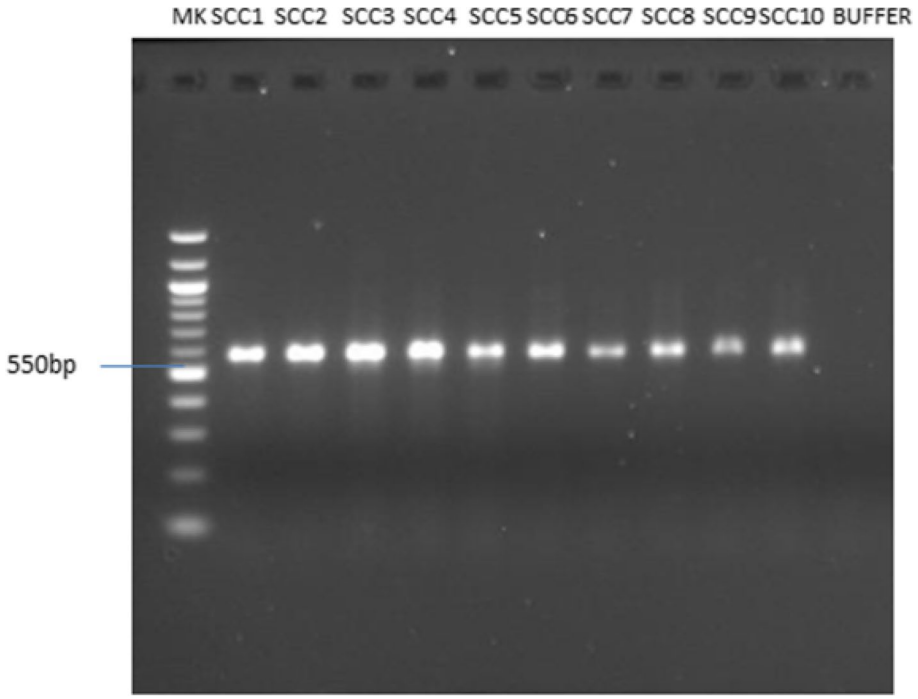

Fig. 1 Agarose gel showing positive amplification of the BAX gene

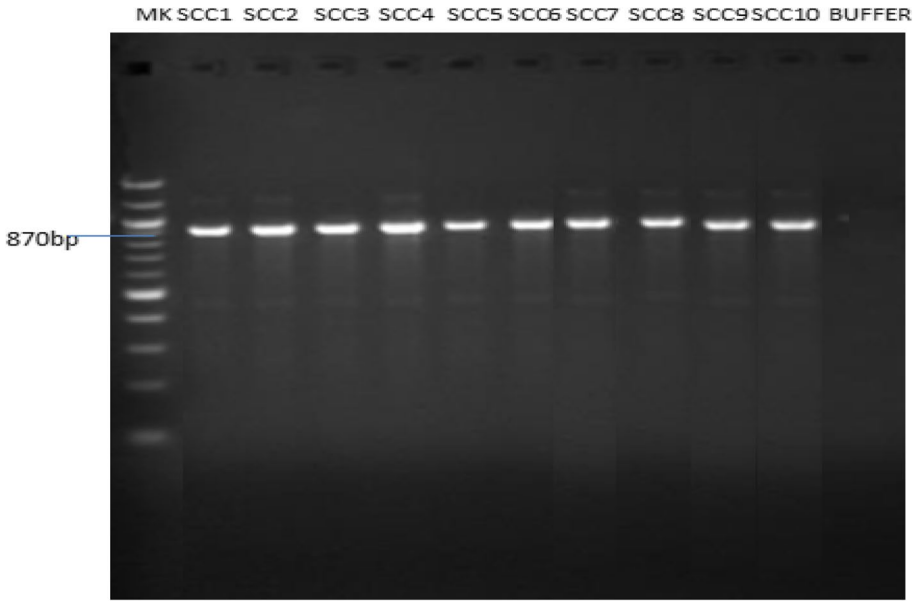

Fig. 2 Agarose gel showing BCL-2 gene amplification

99.82%, 99.64%, 99.82%, and 99.82%, respectively, of the fragments amplified with the BAX gene in each sample. Similarly, Table 3 shows the NCBI BLAST results for the fragment amplified with the Bcl-2-specific primers, which indicated that the sequence of the Bcl-2 gene amplified from the squamous cell carcinoma sample had 100% Query coverage and 99% identity, suggesting that the primers used for this investigation were effective.

Table 4 displays the percentage occurrence of the mutational signature along the BAX and Bcl-2 gene segments. The results showed that the prevalence of the

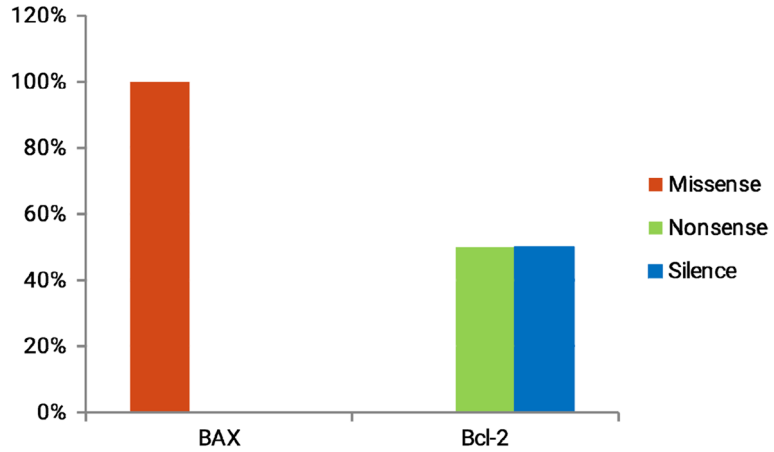

Fig. 3 Functional mutations of the BAX and Bcl-2 genes in squamous cell carcinoma

Table 1 Demographic characteristics of the subjects

Variable	Number (n)	Percentage (%)
Age (years)		
20 – 30	2	20.0
31 – 40	3	30.0
41 – 50	5	50.0
Marital status		
Single	2	20.0
Married	7	70.0
Divorced	1	10.0
Educational status		
Primary	3	30.0
Secondary	4	40.0
Tertiary	3	30.0
Occupation		
Government employed	4	40.0
Self employed	4	40.0
Unemployed	2	20.0
Religion		
Christian	7	70.0
Muslim	2	20.0
African Traditional Religion	1	10.0
Residence		
Urban	6	60.0
Rural	4	40.0
Parity		
Nulliparous	3	30.0
1 – 2 births	6	60.0
3 & above	1	10.0

mutational signature within the BAX gene region was equal, including A > (16.67%), T > (16.67%), G > (16.67%), A > C (16.67%), T > G (16.67%), and T > C (16.67%), while that of the Bcl-2 gene was A > G (50%), C > T (33.33%), and G > A > T (16.67%). Table 5 presents the frequency of SNPs in the Bcl-2 and BAX genes in patients with squamous cell carcinoma. The only SNP mutation in the Bcl-2 gene was a transition mutation (100%), while the corresponding mutations in the BAX gene were Indel (50%), Transversion (34.4%), and Transition (16.6%).

Discussion

In this study, we observed point mutations in the Bcl-2 gene of patients with HBV-associated squamous cell carcinoma of the cervix at positions 10, 270, 300, 410, and 850. The BAX gene also showed mutations at positions 10–340. This suggests that dysregulation of the BAX and Bcl-2 genes in cervical cells can disrupt the apoptotic pathway, leading to increased cell survival and potentially contributing to tumor formation (Kale et al. 2018). This finding is consistent with previous studies that reported mutations in the BAX and Bcl-2 genes in various cancers (Kale et al. 2018; Pal & Kundu 2020; Ramachandran & Dork 2021; Feng et al. 2022). Changes in BAX gene expression or mutations have been shown to affect the immune system's ability to recognize and eliminate cancer cells, potentially influencing the efficacy of immunotherapy (Murthy et al. 2021). The BAX and Bcl-2 gene mutations observed in cervical carcinoma are associated with inactive p53 (Ekundina et al. 2023), either due to mutations in C-33A and HT-3 or via complexation and degradation with the HPV 16/18 E6 protein (HeLa and CaSki). Increased Bcl-2 expression under conditions of p53 inactivation may provide cells with a selective advantage for survival and consequently play a role in the development of cervical carcinogenesis (Pal & Kundu 2020).

Table 2 NCBI BLAST results showing the identity of the fragment amplified with BAX-specific primers

Sample ID	Description	Query Cover	E value	% Identity
SCC1	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	99.64%
SCC2	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	99.64%
SCC3	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	99.82%
SCC4	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	100.00%
SCC5	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	99.82%
SCC6	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	100.00%
SCC7	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	100.00%
SCC8	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	99.64%
SCC9	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	99.82%
SCC10	Homo sapiens BCL2 associated X, apoptosis regulator (BAX)	100%	0	99.82%

Table 3 NCBI BLAST results showing the identity of the fragment amplified with Bcl-2-specific primers

Sample ID	Description	Query Cover	E value	Percentage Identity
SCC1	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC2	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC3	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC4	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC5	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC6	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC7	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC8	Homo sapiens BCL2 (BCL2) gene	100%	0	99.77%
SCC9	Homo sapiens BCL2 (BCL2) gene	100%	0	99.54%
SCC10	Homo sapiens BCL2 (BCL2) gene	100%	0	99.66%

Table 4 Percent occurrence of mutational signatures along the BAX and Bcl-2 gene segments

Gene	Mutational Signature	Number of occurrence (frequency)	Percentage (%) of Mutation Signature
BAX	A	1	16.67%
	T	1	16.67%
	G	1	16.67%
	A > C	1	16.67%
	T > G	1	16.67%
	T > C	1	16.67%
Bcl-2	A > G	3	50%
	G > A > T	1	16.67%
	C > T	2	33.33%

Furthermore, the specific mutational signatures in the BAX gene associated with cervical cancer were A > (16.67%), T > (16.67%), G > (16.67%), A > C (16.67%), T > C (16.67%), and T > G (16.67%), while the mutational signatures of the Bcl-2 gene were A > G (50%), G > A > T (16.67%), and C > T (33.33%). These findings are consistent with previous studies (Halle et al. 2021; Niyazi et al. 2023) indicating the occurrence of specific mutations at certain positions within the BAX and Bcl-2 genes in cervical cancer. However, it is important to note that mutational signatures can differ significantly depending on various factors, such as the population studied, mutation prevalence in samples, and specific cancer types (Igor et al. 2018; Hu et al. 2023).

Our study of cervical cancer patients revealed that mutations in the Bcl-2 gene were exclusively transition mutations, which involve interchanges of purine bases (adenine [A] or guanine [G]) or pyrimidine bases (cytosine [C] or thymine [T]) within the same chemical category. This suggests that the SNPs in the Bcl-2 gene follow a transition mutation pattern. Conversely, the greater percentage of Indel mutations in the BAX gene compared to transversion may indicate that transversion has a greater regulatory effect than does transition. This could be because transversion mutations alter the minor groove width and roll of DNA more than do transition mutations, leading to a greater impact on the binding of

Table 5 Summary table showing gene mutations in the BAX and Bcl-2 genes

Gene type	Mutation type	Description	Location/frequency	Mutation type
BAX	Indel (50%)	A		Deletion
		T	112 (1:1)	Cysteine deletion
		G	223 (4:1)	Valine deletion
	Substitution (0%)			
	Transversion (33.4%)	A:C	4 (7:3)	Missense mutation changing Threonine to proline
		T:G	119 (4:1)	Missense mutation changing Isoleucine to Arginine
	Transition (16.6%)	T:C	76 (3:2)	Missense mutation changing tyrosine to histidine
Bcl-2	Transistion (100%)	A:G	6 (9:1)	Silent mutation retaining threonine
		G:A:T	306(8:1:1)	Silent mutation retaining threonine
		A:G	412(3:7)	Missense mutation changing threonine to Alanine
		A:G	855(1:4)	Silent mutation retaining Glutamine
		C:T	266(1:9)	Missense mutation changing valine to Alanine
		C:T	305(9:1)	Missense mutation changing threonine to Isoleucine

transcription factors, as supported by previous findings (Pal & Kundu 2020; Ekundina et al. 2023). Transversion mutations are more detrimental because they are more likely to cause substitutions that radically alter the biochemical properties of the original amino acid (Ekundina et al. 2023).

In this study, we found that missense mutations were the only functional mutations observed in the BAX gene of HPV-associated squamous cell carcinoma of the cervix, while both missense and silent mutations were found in the Bcl-2 gene. This finding is consistent with previous studies (Konig et al. 2019; Kumari & Rameshwari 2022; Su et al. 2022) reporting that missense mutations can potentially impact the function of the BAX and Bcl-2 genes, leading to changes in the regulation of programmed cell death and the production of new proteins (Su et al. 2022). A specific amino acid substitution resulting from a missense mutation can influence protein structure and function, potentially leading to abnormal cellular processes and contributing to the development or progression of squamous cell carcinoma of the cervix (Liu et al. 2021). On the other hand, silent mutations do not alter the protein sequence but can still have functional consequences through other mechanisms, such as affecting RNA splicing, stability, or translation efficiency (Patel et al. 2019).

Conclusion

In conclusion, our findings revealed various genomic mutations in the BAX and Bcl-2 genes in HPV-associated squamous cell carcinoma of the cervix. This discovery should motivate further research aimed at gaining a better understanding of these mutations and utilizing them for clinical purposes. Studying BAX and Bcl-2 gene mutations in these patients offers valuable insights into the molecular mechanisms underlying the development and progression of cervical cancer, as well as its prognosis and potential therapeutic options.

Author's contributions

The conceptualization, design and analysis were performed by VOE, and EAO was responsible for the original draft and analysis. Review and editing were performed by VOE and EAO. Both authors (VOE and EAO) read and approved the final manuscript.

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Availability of data and materials

The data from this study will be available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Health Research Ethics Committee, University College Hospital (UCH) Ibadan, Oyo State, Nigeria.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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